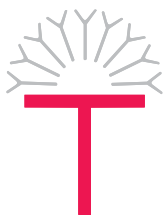**Microxpress®**
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A Handbook of
BEST PRACTICES

Committed to the advancement of Clinical & Industrial Disinfection & Microbiology

Foreword

Microxpress® and **BioShields**® are company divisions of the leading manufacturer and exporter of in-vitro diagnostics kits and reagents, **Tulip Diagnostics (P) Ltd.**

With14 manufacturing plants and exports to over 88 countries world wide, **Tulip Diagnostics** is foraying into newer areas of diagnostics.

While its division **Microxpress**® is into manufacture of microbiology products such as dehydrated culture media, bases, supplements, antibiotic sensitivity discs, ready prepared media, stains, analytical reagents etc., **BioShields**® is dedicated to manufacturing high technology disinfectant products.

The Journal of Hygiene Sciences is a bimonthly newsletter jointly presented by **Microxpress**® and **BioShields**® to provide customers with updated knowledge related to areas of disinfection in Clinical & Industrial segments as well as other important microbiology aspects. Some important topics covered in this bimonthly are current trends, mini review, in profile, bug of the month and best practices to name a few. This newsletter is freely available on our website: www.microxpress in

A Handbook of Best Practices is a compilation of well researched articles on Best Practices covering Microbiology, Disinfection and General Hygiene section presented in the Journal of Hygiene issues.

We hope the contents of this handbook is informative, interesting and educational.
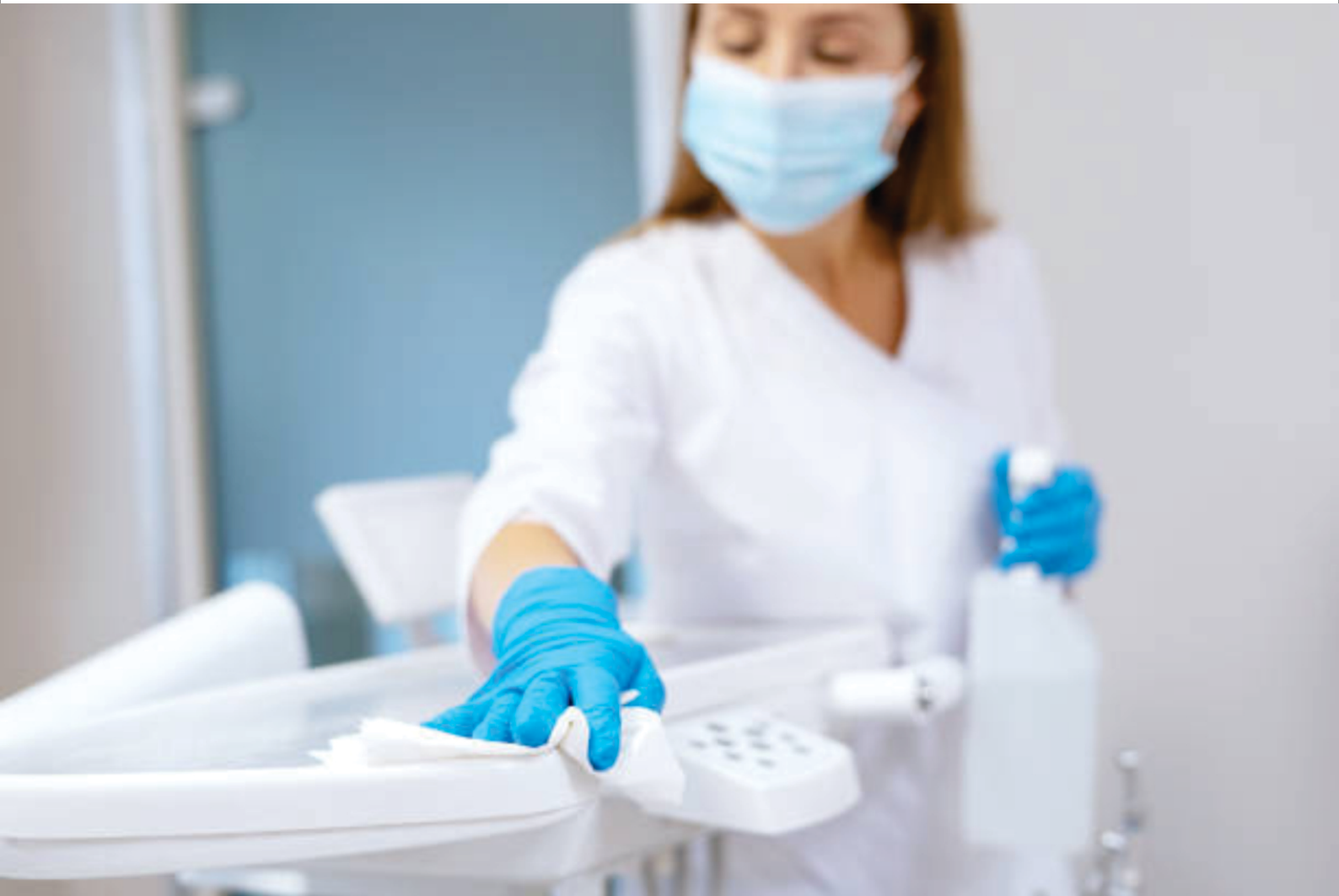
Happy Reading!

A Handbook of
BEST PRACTICES

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DISINFECTION

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Hand Hygiene Techniques (I)

Hand hygiene is a means of achieving a reduction in, or removal of visible soiling, transient or resident microbes and or other hazardous or toxic substances.

Historical perspective

For over a century, skin hygiene, particularly of the hands, has been accepted as a primary mechanism to control the spread of infectious agents. The concept of cleansing hands with an antiseptic agent probably emerged in the early 19th century. In 1846, Ignaz Semmelweiss realized the significance of hand transfer pathogenic bacteria during an outbreak of puerperal fever at obstetrics ward of General Hospital of Vienna. At the same time Dr. Oliver Wendell Holmes had concluded health care practitioners transmitted that puerperal fever. As a result of the seminal studies by Semmelweiss and Holmes, hand washing generally became accepted as one of the most important measures for preventing transmission of pathogens in health care facilities. But hand hygiene awareness started growing exponentially since the early 20th century. Then CDC recommended a guideline for hand washing with non antimicrobial soap between the majority of patient contacts and washing with antimicrobial soap during invasive procedures in 1975 & 1985. Recent studies and developments are going on to improve hand hygiene practices in health care facilities.

Physiology and microflora of the skin

In order to understand the principles of safe hand washing, one must understand the physiology and normal flora of the skin. The primary function of the skin is to reduce water loss, provide protection against abrasive action and microorganisms act as a permeability barrier to the environment. Skin basically composed of four layers, considering from outer layer to inner most layer, the superficial region, the viable epidermis, the dermis and hypodermis. The dermis and subcutaneous tissues are free from micro flora. Bacterial floras are on and within the epidermis and can become established in the hair follicles and in the sweat and sebaceous glands.

Normal human skin is colonized with microorganisms and the skin provides nutrients for selected colonizing microbes in the form of lipids and proteins (keratin). Micro flora of the skin can be classified as resident flora and transient flora.

Resident floras are considered as permanent inhabitants of the skin and are more resistant to removal. Coagulase negative Staphylococci, members of the *Corynebacterium*, *Propionibacterium* and *Acinetobacter* and certain members of the Enterobacterales family reside to the resident micro flora. The presence of resident micro flora on the skin prevents to colonize other pathogenic microorganism. Transient micro flora are those, which are picked up during daily activities and may be shed on skin scales. This flora mainly colonize the superficial layers of the skin and can be effectively removed or substantially reduced to a low level by hand washing or by using some antiseptic hand rub. Transient microorganisms can be of any type, from any source with which the body has had contact. This flora mainly includes *Escherichia coli*, *Salmonella*, *Shigella*, *Clostridium perfringens* and Hepatitis A virus. High level of transient microorganisms attaches to hand, fingertip and fingernail surfaces when hands are not properly washed. A resident flora is considered as permanent inhabitants of the skin and is more resistant to removal.

Significance of Hand Hygiene

Health care associated infections are an important cause of morbidity and mortality among hospitalized patients worldwide. Transmission of health care associated pathogens most often occurs via the contaminated hands of health care workers. Effective hand hygiene removes transient microorganisms, dirt and decreases the risk of cross contamination from patients, patient care equipment and the environment. Hand hygiene is the single most important strategy to reduce the risks of transmitting organisms from one person to another or from one site to another on the same patient. Cleaning hands promptly and thoroughly between patient contact and after contact with blood, body fluids, secretions, excretions, equipment and potentially contaminated surfaces is an important strategy for preventing health care associated infections.

Indications and Guidelines for Hand Hygiene Decision Making

Following indications and guidelines are given for proper hand hygiene decision-making. Hands must be decontaminated:

- Immediately before and after each and every episode of direct patient contact.
- After contact with body fluids or excretions, mucous membranes and wound dressings.
- On arrival to and before leaving the work place.
- Before and after manipulating any invasive device.
- Before and after undertaking clinical procedures.
- Before donning sterile gloves and also after removing gloves.
- Before and after handling food.
- After using restroom.

The choice of using alcohol based hand rub, anti microbial soap or surgical hand preparation is based on:

- The degree of hand contamination.
- The degree of activity which requires less or reduced bio burden.
- Transmission and patient risk factors.
- Invasive or surgical procedure.

Alcohol based hand rub is used to destroy transient flora and part of resident flora on unsoiled hands. Anti microbial soap can be used to remove soil and transient flora. For surgical hand asepsis it is necessary to remove transient flora and reduce resident flora by using either anti microbial disinfectant or alcohol and chlorhexidine based preparation.

Techniques of Hand Hygiene

Different types of hand hygiene techniques are described below.

Procedure for hand washing:

i. Hand washing takes only 1-1.5 minutes. Person should stand near sink, but should avoid touching it, as the sink itself may be a source of contamination.

ii. In case of lever operated paper towel dispenser person should dispense a portion of towel before washing hands.

iii. Washing hands with high level disinfectant is recommended.

iv. Person should wet their hand with tepid water. Splashing should be avoided. Hands, sleeves and clothing should keep away from moisture. Use of hot water is avoided because repeated exposure to hot water may increase the risk of dermatitis.

v. Person should apply the amount of product necessary to cover all hand surfaces.

- Hands are rubbed palm to palm.
- Right palm over left dorsum with interlaced fingers interlocked.
- Palm to palm with fingers interlaced.
- Backs of fingers to opposing palms with fingers interlocked.
- Rotational rubbing of left thumb clasped in right palm and vice versa.
- Hands are rinsed with water.
- Hands are dried thoroughly with a single use/disposable towel.
- Towel should be used to turn off the faucet for handle-operated faucets to prevent contaminating your hands.

Procedure for using alcohol based hand rub:
Onto hands that are not visibly soiled, the person should apply product to palm of one hand and rubs hand together, covering all surfaces of hands fingers until hands get dry. Manufacturer's recommendations should be followed for product volume and this procedure should take approximately 20 seconds.

Procedure for surgical hand antisepsis:
i.   All the jewelleries and watches should be removed before beginning the surgical hand scrub.
ii. Debris is removed from underneath fingernails using a disposable nail cleaner under running water.
iii. Surgical hand antisepsis using an anti microbial soap or an alcohol based hand rub with persistent activity is recommended before donning sterile gloves when performing surgical procedures.
iv. Contact time of scrubbing should be 2-6 minutes. Long scrub time is not necessary.
v.   The manufacturer's instructions are followed while using an alcohol based hand rub with persistent activity. Before applying the alcohol solution pre wash of hands and forearms should be done with a non-anti microbial soap and dried completely.
vi. After application of the alcohol-based product as recommended hands and forearms should allow drying thoroughly before donning sterile gloves.

Selection of Hand Hygiene agents and facilities for its potential use

While selecting a hand hygiene agent following things should be considered:
▪ Personnel should provide with efficacious hand hygiene products that have low irritancy potential, particularly when these products are used multiple times per shift. This recommendation applies to products used for hand antisepsis before and after patient care in clinical areas and to products used for surgical hand antisepsis by surgical personnel.
▪ To maximize the acceptance of hand hygiene by personnel the product is included with some special characteristics like fragrance, colours and consistency (i.e. feel). For soaps, ease of lathering also may affect user performance.
▪ The product should be provided with skin emollients to reduce the skin irritancy and dryness.

Following facilities should be provided to increase the potentiality of the hand hygiene products:
▪ Hand washbasins should be easily accessible.
▪ Warm water must be available for hand washing in all clinical areas, by means of mixer taps or temperature controlled water.

▪ Liquid dispensers should function properly and deliver an appropriate volume of product.
▪ Disposable hand towels should be used instead of using communal hand towels or hot air driers.

Some administrative measures should be taken to increase the potentiality of the procedure:
▪ Improved hand-hygiene adherence should be made as an institutional priority and appropriate administrative support and financial resources should be provided.
▪ A multidisciplinary program should be implemented to improve adherence of health personnel to recommended hand-hygiene practices.
▪ As part of a multidisciplinary program to improve hand-hygiene adherence, HCWs should be provided with a readily accessible alcohol-based hand-rub product.
▪ To improve hand-hygiene adherence among personnel who work in areas in which high workloads and high intensity of patient care are anticipated, an alcohol-based hand rub should be made available at the entrance to the patient's room or at the bedside, in other convenient locations, and in individual pocket-sized containers to be carried by HCWs.

The following performance indicators are recommended for measuring improvements in HCWs' hand-hygiene adherence:
(a) Periodically monitor and record adherence should be done as the number of hand-hygiene episodes performed by personnel/number of hand-hygiene opportunities, by ward or by service. Feedback should be to personnel regarding their performance.
(b) The volume of alcohol-based hand rub (or detergent used for handwashing or hand antisepsis) should be monitored used per 1,000 patient-days.
(c) Monitor adherence to policies dealing with wearing of artificial nails.
(d) When outbreaks of infection occur, assess the adequacy of health-care worker hand hygiene.

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Hand Hygiene Techniques (II)

Pathogens transferred not only from infected or draining wound but also from frequently colonized areas of normal, intact patient skin. The perineal or inguinal areas tend to be most heavily colonized, but the axillae, trunk, and upper extremities (including the hands) are also frequently colonized.

The number of organisms such as *S. aureus, Proteus mirabilis, Klebsiella* spp., and *Acinetobacter* spp. present on intact areas of the skin of some patients which can vary from 100 to 106 CFU/cm^2. Nearly 10^6 skin squames containing viable microorganisms are shed daily from normal skin. Patient gowns, bed linen, bedside furniture and other objects in the immediate environment of the patient become contaminated with patient flora. Such contamination is most likely to be due to Staphylococci, Enterococci or *Clostridium difficile* which are more resistant to desiccation. Certain Gram-negative rods, such as *Acinetobacter baumannii*, can also play an important role in environmental contamination due to their long-time survival capacities. This implies the potential importance of skin and environmental contamination on microbial cross contamination and pathogen spread.

Health Care Workers (HCWs) hands typically touch a continuous sequence of surfaces and substances including inanimate objects, patients' intact or non-intact skin, mucous membranes, food, waste, body fluids, and the HCWs own body. With each hand-to-surface exposure, a bidirectional exchange of microorganisms between hands and the touched object occurs and the transient hand-carried flora is thus continually changing. In this manner, microorganisms can spread throughout a healthcare environment and between patients within a few hours.

The burden of Health care associated infections is severe in immune compressed patients; prolonged hospital stay; invasive devices and procedures such as catheter, ventilators; Surgical site infections and also much more severe in high-risk populations such as adults housed in ICUs and neonates.

Several studies have shown the ability of microorganisms to survive on hands for differing times and inadequacy in hand hygiene leads to risk of cross-transmission of pathogens. Handwashing with soap and water has been considered a measure of personal hygiene for centuries and has been generally embedded in religious and cultural habits. Nevertheless, the link between handwashing and the spread of disease was established only two centuries ago, although this can be considered as relatively early with respect to the discoveries of Pasteur and Lister that occurred decades later.

Indications for hand hygiene-
A. Wash hands with soap and water when visibly dirty or visibly soiled with blood or other body fluids or after using the toilet.
B. If exposure to potential spore-forming pathogens is strongly suspected or proven, including outbreaks of *Clostridium difficile*, hand washing with soap and water is the preferred means.
C. In all clinical situations use an alcohol-based handrub as the preferred means for routine hand antisepsis, if hands are not visibly soiled. If alcohol-based handrub is not obtainable, wash hands with soap and water.
D. Before handling medication or preparing food perform hand hygiene using an alcohol-based handrub or wash hands with either plain or antimicrobial soap and water.

Five moments for hand hygiene–
Moment 1- Before touching a patient
Hand hygiene at this moment will mainly prevent colonization of the patient with health care-associated microorganisms, resulting from the transfer of organisms from the environment to the patient through unclean hands, and exogenous infections in some cases.

Moment 2- Before a clean/aseptic procedure
Once within the patient zone, very frequently after a hand exposure to the patient's intact skin, clothes or other objects, the HCW may engage in a clean/aseptic procedure on a critical site with infectious risk for the patient, such as opening a venous access line, giving an injection, or performing wound care. Importantly, hand hygiene required at this moment aims at preventing HCAI.

Moment 3- After body fluid exposure risk
After a care task associated with a risk to expose hands to body fluids, e.g. after accessing a critical site with body fluid exposure risk or a critical site with combined infectious risk (body fluid site), hand hygiene is required instantly and must take place before any next hand-to-surface exposure, even within the same patient zone. This hand hygiene action has a double objective. First and most importantly, it reduces the risk of colonization or infection of HCWs with infectious agents that may occur even without visible soiling. Second, it reduces the risk of a transmission of microorganisms from a "colonized" to a "clean" body site within the same patient.

Moment 4- After touching a patient
When leaving the patient zone after a care sequence, before touching an object in the area outside the patient zone and before a subsequent hand exposure to any surface in the health-care area, hand hygiene minimizes the risk of dissemination to the health-care environment, substantially reduces contamination of HCWs' hands with the flora from patient X, and protects the HCWs themselves.

Moment 5-After touching patient surroundings
The fifth moment for hand hygiene is a variant of Moment 4: it occurs after hand exposure to any surface in the patient zone, and before a subsequent hand exposure to any surface in the health-care area, but without touching the patient. This typically extends to objects contaminated by the patient flora that are extracted from the patient zone to be decontaminated or discarded. Because hand exposure to patient objects, but without physical contact with the patients, is associated with hand contamination, hand hygiene is still required.

Considering its importance and to empower hand hygiene in health care system, WHO has given guidelines on hand hygiene.

WHO guidelines on Hand hygiene -
A. Apply a palmful of alcohol-based handrub and cover all surfaces of the hands. Rub hands until dry.
B. When washing hands with soap and water, wet hands with water and apply the amount of product necessary to cover all surfaces. Rinse hands with water and dry thoroughly with a single-use towel. Use clean, running water whenever possible. Avoid using hot water, as repeated exposure to hot water may increase the risk of dermatitis.
Use towel to turn off tap/faucet. Dry hands thoroughly using a method that does not recontaminate hands. Make sure towels are not used multiple times or by multiple people.

Recommendations for surgical hand preparation
A. Remove rings, wrist-watch, and bracelets before beginning surgical hand preparation. Artificial nails are prohibited.
B. Sinks should be designed to reduce the risk of splashes.
C. If hands are visibly soiled, wash hands with plain soap before surgical hand preparation. Remove debris from underneath fingernails using a nail cleaner, preferably under running water.

Hand Hygiene Technique with Alcohol-Based Formulation

Duration of the entire procedure: 20-30 seconds

1a Apply a palmful of the product in a cupped hand, covering all surfaces;

1b

2 Rub hands palm to palm;

3 Right palm over left dorsum with interlaced fingers and vice versa;

4 Palm to palm with fingers interlaced;

5 Backs of fingers to opposing palms with fingers interlocked;

6 Rotational rubbing of left thumb clasped in right palm and vice versa;

7 Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa;

8 Once dry, your hands are safe.

Hand Hygiene Technique with Soap and Water

Duration of the entire procedure: 40-60 seconds

0 Wet hands with water;

1 Apply enough soap to cover all hand surfaces;

2 Rub hands palm to palm;

3 Right palm over left dorsum with interlaced fingers and vice versa;

4 Palm to palm with fingers interlaced;

5 Backs of fingers to opposing palms with fingers interlocked;

6 Rotational rubbing of left thumb clasped in right palm and vice versa;

7 Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa;

8 Rinse hands with water;

9 Dry hands thoroughly with a single use towel;

10 Use towel to turn off faucet;

11 Your hands are now safe.

D. Brushes are not recommended for surgical hand Preparation. Surgical hand antisepsis should be performed using either a suitable antimicrobial soap or suitable alcohol-based handrub, preferably with a product ensuring sustained activity, before donning sterile gloves.

F. If quality of water is not assured in the operating theatre; surgical hand antisepsis using an alcohol-based handrub is recommended before donning sterile gloves when performing surgical procedures.

G. When performing surgical hand antisepsis using an antimicrobial soap, scrub hands and forearms for the length of time recommended by the manufacturer, typically 2–5 minutes. Long scrub times (e.g. 10 minutes) are not necessary.

H. When using an alcohol-based surgical handrub product with sustained activity, follow the manufacturer's instructions for application times. Apply the product to dry hands only. Do not combine surgical hand scrub and surgical handrub with alcohol-based products sequentially.

I. When using an alcohol-based handrub, use sufficient product to keep hands and forearms wet with the handrub throughout the surgical hand preparation procedure.

J. After application of the alcohol-based handrub as recommended, allow hands and forearms to dry thoroughly before donning sterile gloves.

Other aspects of hand hygiene
A. Do not wear artificial fingernails or extenders when having direct contact with patients.
B. Keep natural nails short (tips less than 0.5 cm long or approximately ¼ inch)

These guidelines will help to achieve the goal of infection control in health-care system.

Selection and handling of hand hygiene agents
A. Provide HCWs with efficacious hand hygiene products that have low irritancy potential.
B. To maximize acceptance of hand hygiene products by HCWs, solicit their input regarding the skin tolerance, feel, and fragrance of any products under consideration.
C. When selecting hand hygiene products:
1. Determine any known interaction between products used to clean hands, skin care products, and the types of glove used in the institution.
2. Solicit information from manufacturers about the risk of product contamination.
3. Ensure that dispensers are accessible at the point of care.
4. Ensure that dispensers function adequately and reliably and deliver an appropriate volume of the product.
5. Ensure that the dispenser system for alcohol-based handrubs is approved for flammable materials;
6. Solicit and evaluate information from manufacturers regarding any effect that hand lotions, creams, or alcohol-based handrubs may have on the effects of antimicrobial soaps being used in the institution
7. Cost comparisons should only be made for products that meet requirements for efficacy, skin tolerance, and acceptability
8. Do not add soap or alcohol-based formulations to a partially empty soap dispenser. If soap dispensers are reused, follow recommended procedures for cleansing.

WHO recommends alcohol-based handrubs which are the only known means for rapidly and effectively inactivating a wide array of potentially harmful microorganism, at present.

WHO - World Health Organization
HCW - Healthcare Worker
HCAI - Healthcare Associated Infections

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Tackling antibiotic resistance through hand hygiene practices

Antimicrobial resistance occurs when a microorganism is no longer destroyed or stopped from reproducing by an anti-microbial medicine to which it was originally sensitive - quite simply, "the drugs don't work anymore". In recent years, some common pathogens have demonstrated multi-drug resistance and have caused infections, including those of urinary tract, bloodstream and wounds. More recently, microbes have started to show resistance to a group of antibiotics called carbepenemases; yet another group of antibiotics no longer work as effectively to clear infections. The number of antibiotics that can treat patients is shrinking, and there are rare cases of microbes that have become resistant to almost all antibiotics in use, potentially taking us back to a pre-antibiotic age.

Tackling Antimicrobial resistance (AMR) requires action on multiple levels, the prudent use of antibiotics being one of the key actions.

The importance of infection prevention and control in general and hand hygiene in particular in preventing the spread of microorganisms has been repeatedly highlighted. One way to control AMR is ensuring that resistant microorganisms are not spread via the hands of (mainly) healthcare workers, and do not have the opportunity to invade vulnerable patients' bodies. Hand hygiene helps to make this potential threat into an avoidable one.

Hand hygiene undertaken at the right time - the WHO's 5 Moments for Hand Hygiene - prevents the spread of resistant or sensitive organisms that can be present on or in patients or in our environment.

Antibiotic Resistance

When exposed to antibiotics, bacteria change to reduce or eliminate their susceptibility to a specific antibiotic. By developing resistance, the bacteria become more difficult or even impossible to treat. Any time bacteria are exposed to an antibiotic, some organisms are unaffected while others die. These resistant strains then multiply and become more prevalent. Overexposure or improper antibiotic use promotes bacterial resistance. Antibiotics are only effective against bacteria, not viruses.

Emergence of resistant organisms
Staphylococcus aureus

Staphylococcus aureus causes a variety of infections, from superficial skin infections to deep tissue infections or more life-threatening infections such as pneumonia, sepsis, and endocarditis. Treatment is with semi-synthetic penicillins and a wide range of antibiotic agents.

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a strain that is no longer sensitive to methicillin (oxacillin/nafcillin) due to alterations in penicillin-binding proteins located in bacterial cell walls. MRSA is not more virulent than Methicillin-Sensitive *S. aureus* (MSSA), but may be more difficult to treat due to limited antibiotic choices. The majority of MRSA isolates are also resistant to most other antibiotics, necessitating the use of the glycopeptide antibiotic, vancomycin.

Methicillin-resistant Staphylococcus aureus

Methicillin-resistant *Staphylococcus aureus* first emerged in England in 1960, soon after methicillin was introduced for the treatment of penicillin-resistant Staphylococci. Colonization with MRSA may occur in the nares, axillae, chronic wounds or pressure ulcer surfaces, perineum, around gastrostomy and tracheostomy sites, and in the sputum or urine.

One of the most common sites of colonization in both patients and employees is the nose (anterior nares). As with susceptible strains of *S. aureus*, personnel may become colonized with MRSA but rarely develop infections.

MRSA has become a prevalent nosocomial pathogen in hospitals; the most important reservoirs of MRSA are infected or colonized patients. Although hospital personnel can serve as reservoirs for MRSA and may harbor the organism for many months, they have been more commonly identified as a link for transmission between colonized or infected patients. The main mode of transmission is via hands (especially healthcare workers' hands) which may become contaminated by contact with a) colonized or infected patients, b) colonized or infected body sites of the personnel themselves, or c) devices, items, or environmental surfaces contaminated with body fluids containing MRSA.

Vancomycin-resistant Staphylococcus aureus

In June 2002, the first clinical isolate of *S. aureus* that is fully resistant to vancomycin (VRSA) was reported in the United States. The vancomycin-resistant genetic material from a co-infecting enterococci strain apparently had transferred to *Staphylococcus aureus* within the patient. The patient was successfully treated with trimethoprim sulfamethoxazole and the isolate was susceptible to several other drugs.

In the healthcare setting, the CDC recommends a patient with VRSA be placed in a private room and have dedicated patient care items. Health care workers providing care to such patients must follow Contact Precautions.

Enterococcus

Enterococci are part of the normal flora found in the gastrointestinal and female genital tracts. Most Enterococcal infections have been attributed to endogenous flora within the individual patient. However, patient-to-patient transmission can and does occur via direct contact, or indirectly via hands of personnel, contaminated equipment or environmental surfaces.

In the past, all Enterococci were sensitive to antibiotics such as ampicillin and vancomycin.

Vancomycin-resistant Enterococcus (VRE)

Vancomycin-resistant enterococci (VRE) are no more virulent than antimicrobial-sensitive enterococci but VRE poses distinct problems. These include the lack of available antibacterials and the possibility that vancomycin-resistant genes may be transferred to other gram-positive microorganisms.

Infections by multidrug-resistant organisms (MDROs) are increasing worldwide.

Prevention of spread and control of MDROs in health-care settings are critical and urgent as the number of antibiotics available to treat these infections is extremely limited and development of new antibiotics is not forthcoming in the foreseeable future. Worldwide, the most common bacteria causing health-care associated infections (HAIs) are:

MRSA Methicillin **r**esistant *Staphyloccous aureus*

VRE **V**ancomycin **r**esistant *Enterococci* spp.
ESBL **E**xtended-**s**pectrum **b**eta (β)-**l**actamase gram-negative organisms
CRE **C**arbapenems **r**esistant **Enterobacterales**
MRAB **M**ulti-**r**esistant *Acinetobacter* **b**aumannii
The emergence of resistance in these microorganisms has mainly been caused by an inappropriate use of antibiotics in general and use of broad spectrum antibiotics in particular.
In addition the spread of MDROs in health-care settings is common and occurs mostly via health-care workers' (HCWs) contaminated hands, contaminated items/equipment and environment often leading to outbreaks and serious infections especially in critically ill patients.
Therefore, implementation of standard precautions for *all* patients *all* the time is key to preventing spread of all microorganisms and MDROs in particular. Hand hygiene performance according to recommendations is the most important measure among standard precautions.

Health care associated infections (HAIs)
Health care associated infections are drawing increasing attention from patients, insurers, governments and regulatory bodies. This is not only because of the magnitude of the problem in terms of the associated morbidity, mortality and cost of treatment, but also due to the growing recognition that most of these are preventable. The medical community is witnessing in tandem unprecedented advancements in the understanding of pathophysiology of infectious diseases and the global spread of multi-drug resistant infections in health care set-ups. These factors, compounded by the paucity of availability of new antimicrobials have necessitated a re-look into the role of basic practices of infection prevention in modern day health care. There is now undisputed evidence that strict adherence to hand hygiene reduces the risk of cross-transmission of infections. With "Clean Care is Safer Care" as a prime agenda of the global initiative of WHO on patient safety programmes, it is time for developing countries to formulate the much-needed policies for implementation of basic infection prevention practices in health care set-ups.

Normal flora of hands
There are two types of microbes colonizing hands: the resident flora, which consists of microorganisms residing under the superficial cells of the stratum corneum and the transient flora, which colonizes the superficial layers of the skin, and is more amenable to removal by routine hand hygiene. Transient microorganisms survive, but do not usually multiply on the skin. They are often acquired by health care workers (HCWs) during direct contact with patients or their nearby contaminated environmental surfaces and are the organisms most frequently associated with HCAIs.

Colonization of hands with pathogens and their role in transmission
The hands of HCWs are commonly colonized with pathogens like Methicillin resistant *S. aureus* (MRSA), Vancomycin resistant *Enterococcus* (VRE), MDR-Gram Negative bacteria (GNBs), *Candida* spp. and *Clostridium diffcile*, which can survive for as long as 150 h. Approximately 106 skin epithelial cells containing viable microorganisms are shed daily from the normal skin, which can contaminate the gowns, bed linen, bedside furniture, and other objects in the patient's immediate environment. Hand carriage of resistant pathogens has repeatedly been shown to be associated with nosocomial infections. The highest rates of hand contamination are reported from critical care areas, which also report most cases of cross-transmission. The hands may become contaminated by merely touching the patent's intact skin or inanimate objects in patients' rooms or during the "clean" procedures like recording blood pressure

Importance of hand hygiene
Proper hand hygiene is the single most important, simplest, and least expensive means of reducing the prevalence of HAIs and the spread of antimicrobial resistance. Several studies have demonstrated that handwashing virtally eradicates the carriage of MRSA which invariably occurs on the hands of HCPs working in ICUs. An increase in handwashing compliance has been found to be accompanied by a fall in MRSA rates. The hand hygiene liason group identified nine controlled studies, all of which showed significant reductions in infection related outcomes, even in settings with a high infection rates in critically ill patients. Transmission of Health-care-associated *Klebsiella* sp. has also been documented to reduce with improvement in hand hygiene. The evidence suggests that adherence to hand hygiene practices has significantly reduced the rates of acquisition of pathogens on hands and has ultimately reduced the rates of HAIs in a hospital.

Indications for hand hygiene during patient care
Wash hands with soap and water when (*i*) visibly dirty or contaminated with proteinaceous material, blood, or other body fluids and if exposure to *Bacillus anthracis* is suspected or proven (since the physical action of washing and rinsing hands in such circumstances is recommended because alcohols, chlorhexidine, iodophors, and other antiseptic agents have poor activity against spores); (*ii*) After using a restroom, wash hands with a non-antimicrobial soap and water or with an antimicrobial soap and water; and (*iii*) before and after having food.
In all other clinical situations described below, when hands are not visibly soiled, an alcohol-based hand rub should be used routinely for decontaminating hands. (*i*) Before having direct contact with patients. (*ii*) Before donning sterile gloves when inserting a central intravascular catheter. (*iii*) Before inserting indwelling urinary catheters, peripheral vascular catheters, or other invasive devices that do not require a surgical procedure. (*iv*) After contact with a patient's intact skin (*e.g.*, when taking a pulse or blood pressure or lifting a patient). (*v*) After contact with body fluids or excretions, mucous membranes, nonintact skin, and wound dressings if hands are not visibly soiled. (*vi*) After contact with inanimate objects (including medical equipment) in the immediate vicinity of the patient. (*vii*) After removing gloves. (*viii*) If moving from a contaminated body site to a clean body site during patient care.
The WHO "SAVE LIVES: Clean Your Hands" programme reinforces the "My 5 Moments for Hand Hygiene" approach as key to protect the patients, HCWs and the health-care environment against the spread of pathogens and thus reduce HAIs. This approach encourages HCWs to clean their hands: before touching a patient, before clean/aseptic procedures, after body fluid exposure/risk, after touching a patient and after touching patient surroundings.
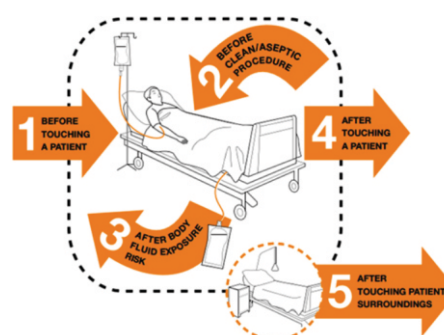
Methods used to improve hand hygiene compliance

Multimodal strategies have been shown to be more successful in improving rates of adherence with hand hygiene in HCWs than single interventions. Targeted, multi-faceted approaches focusing on system change, administrative support, motivation, availability of alcohol-based hand rubs, training and intensive education of HCWs and reminders in the workplace have been recommended for improvement in hand hygiene.

Recent studies support the fact that interactive educational programmes combined with free availability of hand disinfectants significantly increased the hand hygiene compliance. The four member States of the European Union, which implemented National Hand Hygiene Campaigns found the following strategies to be extremely useful in their countries: Governmental support, the use of indicators for hand hygiene benchmarking, developing national surveillance systems for auditing alcohol based hand rub consumption and auditing hand hygiene compliance[4]. Trampuz *et al* advocated simple training sessions for HCWs to be held in each ward to introduce the advantage of alcohol hand rubs over hand washing.

Other factors like positive role modeling (hand hygiene behaviour of senior practitioners) and the use of performance indicators also remarkably improve adherence to hand hygiene. There should be adequate supply of hand hygiene products, lotions and creams, disposable towels and facilities for hand washing, where necessary. Alcohol hand rubs should be available at the point of care in sufficient quantities. It needs to be emphasized that wearing gloves does not replace the need for hand hygiene and that contamination may occur during glove removal. Studies by Pitet showed a remarkable and long lasting improvement in hand hygiene compliance using a multimodal strategy, which has been adopted by the first Global Patient Safety Challenge of WHO to develop hand hygiene strategies. The availability of individual, pocket carried bottles also increased compliance.

Apart from this, all hospitals should have a dynamic infection control team, robust surveillance system, adequate staff to disseminate evidence-based knowledge in an easily comprehensible way to all cadres of staff. At a more local or regional level, there is a need for institutional frameworks or programmes to deal with HAIs.

According to WHO, convincing evidence that improved hand hygiene practices lead to a reduction of infections caused by multidrug resistant bacteria in health facilities has been presented in a new report. For example, when hand hygiene compliance in health facilities increases from poor (<60%) to excellent (90%), there can be a 24% reduction in Methicillin-Resistant *Staphylococcus aureus* (MRSA) acquisition.

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Importance of Automated Liquid / Gel Dispenser for Hands in Hospitals, Pharmaceuticals and Food Industries

The relations among between the environment, hygiene, and hospital-acquired infections (HAIs) were first detailed in Ignaz Semmelweis's landmark 1846 study regarding infection in a Vienna maternity hospital. Semmelweis observed that the significantly higher postpartum maternal infection and mortality rate in one obstetrics ward was likely related to the poor hand hygiene practices of its healthcare workers (HCWs), who often came directly from the morgue after performing autopsies. He believed that the HCWs were transmitting "cadaverous particles" from the autopsy suite to the obstetrics ward via hand contact, resulting in puerperal sepsis. A subsequent change to more rigorous and mandatory hand hygiene greatly decreased the infection and mortality rates. Hand hygiene has since grown to be regarded as the major weapon against HAI.

Today, HAI continues to be a substantial problem, accounting for an estimated 1.7 million infections and 99,000 deaths each year in the United States. Such infections can be spread by direct person-to-person contact, as well as via contaminated inanimate objects in the environment, known as fomites. Fomites act as reservoirs for pathogens that can then be passed to the hands of HCWs, who, in turn, act as vectors in the spread of organisms to patients.

Fomites can be found throughout the hospital. Better-known fomites are the bed linens, bed rails, furniture, countertops, and floors of patient rooms. Door handles andcurtains have been found to harbour pathogens. Mobile fomites that may themselves act as vectors include stethoscopes, blood pressure cuffs, phlebotomy tourniquets, pens, staff identification badges, and cellular telephones.

Pathogens can survive on their inanimate hosts for long periods of time and may be difficult to eradicate despite conventional cleaning. The most common nosocomial pathogens may survive for months on dry, inanimate surfaces, with longer persistence associated with humid cool conditions, higher inoculum, and certain surface characteristics. Efficient transfer of pathogens from fomites to the hands of HCWs has been demonstrated. Finally, the subsequent transfer to patients resulting in HAI has been shown.

"Touchless technology is a good idea, because hard surfaces are significant transfer points for bacteria and viruses," says Charles Gerba, PhD, a microbiologist at the University of Arizona, Tucson. "Much of what people put down on a surface can be picked up by the next person who comes along, and in an age where people share more spaces and surfaces than ever before, touchless technology can help prevent cross-contamination."

It is intuitive to think that the less a potentially contaminated surface is touched, the better, so the advent of automated sink fixtures as well as soap and towel dispensers has been heralded as an important way to reduce the opportunities for cross contamination and hand carriage of pathogenic microorganisms. But how many clinicians consider the role that handwashing stations play in opportunities for cross-contamination?

As experts debate the role inanimate objects play in the transmission of infectious agents, few would doubt that the contamination of

environmental surfaces such as handwashing sinks is a major issue. "Clearly inanimate surfaces play a role, particularly with organisms such as *vancomycin-resistant Enterococcus* (VRE) and *Clostridium difficile*," says Columbia University's Elaine Larson, RN, PhD, FAAN, CIC. "But it seems pretty clear that direct contact (i.e., person-to-person touching) remains the most important mode of cross transmission. Nevertheless, housekeeping and environmental cleaning seem to have taken too much of a back seat and we need to re-emphasize the great importance of keeping the healthcare setting (as well as the people) free of a large microbial bioload."

Supporters of touchless technology frequently point to a study by Larson et al. that compared the frequency of use of manually operated and touch-free dispensers of alcohol sanitizer installed in the emergency department and an intensive care unit of a large paediatric hospital for two, two-month periods for each type of dispenser. Counting devices installed in each dispenser and direct observations were used to determine actual frequency of and indications for hand hygiene. Larson et al. found that the touch-free dispensers were used significantly more often than were the manual dispensers. The means for the number of episodes of hand hygiene per hour were 4.42 for the touch-free dispensers and 3.33 for the manual dispensers (P = .04); the means for the number of episodes per patient per hour were 2.22 and 1.79, respectively (P = .004); and the means for the number of uses of the dispenser per day were 41.2 and 25.6, respectively (P = .02). However, the overall compliance rate was 38.4 percent (2,136 episodes of hand hygiene per 5,568 indications for hand hygiene).

The researchers concluded that while the type of dispensing system influenced hand hygiene behaviour, overall compliance remained low and that in order for interventions to have a major effect on hand hygiene, multiple factors must be considered.

Kampf,Girard, Bischoffand Pittet concur that hand hygiene compliance is boosted when convenient, readily accessible dispensers are installed, although Muto et al. found that compliance did not improve when alcohol dispensers were placed by every patient's door in two units. While every hospital's experience with touchless dispensers will undoubtedly be different, the hope of decreased cross-contamination and improved hand hygiene compliance is usually the biggest reason why healthcare facilities embrace this technology.

Larson et al write, "Although no evidence indicates that devices that must be manually pressed to dispense cleanser increase the risk of transferring microbes, healthcare staff may express concern about the safety of touching dispensers and may prefer dispensers that are more accessible and easier to use than the manual ones are. Such concerns may be a deterrent to using manual dispensers." Larson et al. write further, "Our finding that the number of hand hygiene episodes overall was higher for the touch-free dispenser than for the manual dispenser is consistent with the hypothesis that the delivery system has an effect on behaviour and that a touch-free dispenser may be preferred by healthcare professionals, food and pharmaceutical industries."

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Antiseptics and Areas of concern (I)

Alcohols

Although several alcohols have been shown to be effective antimicrobials, ethyl alcohol (ethanol), isopropyl alcohol (isopropanol, propan-2-ol) and n-propanol are the most widely used. Alcohols exhibit rapid broad-spectrum antimicrobial activity against vegetative bacteria (including Mycobacteria), viruses, and fungi but are not sporicidal. They are, however, known to inhibit sporulation and spore germination, but this effect is reversible. Because of the lack of sporicidal activity, alcohols are not recommended for sterilization but are widely used for both hardsurface disinfection and skin antisepsis. Lower concentrations may also be used as preservatives and to potentiate the activity of other biocides. Many alcohol products include low levels of other biocides (in particular chlorhexidine), which remain on the skin following evaporation of the alcohol, or excipients (including emollients), which decrease the evaporation time of the alcohol and can significantly increase product efficacy. In general, isopropyl alcohol is considered slightly more efficacious against bacteria and ethyl alcohol is more potent against viruses; however, this is dependent on the concentrations of both the active agent and the test microorganism. For example, isopropyl alcohol has greater lipophilic properties than ethyl alcohol and is less active against hydrophilic viruses (e.g., poliovirus). Generally, the antimicrobial activity of alcohols is significantly lower at concentrations below 50% and is optimal in the 60 to 90% range. Little is known about the specific mode of action of alcohols, but based on the increased efficacy in the presence of water; it is generally believed that they cause membrane damage and rapid denaturation of proteins, with subsequent interference with metabolism and cell lysis. This is supported by specific reports of denaturation of *Escherichia coli* dehydrogenases and an increased lag phase in *Enterobacter aerogenes*, speculated to be due to inhibition of metabolism required for rapid cell division.

Anilides

The anilides have been investigated primarily for use as antiseptics, but they are rarely used in the clinic. Triclocarban (TCC; 3,4,49-triclorocarbanilide) is the most extensively studied in this series and is used mostly in consumer soaps and deodorants. TCC is particularly active against gram-positive bacteria but significantly less active against gram-negative bacteria and fungi and lacks appreciable substantivity (persistency) for the skin. The anilides are thought to act by adsorbing to and destroying the semipermeable character of the cytoplasmic membrane, leading to cell death.

Chlorhexidine

Chlorhexidine is probably the most widely used biocide in antiseptic products, in particular in handwashing and oral products but also as a disinfectant and preservative. This is due in particular to its broad-spectrum efficacy, substantivity for the skin, and low irritation. Of note, irritability has been described and in many cases may be product specific. Despite the advantages of chlorhexidine, its activity is pH dependent and is greatly reduced in the presence of organic matter. A considerable amount of research has been undertaken on the mechanism of the antimicrobial action of this important bisbiguanide, although most of the attention has been devoted to the way in which it inactivates nonsporulating bacteria. Nevertheless, sufficient data are now available to examine its sporostatic and mycobacteriostatic action, its effects on yeasts and protozoa, and its antiviral activity. Chlorhexidine is a bactericidal agent. Its interaction and uptake by bacteria were studied initially by

Hugo et al., who found that the uptake of chlorhexidine by *E. coli* and *S. aureus* was very rapid and depended on the chlorhexidine concentration and pH. More recently, by using [14C] chlorhexidine gluconate, the uptake by bacteria and yeasts was shown to be extremely rapid, with a maximum effect occurring within 20s. Damage to the outer cell layers takes place but is insufficient to induce lysis or cell death. The agent then crosses the cell wall or outer membrane, presumably by passive diffusion, and subsequently attacks the bacterial cytoplasmic or inner membrane or the yeast plasma membrane. In yeasts, chlorhexidine "partitions" into the cell wall, plasma membrane, and cytoplasm of cells. Damage to the delicate semipermeable membrane is followed by leakage of intracellular constituents, which can be measured by appropriate techniques. Leakage is not per se responsible for cellular inactivation but is a consequence of cell death. High concentrations of chlorhexidine cause coagulation of intracellular constituents. As a result, the cytoplasm becomes congealed, with a consequent reduction in leakage, so that there is a biphasic effect on membrane permeability. An initial high rate of leakage rises as the concentration of chlorhexidine increases, but leakage is reduced at higher biocide concentrations because of the coagulation of the cytosol. Chlorhexidine was claimed by Harold et al. to be an inhibitor of both membrane-bound and soluble ATPase as well as of net K1 uptake in *Enterococcus faecalis*. However, only high biguanide concentrations inhibit membrane bound ATPase, which suggests that the enzyme is not a primary target for chlorhexidine action. Although chlorhexidine collapses the membrane potential, it is membrane disruption rather than ATPase inactivation that is associated with its lethal effects. The effects of chlorhexidine on yeast cells are probably similar to those previously described for bacteria. Chlorhexidine has a biphasic effect on protoplast lysis, with reduced lysis at higher biguanide concentrations. Furthermore, in whole cells, the yeast cell wall may have some effect in limiting the uptake of the biguanide. The findings presented here and elsewhere demonstrate an effect on the fungal plasma membrane but with significant actions elsewhere in the cell. Increasing concentrations of chlorhexidine (up to 25 mg/ml) induce progressive lysis of *Saccharomyces cerevisiae* protoplasts, but higher biguanide concentrations result in reduced lysis. Work to date suggests that chlorhexidine has a similar effect on the trophozoites of *Acanthameoba castellanii*, with the cysts being less sensitive. Furr reviewed the effects of chlorhexidine and other biocides on *Acanthameoba* and showed that membrane damage in these protozoa is a significant factor in their inactivation. Mycobacteria are generally highly resistant to chlorhexidine. Little is known about the uptake of chlorhexidine (and other antiseptics and disinfectants) by Mycobacteria and on the biochemical changes that occur in the treated cells. Since the MICs for some mycobacteria are on the order of those for chlorhexidine-sensitive, gram-positive cocci, the inhibitory effects of chlorhexidine on mycobacteria may not be dissimilar to those on susceptible bacteria. *Mycobacterium aviumintracellulareis* considerably more resistant than other mycobacteria. Chlorhexidine is not sporicidal. Even high concentrations of the bisbiguanide do not affect the viability of *Bacillus* spores at ambient temperatures, although a marked sporicidal effect is achieved at elevated temperatures. Presumably, sufficient changes occur in the spore structure to permit an increased uptake of the biguanide, although this has yet to be shown experimentally. Little is known about the uptake of chlorhexidine by bacterial spores, although coatless forms take up more of the

compound than do "normal" spores. Chlorhexidine has little effect on the germination of bacterial spores but inhibits outgrowth. The reason for its lack of effect on the former process but its significant activity against the latter is unclear. It could, however, be reflected in the relative uptake of chlorhexidine, since germinating cells take up much less of the bisbiguanide than do outgrowing forms. Binding sites could thus be reduced in number or masked in germinating cells. The antiviral activity of chlorhexidine is variable. Studies with different types of bacteriophages have shown that chlorhexidine has no effect on MS2 or K coliphages. High concentrations also failed to inactivate *Pseudomonas aeruginosa* phage F116 and had no effect on phage DNA within the capsid or on phage proteins; the transduction process was more sensitive to chlorhexidine and other biocides than was the phage itself. This substantiated an earlier finding that chlorhexidine bound poorly to F116 particles. Chlorhexidine is not always considered a particularly effective antiviral agent, and its activity is restricted to the lipid-enveloped viruses. Chlorhexidine does not inactivate nonenveloped viruses such as rotavirus, HAV, or poliovirus. Its activity was found by Ranganathan to be restricted to the nucleic acid core or the outer coat, although it is likely that the latter would be a more important target site.

Alexidine

Alexidine differs chemically from chlorhexidine in possessing ethylhexyl end groups. Alexidine is more rapidly bactericidal and produces a significantly faster alteration in bactericidal permeability. Studies with mixed-lipid and pure phospholipid vesicles demonstrate that, unlike chlorhexidine, alexidine produces lipid phase separation and domain formation. It has been proposed that the nature of the ethylhexyl end group in alexidine, as opposed to the chlorophenol one in chlorhexidine, might influence the ability of a biguanide to produce lipid domains in the cytoplasmic membrane.

Triclosan

Triclosan (2,4,49-trichloro-29-hydroxydiphenyl ether; exhibits particular activity against grampositive bacteria. Its efficacy against gram-negative bacteria and yeasts can be significantly enhanced by formulation effects. For example, triclosan in combination with EDTA caused increased permeability of the outer membrane. Reports have also suggested that in addition to its antibacterial properties, triclosan may have anti-inflammatory activity. The specific mode of action of triclosan is unknown, but it has been suggested that the primary effects are on the cytoplasmic membrane. In studies with *E. coli*, triclosan at subinhibitory concentrations inhibited the uptake of essential nutrients, while higher, bactericidal concentrations resulted in the rapid release of cellular components and cell death. Studies with a divalent-ion-dependent *E. coli* triclosan mutant for which the triclosan MIC was 10-fold greater than that for a wildtype strain showed no significant differences in total envelope protein profiles but did show significant differences in envelope fatty acids. Specifically, a prominent 14:1 fatty acid was absent in the resistant strain, and there were minor differences in other fatty acid species. It was proposed that divalent ions and fatty acids may adsorb and limit the permeability of triclosan to its site of action. Minor changes in fatty acid profiles were recently found in both *E. coli* and *S. aureus* strains for which the triclosan MICs were elevated; however, the MBCs were not affected, suggesting, as for other phenols, that the cumulative effects on multiple targets contribute to the bactericidal activity.

Antiseptics and Areas of concern (II)

Polymeric biguanide (PHMB)

Polymeric biguanide is a hetero disperse mixture of polyhexamethylene biguanides (PHMB) with a molecular weight of approximately 3,000. Earlier Polymeric biguanides have found use as general disinfecting agents in the food industry and, very successfully, for the disinfection of swimming pools. But presently it is found to be very effective antiseptic for acute as well as chronic wounds. This is active against gram-positive and gram-negative bacteria and also sporicidal. PHMB is a membrane-active agent that also impairs the integrity of the outer membrane of gramnegative bacteria, although the membrane may also act as a permeability barrier. Activity of PHMB increases on a weight basis with increasing levels of polymerization, which has been linked to enhanced inner membrane perturbation. Unlike chlorhexidine but similar to alexidine, PHMB causes domain formation of the acidic phospholipids of the cytoplasmic membrane. Permeability changes ensue, and there is believed to be an altered function of some membrane-associated enzymes. The proposed sequence of events during its interaction with the cell envelope of *E. coli* is as follows: (i) there is rapid attraction with strong and specific adsorption to phosphate-containing compounds; (ii) the integrity of the outer membrane is impaired, and PHMB is attracted to the inner membrane; (iii) binding of PHMB to phospholipids occurs, with an increase in inner membrane permeability (K1 loss) accompanied by bacteriostasis; and (iv) complete loss of membrane function follows, with precipitation of intracellular constituents and a bactericidal effect.

Iodine and iodophors

Although less reactive than chlorine, iodine is rapidly bactericidal, fungicidal, tuberculocidal and virucidal. Although aqueous or alcoholic (tincture) solutions of iodine have been used for 150 years as antiseptics, they are associated with irritation and excessive staining. In addition, aqueous solutions are generally unstable; in solution, at least seven iodine species are present in a complex equilibrium, with molecular iodine (I2) being primarily responsible for antimictrobial efficacy. These problems were overcome by the development of iodophors ("iodine carriers" or "iodine-releasing agents"); the most widely used are povidoneiodine and poloxamer-iodine in both antiseptics and disinfectants. Iodophors are complexes of iodine and a solubilizing agent or carrier, which acts as a reservoir of the active "free" iodine. Although germicidal activity is maintained, iodophors are considered less active against certain fungi and spores than are tinctures. Similar to chlorine, the antimicrobial action of iodine is rapid, even at low concentrations, but the exact mode of action is unknown. Iodine rapidly penetrates into microorganisms and attacks key groups of proteins (in particular the freesulfur amino acids cysteine and methionine, nucleotides, and fatty acids, which culminates in cell death. Less is known about the antiviral action of iodine, but nonlipid viruses and parvoviruses are less sensitive than lipid enveloped viruses. Similarly to bacteria, it is likely that iodine attacks the surface proteins of enveloped viruses, but they may also destabilize membrane fatty acids by reacting with unsaturated carbon bonds.

Silver Compounds

In one form or another, silver and its compounds have long been used as antimicrobial agents. The most important silver compound currently in use is silver sulfadiazine (AgSD), although silver metal, silver acetate, silver nitrate, and silver protein, all of which have antimicrobial properties, are listed in Martindale, The Extra Pharmacopoeia. In recent years, silver compounds have been used to prevent the infection of burns and some eye infections and to destroy warts.

Silver nitrate

The mechanism of the antimicrobial action of silver ions is closelymrelated to their interaction with thiol (sulfydryl,™SH) groups, although other target sites remain a possibility. Liau et al demonstrated that amino acids such as cysteine and other compounds such as sodium thioglycolate containing thiol groups neutralized the activity of silver nitrate against *P. aeruginosa*. By contrast, amino acids containing disulfide (SS) bonds, nonsulfurcontaining amino acids, and sulfur-containing compounds such as cystathione, cysteic acid, L-methionine, taurine, sodium bisulfite, and sodium thiosulfate were all unable to neutralize Ag1 activity. These and other findings imply that interaction of Ag1 with thiol groups in enzymes and proteins plays an essential role in bacterial inactivation, although other cellular components may be involved. Hydrogen bonding, the effects of hydrogen bondbreaking agents, and the specificity of Ag1 for thiol groups were discussed in greater detail by Russell and Hugo. Virucidal properties might also be explained by binding to ™SH groups. Lukens proposed that silver salts and other heavy metals such as copper act by binding to key functional groups of fungal enzymes. Ag1 causes the release of K1 ions from microorganisms; the microbial plasma or cytoplasmic membrane, with which is associated many important enzymes, is an important target site form Ag1 activity. In addition to its effects on enzymes, Ag1 produces other changes in microorganisms. Silver nitrate causes marked inhibition of growth of *Cryptococcus neoformans* and is deposited in the vacuole and cell wall as granules. Ag1 inhibits cell division and damages the cell envelope and contents of *P. aeruginosa*. Bacterial cells increase in size, and the cytoplasmic membrane, cytoplasmic contents, and outer cell layers all exhibit structural abnormalities, although without any blebs (protuberances). Finally, the Ag1 ion interacts with nucleic acids; it interacts preferentially with the bases in DNA rather than with the phosphate groups, although the significance of this in terms of its lethal action is unclear.

Silver sulfadiazine

AgSD is essentially a combination of two antibacterial agents, Ag1 and sulfadiazine (SD). The question whether the antibacterial effect of AgSD arises predominantly from only one of the compounds or via a synergistic interaction has been posed repeatedly. AgSD has a broad spectrum of activity and, unlike silver nitrate, produces surface and membrane blebs in susceptible (but not resistant) bacteria. AgSD binds to cell components, including DNA. Based on a chemical analysis, Fox proposed a polymeric structure of AgSD composed of six silver atoms bonding to six SD molecules by linkage of the silver atoms to the nitrogens of the SD pyrimidine ring. Bacterial inhibition would then presumably be achieved when silver binds to sufficient base pairs in the DNA helix, thereby inhibiting transcription. Similarly, its antiphage properties have been ascribed to the fact that AgSD binds to phage DNA. Clearly, the precise mechanism of action of AgSD has yet to be solved.

Bis-Phenols

The bis-phenols are hydroxy-halogenated derivatives of two phenolic groups connected by various bridges. In general, they exhibit broad-spectrum efficacy but have little activity against *P.*

aeruginosa and molds and are sporostatic toward bacterial spores. Triclosan and hexachlorophane are the most widely used biocides in this group, especially in antiseptic soaps and hand rinses. Both compounds have been shown to have cumulative and persistent effects on the skin.

Hexachlorophene

Hexachlorophene (hexachlorophane; 2,29-dihydroxy-3, 5, 6, 39, 59, 69-hexachlorodiphenylmethane) is another bis-phenol whose mode of action has been extensively studied. The primary action of hexachlorophene, based on studies with *Bacillus megatherium*, is to inhibit the membranebound part of the electron transport chain, and the other effects noted above are secondary ones that occur only at high concentrations. It induces leakage, causes protoplast lysis, and inhibits respiration. The threshold concentration for the bactericidal activity of hexachlorphene is 10 mg/ml (dry weight), but peak leakage occurs at concentrations higher than 50 mg/ml and cytological changes occur above 30 mg/ml. Furthermore, hexachlorophene is bactericidal at 0°C despite causing little leakage at this temperature. Despite the broad-spectrum efficacy of hexachlorophene, concerns about toxicity, in particular in neonates, have meant that its use in antiseptic products has been limited.

Halophenols

Chloroxylenol (4-chloro-3,5-dimethylphenol; p-chloro-mxylenol) is the key halophenol used in antiseptic or disinfectant formulations. Chloroxylenol is bactericidal, but *P. aeruginosa* and many molds are highly resistant. Surprisingly, its mechanism of action has been little studied despite its widespread use over many years. Because of its phenolic nature, it would be expected to have an effect on microbial membranes.

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Disinfection of Linen

The rise in the occurrence of nosocomial or hospital acquired infections (HAI) is becoming a huge problem in the hospital / health care sector. The incidence, type and magnitude of HAI varies from hospital to hospital. It is estimated to be around 10% of hospital admissions in South-East Asia. Given the prevailing conditions in the hospitals in developing countries, this is likely to increase.

In a developing country like India, communicable diseases especially infectious, parasitic, gastrointestinal, respiratory diseases and tuberculosis are among the leading causes of deaths. The number of patients with HIV/AIDS is also increasing. In order to fight such a grave situation, hospitals need to develop a programme for the implementation of good infection control measures to ensure the well being of both patients and staff by preventing and controlling HAI.

Three most important components which play an important role for an infection to occur in the hospital are - a susceptible host, a pathogenic micro-organism and an environment that is congenial for the multiplication of the pathogen. The inanimate hospital environment capable of spreading infections comprises of
- air, water, food and medicaments
- Used equipments and instruments
- **Hospital linen**
- Bio medical waste

Out of these used/dirty hospital linen (bed sheets, pillow covers, blankets, gowns, aprons, surgical drapes, scrub suits, towels, uniforms, lab coats etc) is a major potential source of infection as it is likely to be contaminated with a large number of pathogens and may contain sufficient moisture to allow these pathogens to continue to multiply in the warm hospital environment. Irrespective of patients diagnosis, all the used linen of a hospital/ health care center is potentially contaminated. Contaminated linen does not only pose a threat to the patients admitted in a health care facility and the health care workers, it is also dangerous for the laundry personnel responsible for processing hundreds of thousands of kilograms of contaminated reusable linens annually.

Careful management - handling and processing of linen, can therefore, play an important role in stopping or reducing actual pathogen transmission from linen to people or to the environment and cause diseases. Linen management is a complex procedure which requires
- Collection and transportation of contaminated linen
- Sorting of contaminated linen
- Washing and appropriate disinfection of contaminated linen
- Proper storage and distribution of clean linen

Linen management may vary on the basis of the climate, culture, systems and procedures of the individual organization. But it is important that the contaminated linen
- is handled with minimal agitation to avoid aerosolization of pathogenic micro-organisms.
- is placed in closed bags without sorting or washing at the location of use (CDC 1988; OSHA 1991).
- is rolled or folded to contain the heaviest soil in the center of the bundle.
- wet and foul linen/laundry is placed and transported in leak-proof closed bags or containers.
- from different areas, such as wards, surgical units, uniforms are separately collected and processed.
- is transported to laundry-processing area by trolleys / carts / containers with lids and never carried near the body.
- and clean linen is transported in separate and covered trolleys.

Trained and supervised hospital staff who understand the importance of safe handling of contaminated linen can minimize the spreading of pathogens. Measures like use of protective aprons, facial masks, gloves and implementation of effective hand hygiene for the staff handling and processing linen, reduces the risk of exposure to infectious materials and acquiring work-related infections. Collection of soiled linen is a tedious and long process. It may not be possible or practical for the linen handling staff to wash their hands properly with soap and water periodically. Effective hand hygiene can still be achieved by using a liquid quick drying antiseptic handrub which is capable of rapid and powerful cidal action and has residual activity. Thorough cleaning and disinfection of the bags / containers / trolleys / carts used to transport soiled linen after every use with a broad spectrum surface disinfectant can further minimize the chances of contamination.

Collected hospital linen is transported to a sorting area where it is mainly segregated in two categories – foul linen (wet and contaminated with blood or body fluids) and soiled linen (not visibly dirty and not contaminated with blood or body fluids) before washing and is treated separately. Linen from patients suffering from resistant pathogens and linen to be hand washed are also segregated. Sorting of linen before washing is also important to ensure that there are no scalpels, sharp-tipped scissors, hypodermic and suture needles, sharp-tipped towel clips or soiled dressings. Storing of contaminated linen in the laundry area for long is not advisable.

Washing of contaminated linen is the most important step in linen processing. Proper washing using thermal and chemical measures (chemical agents/disinfectants capable of killing / eliminating the micro-organisms) can ensure disinfection of used linen. The process of eliminating the micro-organisms is known as disinfection/ sterilization. It is important to understand the difference between disinfection and sterilization. Disinfection in general refers to the elimination of only pathogenic micro-organisms and not more resistant spores while sterilization means complete elimination of pathogens as well as spores.

Many micro-organisms are physically removed from the linen, by the detergent and water, during the proper washing cycle. Washing linen at high temperature (71°C) in washing machines for at least 25 minutes with a preheating period for at least 5 minutes, is generally recommended as it kills most vegetative bacteria and viruses.

Recent studies have shown that a satisfactory reduction of microbial contamination can also be achieved at lower water temperatures (22-50°C) by monitoring and controlling the cycling of the washer, detergent and certain additives for adjusting the pH of the water and facilitating inactivation of pathogens present in the linen. Addition of a mild acidic "souring" agent neutralizes the alkalinity from the fabric, water and detergent. This shift in pH from approximately 12 to 5 may inactivate any remaining bacteria and reduce the potential for skin irritation.

Plain detergents (bar, liquid or powder) only lower surface tension and help in the removal of dirt/ debris but friction (scrubbing) is required to mechanically remove microorganisms. These detergents do not kill micro organisms. Addition of disinfectants for washing at higher temperatures may not be required. But if hand washing is the only option then contaminated linen needs to be pre-soaked in water, soap and disinfectant. This step helps in loosening and removing excess dirt/debris as well as decontaminating/disinfecting the linen.

Cleaning products having antimicrobial agents both clean and disinfect the linen. In most places along with plain detergents, chlorine releasing agents such as sodium hypochlorite and hydrogen peroxide are used as disinfectants. Use of washing soda and bleaching agents is not recommended as these chemical agents get rapidly neutralized in the presence of organic matter, blood, body fluids, soaps etc and become ineffective. Repeated use of chlorine releasing agents also causes the fabric to decolorize and deteriorate more quickly.

Hospital linen processing also needs to remove blood and body fluid stains from the linen. Therefore, use of cleaning agents is required which are not neutralized in the presence of organic matter, biodegradable, eco friendly, can clean, and disinfect linen without having any adverse effect on them. New quaternary ammonium compounds like Didecyl dimethyl ammonium chloride (DDAC) with broad spectrum disinfection power and additional surfactant action are good option as disinfectant and cleaner.

Spreading linen to dry in sunlight and open air (away from any source of contamination or pollution) allows exposure to ultraviolet sun rays, which produces natural disinfection. In places where drying the washed linen in sunlight is not possible electric dryers are used. Dryer heat is effective in ensuring disinfection. After drying linen is subjected to ironing which also has the same effect. This complete procedure cleans and disinfects the soiled and contaminated linen.

There are areas in a hospital, such as surgical units, which require sterilized linen (drapes, gowns etc). Sterilization of such linens is achieved with the help of steam sterilization/autoclaving following the normal washing and drying cycle to kill any residual spores. Re sterilization of previously sterilized linen requires laundering to rehydrate it.

Infection can be easily transferred between contaminated and uncontaminated items of clothing, laundry and the environment in which they are stored. It is important to store clean linen and supplies in a separate place away from the soiled linens. Shelves used for storing clean linen need to be cleaned periodically. Clean linen should be handled as little as possible.

While addressing one of the most important aspects of linen disinfection, that of preventing Nosocomial or Hospital Acquired Infections and the prevention of the dissemination of pathogens, does not mean that domestic linen should be neglected or only disinfected when visibly soiled.

Domestic linen too gathers dust, microbes and also has the presence of dead skin cells that are shed as a result of body friction with the linen. This linen too has to be cleansed properly as often as possible because the presence of dust particles and mites can cause sensitive individuals to get rashes and other allergic reaction, ranging from skin inflammations to respiratory tract disorders.

While considering linen disinfection, even linen that is soiled by infants and neonates has to be handled with precaution and care, especially in those cases when the infant is sick or shows symptoms of flu, dysentery, skin lesions including scabies, in such cases the linen has to be disinfected thoroughly before the reuse of such linen to avoid further disease progression.
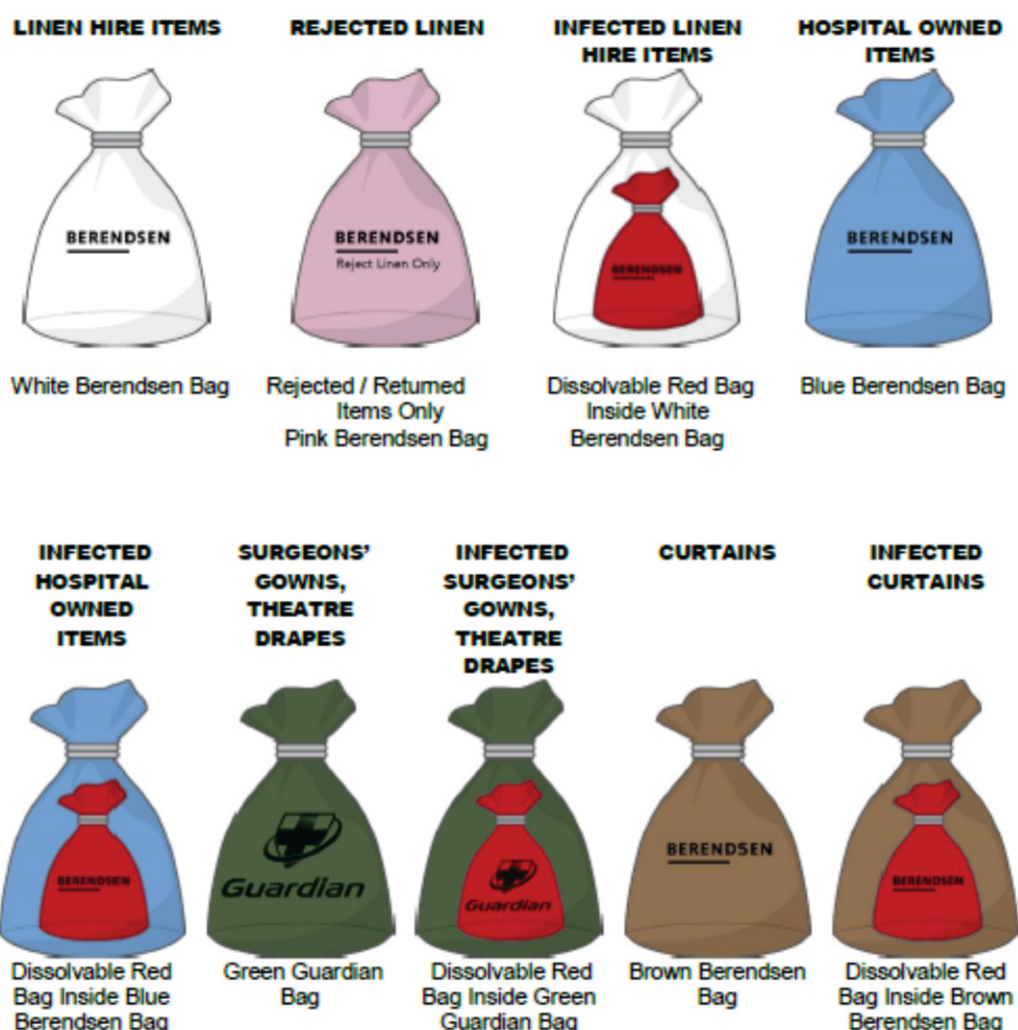
Similar precautions have to be taken for sick, ailing adults. Especially those that are bedridden or having sores. Proper hygiene in such cases is indispensable since the accumulation of microbes on the linen can aggravate the disease and the sores.

Hygiene can not only ensure good health, it can also prevent infection and frequent infections in highly sensitive persons.

Best practices for Linen Management in Hospitals

QUICK REFERENCE GUIDE

1. It is the responsibility of the person disposing of the linen to ensure that it is segregated into the 3 correct categories.
2. It is the responsibility of both the laundry contractor and hospital staff to ensure linen is clean and in a good state of repair.
3. Linen must be stored in a dedicated closed cupboard or fully enclosed mobile linen trolley secured from unauthorized persons.
4. Specific ward/department items must be appropriately labeled before sending to contracted laundry service.
5. It is staff responsibility to ensure instruments, sharps and non-laundry items such as pillows, disposable curtains and patient belongings are not disposed of with linen.
6. Patients' relatives and carers should be encouraged to wash all personal laundry at home and correct washing instructions provided.
7. Advice should be requested from Infection Prevention and Control team regarding infected linen being placed in the infectious waste stream.
8. Infection Prevention and Control policy must be adhered to when handling both clean and soiled linen.
9. Linen items should not be used for other than the intended purpose i.e. not used to mop up spills.

LINEN HIRE ITEMS — White Berendsen Bag

REJECTED LINEN — Rejected / Returned Items Only Pink Berendsen Bag

INFECTED LINEN HIRE ITEMS — Dissolvable Red Bag Inside White Berendsen Bag

HOSPITAL OWNED ITEMS — Blue Berendsen Bag

INFECTED HOSPITAL OWNED ITEMS — Dissolvable Red Bag Inside Blue Berendsen Bag

SURGEONS' GOWNS, THEATRE DRAPES — Green Guardian Bag

INFECTED SURGEONS' GOWNS, THEATRE DRAPES — Dissolvable Red Bag Inside Green Guardian Bag

CURTAINS — Brown Berendsen Bag

INFECTED CURTAINS — Dissolvable Red Bag Inside Brown Berendsen Bag

1. INTRODUCTION

The provision of clean linen is a fundamental requirement for patient care. Incorrect procedures for handling or processing of linen can present an infection risk both to staff handling and laundering linen, and to patients who subsequently use it.

2. PURPOSE

This policy defines the responsibility of managers and staff to ensure correct, safe handling and disposal of contaminated laundry, and the correct, safe distribution and storage of clean linen to minimise infection risk throughout hospitals.

3. SCOPE

This Policy applies to all staff, both clinical and non-clinical, employed by hospitals, who handle linen and also to all visiting staff including tutors, students and agency/locum staff, who handle linen.

'In the event of an infection outbreak, flu pandemic or major incident, the hospital recognizes that it may not be possible to adhere to all aspects of this document. In such circumstances, staff should take advice from their manager and all possible action must be taken to maintain ongoing patient and staff safety'

4. **DEFINITIONS**

    Clean / Unused Linen: Any linen that has not been used since it was last laundered and that has not been in close proximity to a patient or stored in a contaminated environment.

    Used Linen: All linen used in the ward/department setting that is not contaminated with either blood or body fluids.

    Infected linen: Any used linen that is soiled with blood or any other body fluid or any linen used by a patient with a known infection (whether soiled or not).

5. **DUTIES AND RESPONSIBILITIES**

    Infection Prevention Team:
    *   Review and update the Linen Handling and Laundry policy
    *   Give additional advice regarding laundry and risk assessments
    *   Include safe linen handling in all induction and update training for clinical staff
    *   Promote good practice and challenge poor practice

    Matrons / Senior and Ward Sisters:
    *   Must establish a cleanliness culture across their units and promote compliance with infection prevention guidelines, including linen handling and laundry
    *   Promote good practice and challenge poor practice

    All Healthcare Staff:
    *   Must be familiar with and adhere to the relevant infection prevention policies to reduce the risk of cross infection of patients
    *   Must adhere to the full terms and conditions of the linen handling and laundry policy
    *   Promote good practice and challenge poor practice
    *   Refer to the infection prevention team if unable to follow the policy guidelines

6. **PROCESS**

    6.1 Segregation of Linen:

    It is the responsibility of the person disposing of the linen to ensure that it is segregated appropriately. All linen may be segregated into the following three categories:
    *   Clean / Unused Linen
    *   Dirty / Used Linen
    *   Soiled / Infected Linen

    6.1.1 Clean / Unused Linen:

    Clean linen must be in a state of good repair, as tearing or roughness can damage the patient's skin. The condition of the linen in use should be monitored by the laundry contractor and by staff. Linen should also be free from stains and excessive creasing and should be acceptable to both patients and staff. See Section 6.7 for procedure on clean linen not fit for use

    6.1.2 Handling of Clean Linen:

    Once laundry has been decontaminated, every effort must be made to maintain its quality and cleanliness.

    6.1.3 Delivery:

    Laundry should be delivered to the wards/departments in clean covered containers. Clean laundry should not be transported in containers used for used / soiled laundry.

    6.1.4 Storage:

    All clean linen **must** be:
    *   stored in a clean, closed cupboard (either a dedicated linen cupboard or dedicated, fully enclosed mobile linen trolley). **Not** on top of a trolley as it is a potential fire risk.
    *   stored off the floor
    *   stored with the linen cupboard/trolley doors closed to prevent airborne contamination
    *   stored in a clean, dust free environment
    *   segregated from used / soiled linen.

    Clean linen **must not** be stored in unsuitable areas e.g. the sluice, bathrooms, in bed spaces or in corridors.

    6.1.5 Local Use:
    *   Clean linen should not be decanted onto open trolleys unless for immediate use
    *   Linen taken into an isolation room/cohort area and not used must be treated as used linen and laundered before use.
    *   Linen items should not be used for other than the intended purpose i.e. not used to mop up spills

    6.1.6 Dirty / Used Linen:

    Linen which is used but dry: Dirty / Used linen **must not have been:**
    *   visibly soiled with blood or bodily fluids
    *   used on source-isolated patients.

    Dirty / Used linen should be placed directly into a clear plastic laundry bag.
    *   Linen bags should be no more than 2/3 full.

    6.1.7 Soiled / Infected Linen:

    Any used linen that is soiled with blood or any other body fluid or any linen used by a patient with a known infection (whether soiled or not).

    This includes patients with or suspected:
    *   MRSA
    *   Extended Spectrum beta-lactamase (ESBL) or Carbapenemase producing organisms
    *   Human Immunodeficiency Virus (HIV)
    *   Hepatitis A, B or C
    *   Draining Tuberculosis (TB) lesions and open pulmonary TB
    *   Enteric Fever
    *   Dysentry (*Shigella* spp)
    *   *Salmonella*
    *   Norovirus
    *   *Clostridium difficile*
    *   Chickenpox
    *   Head or body lice, scabies
    *   Other notifiable diseases

    Soiled / Infected linen should be placed directly into a RED water-soluble alginate bag and secured, then placed into a WHITE (hire items), BLUE (hospital owned items), GREEN (Surgical gowns/drapes) or BROWN (curtains) outer bag.
    *   Linen bags should be no more than 2/3 full
    *   Never rinse or sluice contaminated laundry
    *   Dirty or soiled linen bags should be stored in 'dirty' linen cages and not on floors or obstructing public thoroughfares or the ward/department environment.

    6.2 Trust Owned Return to Sender Items:
    *   Return to Sender items that belong to specific wards/departments (e.g. slings, slide sheets, duvets, neonatal/paediatric blankets, posy mitts, dressing gowns etc) must be placed in a BLUE bag

- All items must have the hospital and ward/department name on them.
- All return to sender items should be listed on the laundry triplicate tickets (supplied by Linen Rooms). The sender should keep the bottom copy of the ticket and send the top 2 copies to the laundry in the bag. The laundry will then return the item with a copy of the ticket for matching
- Soiled / Infected return to sender items should be placed as normal directly into a RED water-soluble alginate bag and secured, then placed into a BLUE bag.

6.3 Theatre Linen:
- Dirty / Used Operating Theatre staff clothing should be placed into a GREEN plastic laundry bag
- Soiled / Infected Operating Theatre linen and staff clothing should be placed into a red water-soluble alginate bag, then placed into a GREEN outer bag
- Care should be taken to ensure that theatre instruments and sharps are not accidentally disposed of in linen.

6.4 Patient's Personal Laundry:
- Safe return of personal laundry processed off site cannot be guaranteed
- Patients / Relatives / Carers should be encouraged to wash personal laundry at home
- Many micro-organisms will be physically removed from linen by detergent and water, and most are destroyed by a high temperature wash. Any remaining micro-organisms are likely to be destroyed by tumble drying and ironing
- Patient's personal laundry should be placed in a clear plastic bag, not a water-soluble alginate bag (as private laundry facilities will not reach the required temperature to melt the bag, which may lead to damage or blocking of the domestic washing machine). The clear plastic bag should then be placed into a patient's property bag to protect the patient's dignity
- Laundry should be taken home and placed directly into a washing machine
- Clothes should be processed at the hottest wash recommended by the manufacturers' instructions
- Persons handling the laundry must be advised to wash their hands after handling the pre-washed laundry
- Relatives / carers must be advised before they take home personal laundry if it is heavily contaminated.

6.5 Infested Laundry:
Laundry that is potentially infested with parasites (e.g. bed or body lice, scabies).
- Place into a RED water-soluble alginate bag and secure, then place the alginate bag into a WHITE plastic bag
Marking of laundry as infested is not required.

6.6 Laundry which would remain hazardous following normal processing or for which additional precautions are required:
Laundry thought to be contaminated with any of the following micro-organisms must be placed in the i-hazardous waste stream in double bags and incinerated and **not** sent to the laundry service. Advice should be requested from the Infection Prevention Team.
- *Bacillus anthracis* (Anthrax)
- Viral Haemorrhagic Fevers (e.g. Lassa fever, Marburg disease, Ebola fever)
- Rabies

- Tropical pyrexia of unknown origin
- Lepromatous Leprosy
- Bioterrorism agents e.g. Smallpox
- CJD where CSF or other body fluids have leaked onto laundry items

6.7 Linen Unfit for Use
Linen deemed not fit for purpose (heavily stained, torn, rough) should be placed in a separate rejected Item pink plastic bag, labelled as 'unfit for use' and returned to the hospital laundry. Linen deemed not fit for purpose should **not** be placed in the same bag as other linen for laundry as it will remain in general use and the hospital will be recharged for its handling.

6.8 Procedure for Water-soluble Alginate Bags:
This procedure is to be used in all situations where linen is placed in water-soluble alginate bags.
1. Place the linen inside the alginate bag
2. Items that are soaking wet should be wrapped inside drier dirty laundry
3. Do not overfill the water-soluble alginate bag
4. Seal the alginate bag using the neck tie – do not knot the bag
5. Place the water-soluble alginate bag inside the appropriate coloured outer linen bag.

6.9 General Principles:
These general principles should be adhered to when handling all linen and laundry.

6.9.1 Handling Linen:
All dirty linen must be handled with care, to minimise transmission of micro-organisms via dust and skin scales.
- All dirty linen must be placed carefully and directly into the appropriate laundry bag on removal from the bed or patient. Bags should be no more than two-thirds filled
- The used linen skip should be at the bedside. **Used linen should not be carried to avoid contamination of uniforms**
- Dirty linen must never be transported around the care environment unless within an appropriately colour coded linen bag
- Vigorous, enthusiastic bed stripping and changing of curtains is microbiologically hazardous as large numbers of organisms (mainly skin flora) are dispersed. Care should be taken to minimise contamination of equipment and the near patient environment
- **Do not place used linen on the floor** or any other surfaces e.g. a locker/table top
- When beds or curtains are changed all open wounds/drains etc need to be temporarily covered during linen changes.
- Do not shake linen into the environment
- Do not change linen during wound dressings in the same area
- **Use PPE when handling dirty linen.**
Care must be taken to ensure that **no sharps** or non-laundry items are included with dirty linen before it is placed ready for laundering. Such items are potentially dangerous to staff handling the laundry.

6.9.2 Hand Hygiene:
- Hands should be decontaminated before handling clean linen and after handling used laundry.

6.9.3 Personal Protective Equipment:
- Plastic aprons should be worn by all HCWs for all bed making – this includes beds where the patient has been

discharged and patient occupied beds. Plastic aprons must be changed between beds.

- Gloves must be worn when handling laundry from an infected patient or laundry contaminated with blood and body fluids
- Face protection / eye protection must be worn where there is significant contamination with blood and body flushes likely to cause a splash injury.

6.9.4 Accidental Spillage from Used Linen:

- Gloves and apron must be worn
- Re-bag into the appropriate bag. If the appropriate bag is not obvious then the linen should be treated as infected and placed in a red alginate bag, then into a white outer bag and tied securely.
- Clean area with appropriate disinfectant if necessary.

6.9.5 Local Cleaning:

- All hospital linen should be laundered by an external laundry contractor (with the exception of specific wards authorised to have industrial washing machines)
- The washing process should have a disinfection cycle in which the load temperature is maintained at 65°C for not less than 10 minutes or preferably at 71°C for not less than 3 minutes
- Manual soaking / washing / sluicing of soiled items must never be carried out in the clinical areas by staff. This is a contamination and splash injury risk. Solid contaminants should be disposed of in the appropriate clinical waste stream and laundry bagged as per policy
- Patient's personal clothing should be bagged and sent home for cleaning. (See Section 6.4 Patients Personal Laundry)

6.9.6 Storage and Removal:

- All dirty/used linen should be removed from clinical areas as frequently as circumstances demand
- Soiled linen must be kept away from public areas
- Storage areas must remain closed and kept secure from unauthorised persons

6.9.7 Curtains:

- Curtains require washing when visibly dirty, or at least every six months.
- Curtains should be routinely changed when discharging or transferring a patient with a known transmissible infection from the area or during outbreaks
- Removal and changing of curtains result in aerosolisation of ingrained organisms which may be harmful to patients and contaminate the near patient environment. For this reason, curtains should not be changed at key times e.g. during wound dressing changes.
- Disposable curtains should not be sent for laundering but should be disposed of in the appropriate waste stream (i.e. offensive or infectious).

6.9.8 Pillows:

- All pillows used in clinical areas must have sealed intact impermeable covers
- All pillows used in clinical areas can be cleaned with chlorine-based disinfectant and re-used providing there is no tear, split or staining.
- Any pillow torn, split or stained must be discarded into the appropriate waste stream. Discarded pillows should never be placed with dirty laundry items
- New pillows can be ordered.
- Spare pillows must be returned to the pillow store.

7. TRAINING REQUIREMENTS

It is individual ward's/department's responsibility to ensure all staff have read and adhere to the linen handling and laundry policy

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Surface Disinfection

Introduction

Surface disinfection covers a very broad spectrum of objects that surround us, whether they are while we travel, work, learn and touch to even objects which we use to sit, lay down or lean against. All these surfaces are what we are referring to and possibly others.

There is a need to maintain all dairies, abattoirs, breweries and food processing plants as clean and hygienic as possible. When dirty equipment is not in use, a rapid build – up of microorganisms occurs which can result in severe contamination of the foodstuff when the equipment is re – used. If proper attention is not given to the use of clean equipment and reduction of contamination, the foodstuff will spoil rapidly. Proper sanitization will reduce the number of bacteria in all work areas and on equipment in particular.

Sterilizing should not be confused with sanitizing or disinfection. To sterilize means to destroy all forms of life; applied specially to microorganisms, including bacterial and mold spores. There are no degrees of sterilization, a reference item so to speak is either sterile or it is not.

To disinfect is to literally free from infection. This term has come to imply chemical treatment of an inanimate surface or substance to rid it of harmful micro – organisms. Disinfectants are frequently expected to perform their function in the presence of significant quantities of dirt and / or organic matter. Though organic matter may increase bioburden for a particular disinfectant to work, it still has to have the capacity to function adequately.

Sanitizing is reducing the number of bacterial contaminants to levels judged safe by Public Health authorities. It implies a degree of physical cleanliness, i.e. the sanitizer is applied to a pre–cleaned surface. In commercial eateries, industries, farms etc, disinfection is carried out mainly using sanitizing solutions which suit the purpose and serve the benefit of the user as well as the consumer or the customer.

Sanitizing of equipment and utensils is best carried out just prior to use. It is one of the most important step in the general sanitation operation for the following reasons:

- A variety of microorganisms remain on food processing equipment after it has been washed, even though it may appear clean. The organisms may be types which have been slowly accumulating on the equipment and or in the product during the processing operation. These can be removed, after cleaning the equipment, by thorough sanitizing.

- During the period that the processing equipment is unused, large numbers of bacteria may develop even though the equipment was cleaned and sanitized. This is especially true of surfaces which are difficult to dry. There are usually sufficient nutrients to support bacterial growth even on a clean surface and if it is moist, the increase in bacteria before the next usage may be tremendous.

- There may possibly be opportunity for insects or even rodents to contact idle equipment and this may result in appreciable contamination and sometimes even spread of diseases.

- Water supplies occasionally become contaminated and even Municipal supplies are sometimes of questionable quality. When such water is employed for washing or rinsing equipment, spoilage organisms may contaminate the equipment. Thus the use of a sanitizing agent in the water used to rinse equipment helps prevent such contamination.

- A programme of effective sanitizing can make an appreciable and measurable contribution to the quality and shelf life of food products.

Selection of Sanitizers

There are many types of chemical compounds used in the formation of disinfectants and sanitizers. However in the food industry the number of varieties which can be used is severely limited for a number of reasons.

- The compounds used for the purpose must not be toxic to humans in as much as their residues on food must not be harmful in any way to the quality of food and the consumer.

- They must not taint the product and must therefore be completely odorless.

- They must not color the product in any way.

- They must be relatively safe to use for hand cleaning situations.

- Most essential is high bactericidal activity.

When selecting a sanitizer, the six most popular types should be considered for their respective merits. Known by their primary ingredients they are chlorine compounds, iodophores or iodine compounds, quaternary ammonium compounds (QATS), acid anionic surfactant germicides, hydrogen peroxide and phenol.

Chlorine based sanitizers

Are most commonly used. Proven in use and acceptance over the years, they have excellent germicidal power against a wide range of bacteria. In properly blended products, they are relatively non – toxic at use concentrations (200 ppm), colorless, non – staining, easy to prepare and apply. Generally they are also the most economical. Effective cleaning is essential when using these sanitizers as some of the available chlorine may be readily consumed by organic matter other than bacteria. Possible flavor problems associated with these products should be borne in mind in the brewing industry.

Chlorine is highly corrosive to a number of metals and its use is best confined to equipment fabricated in stainless steel. Temperature is another important parameter as the effectiveness of chlorine increases with increase in temperature. However above 50°C the liberated chlorine is rapidly lost to the atmosphere, reducing the effectiveness of the solution in situ.

Chlorine compounds should therefore not be used above 50°C neither should they be used where smoked products are being handled. This is because the phenolic compounds in the smoke may react with the chlorine, producing chlorophenols which have a very strong odor, considered offensive in consumables.

Iodophores

Are basically a combination of iodine and a solubilizing agent that releases free iodine when diluted with water. They possess quick microbial action against a wide variety of microorganisms. At use concentrations, they are non staining, relatively non toxic, non

irritating and stable. No potable rinse is required if use concentration does not exceed 25 ppm available iodine.

Iodophores penetrate soil rapidly and are highly germicidal at virtually all concentrations. Many iodophores are approved for 'no rinse' sanitizing applications at 25 ppm. Iodophore-use solution temperatures should not exceed $48^{o}C$ or they will begin to 'gas – off'. The germicidal performance of different iodophore formulations may differ greatly. Products yielding the same pH and iodine concentration may yield vastly different germicidal activities at equivalent dilutions. Iodophores can be used in very hard water.

QATS

The quaternary ammonium compounds are types of cationic detergents possessing good antibacterial activity. Unfortunately their detergency properties are very poor, but they are good wetting agents. They are widely used throughout the food and meat industries and commonly formulated with detergents to form detergent / sanitizers, which clean and kill bacteria in one operation. They can also be used on their own. Although extremely effective for killing a wide spectrum of bacteria, some groups of bacteria are resistant to them. In use concentrations (200 ppm) QATS are odorless, colorless and non toxic. They are stable when heated and in the presence of organic soil. No potable water rinse is required if concentration is at or below 200 ppm active ingredient. They should not be used on processing equipment in a brewery because of possible adverse effects on head retention and flavor.

The bacteriostatic properties of the QATS plus their stability and the property of being absorbed onto surfaces results in such remaining sanitized for many hours after treatment.

QATS have generally been applied in preference to chlorine under conditions of heavy organic contamination where, to overcome the presence of the organic material, the strength of the chlorine would have to assume corrosive proportions. Generally they are combined with specific non ionic detergents for sanitizing dairy equipment.

QATS can be adversely affected by water hardness and may be incompatible with other compounds. They are completely inactivated by anionic compounds such as soaps. Acidity decreases the efficiency of many QATS to such an extent that at pH 3 their germicidal activities almost disappear while at pH 10, they show greatly improved activity. Temperature also affects their activity and an increase of about $20^{o}C$ normally doubles it.

Acid anionics

Acid anionic surfactant germicides are combinations of organic and inorganic acids with surface active agents. The acid is usually phosphoric. The germicidal effect is provided by the low pH as well as the activity of the surfactant. The acidity of this type of germicide is effective in removing or controlling the formation of microbial films. Acid anionics are low foaming agents. They are effective in hard and soft water and eliminate the need for acid rinsing. They are also non corrosive to stainless steel.

Peroxide

Hydrogen peroxide sanitizers can be used in dairies, breweries and food production plants. Using this sanitization method does away with many of the disadvantages held by other sanitization. Hydrogen peroxide containing sanitizers supersede conventional halogen sanitizers (Chlorine, Iodine, etc.) and cause the disinfection action to be rapid. They are not detrimental to the environment as when hydrogen peroxide decomposes, hydrogen and oxygen are formed. It is a broad spectrum, fast acting, sanitizer with extremely low toxicity.

Phenols

Phenol based disinfectants should not be used inside a food processing plant, as they have a strong odor which will contaminate foods, they have good cleaning and disinfecting properties and should be used in stables, poultry growing houses, toilets, drains and compounds. They should be used diluted with warm or preferably hot water. They also have good deodorizing properties.

It is noteworthy to mention that all cleaning and disinfecting chemicals should be used in concentrations mentioned by the manufacturer. The temperature at which they are used should be checked for effective disinfection and hazard – free usage.

Certain chemicals, when mixed with other compounds with which they are not compatible may liberate dangerous toxic vapors and gases. For example acid compounds should never be mixed with strong alkaline or caustic compounds. Serious burns or even death may result.

All disinfectants / sanitizers have a recommended contact time. This is the time required for them to kill the majority of bacteria they come in contact with before manufacturing operations can begin again. As this time may vary from product to product, the manufacturers instruction must be followed strictly.

Some do's and don'ts with sanitizers
DO's:
- Take the time to measure the sanitizer correctly.
- Add the sanitizer to the correct amount of water to make the correct solution for use.
- Use a clean, dry container or bucket for the solution.
- Wash away all the dirt before using the sanitizer.
- Discard the solution when the day's work is finished.

DON'Ts
- Use a sanitizer for sterilization.
- Store instruments or cleaning tools in a sanitizer solution.
- Top up sanitizer solution.
- Use yesterday's sanitizer solution.
- Mix sanitizers and detergents it may inactivate both.

Effective disinfection is a key ingredient which can help prevent or at least avoid the transmission of many microbes, more specifically pathogens.

Surfactants: Surface active agents

Surfactants are compounds that lower the surface tension (or interfacial tension) between two liquids or between a liquid and a solid. Surfactants may act as detergents, wetting agents, emulsifiers, foaming agents, and dispersants.

Surfactants lower the surface tension of an aqueous solution and are used as wetting agents, detergents, emulsifiers, antiseptics, and disinfectants. As antimicrobials, they alter the energy relationship at interfaces. Based on the position of the hydrophobic moiety in the molecule, surfactants are classified as anionic or cationic.

Composition and structure
Surfactants are usually organic compounds that are amphiphilic, meaning they contain both hydrophobic groups (their tails) and hydrophilic groups (their heads). Therefore, a surfactant contains both a water insoluble (or oil soluble) component and a water soluble component. Surfactants will diffuse in water and adsorb at interfaces between air and water or at the interface between oil and water, in the case where water is mixed with oil. The water-insoluble hydrophobic group may extend out of the bulk water phase, into the air or into the oil phase, while the water-soluble head group remains in the water phase.

World production of surfactants is estimated at 15 Mton/y, of which about half are soaps. Other surfactants produced on a particularly large scale are linear alkylbenzenesulfonates (1700 kton/y), lignin sulfonates (600 kton/y), fatty alcohol ethoxylates (700 ktons/y), and alkylphenol ethoxylates (500 kton/y).

Types of Surfactants
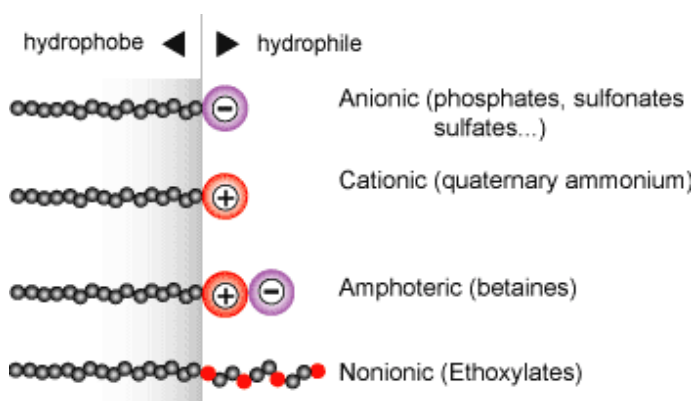
Anionic Surfactants
Soaps are dipolar anionic detergents with the general formula RCOONa/K, which dissociate in water into hydrophilic K^+ or Na^+ ions and lipophilic fatty acid ions. Because NaOH and KOH are strong bases (whereas most fatty acids are weak acids), most soap solutions are alkaline (pH 8–10) and may irritate sensitive skin and mucous membranes. Soaps emulsify lipoidal secretions of the skin and remove, along with most of the accompanying dirt, desquamated epithelium and bacteria, which are then rinsed away with the lather. The antibacterial potency of soaps is often enhanced by inclusion of certain antiseptics, eg, hexachlorophene, phenols, carbanilides, or potassium iodide. They are incompatible with cationic surfactants.

Cationic Surfactants
Cationic detergents are a group of alkyl- or aryl-substituted quaternary ammonium compounds (eg, benzalkonium chloride, benzathonium chloride, cetylpyridinium chloride) with an ionizable halogen, such as bromide, iodide, or chloride. The major site of action of these compounds appears to be the cell membrane, where they become adsorbed and cause changes in permeability. Their activity is reduced by porous or fibrous materials (eg, fabrics, cellulose sponges) that adsorb them. They are inactivated by anionic substances (eg, soaps, proteins, fatty acids, phosphates). Therefore, they are of limited value in the presence of blood and tissue debris. They are effective against most bacteria, some fungi (including yeasts), and protozoa but not against viruses and spores. Aqueous solutions of 1:1,000 to 1:5,000 have good antimicrobial activity, especially at slightly alkaline pH. When applied to skin, they may form a film under which microorganisms can survive, which limits their reliability as antiseptics. Concentrations >1% are injurious to mucous membranes.

Among the classical cationic surfactants, quaternary ammonium compounds (QACs) are the most useful antiseptics and disinfectants. QACs are membrane active agents and cause lysis of spheroplasts and protoplasts suspended in sucrose. The cationic agents hypothetically react with phospholipid components in the cytoplasmic membrane, thereby producing membrane distortion and protoplast lysis under osmotic stress. On the other hand, the positive charge on microbial cells has often been correlated to the biocidal action. The deposition of organic monolayers onto solid surfaces containing quaternary ammonium groups has been shown to prevent deposition and growth of bacterial biofilms. Molecules with a net positive charge are able to kill microorganisms both in solution and upon attachment or adsorption to surfaces, particles, liposomes or bilayers. Various cationic architectures have been tested such as polyelectrolyte layers and hyper branched dendrimers.

Non-ionic surfactants
Non-ionic surfactants are also found in many cleaning products, including carpet products. Non-ionics have no charge on their hydrophilic end, which helps make them superior oily soil emulsifiers.

Some non-ionics are high foamers (like anionics), while others do not generate much foam. Because of their lower foam profile and strong emulsifying potential, these surfactants are the preferred choice when formulating extraction cleaners and pre sprays.

However, unlike anionic surfactants, non-ionics are thick liquids or syrups that are sticky or "gooey" to the touch. When left in the carpet, non-ionic surfactants are the primary contributors to rapid resoiling.

Even with that being the case, their importance as cleaners outweighs this negative, and the cleaner or technician must take care to remove as much of the detergent residue as possible from the carpet in order to get the cleaning benefits of non-ionics without their negatives.

Non-ionic surfactants include:
- Ethoxylates
- Alkoxylates
- Cocamide

Amphoteric surfactants
Probably the least talked about surfactants are the amphoterics. These unique molecules possess both a positive and a negative charge on their hydrophilic end, giving them a net charge of zero.

Amphoteric surfactants have little utility on their own, but work extremely well in enhancing the cleaning effect of both anionic and nonionic surfactants. They can serve as "coupling agents," which hold the surfactants, solvents and inorganic salt components of a formula together.

Amphoterics are usually named in some way to indicate that they are amphoterics, as in amphoterge. Other examples of amphoterics are betaines and amine oxides.

Schematic of how surfactants work:
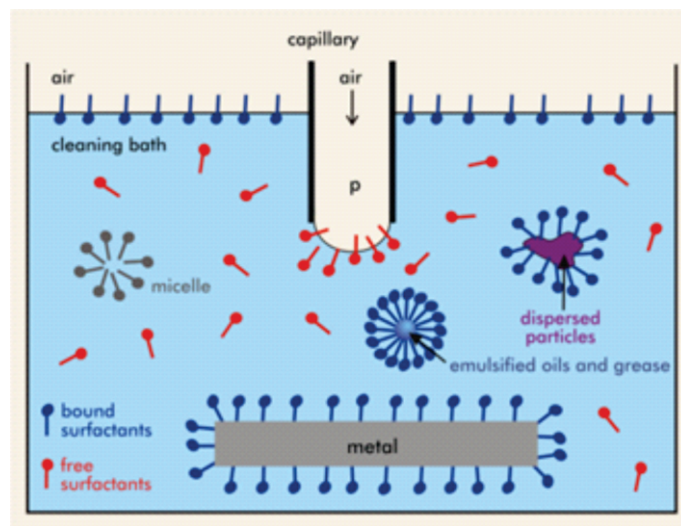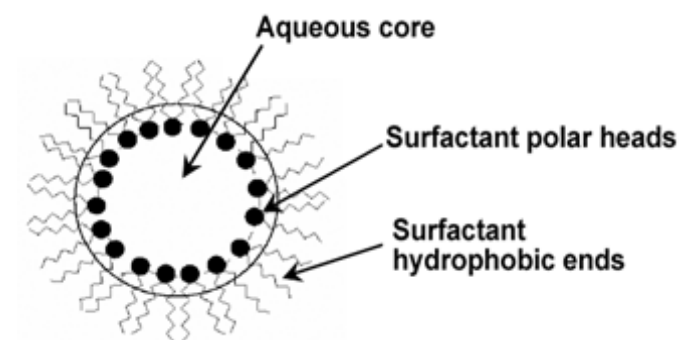
Blood Spills Disinfection in Healthcare

OSHA Blood borne Pathogen Standard

OSHA promulgated a standard entitled "Occupational Exposure to Blood borne Pathogens" to eliminate or minimize occupational exposure to blood borne pathogens. One component of this requirement is that all equipment and environmental and working surfaces be cleaned and decontaminated with an appropriate disinfectant after contact with blood or other potentially infectious materials. Even though the OSHA standard does not specify the type of disinfectant or procedure, the OSHA original compliance document suggested that a germicide must be tuberculocidal to kill the HBV.

To follow the OSHA compliance document a tuberculocidal disinfectant (e.g., chlorine) would be needed to clean a blood spill. OSHA amended its policy and stated that EPA-registered disinfectants labeled as effective against HIV and HBV would be considered as appropriate disinfectants ". . . provided such surfaces have not become contaminated with agent(s) or volumes of or concentrations of agent(s) for which higher level disinfection is recommended." When bloodborne pathogens other than HBV or HIV are of concern, OSHA continues to require use of EPA-registered tuberculocidal disinfectants or hypochlorite solution (diluted 1:10 or 1:100 with water). Studies demonstrate that, in the presence of large blood spills, a 1:10 final dilution of EPA-registered hypochlorite solution initially should be used to inactivate blood borne viruses to minimize risk for infection to health-care personnel from percutaneous injury during cleanup.

Organic and Inorganic Matter

Organic matter in the form of serum, blood, pus, or faecal or lubricant material can interfere with the antimicrobial activity of disinfectants in at least two ways. Most commonly, interference occurs by a chemical reaction between the germicide and the organic matter resulting in a complex that is less germicidal or non-germicidal, leaving less of the active germicide available for attacking microorganisms.

Chlorine and iodine disinfectants, in particular, are prone to such interaction. Alternatively, organic material can protect microorganisms from attack by acting as a physical barrier.

The effects of inorganic contaminants on the sterilization process were studied. These and other studies show the protection by inorganic contaminants of microorganisms to all sterilization processes results from occlusion in salt crystals. This further emphasizes the importance of meticulous cleaning of medical devices before any sterilization or disinfection procedure because both organic and inorganic soils are easily removed by washing.

If a sharps injury is possible, the surface initially should be decontaminated then cleaned and disinfected (1:10 final concentration). Extreme care always should be taken to prevent percutaneous injury. At least 500 ppm available chlorine for 10 minutes is recommended for decontaminating CPR training manikins. Full strength bleach has been recommended for self-disinfection of needles and syringes used for illicit-drug injection when needle-exchange programs are not available. The difference in the recommended concentrations of bleach reflects the difficulty of cleaning the interior of needles and syringes and the use of needles and syringes for parenteral injection.

The use of powders, composed of a mixture of a chlorine-releasing agent with highly absorbent resin, for disinfecting spills of body fluids has been evaluated by laboratory tests and hospital ward trials.

The inclusion of acrylic resin particles in formulations markedly increases the volume of fluid that can be soaked up because the resin can absorb 200–300 times its own weight of fluid, depending on the fluid consistency. When experimental formulations containing 1%, 5%, and 10% available chlorine were evaluated by a standardized surface test, those containing 10% demonstrated bactericidal activity. One problem with chlorine-releasing granules is that they can generate chlorine fumes when applied to urine.

Health services should have management systems in place for dealing with blood and body substance spills. Protocols should be included in procedural manuals, and emphasised in ongoing education or training programs.

The basic principles of blood and body fluid/substance spills management are:

- standard precautions apply, including use of personal protective equipment (PPE), as applicable
- spills should be cleared up before the area is cleaned (adding cleaning liquids to spills increases the size of the spill and should be avoided)
- generation of aerosols from spilled material should be avoided.

Using these basic principles, the management of spills should be flexible enough to cope with different types of spills, taking into account the following factors:

- the nature (type) of the spill (for example, sputum, vomit, faeces, urine, blood or laboratory culture)
- the pathogens most likely to be involved in these different types of spills – for example, stool samples may contain viruses, bacteria or protozoan pathogens, whereas sputum may contain *Mycobacterium tuberculosis*
- the size of the spill – for example, spot (few drops), small (<10 cm)="" or="" large="">10cm)
- the type of surface – for example, carpet or impervious flooring
- the location involved – that is, whether the spill occurs in a contained area (such as a microbiology laboratory), or in a public or clinical area of a health service, in a public location or within a community premises
- whether there is any likelihood of bare skin contact with the soiled (contaminated) surface.

Cleaning spills – equipment

Standard cleaning equipment, including a mop, cleaning bucket and cleaning agents, should be readily available for spills management. It should also be stored in an area known to all. This is particularly important in clinical areas.

To help manage spills in areas where cleaning materials may not be readily available, a disposable 'spills kit' could be used, containing a large (10 L) reusable plastic container or bucket with fitted lid, containing the following items:

- appropriate leak-proof bags and containers for disposal of waste material
- a designated, sturdy scraper and pan for spills (similar to a 'pooper scooper')
- about five sachets of a granular formulation containing 10,000 ppm available chlorine or equivalent (each sachet should contain sufficient granules to cover a 10-cm diameter spill)
- disposable rubber gloves suitable for cleaning (vinyl gloves are not recommended for handling blood)
- eye protection (disposable or reusable)

- a plastic apron
- a respiratory protection device, for protection against inhalation of powder from the disinfectant granules or aerosols (which may be generated from high-risk spills during the cleaning process).
- destroyed by incineration
- immersed in sodium hydroxide or sodium hypochlorite for 1 hour, rinsed and placed in a pan of clean water, and sterilised on an 18-minute cycle.

Single-use items in the spills kit should be replaced after each use of the spills kit.

With all spills management protocols, it is essential that the affected area is left clean and dry.

Sodium hydroxide (caustic soda) spills kits should be available for areas at risk for higher-risk Creutzfeldt–Jakob disease (CJD) spills, such as in neurosurgery units, mortuaries and laboratories.

Cleaning spills – procedures
In clinical areas, blood and body fluid/substance spills should be dealt with as soon as possible. In operating rooms, or in circumstances where medical procedures are under way, spills should be attended to as soon as it is safe to do so.

Care should be taken to thoroughly clean and dry areas where there is any possibility of bare skin contact with the surface (for example, on an examination couch).

PPE should be used for all cleaning procedures, and disposed of or sent for cleaning after use. Hands should be washed and dried after cleaning.

Where a spill occurs on a carpet, shampoo as soon as possible. Do not use disinfectant. Steam cleaning may be used instead.

Wash hands thoroughly after cleaning is completed.

Cleaning spots or small spills
Spots or drops of blood or other small spills (up to 10 cm) can easily be managed by wiping the area immediately with paper towels, and then cleaning with warm water and detergent, followed by rinsing and drying the area. Dry the area, as wet areas attract contaminants.

A hospital-grade disinfectant can be used on the spill area after cleaning.

Cleaning large spills
Where large spills (more than 10 cm) have occurred in a 'wet' area, such as a bathroom or toilet area, the spill should be carefully washed off into the sewerage system using copious amounts of water and the area flushed with warm water and detergent.

Large blood spills that have occurred in 'dry' areas (such as clinical areas) should be contained and generation of aerosols should be avoided.

Granular formulations that produce high available chlorine concentrations can contain the spilled material and are useful for preventing aerosols. A scraper and pan should be used to remove the absorbed material. The area of the spill should then be cleaned with a mop, and bucket of warm water and detergent. The bucket and mop should be thoroughly cleaned after use and stored dry.

Sodium hypochlorite (bleach)
Hypochlorites are corrosive to metals and must be rinsed off after 10 minutes and the area dried.

Cleaning spills that contain Creutzfeldt–Jakob disease prions
If a spill of tissue that is definitely or potentially infected with CJD prions occurs (for example, brain tissue), the contaminated item should either be:

The items should then be cleaned following routine cleaning and sterilisation procedures.

Surface spills should be cleaned up using paper towels before the surface is wiped with either sodium hydroxide or sodium hypochlorite, left for 1 hour (if possible, or as long as possible, with the area cordoned off), the solution wiped off and the surface cleaned by following routine cleaning procedures.

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https://www2.health.vic.gov.au/public-health/infectious-diseases/infection-control-guidelines/manage-blood-body-fluid-spills

Laboratory Decontamination Tools And Practices

Tracking the number of laboratory-acquired infections (LAIs) in a country is no easy task. Not all incidents are reported, and sometimes the victim may not be aware of the source of the infection. Despite that, the Bureau of Labor Statistics (BLS) does collect available information about those reported exposures in labs. The numbers have been declining over the past decade, but exposures do still occur.

It is possible to become infected with a pathogen simply by touching contaminated surfaces in the laboratory without personal protective equipment (PPE). According to the Clinical and Laboratory Standards Institute (CLSI), in the laboratory "telephones, doorknobs and handles, computer terminals, and other surfaces are considered contaminated." One important way to minimize this exposure risk to lab employees is to utilize good decontamination practices.

Decontamination is the act of minimizing the overall pathogenic microbial presence. For labs that process routine patient specimens, that microbial presence may exist in the form of bloodborne pathogens, bacteria, fungi, and even prions. The purpose of the decontamination process is to reduce the number of these contaminants in order to minimize the possibility of transmission or infection.

Routine Decontamination

Routine decontamination of lab counter tops or work areas should occur frequently. Lab benches should be cleaned at a minimum after each working shift and after spills occur. This routine decontamination process should be documented as well. It is also a good idea to regularly include telephones, computer terminals, and other frequently handled surfaces in the lab in the disinfection process.

Typically, chemical germicides are recommended for the surface decontamination in the lab setting. These germicides are classified as high-level, intermediate, or low-level disinfectants based on their activity and strength. The most commonly recommended disinfectant for lab surfaces is a 10-percent solution of sodium hypochlorite (or bleach), which is considered an intermediate-strength chemical germicide. Intermediate-level disinfection will eliminate most bacteria (including *Mycobacterium tuberculosis*) and all fungi, and it inactivates viruses.

Laboratories should use an EPA-registered or approved disinfectant. Manufacturers list their products with the U.S. Environmental Protection Agency (EPA), and the agency verifies that the disinfectant is effective against common pathogens and complies with Occupational Safety and Health Administration (OSHA) Bloodborne Pathogen standard requirements. These requirements include the ability of the product to effectively decontaminate contaminated surfaces and spills.

Many manufacturers offer pre-made bleach cleaning products, but many laboratories still make their own dilutions from concentrated bleach. That is an acceptable practice, but fresh bleach solutions should be made daily, as the efficacy of the solution wanes quickly. It is also important when making mixtures to consider the concentration of the purchased bleach. Traditionally, labs made a 1:10 solution, as most bleach products were sold in the past at a 5.25 percent concentration. Since many available commercial bleach concentrations now are at 8.25 percent, labs should make a 1:16 solution for disinfection purposes if they are using that bleach. Laboratorians should be sure to check the label of the bleach product purchased before making dilutions. Sodium hypochlorite is a corrosive chemical, and even in its diluted form it can cause damage to some surfaces over time. In order to prevent this, the disinfected surface should be rinsed off with sterile water or 70 percent ethanol (another intermediate chemical germicide).

Formaldehyde Use

In the Anatomical Pathology section of the laboratory, formaldehyde is sometimes used as a disinfectant. Depending on its concentration, formaldehyde can be considered a high-level disinfectant. The chemical, a known carcinogen, is placed inside a cryostat, and fumes generated buy a heat source are used to decontaminate the inside of the apparatus. However, the effectiveness of the disinfection is unclear, and the dangers of using a carcinogen mark this method as less than satisfactory. Check with the cryostat manufacturer for cleaning guidelines, but many instruments can be disinfected using 70 percent alcohol.

Chemical Concentration

While concentration of chemicals plays a role in effectiveness against pathogens, one final consideration with all lab disinfectant products is the contact time with the pathogen. Most disinfectants do not instantly kill the organisms on contact; the product must be left on the contaminated surface for a specified amount of time in order to complete its action. EPA-registered disinfectants all have designated contact times printed on the label. For example, a surface disinfectant may have a contact or kill time for hepatitis B virus of one to three minutes; however, its tuberculocidal contact time may be 10 minutes. Be sure to check labels and understand the contact time requirements. Using these products properly is the only way to ensure proper disinfection.

A Dangerous Place…

The laboratory setting is an intrinsically dangerous place where bloodborne and airborne pathogens are omnipresent, and specific actions must be taken in order to protect the people working in the department. In order to prevent LAIs, engineering controls, work practice controls, and PPE should always be employed. Decontamination of surfaces is another significant control that must be utilized properly. Understanding the proper types, concentrations, and contact times of the appropriate lab disinfectants is a practice that will reduce those threatening pathogens and make the laboratory a safer place to work. A list of BioShields' Disinfectants can be used in Laboratory Area:

S. No.	COMPOSITION	APPLICATION
1.	PHMB & DDAC (Novacide)	Cold Sterilant for Instruments
2.	Silver Nitrate & Hydrogen Peroxide (Silvicide)	Aerial Fumigant
3.	Sodium Dichloroisocynurate (NaDCC) in Powder Form (Puresafe)	Blood Spillage
4.	Benzalkonium Chloride (Microlyse)	Floor Mopping (Non-Critical Area)
5.	Glutaraldehyde & Benzalkonium Chloride (Acitar)	Floor Mopping (Critical Area)
6.	CHG, Triclosan, and Isopropyl Alcohol (Sterimax)	Hand Sanitizer
7.	BKC & Isopropyl Alcohol (Aerosept)	Surface Sprayable Disinfectant
8.	DDAC (Linosafe)	Linen Disinfectant
9.	Benzalkonium Chloride (Maxishine)	Glass window Sprayable Disinfectant
10.	Hydrochloric Acid (Exit)	Ready-To-Use Toilet Bowl Cleaner

References

U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories (BMBL). 5th ed. Washington, DC: US Department of Health and Human Services; 2011.
BioShields Data

Disinfection of some important medical/surgical equipment

Endoscopic instruments and their accessories

Fiber-optic endoscopic instruments can be divided into rigid endoscopes (laparoscopic instruments) and flexible endoscopes. There are significant processing and usage differences between rigid and flexible endoscopes.

However, both rigid and flexible endoscopes (and their accessories) require meticulous cleaning prior to undergoing the appropriate sterilization, or high level disinfection, process.

Rigid endoscopes are categorized as 'critical' items (refer to 'Spaulding's classification'), and must be sterilized between uses. Flexible endoscopes are categorized as either 'critical' or 'semi-critical' in relation to the nature of their use. Those classified as 'critical' must be sterilized. In the case of 'semi-critical' flexible endoscopes, sterilization is preferred but not mandatory. Where sterilization of 'semi-critical' flexible endoscopes is not possible, high level chemical disinfection is required.

Both rigid and flexible endoscopes can be sterilized by low temperature processes, although some methods may be unsuitable for some flexible endoscopes. Many rigid endoscopes can also withstand steam sterilization. Endoscopes with no attached lenses, fiber-optic light carriers or cables, and suitable accessories, may be sterilized by steam (refer to 'steam sterilization' and 'low temperature sterilization processes'). In all instances, the instrument manufacturer's instructions as to the preferred method of sterilization should be followed.

A wide range of accessories is available for both invasive and non-invasive endoscopes, including forceps, laparoscopic scissors, diathermy, snares, sphincterotomy knives, and lasers. Accessories used in conjunction with rigid and flexible endoscopes should be treated as 'critical' items and sterilized.

Flexible Endoscope

Rigid endoscopes

Invasive ('critical') endoscopes are mainly laparoscopes, and rigid instruments with no operating channel. Arthroscopes and laparoscopes which are inserted into sterile body cavities shall be sterile. Rigid endoscopes are classified as 'critical' (refer 'Spaulding's classification') and must be sterilized. Although high level disinfection has been used in the past, it is now considered inadequate.

Sterilization of rigid endoscopes

Sterilization of invasive devices attains a higher standard of infection control than high level disinfection can achieve, is more thoroughly controlled, and cycle times are comparable with immersion in disinfectants. Low temperature sterilization methods (ethylene oxide, peracetic acid or hydrogen peroxide plasma sterilization processes) or steam sterilization may be used for rigid endoscopes. Low temperature methods are preferable as they may reduce instrument damage caused by repeated exposure to steam (even when the instrument is steam compatible). Refer to 'steam sterilization' and 'low temperature sterilization processes'. Laparoscopes and accessory instrumentation shall be dismantled, cleaned (specially designed ultrasonic irrigators are available to assist cleaning), dried thoroughly and then reassembled prior to sterilization.

Flexible endoscopes

Flexible endoscopes can be classified according to Spaulding's classification as 'critical' (e.g. invasive) instruments that penetrate the skin or are inserted into a sterile cavity, or as 'semi-critical' (e.g. non-invasive) items that are in contact with intact mucous membranes. However, even in cases where items are not classified as 'critical', sterilization is preferable to high level disinfection.

Sterilization of flexible endoscopes

Steam sterilization is unsuitable for flexible endoscopes because they are unable to tolerate temperatures greater than 60°C. Low temperature sterilization methods are required when sterilization of flexible endoscopes is required (i.e.: flexible endoscopes categorized as 'critical') and, where sterilization is desired (i.e.: flexible endoscopes categorized as 'semi-critical') but not mandatory. Low temperature systems such as ethylene oxide, and peracetic acid sterilization systems may be used to sterilize flexible endoscopes. However, the hydrogen peroxide plasma sterilization process is not commonly used for reprocessing flexible endoscopes due to the following technical problems:

- Very long narrow lumens and those closed at one end are unsuitable for sterilization using the hydrogen peroxide plasma process
- Process compatible packaging must be used
- Biological indicators are required for routine monitoring with lengthy incubation periods
- Entire cycle takes 75 minutes, making hydrogen peroxide plasma impractical for routine processing of most gastroenterological endoscopes

High level disinfection of flexible endoscopes

Non-invasive or 'semi-critical' items may either be sterilized or high level disinfected, however sterilization is the preferred method whenever possible. In the case of bronchoscopes that are high-level disinfected, care must be taken to observe the contact time required to inactivate Mycobacterium tuberculosis.

High level disinfection is a suitable process for non-invasive ('semi-critical') endoscopes such as gastroscopes, duodenoscopes, sigmoidoscopes, proctoscopes, colonoscopes, bronchoscopes, and laryngoscopes.

Reprocessing of anaesthetic and respiratory equipment

Most anaesthetic machines and breathing systems are contaminated to a minor degree with microorganisms. Equipment that is in direct contact with the patient (breathing circuits and masks) becomes more heavily contaminated.

All anaesthetic equipment that comes into contact with a patient's body fluids (including saliva) must be changed, cleaned and thermally disinfected before use on another patient.

This includes equipment that has come into indirect contact with the anaesthetist's hands, which may be contaminated with blood, saliva or other body fluid. Additionally, unused items introduced into the anaesthetic work-field should be regarded as dirty and reprocessed.

To protect staff from aerosols generated during manual cleaning processes, preference should be given to the use of washer/disinfectors for the washing, disinfecting, rinsing and drying of respiratory apparatus. Most units automatically process through pre-wash, disinfection, rinse and drying cycles.

Endotracheal tube

Anaesthetic equipment: cleaning methods

Thorough cleaning of all instruments and equipment is an essential prerequisite in disinfection and sterilization processes:

- All systems must be disassembled completely to allow unrestricted contact of all parts with the cleaning and disinfection process
- measuring instruments and pressure gauges must be processed separately according to the recommendations of the manufacturer
- lumens of non-disposable endotracheal tubes, airways, facemasks, laryngeal masks, anaesthetic breathing circuits and cobbs connectors are to be placed over the appropriate nozzle/water jet on the washer/disinfector to ensure proper

Cleaning and rinsing

- The manufacturer's instructions for cleaning and reprocessing of anaesthetic equipment should be followed
- Mechanical washer/disinfectors must not be overloaded

The cleaning of external surfaces of anaesthetic machines and associated equipment should occur on a regular basis; the use of detergent and water is not sufficient. The internal components do not require routine cleaning and disinfection.

Anaesthetic equipment: disinfection requirements

Anaesthetic respiratory equipment is classified as 'semi-critical' (refer to 'Spaulding's classification') and requires thermal disinfection for reprocessing. Sterilization of anaesthetic and ventilator equipment is generally unnecessary.

Water temperatures for thermal disinfection

Rinse water temperature shall be between 80°C and 86°C (>80°C). Refer to 'Water temperature for thermal disinfection'.

Monitoring of washer/disinfectors

The requirement for routine microbiological monitoring of washer/disinfectors is unwarranted, as there are no current standards to determine if the washer/disinfector is microbiologically safe. Washer/disinfectors and instruments should be visually inspected and cycle parameters monitored to determine if the machine is functioning correctly:

- perform visual inspection and documentation of time at temperature.

- Instruments and equipment should be free from disinfectant and rinse additive residues
- presence of chemical residue (if the washing machine is functioning as designed with temperature, detergent, wash and rinse pressure all at the correct levels, there will be virtually no chemical residue left on instruments)

Standardized test devices are available for testing the effectiveness of wash processes.

These tests are based on a visual indication of soil removal effectiveness.

Perform regular thermocouple testing of disinfection temperatures:

- rinsing 40°C to 50°C;
- washing 50°C to 60°C;
- disinfecting 80°C to 95°C, for up to 10 minutes

Drying

Drying reduces the risk of contamination during inspection and assembly of instruments:

- drying cabinets should be used for drying anaesthetic equipment. Drying cabinet operating temperatures shall be within the range 65°C to 75°C
- On completion of the cycle, the items shall be removed and placed in the anaesthetic apparatus drying machine (if drying cycle not installed). Tubing and other items with lumens shall be placed over appropriate connectors to ensure hot air dries all surfaces. Drying facilities may be available within the washer/disinfector

Single use items

- use single use sachets of lubricant for insertion into the patient's airway
- avoid using multi-dose vials. Such vials should always be accessed with a clean needle and syringe and dedicated for single patient use only

Soaking or 'cold sterilization'

Soaking or 'cold sterilization' (immersion of the different items in solutions containing disinfectants eg. Aldehydes) has a relatively high failure rate due to dosing errors, insufficient contact time (air trapping) as well as disadvantages of toxicity, skin irritation and allergy, and environmental concerns.

So, soaking/cold sterilization should be done with regulatory body approved biocides with recommended contact time.

Management of patients with confirmed or suspected pulmonary tuberculosis

Ideally, elective operative procedures on patients who have pulmonary tuberculosis should be delayed until the patient is no longer infectious. However, if operative procedures must be performed, they should be done, if possible in operating rooms that have anterooms and staff must observe airborne precautions (refer to 'additional precautions').

For operating rooms with anterooms, the doors to the operating room should be closed, and traffic into and out of the room should be minimal to reduce the frequency with which the door opens and closes.

- A bacterial filter should be placed as close as possible to the patient to help reduce the risk of contamination of the anaesthetic equipment (ventilator and CO_2 absorbers) and prevent the discharge of tubercle bacilli into the ambient air
- Preference may be given to a disposable anaesthetic breathing circuit with appropriate filters.

Best Practices in Endoscope Disinfection (I)

Employee Training and Competency- Employee education and training are critical. An educated employee knows what to do why it is being done, and that it has been done correctly. The importance of this is highlighted in an investigation completed by the Centre for Disease Control and prevention (1999) in which an operator error was indicated in each instance of disease transmission. Flexible endoscopes are complex devices and the verification of acceptable endpoint results for many of the processing steps is still subjective. Therefore, consistently assigning personnel to processing tasks and verification of competency are recommended.

Cleaning Agents & Methods- Selection of the right cleaning products can make the difference between a process that is efficient and effective and one that is not.

Enzymatic agents are formulated for specific soil types: protease breaks down blood and other proteins, lipase breaks fats, and amylase breaks down sugar/ carbohydrates. Commonly used formulations consist of two or more enzymes combined with a detergent. The enzymes break down the protein, fat, and carbohydrate- enzymes do not emulsify soil or remove it. Some work better in warm water, some in cold. All require time at temperature (i:e., soak time) to be effective.

Detergents have a number of characteristics. Perhaps the best known is the pH. Alkaline agents are more effective for organic soils such as fat or protein. Acidic agents are more effective for inorganic soil, such as urine scale. "Neutral" agents (actually slightly alkaline, between pH 7 and 8.5) are used for surfaces such as anodized aluminium or stainless steel that might be discovered or destroyed by a highly acidic or alkaline agent. Because the endoscopes and endoscopic instruments have organic soil, alkaline detergents are more widely used. The detergent formulation also contains one or more additives to provide the following actions:

- Dissolving: solubility of soil in the water or cleaning solution
- Saponifying: chemical degradation of lipids or fats not freely soluble in water
- Peptizing: degradation and dispersion of proteins
- Wetting: lowering surface tension of a liquid to improve contact with the device and the adherent soil

- Emulsifying, dispersing, and suspending : preventing soil from redepositing on surfaces
- Sequestering: preventing minerals from precipitating out onto surface as salts

A detergent for cleaning flexible endoscopes and endoscopic instruments should contain peptizing, wetting, emulsifying,, dispersing, and suspending agents. If the source water has a high mineral content, the addition of a sequestering agent should be considered.

The removal of soil (i:e., cleaning) can be accomplished manually or automatically.

Friction
Friction is defined as the rubbing of one object or surface against another. Using friction to remove soil (e.g., rubbing/scrubbing the soiled area with a brush) is the oldest and still remains one of the best methods.

Because the internal channel is immovable, the friction is created by moving the brush bristles back and forth across the channel surface. To optimize the effectiveness of mechanical brushing, the brush should have concentric bristles, a diameter sized to the lumen, and a ball tip. Other brushes specifically shaped to clean the valve seats and instrument ports enhance the cleaning process.

Variances in brushing occur from employee to employee and from time to time with the same employee. Factors such as how "hard" the employee scrubs and how many times the brush is passed through the channel, as well as the quality or condition of the brush bristles, are not easily documented. Because these inconsistencies alter the end result, the procedure should incorporate methods designed for the worst-case scenario to ensure expected variances do not alter the outcome, thereby increasing the patient's risk of infection.

Fluidics
Fluidics (i.e., fluids under pressure) is used to remove soil and debris from internal channels/ lumens after brushing and when the design does not allow the insertion and passage of a brush through the channel. When used manually, the size of the syringe and the pressure exerted by the employee pushing the solution into the lumen

determine the pressure/force of the solution against the channel wall. When used automatically, a manifold in the unit establishes the pressure/force of the solution. When a fluid pathway must be established between two or more channels with a single attachment, a method to ensure consistent volume at pressure flows into each channel or lumen must be incorporated because fluids always flow through the path of least resistance (i.e., the channel with the largest diameter).

Ultrasonic Cleaners

Ultrasonic cleaners use a process called cavitation in which waves of acoustic energy are propagated in aqueous solutions where they can disrupt the bonds that hold particulate matter to surfaces. More specifically, the sound energy generates minute bubbles from gas nuclei in the solution. These bubbles expand until they can no longer be sustained; they then collapse or implode and create a minute vacuum, which disrupts the adherence of debris to surfaces. The devices must also be able to tolerate vibration. Thus, although ultrasonic cleaning is recommended for the cleaning of flexible endoscopic instruments, it is not recommended for flexible endoscopes because the vibration may break fiber optic bundles or lenses. However, preparation of each flexible endoscopic instrument is very important. There must be a fluid interface to ensure transmission of the sound energy; air-water interfaces do not efficiently transmit sound energy. This problem can be eliminated with a combination ultrasonic cleaner/irrigator in which a fluid pathway is established between the unit and the device, so that cleaning solutions can be instilled and irrigated through the internal channel during the process.

Infection Prevention

The risk of transmitting an infectious agent from one patient to another by an endoscope or endoscopic instrument is influenced by many factors, including the design complexity of the device, the type of soiling, the patient's condition, the processing procedures, and the employees competency. These variables account for the sporadic occurrence of infections. Therefore, a quality-assurance program is necessary to ensure that the endoscope and can be used safely for patient care. The quality-assurance program should provide standards with which to evaluate and thereby control variations in performance, procedures, and process outcomes. Although it may not be possible to eliminate variations completely, process variation can be minimized with appropriate procedures. Some causes of quality failure are inappropriately trained personnel, inadequate inventory, procedures that are not clear or complete, malfunctioning equipment, equipment being used for a purpose other than intended, and changes in the environmental conditions in which the process is performed. Inspection and functional testing should, as much as possible, be an integral part of the process.

A comprehensive audit of the external environment should be performed at least biannually. The purpose of this audit is to identify external changes or shifts that could have an impact on the efficacy and safety of the reprocessing procedure. The annual review should begin with an examination of the assumptions under which the procedures, practices or products were judged to be appropriate. These assumptions are then compared with the current or existing conditions. For example, have the defined attributes of the patient population changed (e.g. age, health status)? Has the procedure changed or procedure time lengthened? Is there more manipulation of body tissues in performing the procedure, or additional devices in the procedure? Have clinical studies been completed in which the results indicate a problem with the practices, procedures, or products? Today, clinical interventions take place more quickly, are much more aggressive, and are more likely to occur in an ambulatory care setting. The audit should provide the information needed to answer the following questions:

- If changes have occurred, will they alter the outcome of the process?
- If changes have occurred, will they require different procedures, practices, or products to ensure the safety and efficacy?
- If changes have occurred, are the indicators still appropriate to detect problems before the patient is compromised?

Patient risk for infection can be significantly reduced, if not eliminated, with a comprehensive program that assesses and takes appropriate action to accommodate variations in the patient population, the type of procedure, the processing procedures and practices, and the competence of the processing employee.

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Best Practices in Endoscope Disinfection (II)
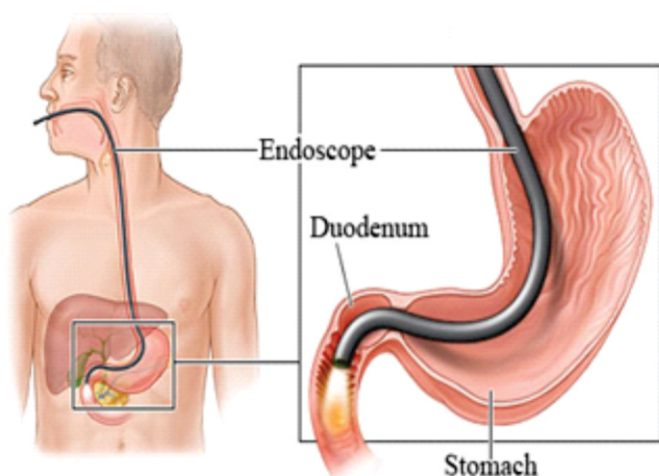
Inventory

Conduct an endoscope inventory to identify all endoscopes and method of reprocessing in use by the facility. Information reviewed for each endoscope should include but is not limited to the:

a. Endoscope manufacturer and model
b. Location of use
c. Number of procedures performed
d. Location of the endoscope manufacturer's IFUs
e. Location for reprocessing
f. Equipment used for HLD and/or sterilization
g. Status of the endoscope (i.e., retired, out for repair, in use)

Ensure that each endoscope has a unique identifier to facilitate tracking. Tracking should include the ability to determine when specific endoscopes were used for specific patients, loaned to other units or facilities, reprocessed, or repaired. Tracking is also essential for responding to device or product recalls.

Disinfection/Sterilization Breach or Failure

1. Breaches in adherence to essential disinfection and sterilization steps can be a result of malfunctioning of equipment and/or human error. Each breach is a result of unique circumstances and should be evaluated to determine the risk of disease transmission. A multi-disciplinary team that includes infection prevention, risk management, and endoscopy personnel should review each event carefully to determine the necessary corrective steps and the need for patient notification.
2. There are several resources available to assist in a breach evaluation. The multi-disciplinary team should use one or more of these documents to guide their investigation.
3. When a breach involves a suspicion of patient exposure to an improperly reprocessed endoscope, the decision to notify patients of their potential exposure should be made in consultation with an infection preventionist and state and local health departments.
4. If a healthcare provider suspects persistent bacterial contamination of an endoscope following reprocessing, either because of an increase in infections after endoscopic procedures or because of the results of microbiological culturing of endoscopes, the healthcare provider should file a voluntary report through MedWatch, the FDA Safety Information and Adverse Event Reporting program.

Discard enzymatic detergents after each use, because these products are not microbicidal and will not retard microbial growth.

Lubricating Agents

Use of lubricating agents is an effective way of extending the use life of some medical devices. Lubricants may reduce the friction commonly associated with metal-on-metal movements and thereby reduce device wear and corrosion.

If applicable, the reprocessing instructions should recommend lubricating agents, or a class of lubricating agents (e.g., water soluble lubricants) that are compatible with the medical device, its intended use, and with any subsequent processing steps such as sterilization. Also, labeling for the reusable device should refer to the lubricating agent labeling for preparation and use instructions of those agents. If your reprocessing instructions specify the use of lubricating agents, you should validate the device reprocessing methods using the lubricating agents under the conditions of use of the device.

Caution should be exercised when using oil-based and silicone based lubricants, as they may coat and protect surface microorganisms and reduce the effectiveness of certain sterilization methods, including steam and EO. They may even provide nutrients for microbial growth.
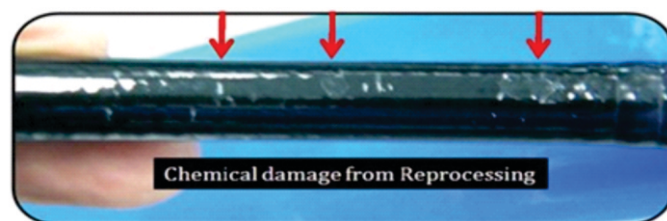

Figure 3. Chemical Damage from Reprocessing

Susceptibility of Resistant Organisms:

Organisms of concern in gastroenterology settings –such as *Clostridium difficile, Helicobacter pylori, Escherichia coli,* Human immunodeficiency virus (HIV), Hepatitis C virus, Hepatitis B virus, multidrug-resistant *M. tuberculosis*, Vancomycin-resistant enterococcus (VRE), and Methicillin-resistant *Staphylococcus aureus* (MRSA)–are susceptible to high-level disinfectants and sterilants (Rutala et al., 2008; ASGE Standards of Practice Committee et al., 2008).

Outbreaks of infection have been traced to lack of adherence to reprocessing guidelines, endoscopes which are damaged or difficult to clean, and AER design problems or failures such as breakdowns in AER water filtration systems (Rutala et al., 2008).

Reports of Carbapenem Resistant (CRE) transmission have led to challenges in achieving effective HLD, requiring added reprocessing steps in all phases.

The complex design of duodenoscopes has prompted manufacturers to implement changes in reprocessing (FDA, 2015).

Prions and other transmissible spongiform encephalopathies (TSE), including Creutzfeldt-Jakob disease (CJD) and variant Creutzfeldt-Jakob disease (v-CJD) are resistant to conventional disinfectants and sterilants. In order for an endoscope or medical/surgical device to act as a vehicle of prion transmission, it must come in contact with infective tissue (Rutala & Weber, 2013). TSEs and CJD are confined to the central nervous system and are transmitted by exposure to infectious brain, pituitary, or eye tissue. Since endoscopes do not come in contact with brain, pituitary, or eye tissue, transmission is highly unlikely (ASGE Standards of Practice Committee et al., 2008; Nelson & Muscarella, 2006; Rutala & Weber, 2013).

Determining Minimum Effective Concentration (MEC)

The high-level disinfectants/sterilants must be monitored to ensure they maintain their effectiveness.

The following factors result in a gradual reduction of the effectiveness of reusable high-level disinfectants/sterilants (Rutala et al., 2008; ASGE Standards of Practice Committee et al., 2008)

1. Decreased concentration because of challenging loads of microbes and organic matter
2. Dilution by rinse water from endoscopes or items not sufficiently dried
3. Aging of the chemical solution

Each solution's minimum effective concentration (MEC) and reuse life are established by the manufacturer. Monitor minimum effective concentration according to the disinfectant/sterilant manufacturer's instructions and maintain a log of test results. Reusable high-level disinfectant/sterilants must be disposed and replaced whenever the MEC fails or the reuse life expires, whichever comes first. Chemical HLD/sterilants that are single use and prepared onsite also need to be tested. It is important to use the product-specific test strip or chemical monitoring device (AAMI, 2015).Because chemical test strips

deteriorate with time, the bottle should be labeled with the manufacturer's expiration date and date when opened, and the strips should be used(or discarded) within the period of time specified by manufacturer. Follow the manufacturer's recommendations regarding the use of quality control procedures to ensure the strips perform properly (Rutala et al., 2008). Document quality control results. If additional chemical solution is added to an AER or basin (if manually disinfected), the reuse life should be determined by the first use/activation of the original solution. The practice of "topping off" of the chemical does not extend the reuse life
(Petersen et al., 2011)

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Endoscope Reprocessing

Endoscope is an instrument for the examination or surgical manipulation (e.g., biopsy, resection, reconstruction) of the interior of a canal or hollow viscus. It is a device that uses fiber optics and powerful lens systems to provide lighting and visualization of the interior of a human body. The portion of the endoscopes inserted into the body may be rigid or flexible, depending upon the medical procedure. Endoscopy is the examination and inspection of the interior of body organs, joints or cavities through endoscopes.

HISTORICAL ASPECTS

In the early 1900s, the first attempts to view inside the body with lighted telescopes were made. These initial devices were often fully rigid. In the 1930s, semi-flexible endoscopes called gastroscopes were developed to view inside of the stomach. Fiberoptic endoscopy entered the realm of practicality in 1957 when South African-born physician Basil Hirschowitz passed the first prototype instrument down his own throat. Widespread use of fiber optic endoscopes began in the 1960s. Many other improvements were made in the field; one of the most important of these is the charge-coupled device camera. Bell Laboratories in the US introduced charge-coupled devices in 1969. They are lightweight, low-powered, extremely sensitive image sensors, and are approximately 15 times more sensitive to light than standard regular photographic film.

TYPES OF ENDOSCOPY

Fiber optic endoscopes now have widespread use in medicine and guide a myriad of diagnostic and therapeutic procedures including:

- Anoscopy - An anoscopy is a procedure that enables a physician to view the anus, anal canal, and lower rectum using a speculum.
- Arthroscopy - Arthroscopy is a method of viewing a joint, and, if needed, to perform surgery on a joint.
- Bronchoscopy - Bronchoscopy is a test to view the airways and diagnose lung disease. It may also be used during the treatment of some lung conditions.
- Chorionic villus sampling (CVS) is a procedure for taking a small piece of placental tissue (chorionic villi) from the uterus in the early stages of pregnancy to check for the presence of genetic defects in the fetus.
- Colonoscopy - Colonoscopy is the examination of the inside of the colon and large intestine to detect polyps, tumors, ulceration, inflammation, colitis diverticula, Chrohn's disease, and discovery and removal of foreign bodies.
- Colposcopy - It is the direct visualization of the vagina and cervix to detect cancer, inflammation, and other conditions.
- Cystoscopy is a procedure that enables your health care provider to view the inside of your bladder and urethra in great detail using a specialized endoscope (a tube with a small camera used to perform tests and surgeries) called a cystoscope.
- Esophagogastroduodenoscopy (EGD) - It is an examination of the lining of the esophagus, stomach, and upper duodenum with a small camera (flexible endoscope) which is inserted down the throat.
- Endoscopic retrograde cholangiopancreatography (ERCP) - It is an endoscopic procedure used to identify stones, tumors, or narrowing in the bile ducts.
- Gastroscopy - It is the examination of the lining of the esophagus, stomach, and duodenum. Gastroscopy is often used to diagnose ulcers and other sources of bleeding and to guide biopsy of suspect GI cancers.
- Laparoscopy - This is the visualization of the stomach, liver and other abdominal organs including the female reproductive organs, for example, the fallopian tubes.
- Proctoscopy, sigmoidoscopy, proctosigmoidoscopy: examination of the rectum and sigmoid colon.
- Thoracoscopy - This is the examination of the pleura (sac that covers the lungs), pleural spaces, mediastinum, and pericardium.

SIGNIFICANCE OF ENDOSCOPE REPROCESSING

Flexible endoscopy procedures are now a routine part of patient diagnosis and treatment in hospitals and surgery centers. The demand for these safe and effective procedures continues to increase. Endoscopies are performed with sophisticated, reusable, flexible instruments that have specific requirements for cleaning, disinfection and sterilization. Because of this, adherence to recommended practices and guidelines for reprocessing is a critical component of infection control and reducing the risk of nosocomial infections. Failure to employ appropriate cleaning and disinfection/sterilization of endoscopes has been responsible for multiple nosocomial outbreaks and serious, sometimes life-threatening, infections. Flexible endoscopes, by virtue of the site of use, have a high bioburden of microorganisms after use. To reduce the bioburden proper reprocessing of endoscope should be done.

STEPS OF MANUAL ENDOSCOPE REPROCESSING

Decontamination of endoscopes is undertaken at the beginning and end of each list, and between patients, by trained staff in a dedicated room. There are six basic steps in cleaning scopes: pre-cleaning, leak testing, cleaning, disinfection/sterilization, drying, and storing.

a) Pre-cleaning- Pre-cleaning is an essential reprocessing step that removes patient biomaterial and microorganisms from the endoscope. Following an endoscopy, biomaterial from the patient is present on the insertion tube and within the internal channels of the endoscope. All channels must be cleaned, even if unused, due to fluid and debris entering these channels at the distal tip. Patient biomaterial provides a nutrient source that will promote the growth of potentially pathogenic microorganisms. Also, when this biomaterial is not removed immediately after a procedure, it will dry and harden. The surface of the hardened material functions as a barrier that prevents the penetration of disinfecting and sterilizing agents that kill microorganisms. Preliminary cleaning should be started before the endoscope is detached from the light source/videoprocessor. Immediately after removing the endoscope from the patient, wipe the insertion tube with the wet clothe or sponge soaked in the freshly prepared enzymatic detergent solution. A reprocessing delay may occur when a patient has both upper and lower procedures performed during the same visit. The endoscope from the first procedure is kept in the procedure room until the second procedure is completed. If pre-cleaning is not initiated within an hour, the endoscope should be soaked in an appropriate enzymatic detergent according to the manufacturer's recommendations, before continuing with mechanical cleaning and then terminal reprocessing. This process will allow for any dried debris to be loosened and ensure its removal during cleaning.

b) Leak testing - Followed by cleaning leak testing should be done. Manufacturer's instructions should be followed for leak testing. The leak tester is attached and pressurized the endoscope before submerging it in water. With the pressurized insertion tube completely submerged, the distal portion of the endoscope is flexed

in all directions, observing for bubbles. The entire endoscope is submerged and observed the head of it, the insertion tube, distal bending section and the universal cord for bubbles coming from the interior of the endoscope. The leak test will detect damage to the interior or exterior of the endoscope. If a leak is detected or the endoscope appears damaged manufacturer's instruction should be followed.

c) <u>Cleaning</u> - Endoscope valves and detachable distal tips are removed from the endoscope prior to manual cleaning. Manual cleaning is a multi-step process that involves accessories for brushing and flushing the endoscope channels and openings. Manual cleaning is carried out in a sink and filled with water to an identified level to ensure correct detergent concentration and temperature in accordance to manufacturers instructions. Detergent and water solutions are discarded after each use to prevent cross contamination. A lowfoaming enzymatic detergent, which is compatible with endoscope, is used at the appropriate dilution and temperature according to manufacturers' guidelines. All accessible channels and parts should be brushed properly. Then the endoscope and all removable parts are thoroughly rinsed to remove the visual debris.

d) <u>Disinfection</u> - FDA recommends the high level disinfection of the endoscopes. The endoscopes and all removable parts are completely immersed in a basin of high-level disinfectants. Disinfectant is injected inside the interior part of the endoscope and a steady flow of HLD solution through the endoscope is necessary. Complete microbial destruction cannot occur unless all surfaces are in complete contact with the chemical. Addition of fresh disinfectant or sterilant to an existing solution will reduce the efficacy of the freshly prepared disinfectant or sterilant. All channels are completely purged with air before removing the endoscope from high-level disinfectant. After that endoscopes are thoroughly rinsed with clean water to get rid off the chemical residues of the disinfectant, which can cause the injury to skin and mucous membrane. Glutaraldehyde can be used for high level disinfection.

e) <u>Drying</u> - An air purge should be completed immediately following the water rinse. Residual water, depending upon the quality used to rinse the endoscope, may contain waterborne organisms. Bacteria like *Pseudomonas aeruginosa* can form biofilms in the interior wall of the endoscope. If sterile water is not used to rinse the endoscope, an additional alcohol purge followed by a forced air purge is required to thoroughly dry the endoscope and prevent recontamination. An alcohol flush is recommended to enhance drying whether or not sterile water is used during the final rinse. Dry the exterior of the endoscope with a soft, clean lint-free towel.

f) <u>Storage</u> - Endoscopes should be stored in a clean, dry, and well-ventilated area to minimize the possibility of recontamination. All valves and the water resistant cap should be removed during storage to facilitate drying. During storage, many facilities use distal tip protectors, most of which are essentially sponges. These protectors will absorb moisture and may harbour microorganisms. To minimize the risk of recontamination, these protectors are typically designed for single-use only.

QUALITY ASSURANCE & SAFETY INSTRUCTIONS
All staff in any setting where gastrointestinal endoscopy is performed must adhere to infection control principles that will maintain a safe environment, free from the possibility of spreading disease to patients and coworkers. All staff involved in decontamination has access to and wear appropriate personal protective equipment including full-face visors, single-use gloves and aprons. During manual cleaning, forearms should be protected. Minimum Effective Concentration (MEC) of the high level disinfectant is monitored to maintain the stability of the product. Endoscope reprocessing should be done in a separate room so that chance of cross contamination is reduced. Following things should be considered for a proper reprocessing area including adequate space for reprocessing activities, proper airflow and ventilation requirements, work flow patterns, work surfaces, lighting, adequate utilities such as electrical support and water, handwashing and eye washing facilities, air drying capability, and storage. Each endoscopy setting should have a spill containment plan specific for the high-level disinfectant or sterilant used. Reprocessing protocol should be reviewed at regular interval of time.

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Storage and Handling of Sterile Instruments/Devices

Medical devices/surgical instruments are used throughout the hospital to perform procedures on patients on a daily basis. These procedures are performed in theatre, the ward, maternity and doctors rooms. Contaminated devices need to be transported safely to the CSSD to be decontaminated. Contaminated devices should be transported in a manner that will ensure the safety of the staff and other patients. For this reason it is best to transport contaminated devices in closed, durable, and easy to decontaminate trolleys. It is not acceptable to transport contaminated items on open trolleys only covered with a piece of linen. Linen is not impermeable and will not contain pathogenic soils and microorganisms.

Once medical devices/surgical instruments have been decontaminated (cleaned, packed and sterilized) they need to transported and stored in a sterile store. The Centre for Disease Control state in their guidelines that medical devices/surgical instruments that have been sterilized must be handled using aseptic techniques in order to prevent contamination. A pack will only stay sterile if it is not exposed to any adverse events. It is difficult to say how long an item will remain sterile for on a shelf as contamination is event related. The chances of a pack becoming contaminated are greater if a pack is handled frequently. Other factors that could compromise sterility include; poor storage conditions, conditions during transport and quality of packing materials. For this reason packs should be stored on shelves that are easy to clean and slotted to allow for adequate circulation of air. Sterile packs and sets should not be compressed. Sets should rather be stored one per shelf, and gowns, linen and bowl packs can be stored on their sides to prevent compression. They should be arranged in a manner that they are easy to locate and handling is reduced.

It is critical that contaminated and sterilized medical devices are transported in safe manner and stored correctly to prevent cross contamination, to protect the staff, the patients and to ensure sterility is not compromised.

Packaging

Once items are cleaned, dried, and inspected, those requiring sterilization must be wrapped or placed in rigid containers and should be arranged in instrument trays/baskets according to the guidelines. These guidelines state that hinged instruments should be opened; items with removable parts should be disassembled unless the device manufacturer or researchers provide specific instructions or test data to the contrary; complex instruments should be prepared and sterilized according to device manufacturer's instructions and test data; devices with concave surfaces should be positioned to facilitate drainage of water; heavy items should be positioned not to damage delicate items; and the weight of the instrument set should be based on the design and density of the instruments and the distribution of metal mass. While there is no longer a specified sterilization weight limit for surgical sets, heavy metal mass is a cause of wet packs (i.e., moisture inside the case and tray after completion of the sterilization cycle). Other parameters that may influence drying are the density of the wraps and the design of the set.

There are several choices in methods to maintain sterility of surgical instruments, including rigid containers, peel-open pouches (e.g., self-sealed or heat-sealed plastic and paper pouches), roll stock or reels (i.e., paper-plastic combinations of tubing designed to allow the user to cut and seal the ends to form a pouch) and sterilization wraps (woven and nonwoven). Healthcare facilities may use all of these packaging options. The packaging material must allow penetration of the sterilant, provide protection against contact contamination during handling, provide an effective barrier to microbial penetration, and maintain the sterility of the processed item after sterilization. An ideal sterilization wrap would successfully address barrier effectiveness, penetrability (i.e., allows sterilant to penetrate), aeration (e.g., allows ETO to dissipate), ease of use, drapeability, flexibility, puncture resistance, tear strength, toxicity, odor, waste disposal, linting, cost, and transparency. Unacceptable packaging for use with ETO (e.g., foil, polyvinylchloride, and polyvinylidene chlorine [kitchen-type transparent wrap]) or hydrogen peroxide gas plasma (e.g., linens and paper) should not be used to wrap medical items.

In central processing, double wrapping can be done sequentially or non-sequentially (i.e., simultaneous wrapping). Wrapping should be done in such a manner to avoid tenting and gapping. The sequential wrap uses two sheets of the standard sterilization wrap, one wrapped after the other. This procedure creates a package within a package. The non-sequential process uses two sheets wrapped at the same time so that the wrapping needs to be performed only once. This latter method provides multiple layers of protection of surgical instruments from contamination and saves time since wrapping is done only once. Multiple layers are still common practice due to the rigors of handling within the facility even though the barrier efficacy of a single sheet of wrap has improved over the years. Written and illustrated procedures for preparation of items to be packaged should be readily available and used by personnel when packaging procedures are performed.

Loading

All items to be sterilized should be arranged so all surfaces will be directly exposed to the sterilizing agent. Thus, loading procedures must allow for free circulation of steam (or another sterilant) around each item. Historically, it was recommended that muslin fabric packs should not exceed the maximal dimensions, weight, and density of 12 inches wide x 12 inches high x 20 inches long, 12 lbs, and 7.2 lbs per cubic foot, respectively. Due to the variety of textiles and metal/plastic containers on the market, the textile and metal/plastic container manufacturer and the sterilizer manufacturers should be consulted for instructions on pack preparation and density parameters.

There are several important basic principles for loading a sterilizer: allow for proper sterilant circulation; perforated trays should be placed so the tray is parallel to the shelf; non-perforated containers should be placed on their edge (e.g., basins); small items should be loosely placed in wire baskets; and peel packs should be placed on edge in perforated or mesh bottom racks or baskets.

Storage

Studies in the early 1970s suggested that wrapped surgical trays remained sterile for varying periods depending on the type of material used to wrap the trays. Safe storage times for sterile packs vary with the porosity of the wrapper and storage conditions (e.g., open versus closed cabinets). Heat-sealed, plastic peel-down pouches and wrapped packs sealed in 3-mil (3/1000 inch) polyethylene overwrap have been reported to be sterile for as long as 9 months after sterilization. The 3-mil polyethylene is applied after sterilization to extend the shelf life for infrequently used items. Supplies wrapped in double-thickness muslin comprising four layers, or equivalent, remain sterile for at least 30 days. Any item that has been sterilized should not be used after the expiration date has been exceeded or if the sterilized package is wet, torn, or punctured.

Although some hospitals continue to date every sterilized product and use the time-related shelf-life practice, many hospitals have switched to an event-related shelf-life practice. This latter practice recognizes that the product should remain sterile until some event causes the item to become contaminated (e.g., tear in packaging, packaging becomes wet, seal is broken). Event-related factors that contribute to the contamination of a product include bioburden (i.e., the amount of contamination in the environment), air movement, traffic, location, humidity, insects, vermin, flooding, storage area space, open/closed shelving, temperature, and the properties of the wrap material. There are data that support the event-related shelf-life practice. One study examined the effect of time on the sterile integrity of paper envelopes, peel pouches, and nylon sleeves. The most important finding was the absence of a trend toward an increased rate of contamination over time for any pack when placed in covered storage. Another evaluated the effectiveness of event-related outdating by microbiologically testing sterilized items. During the 2-year study period, all of the items tested were sterile. Thus, contamination of a sterile item is event-related and the probability of contamination increases with increased handling.

Following the sterilization process, medical and surgical devices must be handled using aseptic technique in order to prevent contamination. Sterile supplies should be stored far enough from the floor (8 to 10 inches), the ceiling (5 inches unless near a sprinkler head [18 inches from sprinkler head]), and the outside walls (2 inches) to allow for adequate air circulation, ease of cleaning, and compliance with local fire codes (e.g., supplies must be at least 18 inches from sprinkler heads). Medical and surgical supplies should not be stored under sinks or in other locations where they can become wet. Sterile items that become wet are considered contaminated because moisture brings with it microorganisms from the air and surfaces. Closed or covered cabinets are ideal but open shelving may be used for storage. Any package that has fallen or been dropped on the floor must be inspected for damage to the packaging and contents (if the items are breakable). If the package is heat-sealed in impervious plastic and the seal is still intact, the package should be considered not contaminated. If undamaged, items packaged in plastic need not be reprocessed.

Quality Control/Monitoring

A quality control program should be established within the practice setting that applies to all aspects of the sterilization process and sterilizer performance, including:
- Sterilizer equipment documentation
- Preventive maintenance
- Mechanical, biological, and chemical monitoring
- Product identification, traceability and recall procedure
- Visual inspection of packaging when applicable
- Residual-air (Bowie-Dick type) testing of prevacuum steam sterilizers

Documentation

Information recorded from each sterilization cycle should include, but not be limited to:
- Identification of the sterilizer used (e.g. sterilizer)
- Type of sterilizer and cycle used
- Load or lot control number
Contents of the load
- Exposure time and temperature if not provided by recording chart
- Name of operator
- Results of biological and chemical monitoring.

Event-Related Shelf Life Policy
- All sterile items will no longer have an expiration date; loss of sterility is event related. These items may be used as long as the integrity of the package is not compromised (e.g., wet, torn, damaged, suspected of being contaminated).
- Place an indefinite shelf-life label on each sterilized item.
- Document each label with at least the sterilizer identification number, load number, operator's initials, and sterilization date.
- Properly wrap and heat sterilize each item to provide an effective barrier to microbes.
- Do not use instrument packs if mechanical or chemical indicators indicate inadequate processing.
- Examine wrapped packages of sterilized instruments before opening them to ensure the barrier wrap has not been compromised during storage. Reclean, repack, and resterilize any instrument package that has been compromised (e.g., dropped, torn, or wet).
- Ensure proper storage of items to reduce package contamination and compromise.
- Maintain stock rotation according to the principle "first in, first out" so that older items are used first.

Minimize Risk of Surgical Site Infection (I)

Surgical site infection is divided into two main groups, incisional and organ–space. Incisional infections are further subdivided into superficial (skin and subcutaneous tissue) and deep (deep soft tissue such as fascia and muscle layers). Organ–space surgical site infection involves any part of the anatomy other than the incision that is opened or manipulated during an operation (Figure 1). The criteria for the different sites of infection are given below.
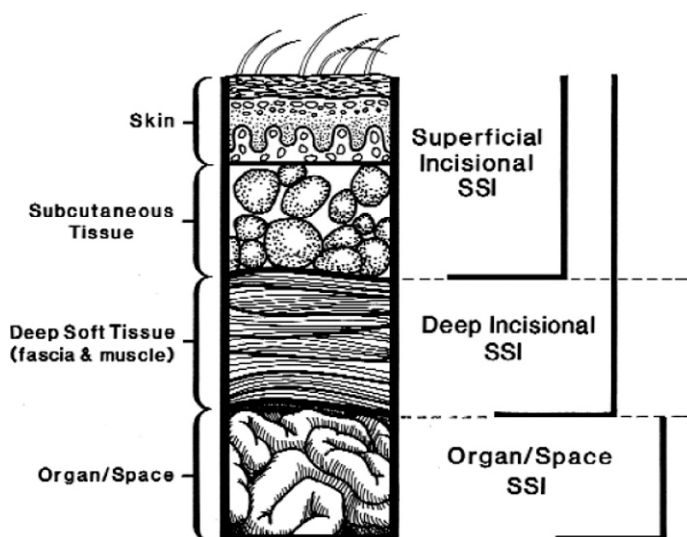

Figure 1 - Cross-section of abdomen depicting classification of surgical site infection according to the Centres for Disease Control and Prevention (United States)

The aim of skin disinfection is to remove and rapidly kill skin flora at the site of a planned surgical incision. The antiseptics that are currently available do not eliminate all microorganisms, and coagulase-negative Staphylococci can be isolated even after three applications of agents such as iodine-alcohol to the skin.

The United States Food and Drug Administration defines a skin disinfectant as a "fast acting, broad-spectrum and persistent antiseptic-containing preparation that significantly reduces the number of microorganisms on intact skin." There is no clear-cut level of bacterial skin load that should be removed or killed before surgery, and 80% of bacteria in surgical site infections originate from the skin of the patient. Therefore, the Food and Drug Administration and authorities in Europe and elsewhere have set standards that a disinfectant for presurgical skin preparation must meet before it can be legally marketed. The Food and Drug Administration requires testing at both 10 minutes and 6 hours: disinfectants should reduce colony-forming units (CFU) by more than 2 log10 at dry sites (e.g. abdominal skin) and by 3 log10 at moist sites (e.g. groin).

Most guidelines recommend a scrub-paint technique for applying a disinfectant. One study indicated, however, that spraying might be sufficient. The number of bacteria expected at a surgical site ultimately determines the number of disinfectant applications. As a general rule, three applications are sufficient; however, in areas with high densities of bacteria, this might not be sufficient to kill all vegetative bacteria.

Before a patient's skin is prepared for a surgical procedure, it should be cleansed of gross contamination (e.g. dirt, soil or any other debris). Although preoperative showering has not been shown to reduce the incidence of surgical site infection, it might decrease bacterial counts and ensure that the skin is clean. The antiseptics used to prepare the skin should be applied with sterile supplies and gloves or by a no-touch technique, moving from the incision area to the periphery. The person preparing the skin should use pressure, because friction increases the antibacterial effect of an antiseptic. For example, alcohol applied without friction reduces bacterial counts by 1.0–1.2 log10 CFU compared with 1.9–3.0 log10 CFU when friction is used. Alcoholic sprays have little antimicrobial effect and produce potentially explosive vapours.

Alcoholic compounds: For centuries, alcohols have been used for their antimicrobial properties. Ethanol and isopropanol act within seconds, are minimally toxic to the skin, do not stain and are not allergenic. They evaporate readily, which is advantageous for most disinfection and antisepsis procedures. The uptake of alcohol by intact skin and the lungs after topical application is negligible. Alcohols have better wetting properties than water due to their lower surface tensions, which, with their cleansing and degreasing actions, make them effective skin antiseptics. Alcoholic formulations used to prepare the skin before invasive procedures should be filtered to ensure that they are free of spores; otherwise, 0.5% hydrogen peroxide should be added.

Alcohols have some disadvantages. If alcoholic antiseptics are used repeatedly, they may dry and irritate the skin. In addition, they are flammable (the flash-point should be considered) and cannot penetrate protein-rich materials. The exact mechanism by which alcohols destroy microorganisms is not fully understood. The most plausible explanation for their antimicrobial action is that they coagulate (denature) proteins, such as enzymatic proteins, thus impairing specific cellular functions. Ethanol and isopropanol at appropriate concentrations have broad spectra of antimicrobial activity that include vegetative bacteria, fungi and viruses. Their antimicrobial efficacies are enhanced in the presence of water, with optimal alcohol concentrations being 60–90% by volume.

Alcohols such as 70–80% ethanol kill vegetative bacteria such as *S. aureus, Streptococcus pyrogenes,* Enterobacterales and *P. aeruginosa* within 10–90 seconds in suspension tests. Isopropanol is slightly more bactericidal than ethanol and is highly effective against Vancomycin-Resistant enterococci. It also has excellent activity against fungi such as *Candida* spp., *Cryptococcus neoformans, Blastomyces dermatitidis, Coccidioides immitis, Histoplasma capsulatum, Aspergillus niger* and dermatophytes and mycobacteria, including *Mycobacterium tuberculosis*. Alcohols generally do not, however, destroy bacterial spores, and fatal infections due to *Clostridium* species have occurred when alcohol was used to sterilize surgical instruments.

Both ethanol and isopropanol inactivate most viruses with a lipid envelope (e.g. influenza virus, herpes simplex virus and adenovirus). Several investigators found that isopropanol had less virucidal activity against naked, nonenveloped viruses. In experiments by Klein and DeForest, 2-propanol, even at 95%, did not inactivate

nonenveloped poliovirus type 1 or coxsackievirus type B within 10 min, whereas 70% ethanol inactivated these enteroviruses. Neither 70% ethanol nor 45% 2-propanol killed hepatitis A virus when their activities were assessed on stainless-steel discs contaminated with faecally suspended virus. Of the 20 disinfectants tested, only three reduced the titre of hepatitis A virus by more than 99.9% in 1 min (2% glutaraldehyde, sodium hypochlorite with > 5000 ppm free chlorine, and a quaternary ammonium formulation containing 23% HCl). Bond et al. and Kobayashi et al. showed that 2-propanol (70% for 10 minutes) or ethanol (80% for 2 minutes) rendered human plasma contaminated with hepatitis B virus at high titre non-infectious for susceptible chimpanzees. Both 15% ethanol and 35% isopropanol readily inactivated human immunodeficiency virus (HIV), and 70% ethanol rapidly inactivated high titres of HIV in suspension, independent of the protein load. The rate of inactivation decreased when the virus was dried onto a glass surface and high levels of protein were present. In a suspension test, 40% propanol reduced the rotavirus titre by at least 4 log10 in 1 min, and both 70% propanol and 70% ethanol reduced the release of rotavirus from contaminated

fingertips by 2.7 log10 units, whereas the mean reductions obtained with liquid soap and an aqueous solution of chlorhexidine gluconate were 0.9 and 0.7 log10 units, respectively. Alcohol is thus the most widely used skin disinfectant. Alcohols used for skin disinfection before invasive procedures should be free of spores; although the risk of infection is minimal, the low additional cost for a spore-free product is justified. One study indicated that isopropanol in a commercial hand rub could be absorbed dermally, transgressing the religious beliefs of some health-care workers, although the results have been put into question by a recent trial. WHO resolved the issue in their most recent guidelines on hand hygiene by carefully analysing the available information and concluding that use of alcoholic compounds for patient care does not transgress religious beliefs. Alcoholic compounds are not suitable for use during surgery at or in close proximity to mucous membranes or the eyes.

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Minimize Risk of Surgical Site Infection (II)

Chlorhexidine: Chlorhexidine gluconate, a cationic bisbiguanide, has been widely recognized as an effective, safe antiseptic for nearly 40 years. Chlorhexidine formulations are used extensively for surgical and hygienic hand disinfection; other applications include preoperative showers (for whole-body disinfection), antisepsis in obstetrics and gynaecology, management of burns, wound antisepsis and prevention and treatment of oral disease (plaque control, pre- and postoperative mouthwash, oral hygiene). When chlorhexidine is used orally, its bitter taste must be masked; it can also stain the teeth. Intravenous catheters coated with chlorhexidine and silver sulfadiazine are used to prevent catheter-associated bloodstream infections.

Chlorhexidine is most commonly formulated as a 4% aqueous solution in a detergent base; however, alcoholic preparations have been shown in numerous studies to have better antimicrobial activity than detergent-based formulations. Bactericidal concentrations destroy the bacterial cell membrane, causing cellular constituents to leak out of the cell and the cell contents to coagulate. The bactericidal activity of chlorhexidine gluconate against vegetative Gram-positive and Gram-negative bacteria is rapid. In addition, it has a persistent antimicrobial action that prevents regrowth of microorganisms for up to 6 hours. This effect is desirable when a sustained reduction in microbial flora reduces the risk for infection, such as during surgical procedures. Chlorhexidine has little activity against bacterial and fungal spores except at high temperatures. Mycobacteria are inhibited but are not killed by aqueous solutions. Yeasts and dermatophytes are usually susceptible, although the fungicidal action varies with the species. Chlorhexidine is effective against lipophilic viruses, such as HIV, influenza virus and herpes simplex virus types 1 and 2, but viruses like poliovirus, coxsackievirus and rotavirus are not inactivated. Blood and other organic material do not affect the antimicrobial activity of chlorhexidine significantly, in contrast to their effects on povidone-iodine. Organic and inorganic anions such as soaps are, however, incompatible with chlorhexidine, and its activity is reduced at extremely acidic or alkaline pH and in the presence of anionic- and nonionic-based moisturizers and detergents.

Microorganisms can contaminate chlorhexidine solutions, and resistant isolates have been identified. For example, Stickler and Thomas found chlorhexidine-resistant *Proteus mirabilis* after extensive use of chlorhexidine over a long period to prepare patients for bladder catheterization. Resistance of vegetative bacteria to chlorhexidine was thought to be limited to certain Gram-negative bacilli such as *P. aeruginosa, Burkholderia (Pseudomonas) cepacia, P. mirabilis* and *S. marcescens*, but genes conferring resistance to various organic cations, including chlorhexidine, have been identified in *S. aureus* clinical isolates.

There are several other limitations to the use of chlorhexidine. When it is absorbed onto cotton and other fabrics, it usually resists removal by washing. Long-term experience with use of chlorhexidine has shown that the incidence of hypersensitivity and skin irritation is low, but severe allergic reactions including anaphylaxis have been reported. Although cytotoxicity has been observed in exposed fibroblasts, no deleterious effects on wound healing have been found in vivo. While there is no evidence that chlorhexidine gluconate is toxic if it is absorbed through the skin, ototoxicity is a concern when chlorhexidine is instilled into the middle ear during operations. High concentrations of chlorhexidine and preparations containing other compounds, such as alcohols and surfactants, may also damage the eyes, and its use on such tissues is not recommended.

Iodophors: Iodophors have essentially replaced aqueous iodine and tincture as antiseptics. These are chemical complexes of iodine bound to a carrier such as polyvinylpyrrolidone (povidone) or ethoxylated nonionic detergents (poloxamers), which gradually release small amounts of free microbicidal iodine. The most commonly used iodophor is povidone-iodine. Preparations generally contain 1–10% povidone–iodine, equivalent to 0.1–1.0% available iodine. The active component appears to be free molecular iodine. A paradoxical effect of dilution on the activity of povidone-iodine has been observed: as the dilution increases, bactericidal activity increases to a maximum and then falls. Commercial povidone–iodine solutions at dilutions of 1:2 to 1:100 kill *S. aureus* and *Mycobacterium chelonae* more rapidly than do stock solutions. *S. aureus* can survive a 2-minute exposure to full-strength povidone-iodine solution but cannot survive a 15-second exposure to a 1:100 dilution of the iodophor. Thus, iodophors must be used at the dilution stated by the manufacturer.

The exact mechanism by which iodine destroys microorganisms is not known. It may react with the microorganisms' amino acids and fatty acids, destroying cell structures and enzymes. Depending on the concentration of free iodine and other factors, iodophors exhibit a broad range of microbiocidal activity. Commercial preparations are bactericidal, mycobactericidal, fungicidal and virucidal but not sporicidal at the dilutions recommended for use. Prolonged contact is required to inactivate certain fungi and bacterial spores. Despite their bactericidal activity, povidone-iodine and poloxamer-iodine solutions can become contaminated with *B. (P.) cepacia* or *P. aeruginosa*, and contaminated solutions have caused outbreaks of pseudobacteraemia and peritonitis. *B. cepacia* was found to survive for up to 68 weeks in a povidone-iodine antiseptic solution. The most likely explanation for the survival of these microorganisms in iodophor solutions is that organic or inorganic material and biofilm provide mechanical protection.

Iodophors are widely used for antisepsis of skin, mucous membranes and wounds. A 2.5% ophthalmic solution of povidone-iodine is more effective and less toxic than silver nitrate or erythromycin ointment when used as prophylaxis against neonatal conjunctivitis (ophthalmia neonatorum). In some countries, povidone-iodine alcoholic solutions are used extensively for skin antisepsis before invasive procedures. Iodophors containing higher concentrations of free iodine can be used to disinfect medical equipment. However, iodophor solutions designed for use on the skin should not be used to disinfect hard surfaces because the concentrations of antiseptic solutions are usually too low for this purpose.

The risk of side-effects, such as staining, tissue irritation and resorption, is lower with use of iodophors than with aqueous iodine. Iodophores do not corrode metal surfaces; a body surface treated with iodine or iodophor solutions may absorb free iodine, however. Consequently, increased serum iodine (and iodide) levels have been found in patients, especially when large areas were treated for a long period. For this reason, other disinfectants should be considered for

patients with hyperthyroidism and other disorders of thyroid function. Because severe local and systemic allergic reactions have been observed, iodophors and iodine should not be used in patients with allergies to these preparations. Iodophores have little if any residual effect; however, they may have residual bactericidal activity on the skin surface for a limited time, because free iodine diffuses into deep regions and also back to the skin surface. The antimicrobial efficacy of iodophors is reduced in the presence of organic material such as blood.

Triclosan and chloroxylenol (para-chlorometaxylenol): Triclosan (Irgasan DP-300, Irgacare MP) has been used for more than 30 years in a wide array of skin-care products, including handwashes, surgical scrubs and consumer products. A review of its effectiveness and safety in health-care settings has been published. A concentration of 1% has good activity against Gram-positive bacteria, including antibiotic-resistant strains, but is less active against Gram-negative organisms, mycobacteria and fungi. Limited data suggest that triclosan has a relatively broad antiviral spectrum, with high-level activity against enveloped viruses such as HIV-1, influenza A virus and herpes simplex virus type 1. The nonenveloped viruses proved more difficult to inactivate.

Clinical strains of bacteria resistant to triclosan have been identified, but the clinical significance remains unknown. Triclosan is added to many soaps, lotions, deodorants, toothpastes, mouth rinses, commonly used household fabrics, plastics and medical devices. The mechanisms of triclosan resistance may be similar to those involved in antimicrobial resistance, and some of these mechanisms may account for the observed cross-resistance of laboratory isolates to antimicrobial agents. Consequently, concern has been raised that widespread use of triclosan formulations in non-health-care settings and products might select for biocide resistance and even cross-resistance to antibiotics. Environmental surveys have not, however, demonstrated an association between triclosan use and antibiotic resistance.

Triclosan solutions have a sustained residual effect against resident and transient microbial flora, which is minimally affected by organic matter. No toxic, allergenic, mutagenic or carcinogenic potential has been identified in any study. Triclosan formulations can help control outbreaks of Methicillin-Resistant *S. aureus* when used for hand hygiene and as a bathing cleanser for patients, although some methicillin-resistant *S. aureus* isolates have reduced triclosan susceptibility. Triclosan formulations are less effective than 2–4%

chlorhexidine gluconate when used as surgical scrub solutions, but properly formulated triclosan solutions can be used for hygienic hand washing. para-Chlorometaxylenol (chloroxylenol, PCMX) is an antimicrobial agent used in hand-washing products, with properties similar to those of triclosan. It is available at concentrations of 0.5–3.75%. Nonionic surfactants can neutralize this compound.

Octenidine: Octenidine dihydrochloride is a novel bispyridine compound and an effective, safe antiseptic agent. The 0.1% commercial formulation compared favourably with other antiseptics with respect to antimicrobial activity and toxicological properties. It rapidly killed both Gram-positive and Gram-negative bacteria as well as fungi in vitro and in vivo. Octenidine is virucidal against HIV, hepatitis B virus and herpes simplex virus. Like chlorhexidine, it has a marked residual effect. No toxicological problems were found when the 0.1% formulation was applied according to the manufacturer's recommendations. The colourless solution is a useful antiseptic for mucous membranes of the female and male genital tracts and the oral cavity, but its unpleasant taste limits its use orally. In a recent observational study, the 0.1% formulation was highly effective and well tolerated in the care of central venous catheter insertion sites, and the results of this study are supported by those of a randomized controlled clinical trial. Octenidine is not registered for use in the United States.

Table II – Antimicrobial agents recommended for surgical skin preparation

Solution	Comment
60-90% isopropanol	Not for use on mucous membranes
7.5-10% povidone-iodine	Can be used on mucous membranes
2-4% chlorhexidine	Not for use on eyes, ears, mucous membranes
Iodine, 3% preparation	Not for use on mucous membranes: can cause skin irritation if left for a long time
para-Chlorometaxylenol (PCMX)	Not for use on newborn babies; penetrates skin

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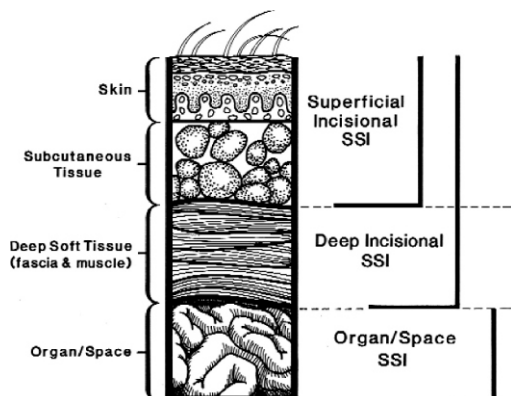
Preventing SSI (Surgical Site Infections)

INTRODUCTION

Surgical patients commonly developed postoperative "irritative fever," followed by purulent drainage from their incisions, overwhelming sepsis, and often death. It was in late 1860s, Joseph Lister introduced the principles of antisepsis, after which postoperative infectious morbidity decreased substantially. Lister's work radically changed surgery from an activity associated with infection and death to a discipline that could eliminate suffering and prolong life.

Definition-A surgical site infection is an infection that occurs after surgery in the part of the body where the surgery took place.

Surgical site infections can sometimes be superficial infections involving the skin only. Other surgical site infections are more serious and can involve tissues under the skin, organs, or implanted material.

Cross-section of abdominal wall depicting CDC classifications of surgical site infection.

Prevalence-SSIs are the third most frequently reported nosocomial infection, accounting for 14% to 16% of all nosocomial infections among hospitalized patients.

SSIs were the most common nosocomial infection, accounting for 38% of all such infections.

When surgical patients with nosocomial SSI died, 77% of the deaths were reported to be related to the infection, and the majority (93%) were serious infections involving organs

Infection occurs within 30 days after the operation and infection involves only skin or subcutaneous tissue of the incision and at least one of the following symptoms:

1. Purulent drainage, with or without laboratory confirmation, from the superficial incision.

2. Organisms isolated from an aseptically obtained culture of fluid or tissue from the superficial incision.
3. At least one of the following signs or symptoms of infection: pain or tenderness, localized swelling, redness, or heat.

Operating Room- A room in an operating suite where operations are performed

Surgical Personnel- Any healthcare worker who provides care to surgical patients during the pre-, intra-, or postoperative periods.

Surgical Team Member -Any healthcare worker in an operating room during the operation who has a surgical care role.

MICROBIOLOGY

The distribution of pathogens isolated from SSIs has not changed markedly during the last decade *Staphylococcus aureus*, coagulase-negative Staphylococci, *Enterococcus* spp., and *Escherichia coli* are mainly the most frequently isolated pathogens. An increasing proportion of SSIs are caused by antimicrobial-resistant pathogens, such as Methicillin-Resistant *S. aureus* (MRSA) or by *Candida albicans*.

Pathogenesis

Most SSIs are believed to be acquired during surgery. Reports demonstrating matching strains of pathogens from the surgeon's fingers and postoperative infection.

Best practices in Preventing SSI
1.   **Surgical Hand Preparation**

"Wait, this one's a lawyer. We'd better wash our hands."

Surgical hand preparation is probably the most important SSI prevention strategy. Its importance is supported by expert opinion experimental studies and success stories of SSI reduction via mere hand hygiene promotion campaigns. It was recognized that the incidence of puerperal fever was high in obstetric clinic. After the compulsory introduction of hand antisepsis for obstetricians using chlorinated lime, he

succeeded in lowering the incidence of this life-threatening, postpartum maternal infection. Either alcohol-based hand rubs or aqueous antiseptic scrubs can then be subsequently used between patients, provided hands are not visibly soiled. However, the rapid antimicrobial action, wider spectrum of activity, lower side effects and the absence of the risk of hand contamination by rinsing water in resource-poor areas might favour alcohol-based solutions. Perform a preoperative surgical scrub for at least 2 to 5 minutes using an appropriate antiseptic. Scrub the hands and forearms up to the elbows. After performing the surgical scrub, keep hands up and away from the body (elbows in flexed position) so that water runs from the tips of the fingers toward the elbows. Dry hands with a sterile towel and don a sterile gown and gloves. Clean underneath each fingernail. Do not wear hand or arm jewellery.

2. **Postponing Elective Surgery in the Case of Symptomatic Remote Infection** - Preparation of Surgical Patients -Eradicate or control all infections remote to the surgical site before elective surgery whenever possible. Screen patients for presence of hyperglycaemia and implement protocol to adequately control the serum blood glucose level (less than 11.1mmol/L) preoperatively and during the first 48 hours postoperatively (10-12). There is evidence for such measures to be applied in patients undergoing cardiothoracic operations, most notably coronary artery bypass graft (CABG).

Minimize the preoperative length of stay of the patients in the hospital, such as completing presurgical assessments and correcting underlying conditions before admission to hospital for operation and performing elective surgery, where possible, in ambulatory day centres.

Educate the patients about the increased risk of smoking on postoperative surgical site infection and encourage patients to stop smoking or taking any tobacco consumption at least 30 days before the operation.

3. **Expertise of the Surgeon** The surgeon's expertise and surgical technique is probably very important, although subjective and difficult to analyze. Furthermore, it is almost impossible to perform a randomized trial on this subject. An excellent surgical technique is believed to reduce SSI by: maintaining effective haemostasis while preserving adequate blood supply; gentle handling of tissue; removal of devitalized tissue; eradication of dead space; and appropriate management of the postoperative incision. These techniques can be learned and it has been suggested since the mid-1980s that surgical simulation has a beneficial impact on surgeons' experience and performance.

Antimicrobial prophylaxis. Administer a prophylactic antimicrobial agent only when indicated, and select it based on its efficacy against the most common pathogens causing SSI for a specific operation.

For high-risk caesarean section, administer the prophylactic antimicrobial agent immediately after the umbilical cord is clamped.

4. **Screening for MRSA Carriage on Admission** The rationale behind this approach is to detect MRSA skin carriage before incision, identify carriers and administer glycopeptides prophylaxis in the case of known carriage.

As an example, Dutch investigators reported that nasal- and or pharynx decontamination with chlorhexidine before cardiac surgery significantly lowered deep SSIs, bacteraemia and lower respiratory tract infections.

5. **Preoperative Bathing or Showering** - There is currently no evidence that preoperative showering with an antiseptic agent reduces SSI rates, despite the fact that it has been shown to reduce skin colonization. The CDC recommends that patients shower or bathe with an antiseptic agent prior to surgery.

6. **Hair Removal** - In one study, SSI rates were 5.6% in patients who had hair removed by razor shave compared to a 0.6% rate among those who had hair removed by depilatory or who had no hair removed. The increased SSI risk associated with shaving has been attributed to microscopic cuts in the skin that later serve as sites for bacterial multiplication. Clipping hair immediately before an operation also has been associated with a lower risk of SSI than shaving or clipping the night before an operation.

7. **Preoperative Skin Preparation** - Skin preparation in the operating theatre immediately before surgery is routinely implemented in daily clinical practice worldwide, based on expert opinion. However, even with optimal preparation, true sterilization of the skin is impossible. To the best of our knowledge, there is no consensus on the best antiseptic agent to be used, although, very recently, a prospective randomized no blinded study revealed a superiority of 2% chlorhexidine combined with 70% isopropyl alcohol versus 10% povidone-iodine for preventing SSI after clean-contaminated surgery. For several decades, povidone-iodine or chlorhexidine have been generally used for skin antisepsis.

In some comparisons of the two antiseptics when used as preoperative hand scrubs, chlorhexidine gluconate achieved greater reductions in skin microflora than did povidone-iodine and also had greater residual activity after a single application. Further, chlorhexidine gluconate is not inactivated by blood or serum proteins.

8. **Gloves & Adhesive Drapes** - Sterile gloves and adhesive drapes are almost always used in the operating theatre. They contribute to prevent site contamination, but also reduce blood-borne pathogen transmission from patients to surgeons. However, many gloves reveal tiny punctures after use that mostly go unnoticed by the operating team and may double the SSI risk. Hence, the use of sterile gloves does not render surgical hand preparation unnecessary.[1] Double-gloving or glove-changing might reduce the risk of punctures but does not guarantee their absence.

9. **Laminar Airflow in the Operating Theatre** - Several drawbacks of laminar air flow in the operating theatre were identified. Many hospitals in resource-rich countries are equipped with relatively expensive vertical or horizontal laminar airflow systems that reduce the bacterial burden in the air.

10. **Other Practices** - Use of staples versus sutures or the use of drains; both are reported to be similar in terms of SSI risk. Some studies concluded that primary closure in dirty abdominal surgery leads to less SSIs than delayed primary closure, whereas others report the opposite. Microbial sealing (with mechanical blockage of pathogen migration to the surgical wound) may be a new approach to reduce wound contamination, but this has yet to prove its effectiveness in reducing SSI rates.

11. **Postsurgical Wound Care** - Hands must be cleaned before and after wound care.

12. **Public (mandatory) Reporting of HAI & SSI Rates** - Public reporting of HAIs or SSIs is mandatory such a reporting method might influence healthcare workers' motivation or how to handle possible unexpected consequences.

13. **Management of infected or colonized surgical personnel** - Work exclusion policies should be enforceable and include a statement of authority to exclude ill personnel, they should also be designed to encourage personnel to report their illnesses and exposures.

14. **Antimicrobial prophylaxis**
Surgical antimicrobial prophylaxis (AMP) refers to a very brief course of an antimicrobial agent initiated just before an operation begins. AMP is not an attempt to sterilize tissues, but a critically timed adjunct used to reduce the microbial burden of intraoperative contamination to a level that cannot overwhelm host defences. AMP does not pertain to prevention of SSI caused by postoperative contamination. Intravenous infusion is the mode of AMP delivery used most often in modern surgical practice. Essentially all confirmed AMP indications pertain to elective operations in which skin incisions are closed in the operating room.

15. **Operative characteristics:**
Intraoperative issues
a. Operating room environment
(1) Ventilation
Laminar airflow and use of UV radiation have been suggested as additional measures to reduce SSI risk for certain operations.. Filter all air, recirculated and fresh, through the appropriate filters per the American Institute of Architects' recommendations. Introduce all air at the ceiling, and exhaust near the floor.
(2) Environmental surfaces Environmental surfaces in U.S. operating rooms (e.g., tables, floors, walls, ceilings, lights) are rarely implicated as the sources of pathogens important in the development of SSIs. Nevertheless, it is important to perform routine cleaning of these surfaces to reestablish a clean environment after each operation.
(3) Environmental Protection Agency (EPA)-approved hospital disinfectant should be used to decontaminate the affected areas before the next operation.
(4) Conventional sterilization of surgical instruments - Inadequate sterilization of surgical instruments has resulted in SSI outbreaks.

Flash sterilization of surgical instruments should only be used for emergency or unplanned cases. Flash sterilization of implant devices should be avoided. Standard procedures and staff proficiency of flash sterilization should be monitored. Flash sterilization record should be maintained and updated.
Scrub suits-Surgical team members often wear a uniform called a "scrub suit" that consists of pants and a shirt.
Masks-The wearing of surgical masks during operations to prevent potential microbial contamination of incisions is a longstanding surgical tradition in combination with protective eye wear, such as goggles or glasses with solid shields, or chin length face shields be worn whenever splashes, spray, spatter, or droplets of blood or other potentially infectious.
Surgical caps/hoods and shoe covers Surgical caps/hoods are inexpensive and reduce contamination of the surgical field by organisms shed from the hair and scalp.
Sterile gloves Sterile gloves are put on after donning sterile gowns Sterile gloves are worn to minimize transmission of microorganisms from the hands of team members to patients and to prevent contamination of team members' hands with patients' blood and body fluids. If the integrity of a glove is compromised (e.g., punctured), it should be changed as promptly as safety permits. Wearing two pairs of gloves (double-gloving) has been shown to reduce hand contact with patients' blood and body fluids when compared to wearing only a single pair.
Gowns and drapes Sterile surgical gowns and drapes are used to create a barrier between the surgical field and potential sources of bacteria. Gowns are worn by all scrubbed surgical team members and drapes are placed over the patient.
Asepsis and surgical technique Rigorous adherence to the principles of asepsis by all scrubbed personnel is the foundation of surgical site infection prevention.
Do not use tacky mats at the entrance to the operating room suite or individual operating rooms for infection control.

Postoperative Issues
a. Incision care -the incision is usually covered with a sterile dressing for 24 to 48 hours. Which has to be changed as directed by the doctor.
b. Discharge planning-Unnecessary prolong stay has to be avoided. Educate the patient and family regarding proper incision care, symptoms of SSI, and the need to report such symptoms.

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WHO Guidelines For Safe Surgery (I)

Confronted with worldwide evidence of substantial public health harm due to inadequate patient safety, the World Health Assembly (WHA) in 2002 adopted a resolution urging countries to strengthen the safety of health care and monitoring systems. The resolution also requested that WHO take a lead in setting global norms and standards and supporting country efforts in preparing patient safety policies and practices. In May 2004, the WHA approved the creation of an international alliance to improve patient safety globally; WHO Patient Safety was launched the following October. For the first time, heads of agencies, policy-makers and patient groups from around the world came together to advance attainment of the goal of "First, do no harm" and to reduce the adverse consequences of unsafe health care. The purpose of WHO Patient Safety is to facilitate patient safety policy and practice. It is concentrating its actions on focused safety campaigns called Global Patient Safety Challenges, coordinating Patients for Patient Safety, developing a standard taxonomy, designing tools for research policy and assessment, identifying solutions for patient safety, and developing reporting and learning initiatives aimed at producing 'best practice' guidelines. Together these efforts could save millions of lives by improving basic health care and halting the diversion of resources from other productive uses. The Global Patient Safety Challenge, brings together the expertise of specialists to improve the safety of care. The area chosen for the first Challenge in 2005–2006, was infection associated with health care. This campaign established simple, clear standards for hand hygiene, an educational campaign and WHO's first Guidelines on Hand Hygiene in Health Care. The problem area selected for the second Global Patient Safety Challenge, in 2007–2008, was the safety of surgical care.

The groundwork for the project began in autumn 2006 and included an international consultation meeting held in January 2007 attended by experts from around the world. Following this meeting, expert working groups were created to systematically review the available scientific evidence, to write the guidelines document and to facilitate discussion among the working group members in order to formulate the recommendations. A steering group consisting of the Programme Lead, project team members and the chairs of the four working groups, signed off on the content and recommendations in the guidelines document. The guidelines were pilot tested in each of the six WHO regions—an essential part of the Challenge—to obtain local information on the resources required to comply with the recommendations and information on the feasibility, validity, reliability and cost–effectiveness of the interventions.

Ten Essential Objectives for Safe Surgery

Surgical care is complex and involves dozens of steps which must be optimized for individual patients. In order to minimize unnecessary loss of life and serious complications, operating teams have 10 basic, essential objectives in any surgical case, which the WHO safe surgery guidelines support.

1. The team will operate on the correct patient at the correct site.
2. The team will use methods known to prevent harm from administration of anaesthetics, while protecting the patient from pain.
3. The team will recognize and effectively prepare for life threatening loss of airway or respiratory function.
4. The team will recognize and effectively prepare for risk of high blood loss.
5. The team will avoid inducing an allergic or adverse drug reaction for which the patient is known to be at significant risk.
6. The team will consistently use methods known to minimize the risk for surgical site infection.
7. The team will prevent inadvertent retention of instruments and sponges in surgical wounds.
8. The team will secure and accurately identify all surgical specimens.
9. The team will effectively communicate and exchange critical information for the safe conduct of the operation.
10. Hospitals and public health systems will establish routine surveillance of surgical capacity, volume and results.

Guide to infrastructure, supplies, and anaesthesia standards at three levels of healthcare facilities

Level 1 - Small hospital or health centre (Should meet at least 'highly recommended' anaesthesia standards)	Level 2 - District or provincial hospital (Should meet at least 'highly recommended' and 'recommended' anaesthesia standards)	Level 3 - Referral hospital (Should meet at least 'highly recommended', 'recommended' and 'suggested' anaesthesia standards)
• Rural hospital or health centre with small number of beds (or urban location in an extremely disadvantaged area); sparsely equipped operating room for 'minor' procedures • Provides emergency measures in the treatment of 90–95% of trauma and obstetrics cases (excluding caesarean section) • Referral of other patients (for example, obstructed labor, bowel obstruction) for further management at a higher level	• District or provincial hospital (e.g. with 100–300 beds) and adequately equipped major and minor operating rooms • Short-term treatment of 95–99% of major life-threatening conditions	• A referral hospital with 300–1000 or more beds and basic intensive care facilities. Treatment aims are the same as for level 2, with the addition of: • Ventilation in operating room and intensive care unit • Prolonged endotracheal intubation • Throacic trauma care • Homodynamic and inotropic treatment • Basic intensive care unit patient management and monitoring for up to 1 week: all types of cases, but possibly with limited provision for: • Multi-organ system failure • Hemodialysis • Complex neurological and cardiac surgery • Prolonged respiratory failure • Metabolic care monitoring
Essential Procedures	**Essential Procedures**	**Essential Procedures**
• Normal delivery • Uterine evacuation • Circumcision • Hydrocele reduction, incision and drainage • Wound suturing • Control of haemorrhage with pressure dressings • Debridement and dressing of wounds • Temporary reduction of factures • Cleaning or sterilization of open and closed fractures • Chest drainage (possibly) • Abscess drainage	Same as level 1 with the following additions: • Caesarean section • Laparotomy (usually not for bowel obstruction) • Amputation • Hernia repair • Tubal ligation • Closed fracture treatment and application of plaster of Paris • Acute open orthopaedic surgery, e.g., internal fixation of fractures • Eye operations, including cataract extraction • Removal of foreign bodies e.g., in the airways • Emergency ventilation and airway management for referred patients such as those with chest and head injuries	Same as level 2 with the following additions: • Facial and intracranial surgery • Bowel surgery • Paediatric and neonatal surgery • Thoracic surgery • Major eye surgery • Major gynaecological surgery, e.g., vesico-vaginal repair
Personnel	**Personnel**	**Personnel**
• Paramedical staff or anaesthetic officer (including on-the-job training) who may have other duties as well • Nurse-midwife	• One or more trained anaesthetists • District medical officers, senior clinical officers, nurses, midwives • Visiting specialists, resident surgeon, obstetrician or gynaecologist	• Clinical officers and specialists in anaesthesia and surgery
Drugs	**Drugs**	**Drugs**
• Ketamine 50 mg/ml injection • Lidocaine 1% or 2% • Diazepam 5 mg/ml injection, 2 ml or midazolam 1 mg/ ml injection, 5 ml	• Same as level 1, but also: • Thiopental 500 mg/g powder or propofol • Suxamethonium bromide 500 mg powder	• Same as level 2 with the following additions: • Propofol • Nitrous oxide

• Pethidine 50 mg/ ml injection, 2 ml • Morphine 10 mg/ml, 1 ml • Epinephrine (adrenaline) 1mg • Atropine 0.6 mg/ml • Appropriate inhalation anaesthetic if vaporizer available	• Pancuronium • Neostigmine 2.5 mg injection • Ether, halothane or other inhalation anaesthetics • Lidocaine 5% heavy spinal solution, 2 ml • Bupivacaine 0.5% heavy or plain, 4 ml • Hydralazine 20 mg injection • Frusemide 20 mg injection • Dextrose 50% 20 ml injection • Aminophylline 250 mg injection • Ephedrine 30/50 mg ampoules • Hydrocortisone • Nitrous oxide	• Various modern neuromuscular blocking agents • Various Modern Inhalation anaesthetics • Various inotropic agents • Various intravenous antiarrhythmic agents • Nitroglycerine for infusion • Calcium chloride 10% 10 ml injection • Potassium chloride 20% 10 ml injection for infusion
Equipment: Capital Outlay	**Equipment: Capital Outlay**	**Equipment: Capital Outlay**
• Adult and paediatric self-inflating breathing bags with masks • Foot-powered suction • Stethoscope, sphygmomanometer, thermometer • Pulse Oximeter • Oxygen concentrator or tank oxygen and a drawover vaporizer with hoses • Laryngoscopes, bougies	Complete anaesthesia, resuscitation and airway management systems including: • Reliable oxygen sources • Vaporizer(s) • Hoses and valves • Bellows or bag to inflate lungs • Face masks (sizes 00–5) • Work surface and storage • Paediatric anaesthesia system • Oxygen supply failure alarm; oxygen analyser • Adult and paediatric resuscitator sets • Pulse oximeter, spare probes, adult and paediatric* • Capnograph* • Defibrillator (one per operating suite or intensive care unit)* • Electrocardiograph monitor* • Laryngoscope, Macintosh blades 1–3(4) • Oxygen concentrator(s) (cylinder) • Foot or electric suction • Intravenous pressure infusor bag • Adult and paediatric resuscitator sets • Magill forceps (adult and child), intubation stylet or bougie • Spinal needles 25G • Nerve stimulator • Automatic non-invasive blood pressure monitor	Same as level 2 with these additions (per each per operating room or intensive care unit bed, except where stated): • Electrocardiograph monitor* • Anaesthesia ventilator, reliable electric power source with manual override • Infusion pumps (two per bed) • Pressure bag for intravenous infusion • Electric or pneumatic suction • Oxygen analyser* • Thermometer (temperature probe*) • Electric warming blanket • Electric overhead heater • Infant incubator • Laryngeal mask, airways sizes 2, 3, 4 (three sets per operating room) • Intubating bougies, adult and child (one set per operating room) • Anaesthetic agent (gas and vapour) analyser • Depth of anaesthesia monitors are being increasingly recommended for cases at high risk of awareness but are not standard in many countries. * It is preferable to combine these monitoring modalities in one unit.
Equipment: Disposable	**Equipment: Disposable**	**Equipment: Disposable**
• Examination Gloves • Intravenous infusion and drug injection equipment • Suction catheter size 16FG • Airway support equipment, including airways and tracheal tubes • Oral and nasal airways	• Electrocardiograph electrodes • Intravenous equipment (minimum fluids: normal saline, Ringer lactate and dextrose 5%) • Paediatric giving sets • Suction catheter size 16FG • Suction gloves sizes 6–8 • Nasogastric tubes sizes 10–16 FG • Oral airways sizes 000–4 • Tracheal tubes sizes 3–8.5 mm • Spinal needles sizes 22 G and 25G batteries size C.	Same as level 2 with these conditions: • Ventilator circuits • Yankauer suckers • Giving sets for intravenous infusion pumps • Disposables for suction machines • Disposables for capnography, oxygen analyser in accordance with manufacturer's specifications • Sampling lines • Water traps • Connectors • Filters and fuel cells.

WHO Guidelines For Safe Surgery (II)

Recommendations to achieve 10 basic and essential objectives in any surgery.

(1) To correct patient at the correct site.
- Before induction of anaesthesia, a member of the team should confirm that the patient is correctly identified, usually verbally with the patient or family member and with an identity bracelet or other appropriate means of physical identification. Identity should be confirmed from not just the name but also a second identifier (e.g. date of birth, address, hospital number).
- A team member should confirm that the patient has given informed consent for the procedure and should confirm the correct site and procedure with the patient.
- The surgeon performing the operation should mark the site of surgery in cases involving laterality or multiple structures or levels (e.g. a finger, toe, skin lesion, vertebra). Both the anaesthetist and the nurse should check the site to confirm that it has been marked by the surgeon performing the operation and reconcile the mark with the information in the patient's records. The mark should be unambiguous, clearly visible and usually made with a permanent marker so that it does not come off during site preparation. The type of mark can be determined locally (signing, initialling or placing an arrow at the site). A cross or 'X' should be avoided, however, as this has been misinterpreted to mean that the site is the one not to be operated on.
- As a final safety check, the operating team should collectively verify the correct patient, site and procedure during a 'time out' or pause immediately before skin incision. The surgeon should state out loud the patient's name, the operation to be performed, and the side and site of surgery. The nurse and anaesthetist should confirm that the information is correct.

(2) To methods known to prevent harm from administration of anaesthetics, while protecting the patient from pain.
- The first and most important component of peri-anaesthetic care is the continuous presence of a vigilant, professionally trained anaesthesia provider. If an emergency requires the brief temporary absence of the primary anaesthetist, judgement must be exercised in comparing the threat of an emergency to the risk of the anaesthetized patient's condition and in selecting the clinician left responsible for anaesthesia during the temporary absence.
- Supplemental oxygen should be supplied for all patients undergoing general anaesthesia. Tissue oxygenation and perfusion should be monitored continuously using a pulse oximeter with a variable-pitch pulse tone loud enough to be heard throughout the operating room.
- The adequacy of the airways and of ventilation should be monitored continuously by observation and auscultation. Whenever mechanical ventilation is employed, a disconnect alarm should be used.
- Circulation should be monitored continuously by auscultation or palpation of the heart beat or by a display of the heart rate on a cardiac monitor or pulse oximeter.
- Arterial blood pressure should be determined at least every 5 minutes and more frequently if indicated by clinical

circumstances.
- A means of measuring body temperature should be available and used at frequent intervals where clinically indicated (e.g. prolonged or complex anaesthesia, children).
- The depth of anaesthesia (degree of unconsciousness) should be assessed regularly by clinical observation.

(3) To recognize and effectively prepare for life threatening loss of airway or respiratory function.
- All patients should undergo an objective evaluation of their airway before induction of anaesthesia, even when intubation is not anticipated, in order to identify potential difficulties in airway management.
- The anaesthetist should have a planned strategy for managing the airways and be prepared to execute it, even if airway loss is not anticipated.
- When the anaesthetist suspects a difficult airway, assistance during induction should be immediately available and a backup plan for airway management should be clearly identified.
- When a patient is known to have a difficult airway, alternative methods of anaesthesia should be considered, including regional anaesthesia or awake intubation under local anaesthetic.
- All anaesthetists should maintain their airway management skills and be familiar with and proficient in the multiple strategies for dealing with difficult airways.
- After intubation, the anaesthetist should always confirm endotracheal placement by listening for breath sounds as well as gastric ventilation and monitoring the patient's oxygenation with a pulse oximeter.
- Patients undergoing elective surgery should be fasting prior to anaesthesia. Those at risk of aspiration should be pre-treated to reduce gastric secretion and increase pH.

(4) To recognize and effectively prepare for risk of high blood loss.
- Before inducing anaesthesia, the anaesthetist should consider the possibility of large-volume blood loss, and, if it is a significant risk, should prepare appropriately. If the risk is unknown, the anaesthetist should communicate with the surgeon regarding its potential occurrence.
- Before skin incision, the team should discuss the risk for large volume blood loss and, if it is significant, ensure that appropriate intravenous access is established.

(5) To avoid inducing an allergic or adverse drug reaction for which the patient is known to be at significant risk.
- Anaesthetists should fully understand the pharmacology of the medication they prescribe and administer, including its toxicity.
- Every patient to whom any drug is administered must first be identified clearly and explicitly by the person administering the drug.
- A complete drug history, including information on allergies and other hypersensitivity reactions, should be obtained before administration of any medication.
- Medications should be appropriately labelled, confirmed and rechecked before administration, particularly if they

are drawn into syringes.

- Before any drug is administered on behalf of another health provider, explicit communication should take place to ensure that the two have a shared understanding of the indications, potential contraindications and any other relevant information.

(6) **To consistently use methods known to minimize the risk for surgical site infection.**

- Prophylactic antibiotics should be used routinely in all clean–contaminated surgical cases and considered for use in any clean surgical case. When antibiotics are given prophylactically to prevent infection, they should be administered within 1 hour of incision at a dose and with an antimicrobial spectrum that is effective against the pathogens likely to contaminate the procedure. Before skin incision, the team should confirm that prophylactic antibiotics were given within the past 60 minutes. (When vancomycin is used, infusion should be completed within 1 hour of skin incision.)
- Every facility should have a routine sterilization process that includes means for verifying the sterility of all surgical instruments, devices and materials. Indicators should be used to determine sterility and checked before equipment is introduced onto the sterile field. Before induction of anaesthesia, the nurse or other person responsible for preparing the surgical trays should confirm the sterility of the instruments by evaluating the sterility indicators and should communicate any problems to the surgeon and anaesthetist.
- Redosing with prophylactic antibiotics should be considered if the surgical procedure lasts more than 4 hours or if there is evidence of excessive intraoperative bleeding. (When vancomycin is used as the prophylactic agent, there is no need for redosing in operations lasting less than 10 hours.)
- Antibiotics used for prophylaxis should be discontinued within 24 hours of the procedure.
- Hair should not be removed unless it will interfere with the operation. If hair is removed, it should be clipped less than 2 hours before the operation. Shaving is not recommended as it increases the risk for surgical site infection.
- Surgical patients should receive oxygen throughout the perioperative period according to individual requirements.
- Measures to maintain core normothermia should be taken throughout the perioperative period.
- The skin of all surgical patients should be prepared with an appropriate antiseptic agent before surgery. The antimicrobial agent should be selected on the basis of its ability to decrease the microbial count of the skin rapidly and its persistent efficacy throughout the operation.
- Surgical hand antisepsis should be assured with an antimicrobial soap. The hands and forearms should be scrubbed for 2–5 minutes. If the hands are physically clean, an alcohol-based hand antiseptic agent can be used for antisepsis.
- The operating team should cover their hair and wear sterile gowns and sterile gloves during the operation.

(7) **To prevent inadvertent retention of instruments and sponges in surgical wounds.**

- A full count of sponges, needles, sharps, instruments and miscellaneous items (any other item used during the procedure that is at risk of being left within a body cavity)

should be performed when the peritoneal, retroperitoneal, pelvic or thoracic cavity is entered.

- The surgeon should perform a methodical wound exploration before closure of any anatomical cavity or the surgical site.
- Counts should be done for any procedure in which sponges, sharps, miscellaneous items or instruments could be retained in the patient. These counts must be performed at least at the beginning and end of every eligible case.
- Counts should be recorded, with the names and positions of the personnel performing the counts and a clear statement of whether the final tally was correct. The results of this tally should be clearly communicated to the surgeon.

(8) **To secure and accurately identify all surgical specimens**

- The team should confirm that all surgical specimens are correctly labelled with the identity of the patient, the specimen name and location (site and side) from which the specimen was obtained, by having one team member read the specimen label aloud and another verbally confirming agreement.

(9) **To effectively communicate and exchange critical information for the safe conduct of the operation.**

- Before skin incision, the surgeon should ensure that team members, in particular nurses, anaesthetists, and surgical assistants are aware of the critical steps of the procedure to be performed, the risk for heavy blood loss, any special equipment needed (such as instruments, implants, intraoperative imaging, frozen section pathology) and any likely deviation from routine practice. The nurse(s) should inform the team members about any critical safety concerns and the lack of availability or preparation of any special equipment. The anaesthetist should inform the team about any critical safety concerns, in particular any difficulty in preparing for resuscitation after heavy blood loss or patient comorbidities that add risk to the anaesthesia.
- In cases of bilaterality, multiple body parts (e.g. fingers or toes) and multiple levels (e.g. spine) or when intraoperative decisions on the extent of surgical resection are to be made in conjunction with radiographic imaging, the team should confirm that the necessary imaging is available and displayed in the operating room.
- Before the patient leaves the room, the surgeon should inform team members of any alterations that were made to the procedure performed, any problems that may occur in the postoperative period and essential postoperative plans (which might include antibiotics, venous thromboembolism prophylaxis, oral intake or drain and wound care). The anaesthetist should summarize the clinical condition of the patient during the operation and any other instructions needed to ensure a safe recovery. The nurse should notify the team of any additional concerns recognized during the operation or for recovery. An accurate, complete, signed surgical record should be maintained. All patient records should be:
  - clear: the patient clearly identified by his or her name and hospital number on each page, written legibly or typed and each entry signed, dated and timed;
  - objective: opinions should be based on recorded facts;
  - contemporary: notes should be written as soon as possible after an event;
  - tamper-proof: attempts to amend records should be

immediately apparent; if computerized systems are used, they should record the date and author of any notes and track any amendments;

– original: records should not be altered or amended once an entry is complete. If a mistake is noticed, amendments or corrections may be added and clearly identified as such. If a change is made to the record, it should be signed and dated, and a note should explain why the change was made.

● Information recorded by the surgeon in the operation note should include, at a minimum, the name of the main procedure performed and any secondary procedures, the names of any assistants, the details of the procedure and the intraoperative blood loss. The information recorded by the anaesthetist should include, at a minimum, intraoperative vital sign parameters recorded at regular intervals, medications and fluids administered intraoperatively and any intraoperative events or periods of patient instability. The information recorded by the nursing team should include, at a minimum, sponge, needle, sharps and instrument counts, the names and positions of the personnel performing the counts, instruments and sponges specifically left inside the patient, any action taken in the event of a count discrepancy, and, if no count was performed, the reasons for not conducting a count. The complete operation record should therefore include the names of all team members involved.

(10) To Hospitals and public health systems will establish routine surveillance of surgical capacity, volume and results.

● For surgical surveillance at the national level, the following data should be collected systematically by WHO Member States:
  – number of operating rooms,
  – number of surgical procedures performed in an operating room,
  – number of trained surgeons and number of trained anaesthetists,
  – day-of-surgery mortality rate and
  – postoperative in-hospital mortality rate.

● For surgical surveillance at hospital and practitioner levels, the following data should be collected systemically by facilities and clinicians:
  – Day-of-surgery mortality rate,
  – Postoperative in-hospital mortality rate.

Disinfection in the Hemodialysis Unit

The combination of peracetic acid and hydrogen peroxide has been used for disinfecting hemodialyzers. The percentage of dialysis centers using a peracetic acid-hydrogen peroxide-based disinfectant for reprocessing dialyzers increased.

Hemodialysis systems include hemodialysis machines, water supply, water-treatment systems, and distribution systems. During hemodialysis, patients have acquired bloodborne viruses and pathogenic bacteria. Cleaning and disinfection are important components of infection control in a hemodialysiscenter. EPA and FDA regulate disinfectants used to reprocess hemodialyzers, hemodialysis machines, and water-treatment systems.

Non critical surfaces (e.g., dialysis bed or chair, countertops, external surfaces of dialysis machines, and equipment [scissors, hemostats, clamps, blood pressure cuffs, stethoscopes]) should be disinfected with an EPA-registered disinfectant unless the item is visibly contaminated with blood; in that case a tuberculocidal agent (or a disinfectant with specific label claims for HBV and HIV) or a 1:100 dilution of a hypochlorite solution (500–600 ppm free chlorine) should be used. This procedure accomplishes two goals: it removes soil on a regular basis and maintains an environment that is consistent with good patient care. Hemodialyzers are disinfected with peracetic acid, formaldehyde, glutaraldehyde, heat pasteurization with citric acid, and chlorine-containing compounds. Hemodialysis systems usually are disinfected by chlorine-based disinfectants (e.g., sodium hypochlorite), aqueous formaldehyde, heat pasteurization, ozone, or peraceticacid. All products must be used according to the manufacturers' recommendations. Some dialysis systems use hot-water disinfection to control microbial contamination.

At its high point, 82% of U.S. chronic hemodialysis centers were reprocessing (i.e., reusing) dialyzers for the same patient using high-level disinfection. However, one of the large dialysis organizations has decided to phase out reuse and, by 2002 the percentage of dialysis facilities reprocessing hemodialyzers had decreased to 63%. The two commonly used disinfectants to reprocess dialyzers were peracetic acid and formaldehyde; 72% used peracetic acid and 20% used formaldehyde to disinfect hemodialyzers. Another 4% of the facilities used either glutaraldehyde or heat pasteurization in combination with citric acid. Infection-control recommendations, including disinfection and sterilization and the use of dedicated machines for hepatitis B surface antigen (HBsAg)-positive patients, in the hemodialysis setting were detailed in two reviews. The Association for the Advancement of Medical Instrumentation (AAMI) has published recommendations for the reuse of hemodialyzers253.

Are disinfectant residues remained after cleaning hemodialysis machine procedure safe for patients?
The dialysis machine shall be cleaned and disinfected after each patient treatment or after every 72 hours break in working. An acceptable disinfectants such as Puristeril plus or Puristeril 340, Citrosteril, Diasteril and Sporotal are used for decontamination. Puristeril 340 is designed for cold disinfection and due to the low pH value, the necessary decalcification of hemodialysis machines is easily achieved. It can be used for all haemodialysis systems like hemodialysis machines, water treatment devices and circuit pipes.

Diluted Puristeril decomposes in a non-toxic way. Degradation products of peracetic acid, which is main component of Puristeril are: hydrogen peroxide and acetic acid. Peracetic acid is widely used for disinfection due to its exceptionally broad spectrum of microbiocidal activity at low concentrations and short exposure times. After use Puristeril is easily removable by rinsing with water. This paper deals with the effect of the Puristeril toxicity on blood as a function of its concentration and incubation time. Concentration range of 3.5-70 ppm was used, with particular emphasis on concentrations close to 5 ppm, a value is the limit of sensitivity of strips of starch potassium iodide, the tests for detection of peracetic acid. There was a strong increase in autohaemolysis and malondialdehyde concentrations with increasing concentration of Puristeril. There were also changes in dependence on the parameters of the incubation time, with the greatest effects obtained after 2 hours incubation with Puristeril. The detection limit of peracetic acid used strips of starch potassium iodide does not guarantee the safety of a patient undergoing hemodialysis. Even the residual concentration of Puristeril plus cause increased lipid peroxidation of membrane, and therefore suggest the routine use of stripes on the lower limit of detection of peracetic acid or implement measurement of hydrogen peroxide residues performed with sensitivity 1 ppm.

Biofilms have been observed in the fluid pathways of hemodialysis machines. The impacts of four biocides used for the disinfection of hemodialysis systems were tested against *Candida parapsilosis* sensustricto and *Candida orthopsilosis* biofilms generated by isolates obtained from a hydraulic circuit that were collected in a hemodialysis unit. Acetic acid was shown to be the most effective agent against *Candida* biofilms. Strategies for effective disinfection procedures used for hemodialysis systems should also seek to kill and inhibit biofilms.

Low-pH cleaning agents have also been used as disinfectants, and 3% hydrogen peroxide (vol/vol) may be used to treat biofilms on implants, on the implant-surrounding tissue, on the skin surface, or on infected wounds without devices.

Checklist for Dialysis Station Routine Disinfection
Using a wiping motion (with friction), disinfect all surfaces in the dialysis station in contact with the patient and/or staff. e.g., dialysis chair or bed; tray tables; blood pressure cuffs; countertops; keyboard, etc.
- Clean dialysis machine from top to bottom.
- If visible contaminant on the machine, wipe off using an absorbent material.
- Clean the machine using wipes/cloths with a disinfectant that is acceptable to the HD machine manufacturer and the HA renal program/infection control.
- Remove excess fluid from the wipes/cloth(s) prior to using to clean machine.
- Clean the monitor.
- If available on machine, activate the wipe screen option (pauses the screen).
- If any residue remains after cleaning, wipe down screen with a clean, dry cloth.
- Clean the top of the machine.
  If the machine has a door(s), clean the front first, then the insides of the doors.

- Clean all components of the main interface (screen) and the back of the machine* unless recommended otherwise by the manufacturer. e.g., sensors and optical detectors.
- Clean exposed surfaces of dialysate, concentrate, and bicarb connectors.
- Clean each side of machine.
- Clean the area between the main interface (screen) and brakes, including the shelf.
- Clean the brakes.

* Frequency of cleaning back of machine is as per HA protocol.

¨ Ensure surfaces are visibly wet with disinfectant but not dripping. Allow surfaces to air-dry.

Air-drying is recommended to allow for sufficient contact time with the disinfectant.

¨ Remove gloves, eye goggles and gown.

¨ Perform hand hygiene.

Appendix 1: Checklist for Dialysis Station Routine Disinfection

Do not bring patient or clean supplies to station until these steps have been completed.

References

Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008

William A. Rutala, Ph.D., M.P.H.1,2, David J. Weber, M.D., M.P.H.1,2, and the Healthcare Infection Control Practices Advisory Committee (HICPAC)3

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Best practices in dialysis unit disinfection (I)

CDC Approach to BSI Prevention in Dialysis Facilities (i.e., the core Interventions for Dialysis Bloodstream infection (BSI) Prevention)

1. Surveillance & Feedback
2. Hand hygiene & glove use observations
3. Catheter and vascular access observations
4. Patient education & engagement
5. Staff education & competency
6. Catheter reduction
7. CHG/alcohol for skin antisepsis
8. Catheter hub cleansing (aka scrub-the-hub)
9. Catheter exit site care: antimicrobial ointment or disk
10. Environmental surface disinfection

Common Themes from Outbreaks

- Patient overlaps in space and time (i.e., transmission from)
- One patient to the next at the same station
- One patient to another at adjacent stations
- Breaches in medication preparation and administration practices
- Preparing medications in potentially contaminated areas
- Mobile medication carts
- Not wiping injection ports prior to accessing
- Breaches in environmental cleaning and disinfection practices
- Surfaces wiped down with patient still at station
- Rushed turnover processes

Recommendations for disinfection of the HD machine:

- After an episode of blood leak in to the dialysate.
- If surveillance cultures show high cfu or endotoxin levels
- Regular disinfection at least once a week.
- After each dialysis session or once a day (optional).
- Bleach or Citrosteril (Combination of Citric, maleic and oxalic acid) or heat or a combination may be used for disinfection of HD machine

Disinfection of the HD machine is mandatory to prevent transmission of infections between patients. The disinfection of the machine may be performed using either bleach or citrosteril or heat. Disinfection with bleach is recommended after each blood leak in to the dialysate or at a regular interval of at least one week. Disinfection with citrosteril may be performed after each dialysis session or at least once daily. The steps for sterilization are detailed below. With standard disinfectant fitted to the rear of the machine, bleach must be administered via the pickup stick (Concentrate connectors) at the front of the machine. The disinfection procedure is performed by the designated personnel in the dialysis unit. Gloves and protective glasses must be worn during the procedure by the operator.

Steps of bleach disinfection:
1. Bleach (Sodium hypochlorite 5%) is used for disinfecting the machine.
2. Bleach should not be heated.
3. If bleach disinfection is required for the blood leak, rinse machine for 15 minutes.
4. Ensure that power and water supply to the machine are operational.
5. Turn on the machines.
6. Press cleaning key.
7. Use up/down arrow keys to select "Cleaning (font supplied)"
8. Select Treatment/Rinse – select chemical mode – confirm.
9. Machine alarm "! Connect disinfectant" displayed.
10. Place PICKUP STICK Concentrate connectors into sodium hypochlorite at the front of the machine.
11. Press conf key, "Please Wait" displayed.
12. On completion "Mandatory rinse end" displayed.
13. Test for residual bleach using Chlorine test strips on completion of cycle.

Steps of citrosteril disinfection:
1. Following conditions/reminder must be fulfilled before activating the cleaning program:
   a. The dialysate lines are connected to the shunt (Rinse bridge).
   b. The shunt door is closed.
   c. The concentrate suction tubes are in the appropriate rinse ports.
   d. The interlock plate of the bigbag® connector (option) is closed.
   e. The optical detector does not sense blood.
2. Citrosteril should be fitted to the rear of the machine
3. Citrosteril should be heated ($\geqslant 60^{\circ}$C) for efficient results.
4. Ensure that power and water supply to the machine are operational.
5. Turn on the machines.
6. Ensure the basic conditions/reminder (mentioned above) has been reviewed.
7. Press cleaning key.
8. Use up/down key to select the desired program- "Hot Disinfection".
9. Press conf key, "-F-HDIS- or –F-HDIS-M-HR-"displayed.
10. On completion "Mandatory Rinse End" will be displayed.
11. It is not necessary to test residual citric acid if Citrosteril is used, since it is a decaying agent which is formulated in a non-toxic solution.

Recommendation for once monthly evaluation and monitoring:

Microbiological monitoring: water for production of dialysate and actual dialysate proportioned and exiting the dialyzer should be monitored for bacterial levels on no less than a monthly basis. Microbiological monitoring is performed to establish ongoing validation of proper disinfection protocols. The sampling should be done at the termination of dialysis at the point where dialysate exits the dialyzer. Results for total microbial counts shall not exceed 2,000 colony forming units per ml.

Assessing trends: Pertinent information, i.e., bacterial levels, conductivity and pH readings, etc., should be logged on a chart across a page so that readings can be examined and compared over an

extended period of time. This tool makes it possible to compare current readings to those taken during the past several days/weeks/months.

Cleaning and disinfection of external surfaces of HD machines

It is recommended to clean and disinfect the external surfaces of the HD machine after each dialysis session. A low-level disinfectant or any EPA-registered disinfectant solution labeled for use in a health-care setting is recommended to be used on non-critical items (including HD machines), and should also be in accordance with the machine manufacturer's recommendations. The presence of bio-burden will reduce the killing/inactivating effect of disinfectants. Therefore, if visible blood spills or other infectious material is present on the external surface of an HD machine, it should be cleaned separately (not to spread) before applying the disinfectant solution. In such cases, it is recommended to use an intermediate-level disinfectant or tuberculocidal agent (with specific label claims for HBV and HIV) or a 1:100 dilution of a hypochlorite solution (500-600 ppm free chlorine). If using disinfectant wipes, one wipe should be used to exclusively clean the blood stain followed by another wipe(s) for disinfection. All external surfaces of the machine, especially the frequently touched front panel, including the intravenous pole, the side, back and base, should be thoroughly cleaned and disinfected using *friction* and be allowed to air dry. All used towels or wipes and gloves that are contaminated with blood should be discarded in a biohazard waste container, and hand hygiene performed after glove removal.

WATER TREATMENT FOR HEMODIALYSIS

Rationale: The average hemodialysis patient is exposed to approximately 25 times the amount of water normally ingested by an individual. In addition he is deprived of the protective barrier of the gastrointestinal tract and the detoxification function of the kidneys, increasing the risk several fold of toxicity caused by the numerous chemical and microbiological contaminants in the water.

The final quality of the water is dependent on the configuration of the treatment system and the quality of the feed water which itself may be highly variable. As processes of hemodialysis evolve with use of high flux dialyzers and hemodiafiltration probably becoming increasingly used many countries in the world have made the use of ultrapure water the goal of every dialysis unit. This requires a unit to design a system capable of delivering this very high quality from the worst feed water and a monitoring system and quality assurance to prevent breakdown of the system. The following guidelines describe the setting up, maintenance and monitoring of a system designed to reliably provide ultrapure water

1. Water treatment to achieve the following water quality (AAMI standards) is mandatory for All hemodialysis units.
2. It is recommended that the hemodialysis units should try to achieve European Standards of purity of water (Table)

Maximum levels of the different water purity grades

Maximum levels	AAMI Water	European Pharmacopoeia		
		Regular water	Ultrapure water	Sterile water
Microbial contamination (CFU/ml)	200	<100	<0.1	<0.000001
Bacterial endotoxins (lU/ml)	< 2	<0.25	<0.03	<0.03

Tracking infections

Surveillance for infections (outcome measures) and monitoring adherence to recommended infection prevention practices (process measures) are important components of an infection prevention program. To enable accurate comparison and analyses of monthly rates within the same facility or meaningful bench-marking with other units/centers, it is important that a standardized and validated surveillance protocol be used uniformly by all dialysis facilities. A centralized surveillance system for health-care-associated infections like the CDC's national health-care safety network (NHSN), which requires all participating facilities to strictly follow every specific surveillance criteria, can provide accurate and reliable data that can be used to identify problem areas as well as measure progress of prevention efforts. Implementation of the CDC's NHSN Dialysis Event Protocol (accessible online: www.cdc.gov/nhsn/dialysis) by other dialysis facilities outside the United States have been demonstrated to be feasible. Dialysis events that should be reported include (a) intravenous antimicrobial starts, (b) positive blood cultures and (c) evidence of local access site infection (pus, redness or increased swelling at the vascular access site), and data collected from these three events can generate four other types of dialysis events: Blood-stream infection (BSI), local access site infection (LASI), access-related bloodstream infection (ARB) and vascular access infection (VAI). The number of maintenance HD out-patients who received HD in the unit/center during the first two working days of the month (including transient HD patients but excluding inpatients and PD patients) should be reported on a monthly basis and according to their vascular access type. This will serve as the denominators for rate calculation. Each patient is counted only once; if the patient has multiple vascular accesses, that patient is counted with the vascular access type of highest infection risk. Rates are calculated by dividing the number of events by the number of patient-months and multiplying the result by 100.

As a means to reduce infection transmission, each dialysis facility should also monitor other parameters like dialysis water and dialysis fluid cultures and endotoxin results, incidence of drug-resistant infections, hospitalizations, as well adherence to standard precautions (hand hygiene, glove use and other PPE, equipment and environmental cleaning, safe injection practices, etc.) and other recommended practices (screening for HBV, HCV, HIV and tuberculosis infections and immunizations). Regular feedback of surveillance results to everyone involved in the health-care delivery (especially the frontline staff) would help to stimulate and encourage active engagement and improve compliance with infection prevention efforts. At least one designated person with training in infection control and epidemiology (infection preventionist) should be responsible for over-sight of the program as well as education of staff and patients related to infection prevention and control.

Steps that should be taken to control spread of infection, especially if there is an incidence of a positive seroconversion or outbreak in the HD unit, include the following: (a) review of the laboratory test results of all patients dialyzing in the same unit to identify any additional case(s), (b) performance of additional tests (c) determination/tracking of potential sources for infection, which includes (i) revision of newly infected patients' recent history of blood transfusion, invasive procedure(s) and/or hospitalization and (ii) high-risk behavior such as history of injection drug use and sexual activity, and (d) revision of HD unit's practices and procedures of infection control.

References

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Best practices in dialysis unit disinfection (II)

Dialysis is a treatment for people whose kidneys are failing. When you have kidney failure, your kidneys don't filter blood the way they should. As a result, wastes and toxins build up in your bloodstream. Dialysis does the work of your kidneys, removing waste products and excess fluid from the blood.

Renal patients have lowered immunity. That makes them more susceptible to infection, and it's much harder for them to recover from it. All this means that keeping a clean dialysis centre is very important.

Preparation for Disinfection of the Dialysis Station
- Gather necessary supplies including:
  - Personal protective equipment (PPE): eye goggles, gown and clean gloves.
  - Properly diluted hospital disinfectant and wipes/cloths (separate wipe(s)/cloth(s) per machine).
  - Biohazard disposal container(s)
- Perform hand hygiene.
- Don gown, eye goggles and clean gloves.
- Disconnect and takedown used blood tubing and dialyzer from the dialysis machine.
- Discard tubing and dialyzers in a leak-proof container (container is brought to the dialyzer station or is placed as near to the station).
- Check that there is no visible soil or blood on surfaces.
- If drain bag is still hanging, remove bag and empty in the soiled utility area.
- Ensure that the patient has left the dialysis station.
  - Patients should not be removed from the station until they have completed treatment and are clinically stable.
  - If a patient cannot be moved safely, delay routine disinfection of the dialysis station.
  - If patients are moved to a separate seating area prior to removing cannulation needles or while trying to achieve hemostasis, disinfect the chairs and armrests in those areas in between patients.
- Discard all single-use supplies. Move any reusable supplies (e.g., clamps) to an area where they will be cleaned and disinfected before being stored or returned to a dialysis station. This may occur before or after the patient has left the station.
- Remove gloves and perform hand hygiene.

Routine Disinfection of the Dialysis Station
- Perform hand hygiene and don clean gloves.
- Using a wiping motion (with friction), disinfect all surfaces in the dialysis station in contact with the patient and/or staff. e.g., dialysis chair or bed; tray tables; blood pressure cuffs; countertops; keyboard, etc.
- Clean dialysis machine from top to bottom.
  - If visible contaminant on the machine, wipe off using an absorbent material.

- Clean the machine using wipes/cloths with a disinfectant that is acceptable to the machine manufacturer and the renal program/infection control.
- Remove excess fluid from the wipes/cloth(s) prior to using to clean machine
- Clean the top of the machine.
- If the machine has a door(s), clean the front first, then the insides of the doors.
- Clean all components of the main interface (screen) and the back of the machine unless recommended otherwise by the manufacturer
- Clean exposed surfaces of dialysate, concentrate, and bicarb connectors.
- Clean each side of machine.
- Clean the area between the main interface (screen) and brakes, including the shelf
- Clean the brakes.
- Ensure surfaces are visibly wet with disinfectant but not dripping. Allow surfaces to air-dry. Air-drying is recommended to allow for sufficient contact time with the disinfectant.
- Remove gloves, eye goggles and gown.
- Perform hand hygiene.

Preparation for Disinfection of the Dialysis Station
- Gather necessary supplies including:
  - Personal protective equipment (PPE): eye goggles, gown and clean gloves.
  - Disinfectant wipes.
  - Disinfectant concentrate (accelerated hydrogen peroxide).
  - Measuring cup.
  - 1-4L water (depending on number of items).
  - 1-4L container (clean).
  - Drying rack or clean absorbent cloths.
- Perform hand hygiene.
- Don gown, eye goggles and clean gloves.
- Discard solutions if containers are not empty.
- Prepare fresh solutions daily.
- Fill clean container with 1-4L of water.
- Add 25 mL of accelerated hydrogen peroxide per 1L of water (100mL of concentrate for 4L of water).
- Collect dirty small items in soiled utility room until ready to disinfect.
- Wipe any soiled items with approved disinfectant wipes.
- Soak small items in solution for 5 minutes.
- Allow small items to air dry in drying rack or on clean absorbent cloth.
- Discard solutions at end of day.

Wound Management in Diabetic Foot Ulcers

DFU wound management

Practitioners must strive to prevent DFUs developing elsewhere on the foot or on the contralateral limb and to achieve limb preservation.

The principle aim of DFU management is wound closure. More specifically, the intention should be to treat the DFU at an early stage to allow prompt healing.

The essential components of management are:
- Treating underlying disease processes,
- Ensuring adequate blood supply,
- Local wound care, including infection control,
- Pressure of floading.

Effective foot care should be a partnership between patients, carers and healthcare professionals. This means providing appropriate information to enable patients and carers to participate in decision making and understand the rationale behind some of the clinical decisions as well as supporting good self-care.

TREATING THE UNDERLYING DISEASE PROCESSES
Practitioners should identify the underlying cause of the DFU during the patient assessment and, where possible, correct or eliminate it.
- Treating any severe ischaemia is critical to wound healing, regardless of other interventions. It is recommended that all patients with critical limb ischaemia, including rest pain, ulceration and tissue loss, should be referred for consideration of arterial reconstruction.
- Achieving optimal diabetic control. This should involve tight glycaemic control and managing risk factors such as high blood pressure, hyperlipidaemia and smoking. Nutritional deficiencies should also be managed.
- Addressing the physical cause of the trauma. As well as examining the foot, practitioners should examine the patient's footwear for proper fit, wear and tear and the presence of any foreign bodies (such as small stones, glass fragments, drawing pins, pet hairs) that may traumatise the foot1 . When possible and appropriate, practitioners should check other footwear worn at home and at work (eg slippers and work boots).

ENSURING ADEQUATE BLOOD SUPPLY
A patient with acute limb ischaemia is a clinical emergency and may be at great risk if not managed in a timely and effective way.

It is important to appreciate that, aside from critical limb ischaemia, decreased perfusion or impaired circulation may be an indicator for

revascularisation in order to achieve and maintain healing and to avoid or delay a future amputation.

OPTIMISING LOCAL WOUND CARE
The European Wound Management Association (EWMA) states that the emphasis in wound care for DFUs should be on radical and repeated debridement, frequent inspection and bacterial control and careful moisture balance to prevent maceration. Its position document on wound bed preparation suggests the following TIME framework for managing DFUs.

1: Wound bed preparation and TIME framework
Wound bed preparation is not a static concept, but a dynamic and rapidly changing one
There are four components to wound bed preparation, which address the different pathophysiological abnormalities underlying chronic wounds
The TIME framework can be used to apply wound bed preparation to practice
- Tissue debridement
- Inflammation and infection control
- Moisture balance (optimal dressing selection)
- Epithelial edge advancement.

Tissue debridement
There are many methods of debridement used in the management of DFUs including surgical/sharp, larval, autolytic and, more recently, hydrosurgery and ultrasonic.

Debridement may be a one-off procedure or it may need to be ongoing for maintenance of the wound bed. The requirement for further debridement should be determined at each dressing change. If the wound is not progressing, practitioners should review the current treatment plan and look for an underlying cause of delayed healing (suchas ischaemia, infection or inflammation) and consider patient concordance with recommended treatment regimens (such as not wearing offloading devices or not taking antidiabetic medication).

Sharp debridement
No one debridement method has been shown to be more effective in achieving complete ulcer healing. However, in practice, the gold standard technique for tissue management in DFUs is regular, local, sharp debridement using a scalpel, scissors and/or forceps. The benefits of debridement include:
- Removes necrotic/sloughy tissue and callus
- Reduces pressure
- Allows full inspection of the underlying tissues
- Helps drainage of secretions or pus
- Helps optimise the effectiveness of topical preparations
- Stimulates healing.

Sharp debridement should be carried out by experienced practitioners (eg a specialist podiatrist or nurse) with specialist training.

Practitioners must be able to distinguish tissue types and understand anatomy to avoid damage to blood vessels, nerves and tendons. They should also demonstrate high-level clinical decision-making skills in assessing a level of debridement that is safe and effective. The procedure may be carried out in the clinic or at the bedside.

Ulcers may be obscured by the presence of callus. After discussing the plan and expected outcome with the patient in advance, debridement should remove all devitalised tissue, callus and foreign bodies down to the level of viable bleeding tissue. It is important to debride the wound margins as well as the wound base to prevent the 'edge effect', whereby epithelium fails to migrate across a firm, level granulation base.

Sharp debridement is an invasive procedure and can be quite radical. Practitioners must explain fully to patients the risks and benefits of debridement in order to gain their informed consent. One small study piloting an information leaflet showed that many patients did not understand the procedure despite having undergone debridement on several previous occasions.

Vascular status must always be determined prior to sharp debridement. Patients needing revascularisation should not undergo extensive sharp debridement because of the risk of trauma to vascularly compromised tissues. However, the 'toothpick' approach may be suitable for wounds requiring removal of loose callus45. Seek advice from a specialist if in doubt about a patient's suitability.

Other debridement methods
While sharp debridement is the gold standard technique, other methods may be appropriate in certain situations:
- As an interim measure (eg by practitioners without the necessary skill sets to carry out sharp debridement; methods include the use of a monofilament pad or larval therapy)
- For patients for whom sharp debridement is contraindicated or unacceptably painful
- When the clinical decision is that another debridement technique may be more beneficial for the patient
- For patients who have expressed another preference.

Larval therapy The larvae of the green bottle fly can achieve relatively rapid, atraumatic removal of moist, slimy slough, and can ingest pathogenic organisms present in the wound69. The decision to use larval debridement must be taken by an appropriate specialist practitioner, but the technique itself may then be carried out by generalist or specialist practitioners with minimal training.

Larval therapy has been shown to be safe and effective in the treatment of DFUs. However, it is not recommended as the sole method of debridement for neuropathic DFUs as the larvae cannot remove callus.

A recent review of debridement methods found some evidence to suggest that larval therapy may improve outcomes when compared to autolytic debridement with a hydrogel.

Hydrosurgical debridement This is an alternative method of wound debridement, which forces water or saline into a nozzle to create a high-energy cutting beam. This enables precise visualisation and removal of devitalised tissue in the wound bed.

Autolytic debridement This is a natural process that uses a moist wound dressing to soften and remove devitalised tissue. Care must be taken not to use a moisture donating dressing as this can predispose to maceration. In addition, the application of moisture-retentive dressings in the presence of ischaemia and/or dry gangrene is not recommended.

Not debriding a wound, not referring a patient to specialist staff for debridement, or choosing the wrong method of debridement, can cause rapid deterioration with potentially devastating consequences.

Reference
National Institute for Health and Clinical Excellence. Diabetic foot problems: inpatient management of diabetic foot problems. Clinical guideline 119. London: NICE, 2011.

Diabetic Foot Treatment

Foot pain is one of the adverse side effects of diabetes. For this reason, foot care is integral to diabetic care and management. Unattended feet can become problematic and painful, sometimes, leading to amputation as well. Depending on the **extent of pain and damage**, medications can be given although the first line of action is to control the blood glucose level.

Pain from Diabetic Peripheral Neuropathy
An estimated 60-70 percent of people with diabetes have some form of neuropathy, making it one of the most common complications of diabetes. The symptoms of diabetic peripheral neuropathy (DPN)—nerve damage in the extremities—are most often felt in the toes, feet, and hands.

Common descriptions of the feelings or sensations for DPN are:
- Burning
- Numbness
- Tingling
- Stinging
- Electrical vibrations
- Shooting pain
- Searing pain

CONTROL OF BLOOD GLUCOSE LEVEL
The primary treatment for **diabetic foot pain** is to **bring blood glucose levels within the normal rang**e. This is essential to hinder further damage to the nerves and related parts. Although blood glucose control can worsen symptoms initially, over time, this helps in reducing the symptoms. Problems with the feet may also force you to consult a foot care specialist depending on the damage.

MEDICAL TREATMENT FOR FOOT PAIN
Medication can be administered for **wounded or infected feet** or if the doctor suspects risk of infection. If antibiotics are prescribed, take the entire course even though positive results can appear within two to three days only. For treating painful **diabetic neuropathy**, Cymbalta and Lyrica are the FDA-approved medications. In case of a severe infection, the doctor can advice hospitalization as giving pills may seem ineffective.

There can be a single dose of antibiotics as a **shot or IV dose** before starting pills. Diabetic foot treatment can also be done at several wound care centers which focus on the lower extremity wounds and

ulcers. Debridement of the wound through surgery, improvement of circulation and special dressings can be some of the other treatment procedures.

Depending on the need, a **combination of treatments** can also be implemented. In some cases where there is a bone-related problem, flat feet, arthritis, etc. you may need to consult a specialist as an orthopedic surgeon. Besides taking care of your existing problem, the specialist can also provide an excellent resource for how to care for your feet on daily basis. Diabetic foot pain treatment may also be dealt with shoe inserts, removal of calluses, etc.
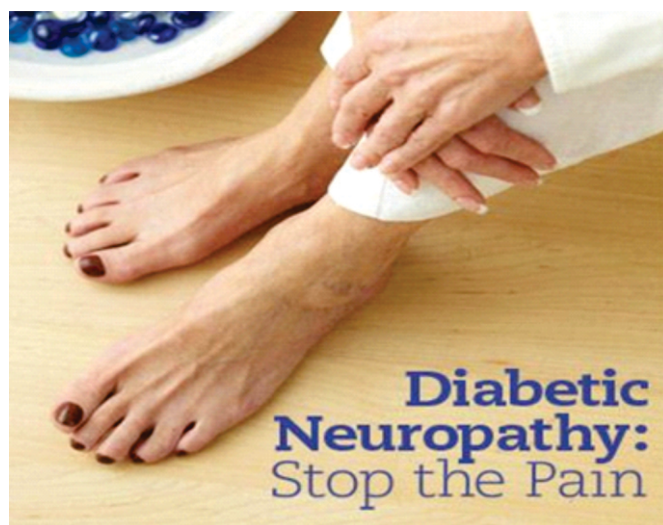
CARE THROUGH LIFESTYLE CHANGES
There are many things which aid **diabetes foot pain treatment** and delay worsening of the damage. Routine examination of the feet helps identify any damage even though it is trivial. In case of any injury, **attend to it immediately as even a small injury** can create havoc for the foot. Use cotton or woolen socks as elastic socks (and even hosiery) can hinder circulation. Besides, choose comfortable shoes to protect the feet. If you are unsure of the fitness of your footwear, consult a podiatrist.

Regular exercising can **keep bones and joints in good health**. It is good to lower blood sugar and consequently manage diabetes complications like foot pain. High impact exercises may not suit all, particularly when you have lost sensation in the feet. Consult the doctor when adopting an exercise program.

Keep nails trimmed to avoid damaging them. Apply a water-based moisturizer regularly to prevent cracking but avoid using it between your toes. Smoking adversely affects blood vessels by increasing the damage already caused.

SELF CARE THROUGH OTHER THINGS
Diabetic foot pain can be treated through other ways depending on the symptoms. Self care through warm baths can relieve you of the pain. Frequent walking can also soothe peripheral nerve pain. If you do not have cuts or wounds, bathing and walking may be helpful in reducing foot pain.

A suited diet can also take care of foot (and nerve) pain by emphasizing on blood sugar control. Try to keep track of what you eat, when and how much you eat. Remembering everything can be cumbersome, so concentrate on the major factors contributing to good glucose control.

Sometimes, creams and over-the-counter pain suppressers can help. **Lidocaine or capsaicin** containing creams may prove useful in controlling pain to certain extent. As far as over the-counter medications are concerned, ibuprofen, naproxen and aspirin may be useful. But remember that over-the-counter pain medicines may not work well can pose (serious) side effects as well.

USING ANTIDEPRESSANTS TO RELIEVE PAIN
Antidepressants may also relieve diabetic nerve pain. Even if there is absence of clinical depression in a patient, doctors may prescribe antidepressants to cater to foot pain and other nerve pain.

Antidepressants have the ability to deal with both pain and depression and can be used as a part of diabetic foot pain treatment.

SWITCHING THERAPIAES FOR BETTER CONTROL
Changing a therapy can, sometimes, be a **helpful technique in combating foot and nerve pain**. A study pointed out that insulin injections used for glucose control can reduce the likelihood of neuropathy.

Therapies like acupuncture, transcutaneous electrical nerve stimulation (TENS), anodyne therapy can also **relieve diabetic foot pain**. Another technique which can be helpful in diabetes foot pain treatment is the Dellon procedure. It is a surgical decompression of multiple peripheral nerves. Administering vitamin B12 can also be helpful, although it has its limitations.
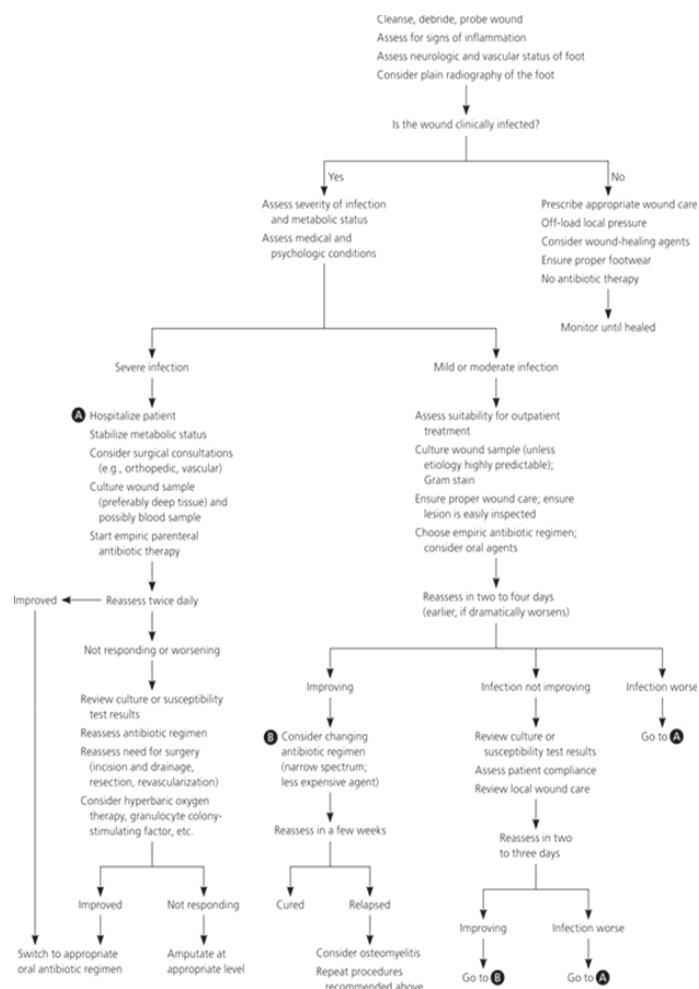
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https://www.google.co.in/search?q=diabetic+foot+treatment+soothing+pictures&source=lnms&tbm=isch&sa=X&ved=0ahUKEwjR06qxuvXaAhVBuY8KHYEbA-wQ_AUICigB&biw=1280&bih=918#imgrc=miYJ8o6BQnJeJM:
http://www.findhealthremedies.com/6-effective-treatment-of-diabetic-foot-pain/
http://www.diabeticlivingonline.com/complications/feet/stop-pain-diabetes-nerve-damage

Best Practices in Wound Care Management (I)

INTRODUCTION:

The use of topical antimicrobial agents in wound management

Wound infection can be financially costly to healthcare organisations and can negatively affect quality of life for patients, families and carers, due to pain, malodour, frequent dressing changes, loss of appetite, malaise, or deterioration of glycaemic control in people with diabetes (WUWHS, 2008).

Cases of surgical site infection (SSI)can double length of hospital stay, and healthcare interventions for a patient with an SSI can cost £814 to £6,626, depending on the surgery type and severity of the infection (NICE, 2008). Pressure ulcers can cost an average of £1,214 (category 1)to £14,108 (category IV) each (Dealey etal, 2012). Venous leg ulcers cost the NHS nearly £200 million annually, and diabetic foot ulcers £300 million a year (Posnett and Franks, 2008). Furthermore, it's estimated up to half these wounds will become infected (Posnett and Franks, 2008), which can, in lower limbs, result in amputation —a life-changing outcome desired by neither clinicians nor patients.

Effectively managing and treating wound infection can challenge clinicians, with myriad products and pharmaceutical interventions available. The results of the Health Protection Agency's Point Prevalence Survey on healthcare-associated infections and antimicrobial use estimated the total number of antimicrobials prescribed as 25,942 for 18,219 patients, with the prevalence of antimicrobial drug and device use being 34.7% (HPA, 2011).

However, indiscriminate use of antimicrobials — in particular, antibiotics — has led to the rising prevalence of resistant organisms, with the potential to jeopardise patient outcomes (EWMA, 2013a).

Professor Dame Sallie Davies, Chief Medical Officer for England, recently highlighted the urgency of reviewing the use of antibiotics and antimicrobials. In her annual report, she stated: 'There is a need for politicians in the UK to prioritise antimicrobial resistance as a major area of concern, including it on the national risk register (specifically, the National Security Risk Assessment)' (Davies, 2013). Prof Davies warned that, during the next 50 years, microorganisms' drug resistance will increase, and new strains with resistance to a wide variety of agents will emerge, rendering antimicrobial drugs ineffective. She further suggested development of new antimicrobial agents has declined, leaving fewer options for treating infections (Davies, 2013).

It is therefore essential that clinicians be able to identify wound infections correctly and, when appropriate, choose the right topical antimicrobial and/or systemic antibiotics for treatment, with the goals of preventing/eradicating infection and promoting wound healing.

Effective management and treatment of wound infections is challenging. This document seeks to provide clinicians with a best practice guide on when — and when not — to use topical antimicrobial agents, comprising the following:

- Assessing the patient and wound.
- Biofilms and wound infection.
- Selecting and using topical antimicrobials.
- Considerations in different wound aetiologies.
- Decision-making algorithm for best practice.

SECTION 1: ASSESSING THE PATIENT AND WOUND

Infection-relatedterminology

The WUWHS (2008) identified the presence of microbes in a wound can result in:

- **Contamination**, in which the microbial burden does not increase or cause clinical problems.
- **Colonisation**, in which the microbes multiply, but wound tissues are not damaged; i.e., the wound is on a normal healing trajectory with no clinical evidence of infection.
- **Critical colonisation or localised infection**, in which microbes multiply and the wound moves from benign colonisation to an infected state with impaired healing but without tissue invasion or host immunological response (Moore et al, 2007). However, there is currently no consensus on how to define or identify critical colonisation (EWMA,2013a).
- **Infection (spreading or systemic)**, in which the bacteria multiply, healing is disrupted and deep tissues are damaged. Bacteria might produce localised problems or cause systemic illness (sepsis).

Key Points:

1. Before prescribing any wound products or medications, the clinician must undertake and document a holistic assessment of the patient.
2. Wound infection assessment should include examination of the wound bed and peri wound area, documenting any signs of redness, unexplained pain or malodour.
3. Accurately assess the wound bed to help differentiate viable tissue from non-viable tissue.
4. Several classic signs and symptoms are easily identifiable as wound infection, but not all wounds will exhibit all these signs at any one time.
5. The value of a surface swab is debated.
6. If infection or colonisation is clinically diagnosed, use TIME to develop a wound management plan.
7. Wound healing is a complex and multi faceted process influenced by intrinsic and extrinsic factors, some of which can be controlled.

INTRODUCTION TO INFECTION

All wounds are contaminated with a variety of microorganisms (Stotts, 2004;WUWHS, 2008). In general, these microbes are harmless skin flora naturally found on the skin's surface. Intact skin provides a physical barrier against these microbes; however, the creation of a wound, acute or chronic, damages this defence mechanism, letting microbes enter the body.

Infections have been categorised into those that affect superficial tissues (skin and subcutaneous layer) of the incision and those that affect the deeper tissues (deep incisional or organ-space) (CDC, 2000). See above in infection-related terminology for further terms associated with microbes and their effect on the wound healing process that will be used throughout the document. Clinicians must be aware of the terminology and confident in their abilities to recognise each.

ASSESSING THE WOUND FOR INFECTION

Before prescribing any wound products or medications, the clinician must undertake and document a holistic assessment of the wound, including examination of the wound bed and periwound area,

documenting any signs of redness, unexplained pain or malodour (Ousey and Cook, 2012). However, the assessment should not comprise the wound and its characteristics in isolation but, rather account for a number of factors.

ASSESSING THE PATIENT'S INFECTION RISK

Wound healing is a complex and multifaceted process influenced by intrinsic and extrinsic factors, some of which can be controlled. Patient assessment should encompass the general medical condition, as immune compromised, neonatal and elderly patients are at greater risk of wound infections (White, 2009). In addition, certain chronic medical conditions (e.g. diabetes), medications (e.g. oncology drugs) and lifestyle factors (e.g. smoking) put patients with wounds at greater risk.

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Best Practices in Wound Care Management (II)

Comprehensive Wound Assessment

A comprehensive wound assessment must consider and document the following aspects:

- Underlying cause
- Wound location and size
- Comorbidities
- Nutritional status of the patient
- Smoking habits
- Drug/alcohol use
- Mobility of the patient
- Circulation
- Infection
- Inflammation
- Odour
- Exudate
- Medication
- Site and type of pain, changes in nature of onset-triggers of pain
- Colour
- Periwound skin
- Wound bed
- Patient-centred concerns
- Patient's psychological status

Aetiological factors and comorbidities

Chronic medical conditions can continually erode the immune system, predisposing patients to complications simultaneously affecting several organs of the body, including the eye, blood vessels, kidneys and the nervous system (Ahmed, 2005).Immunosuppression with increased bacterial virulence can make wound infection more likely (Wounds UK, 2010) and play a significant part in chronicity.

- **Diabetes.** Metabolic disorders associated with diabetes impair immune and inflammatory cells (Falanga, 2000),increasing the risk of wound infection and decreasing the potential for wound healing. Saad et al (2013) stated that neuropathy, peripheral vascular disease and minor trauma could contribute to impaired healing in diabetic foot ulcers, with Novak (2010) warning that intermittent claudication, absent pedalpulses and ischaemic gangrene were more prevalent in patients with diabetes. Diabetes in the presence of elevated blood glucose will further reduce neutrophil activity and interfere with the action of phagocytosis, thus delaying the normal inflammatory response. In addition, associated peripheral neuropathy will mask indicators of wound infection such as inflammation, pain and discomfort (Jones, 2012). Regular inspection of these wounds is paramount.
- **Circulatory disorders.** Oxygen is essential for cell metabolism and critical to all wound-healing processes. It prevents wound infection, induces angiogenesis, increases keratinocytes, enhances fibroblast proliferation and collagen synthesis, and promotes wound contraction (Bishop, 2008; Rodriguez etal, 2008). Systemic conditions such as ageing, diabetes and atherosclerosis can impair vascular flow, setting the stage for poor tissue oxygenation, increased infection risk and delayed healing (Guoand Dipietro, 2010). Poor tissue perfusion due to ischemia also might lower infection resistance. Clinicians should consider using topical antimicrobialsin arterially compromised patients who have non-healing wounds, as reduced blood flow hinders cell, nutrient and oxygen transport to the wound bed (Lipsky and Hoey, 2009).

Lifestyle factors

- **Alcohol consumption.** Wigston et al(2013) identified that alcohol significantly affects non-healing. Excess alcohol consumption inhibits the inflammatory response, and delays collagen and epithelial cell production, and blood vessel growth during the proliferative stage of wound healing (Radek et al,2009). Encourage patients to reduce alcohol consumption during wound healing.
- **Tobacco smoking.** Pharmacologically, smoking's influence on wound healing is multifaceted. The literature has identified smoking as a potential risk factor for wound infection due to delayed re-epithelialisation through nicotine-dependent downregulation of keratinocyte migration or from reduced monocyte and neutrophil oxidative burst activity, leading to a higher bacterial count in the wound bed (Kean, 2010).Smoking leads to tissue ischaemia due to its vasoconstrictive effect. It results in lower oxygen levels from preferential uptake of carbon monoxide, thereby limiting oxygen available for oxidative killing by white cells. Smoking impairs white blood cell migration, resulting in lower numbers of monocytes and macrophages in the wound bed, and reduces neutrophil activity, increasing the risk of wound infection and delayed healing (Ahn et al, 2008). Smoking reduces collagen production and deposition, and might also delay healing, mainly due to its immunosuppressive action (Sørensen et al, 2009). In addition, smokers exhibit delayed epithelisation, resulting in a dampened white cell and inflammatory response, which results in a higher bacterial count in the wound bed (Jones, 2012).
- **Nutrition.** Malnourished patients have higher risk of infection and often experience chronic non-healing wounds with decreased tensile strength (Stechmiller, 2010).

Medications

Certain drugs that are vital to a patient's health status negatively affect the wound healing process. In all cases, liaise with the prescriber to analyse risks and benefits before stopping prescriptions.

- **Antibiotics.** Although antibiotic therapy is sometimes necessary to treat wound infection, these drugs should be used only in clinically infected wounds (Karukonda et al, 2000b) to encourage wound healing. However, antibiotics might also reduce the wound's tensile strength, impeding final wound closure(Diehr et al, 2007).
- **Anticancer drug.** Oncology drugs also negatively affect wound healing (Valls et al, 2009), but cessation is not advisable, so it is important that both the patient and the wound be carefully monitored and reassessed in a timely manner. Chemotherapeutic drugs inhibit cellular metabolism, cell division and angiogenesis and, therefore, inhibit many of wound repair's critical pathways (Guoand Dipietro, 2010). In addition, they weaken the patient's immune functions, thereby impeding the inflammatory phase of wound healing and increasing the risk of wound infection.
- **Antiplatelet drugs.** Certain antiplatelet drugs have been found to hinder wound healing. Acetylsalicylic acid reduces platelet activation by preventing thrombus formation (Karukonda et al,2000a). Patients should refrain from taking these drugs unless doing so is essential.
- **Glucocorticoid steroids.** These anti-inflammatory agents inhibit wound repair and suppress cellular wound responses. However, they are also essential in some autoimmune disorders that lead to wounds. Systemic steroids cause incomplete granulation tissue and reduced wound contraction, resulting in

hard-to-heal wounds (Franz et al,2007). Hydrocortisone and prednisolone stimulate the production of cortisol, which depresses the immune system, depleting either the neutrophils that move to the wound site or the concentration of the cytokines necessary for healing (Glaser et al, 1999).

- **NSAIDs.** Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat inflammation and pain. Short-term NSAID use does not adversely affect wound healing. However, long-term use might decrease fibroblast numbers, weaken skin strength, reduce wound contraction, delay epithelisation and impair angiogenesis (Dvivedi et al,1997; Jones et al, 1999).

The clinician assessing the patient and wound must understand the repercussions of comorbidities, lifestyle factors and medications on the wound. This knowledge will help ensure an appropriate topical antimicrobial treatment plan that's been tailored to the patient is implemented.

TIME
T – Tissue, non-viable or deficient
I – Infection / inflammation
M – Moisture imbalance
E – Edge of wound non-advancing or undermined

DEVELOPING A WOUND MANAGEMENT PLAN
The International Advisory Board on WoundBed Preparation developed a framework— known by the acronym TIME (see above) — to provide a means by which clinicians can approach optimising the wound bed. If infection or colonisation is clinically diagnosed, use TIME to develop a wound management plan that includes removing non-viable tissue, reducing oedema and exudate, reducing the bacterial burden and correcting any abnormalities to promote wound healing (Schultz et al, 2003;Falanga, 2004).

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Best Practices in Wound Care Management (III)

TISSUE MANAGEMENT

Accurately assessing the wound bed will help differentiate between viable (eg granulation and epithelial) tissue and nonviable tissue (eg black eschar/necrosis and slough). Non-viable or devitalised tissue provides an opportunity for anaerobic and aerobic bacteria to grow, which can delay wound healing and result in significant malodour.

INFECTION/INFLAMMATION

There is no hard-scientific test to diagnose infection, so clinical judgement is needed to interpret signs and symptoms. The list of signs and symptoms is itself a topic of debate, so the challenge is to make the best use of the clinical information available at the assessment, create a plan, and reassess regularly to determine treatment response and alter the care plan accordingly.

Several classic signs and symptoms are easily identifiable as wound infection, but not all wounds will exhibit all these signs at any one time. Localised infection is often characterised by the classical signs and symptoms of inflammation, pain, heat, swelling, redness and loss of function; these indicators are more likely to be apparent in acute wound infection than in chronic wound infection. Additional, possibly more sensitive, criteria have been suggested for identifying wound infection, including abscess formation, cellulitis, discharge, delayed healing, discolouration, friable granulation tissue that bleeds easily, unexpected pain, pain that has changed in nature, tenderness, pocketing at the base of the wound, bridging of epithelium or soft tissue, abnormal smell wound breakdown (Cutting et al, 2005). These kinds of so-called secondary wound infection characteristics might be better indicators in chronic wounds, particularly when classic signs are absent (Gardner et al, 2001).

There is little consensus to define whether wound microbiology is of use in guiding clinical decisions (Moore et al, 2007) because swabbing a wound will identify some or all bacteria within the wound but might not always indicate the clinically significant species (Wounds UK, 2010). The value of a surface swab is debatable; tissue biopsy for quantitative microbiology is considered the most appropriate sampling method to identify wound infection and causative organism (Bowler et al, 2001; cited in Moore et al, 2007). This should be done after wound debridement ideally, from the tissue or bone from the base of the wound, or a deep wound swab and before systemic antibiotics are initiated (Saad et al, 2013).

MOISTURE IMBALANCE

High levels of exudate are associated with bacterial colonisation of a wound (Cutting and White, 2002). When a wound becomes infected, exudate will increase rapidly, particularly in those with underlying comorbidities such as diabetes. Discolouration and highly viscose exudate often indicate infection, especially when the exudate changes from pale amber colour to, for example, green (indicative of *P. aeruginosa*). However, a wound can be infected even if thick or discoloured exudate is absent (Wounds UK, 2013b). Further, diagnosis must also rule out conditions, eg lymphoedema or chronic venous insufficiency, that can cause excess exudate. Clinicians must effectively manage exudate to create the optimal moist environment necessary for wound healing and to protect the surrounding skin from the risks of maceration and excoriation. Achieving these goals requires a detailed knowledge of dressing materials and their performance (Wounds UK, 2013b).

EDGE OF WOUND

Lack of improvement in wound dimensions and non-progression of the wound edge indicate failure to heal. The presence of devitalised tissue, such as areas of necrosis or slough, can delay wound healing. Healing rates are a reliable early predictor of complete wound closure; wound margin advance, initial healing rate, percent wound surface reduction and wound healing trajectories are powerful predictors of healing at 12 weeks (Cardinal et al, 2007).

BEST PRACTICE STATEMENT APPLICATION TO PRACTICE: ASSESSING THE PATIENT AND WOUND

Best practice statement	Reason for best practice statement	How to demonstrate best practice
Holistically assess each patient and rule out the need to treat underlying conditions before prescribing any wound products or medications	To prevent inappropriate products or medications being used	Clearly document the assessment process, including a plan of care, review dates for future assessments and the rationale for dressing choice Regularly review medications
Clinicians must ensure they understand the wound-healing process and are competent in accurate assessment	To ensure factors that might impede the complex wound-healing process are identified and, where possible, addressed	Clearly document the assessment process, including the wound bed condition, using an assessment tool (eg TIME) Refer the patient, in a timely manner, to the appropriate member of the multidisciplinary team if there is delayed wound healing or signs of infection

BIOFILMS AND WOUND INFECTION

Key Points:

1. Biofilms have been implicated in infections of many tissues; they are very likely to be implicated in chronic wounds.
2. Biofilms cannot be visualized or detected in the wound.
3. Treatment should anticipate biofilm's presence. The coordinated use of debridement and specific topical antimicrobials is advocated.

Biofilms are complex polymicrobial communities that develop on or near wound surfaces. Biofilms may not present with clinical signs of infection (Phillips et al, 2010), but their presence has been implicated in chronicity (Bjarnsholt et al, 2006; James et al, 2008). They are invisible to the naked eye, cannot be detected by routine cultures and are extremely difficult to eradicate (Phillips et al, 2010). Not all biofilms are harmful, but some communities can be tantamount to wound infection, delaying healing as a result (Wolcott et al, 2008). The host's attempt to rid the wound of a biofilm stimulates a chronic inflammatory response, which releases high levels of reactive oxygen species (ROS) and proteases (MMPs and elastase). Although these substances help break down the attachments between the tissue and the biofilms, the ROS and proteases also damage normal and healing extracellular matrix tissues, potentially delaying ealing (Wolcott et al, 2008).

DEVELOPMENT OF BIOFILMS

The extracellular polymeric substance that contributes to the structure of the biofilm lets microbial species exist in close proximity to one another. This matrix which can be largely impermeable to antibiotics acts as a thick, slimy protective barrier and attaches the biofilm firmly to a living or non-living surface. Biofilms are dynamic and heterogeneous communities. They form quickly within two to four hours and evolve into a fully mature biofilm community within two to four days (Wolcott et al, 2008). They rapidly recover from mechanical disruption and reform mature biofilm within 24 hours. Communities can consist of a single bacterial or fungal species or, more commonly, can be polymicrobial (Dowd et al, 2008).

PREVALENCE OF BIOFILMS

Using electron microscopy and confocal scanning laser microscopy, biofilms have been found in 60% of biopsy specimens from chronic wounds, compared with only 6% of biopsies from acute wounds (James et al, 2008). Because biofilms are thought to significantly contribute to multiple inflammatory diseases, it is likely that almost all chronic wounds have biofilm communities on at least part of the wound bed (Phillips et al, 2010). Although biofilms might be an important contributor to wound chronicity, not all wounds with delayed healing can be assumed to contain biofilm. Further, the distribution of biofilms when they do exist in wounds (49% of wounds in James et al [2008] were without biofilms) seems to depend on the species, with *P. aeruginosa* found in deeper wound areas than *S. aureus* (Fazli et al, 2009). In addition, it is not known whether the presence of a biofilm in a wound will always lead to problems.

WHEN TO SUSPECT A BIOFILM

Chronic skin wounds often lack overt clinical signs of infection and might have low bacterial burdens as measured by standard clinical microbiology laboratory assays (WUWHS, 2008). The term 'biofilm' was developed in an attempt to acknowledge that bacteria play a critical role in the failure to heal of wounds that do not have obvious signs of infection.

MANAGING BIOFILMS

Evidence to date suggests that debridement or vigorous physical cleansing, are the best methods for reducing biofilm burden (Wolcott et al, 2009). Before commencing debridement, however, the patient should be assessed to determine the wound's healing potential. Wound irrigation using sterile saline or tap water can be used to clean chronic wounds to allow assessment and debridement. It is important to remember to not use gauze or cotton wool during cleaning, to avoid leaving debris in the wound bed, which might in turn cause infection. Topical antiseptic agents are considered unnecessary for general wound cleansing, but might be of value when irrigating an infected cavity wound or chronic wounds at risk of infection (Bradbury and Fletcher, 2011).

Active debridement is contraindicated in cases of severe vascular compromise. When indicated, remove non-viable tissue as quickly and efficiently as possible using an appropriate debridement method to assist with assessment, reduce bioburden/biofilm and accelerate healing (Wounds UK, 2013). Clinicians can use autolytic, mechanical, sharp, larval therapy (biosurgical), ultrasonic, hydrosurgical and surgical debridement. Each clinician must be competent, skilled, educated and trained in each technique. The debridement method chosen should be determined by the patient's clinical need and choices, and not limited by the skills of the clinician (Gray et al, 2011).

Debridement with a monofilament fibre pad 'shows the potential to advance mechanical debridement as a viable technique, by providing a rapid, safe and easy-to-use method with limited pain for the patient' according to the EWMA (2013b). However, if this method is not available and the clinician has received no training in specific debridement skills, assistance and advice must be sought from a healthcare professional with expertise in debridement techniques.

There are relatively few wounds that are not safe to debride if the correct method is chosen. As a general rule, if the wound is not covered in granulation tissue, debridement can be performed to progress a wound towards healing (Wounds UK, 2013a).

Keep in mind that no form of debridement or cleansing is likely to remove all biofilm, so remaining bacteria/biofilm could reform into mature biofilm in a matter of days. Topical antimicrobial interventions are potentially more effective at this post-cleansing/post-debridement stage (Wolcott et al, 2009), and should be considered for application to the wound, either as an antiseptic wound cleansing agent with a surfactant component and/or antimicrobial dressing.

Several antimicrobial agents have been shown to inhibit or even prevent biofilms in vitro (EWMA, 2013a). Sustained-release cadexomer iodine has been shown to be more effective than silver (Hill et al, 2010) or PHMB in disrupting mixed biofilms (Phillips et al, 2010); silver absorbent dressings have been shown to prevent biofilm formation by all singled and mixed biofilm cultures (Driffield et al, 2007). However, PHMB has also been shown to have microbiocidal activity on chronic wounds and burns, and to reduce biofilm in wounds exhibiting chronicity (Lenselink and Andriessen, 2011). Inert absorbent dressings have also been shown to exhibit both antifungal and antimicrobial effects, inhibiting *P. aeruginosa*, *K. pneumoniae* and *E. coli* presence in wounds, and significantly reducing *S. aureus* and *C. albicans* (Wiegand et al, 2012).

Use of topical antimicrobial agents in the presence of biofilms should occur only after biofilm disruption. These key steps summarise the management of biofilms in practice (Dowsett, 2013)

- Seek to prevent biofilm development whenever possible.
- Prepare the wound bed, considering the use of cleansing, debridement and topical antimicrobials where appropriate.
- Vigorously clean the wound with products designed to disrupt biofilm.
- Select debridement method based on wound type, best practice and patient preference.
- After debridement, consider topical antimicrobial treatment, as the biofilm is more vulnerable at this stage and can be managed with topical antimicrobial application more effectively than it could have been pre-debridement.

SELECTION AND USE OF TOPICAL ANTIMICROBIALS
Key points:

1. Topical antimicrobials present limited potential for systemic absorption and toxicity.
2. Topical antimicrobials are ideal for providing high and sustained concentration of antimicrobial at the site of the infection, potentially limiting the amount of overall antimicrobial needed in combination with systemic treatment.
3. Topical antimicrobials should be used only when signs and symptoms suggest that wound bioburden is interfering with healing, or when there is an increased risk of serious outcomes.
4. Not all wounds exhibit all symptoms of critical colonisation or infection, and there is not necessarily a standard progression of indicator severity.
5. Clinical colonisation must be determined in the context of all information about the wound and patient.

BEST PRACTICE STATEMENT APPLICATION TO PRACTICE: BIOFILMS AND WOUND INFECTION

Best practice statement	Reason for best practice statement	How to demonstrate best practice
Prevent biofilm development wherever possible	Biofilms can delay healing	Clearly track and document wound progress towards healing
Treatment should aim to disrupt biofilm burden through regular, repeated debridement and/or cleansing	To reduce the presence of biofilm and help prevent the reformation and attachment of biofilm	Patient documentation should reflect clinical rationale for treatment choice
Select debridement or cleansing method based on wound type, the clinician's knowledge and patient preference	To encourage effectiveness of treatment and patient concordance with the treatment chosen	Patient documentation should reflect clinical rationale for treatment choice as well as record of discussion with the patient
Consider topical antimicrobial treatment after cleansing or debridement	To better manage biofilm burden, as it is more vulnerable at this stage	Clearly document the rationale for pursuing treatment with a topical antimicrobial

6. Topical antimicrobials vary according to the concentration and availability of the active ingredients, mode and duration of action, and ability to handle exudate, odour or pain, and should be selected specific to the needs of each wound and patient, weighing the advantages and drawbacks of use.
7. To avoid serious consequences of infection, clinicians must also identify high-risk patients for whom
8. systemic antibiotics might be indicated.
9. Using topical antimicrobials does not guarantee a healing outcome, but is currently a reasonable, practical method for reducing the risks posed by infection at specific times on the wound care pathway.

In clinical practice, attributing either positive (clinical improvement) or negative (treatment failure) outcomes to topical antimicrobial treatment is currently not possible; it is a matter of reasoned opinion based on good clinical assessment. The important elements or treatment goals of using topical antimicrobials in a management plan are the potential to:
- Prevent progression from localised colonisation to more invasive infection states, thereby reducing the antibiotic usage
- Return to normal healing progression
- Treat critical colonisation/local infection without resorting to antibiotics
- Achieve faster resolution of local infection in conjunction with antibiotics (the literature does not prove this outcome advantage, but it is logical to expect more rapid resolution when reducing the wound-base pathogen reservoir and minimising antibiotic-resistant strains in the wound bed)
- Improve the patient experience by correctly diagnosing the cause of and controlling dour, exudate leakage and pain.

Clinically determining the patient's ability to resist bacterial invasion is the most important contributing factor in determining microbial balance (White, 2013). As such, topical antimicrobials should not be used 'just in case' in a wound that is healing as expected, unless clinically justified due to a patient's high risk (Butcher and White, 2013). For critically colonised or locally infected wounds, topical antimicrobials can be used, as part of a treatment plan as determined using the TIME framework, to help control microbial load (eg, biofilm) and protect the wound from further damage or contamination.

WHAT ARE ANTIMICROBIALS?
Antimicrobials are agents capable of killing (biocidal) or inhibiting

(biostatic) microorganisms. They have broad-spectrum activity against potentially infection-causing Gram-positive, Gram-negative, aerobic and anaerobic, planktonic and sessile (Wolcott et al, 2008) bacteria, and fungi and spores commonly found in the wound bioburden. As many antimicrobials can adversely affect human tissue, a compromise between antibacterial efficacy and cytotoxicity might have to be accepted (Müller and Kramer, 2008). The umbrella term includes:
- Disinfectants, substances used to inhibit or kill microbes on inanimate objects (eg dressing trolleys and instruments)
- Antiseptics, agents used to inhibit or kill microorganisms within a wound (biofilm) or on intact skin (eg, iodine)
- Antibiotics, naturally occurring (produced by microorganisms) or synthetically produced substances that can act selectively and can be applied topically (not normally recommended in wound care) or systemically. Microbial resistance is common (Vowden et al, 2011).

According to Lipsky and Hoey (2009), topical antimicrobials are ideal for providing high and sustained concentration of antimicrobial at the site of infection, potentially limiting the amount of overall antimicrobial needed in combination with systemic treatment perhaps eliminating systemic therapy altogether. Further, topical antimicrobials present limited potential for systemic absorption and toxicity (Lipsky and Hoey, 2009). Other benefits include:
- Relatively easy use
- Wide availability
- Generally lower cost than antibiotics
- Less risk for developing resistance (Vowden et al, 2011).

However, because of their surface nature, antimicrobials cannot be used to treat deep-tissue infection and might cause local hypersensitivity or contact dermatitis reactions at the skin and wound bed or alter normal skin flora, interfering with wound healing (Lipsky and Hoey, 2009).
Wound bioburden can also be managed via passive mechanisms without necessarily inhibiting the wound's microbial flora. Modes of action include bacterial sequestration (eg via mechanically modified cellulose fibres and selected gelling agents) within the dressing or binding of wound pathogens to a dressing substrate (eg via dialkylcarbamoylchloride, known as DACC). Bacteria and fungi that are bound in the latter manner are rendered inert on the wound contact layer, so no further replication takes place, and are removed from the wound environment when the dressing is changed.

WHEN ARE TOPICAL ANTIMICROBIALS INDICATED?

There are two broad categories of wounds in which topical antimicrobials should be considered for use. In the first kind of situation, no obvious underlying patient historical or lifestyle factors would compromise wound healing. In the second, underlying comorbidities and patient historical and lifestyle factors are present that might inhibit wound healing.

Situation 1

● Topical antimicrobials should be used only when signs and symptoms suggest that wound bioburden is interfering with healing:

● Cessation of progress, where previously response to that same therapy was evident and when other potential reasons have been explored and eliminated

● Failure to heal despite proper treatment — meaning wound care has included adequate debridement, removal of foreign bodies, pressure offloading (not leg ulcers), appropriate dressings, and treatment of any arterial or venous insufficiency or metabolic derangements (Lipsky and Hoey, 2009)

● Signs and symptoms of critical colonisation or localised infection (covert infection)

● Signs and symptoms of overt local or spreading infection.

CONSIDERATIONS WHEN SELECTING TOPICAL ANTIMICROBIALS

Products vary according to the concentration and availability of the active ingredients, mode and duration of action, and ability to handle exudate, odour or pain, and should be selected specific to the needs of each wound, weighing the advantages and drawbacks of use. To avoid serious consequences of infection, clinicians must also identify high-risk patients, such as those with poor vascularity or compromised immune systems, for whom the systemic antibiotic use might be indicated. For spreading infection, systemic antibiotics are normally selected empirically (EWMA, 2013a).

SUMMARY AND CONCLUSIONS

Promptly diagnosing and managing infection is vital to avoid complications. Clinicians must be knowledgeable of the signs and symptoms of infection, and those patients in whom these might be subtle or absent. It is imperative that clinicians be aware of the impact of comorbidities, medication and therapies on wound healing and infection. Most wounds are colonised by bacteria (ie contain biofilm) and, yet, the majority are not infected, and healing progresses normally (Angel et al, 2011).

Understanding the correct use of antimicrobial therapy is crucial not only in preventing wound infection but also in promoting wound healing for the patient. All wounds are colonised. Critical colonisation/local infection may can delay healing, cause complications and significantly affect daily living for patients, with increased pain and anxiety, exudate with the potential for leakage and odour. Preventing and managing critical colonisation/local infection is closely linked to quality of care and patient safety (EWMA, 2013a).

Guide to topical antimicrobials

This table presents the key points of widely used types of topical antimicrobials, listed alphabetically. Always check manufacturer instructions for use and contraindications. Select the antimicrobial product on an individual basis, customising the agent and dressing choice according to patient, wound and environment needs.

Active control	Mode of delivery	Rationale for use	Wound types	Guidance for use	Contraindications
Enzyme alginogel	Alginate gel	▪ Autolytic debridement ▪ Maintain moisture balance ▪ Reduce microbial burden ▪ Protect wound edges and epithelial cells	▪ Pressure ulcers ▪ Diabetic ulcers ▪ Traumatic wounds ▪ Arterial ulcer ▪ Second-degree burns ▪ Radiotherapy and oncology wounds ▪ Treat pregnant patients, as there is no absorption into the body	▪ Apply to wound and cover with a secondary dressing ▪ Check frequently to ensure correct level of gel ▪ Can be used long-term due to no body absorption	▪ Patients with known sensitivity alginate dressing or polyethylene glycol ▪ Wounds on the eyelid or where there is danger of contact with the eye
Iodine - Povidone iodine - Cadexomer iodine	Solution, cream, ointment, spray or impregnated dressings	▪ Treat localised infection, or spreading infection when healing is delayed ▪ Prevent wound infection or recurrence in susceptible patients ▪ Rapidly kill microorganisms, including MRSA ▪ Prevent bacterial resistance ▪ Suppress biofilm formation	▪ Venous leg ulcers ▪ Diabetic ulcers ▪ Cavity wounds (cadexomer only)	▪ Use initially for one week only, with dressing changes 2 to 3 times weekly ▪ If the wound does not improve after 10 to 14 days, re-evaluate the wound and change the dressing regimen/ systemic treatment	▪ Long-term use (due to perceived issues with toxicity, systemic absorption and delayed healing) ▪ Known or suspected iodine sensitivity ▪ Children ▪ Before after radio-iodine diagnostic tests ▪ Patients with significant renal disease ▪ Patients with thyroid Disease

Active control	Mode of delivery	Rationale for use	Wound types	Guidance for use	Contraindications
Polyhexamethylene biguanide (PHMB)	Solution, gel or impregnated dressings	▪ Cleanse/decontaminate the wound ▪ Suppress biofilm formation ▪ Reduce wound odour ▪ Removal of encrusted dressings (solution only) ▪ Manage wound bioburden ▪ Provide an antimicrobial barrier	▪ Partial-thickness burns ▪ Post-surgical wounds ▪ Traumatic wounds ▪ Skin donor/recipient sites ▪ Leg ulcers ▪ Pressure ulcers ▪ Diabetic foot ulcers ▪ Scleroderma wounds ▪ Paediatric wounds	▪ Apply solution to wound and leave for 10 to 15 minutes (can be warmed to body temperature) ▪ Gel can be applied to deep or tunnelling wounds and cavity wounds. Leave in place and apply secondary dressing ▪ Dressings can be left in place for up to 5 to 7 days	▪ Patients with known PHMB sensitivity ▪ Combined with other wound cleansers (eg Dakin's) or ointments ▪ With caution/under medical supervision in pregnant and lactating women or babies ▪ With peritoneal or joint lavage

Going forward, preventing MRSA bacteraemia through control of MRSA in wounds is also on the government agenda in England. MRSA bacteraemia is being treated essentially as a 'never event' because the policy is zero tolerance — if an orangisation goes over its limit as set by the Department of Health (commonly, the figure is zero cases), a fine will be levied by the commissioners of care (NHS England, 2013). The CCG is also penalised for cases in its commissioning area, as 12.5% of quality premiums will not be paid to the CCG. The full premium can be earned only if no cases of MRSA bacteraemia are assigned to the CCG, and if *C. difficile* cases are at or below defined thresholds for the CCG (NHS England, 2013).

Reducing inappropriate use of antibiotics for wound care will contribute to meeting *C. difficile* and MRSA targets (by not creating further resistance issues) and, therefore, reducing fines levied on providers and helping ensure quality premiums are awarded to CCGs. Using topical antimicrobials appropriately can help prevent selecting for resistant bacteria while promoting factors such as reduced bioburden that encourage wound healing.

Appropriate and effective use of topical antimicrobial agents and dressings is important to meeting clinical and patient needs.

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Clean-Room Techniques

First cleanrooms were in hospitals to prevent disease transmission and infection in operating rooms (over 100 years ago). It is a valuable tool to prevent particulate and bio contamination.

A cleanroom is an environment used in manufacturing or scientific research. It has a low level of environmental pollutants such as dust, airborne microbes, aerosol particles and chemical vapors. More accurately, a cleanroom has a controlled level of contamination that is specified by the number of particles per cubic meter at a specified particle size. To give perspective, the ambient air outside in a typical urban environment contains 35,000,000 particles per cubic meter in the size range 0.5 μm and larger in diameter, corresponding to an ISO 9 cleanroom, while an ISO 1 cleanroom allows no particles in that size range & only 12 particles per cubic meter of 0.3 μm and smaller.

Overview

Cleanrooms can be very large. Entire manufacturing facilities can be contained within a cleanroom with factory floors covering thousands of square meters. They are used extensively in semiconductor manufacturing, biotechnology, the life sciences and other fields that are very sensitive to environmental contamination.

The air entering a cleanroom from outside is filtered to exclude dust, and the air inside is constantly re-circulated through high efficiency particulate air (HEPA) and/or ultra low particulate air (ULPA) filters to remove internally generated contaminants.

HEPA filters are composed of a mat of randomly arranged fibres. The fibres are typically composed of fiberglass and possess diameters between 0.5 and 2.0 micrometer. Key factors affecting function are fibre diameter, filter thickness, and face velocity. The air space between HEPA filter fibres is much greater than 0.3 μm. The common assumption that a HEPA filter acts like a sieve where particles smaller than the largest opening can pass through is incorrect. Unlike membrane filters, where particles as wide as the largest opening or distance between fibres cannot pass in between them at all, HEPA filters are designed to target much smaller pollutants and particles. These particles are trapped (they stick to a fibre) through a combination of the following three mechanisms:

Interception, where particles following a line of flow in the air stream come within one radius of a fibre and adhere to it. Impaction, where larger particles are unable to avoid fibres by following the curving contours of the air stream and are forced to embed in one of them directly; this effect increases with diminishing fibre separation and higher air flow velocity. Diffusion, an enhancing mechanism is a result of the collision with gas molecules by the smallest particles, especially those below 0.1 μm in diameter, which are thereby impeded and delayed in their path through the filter; this behavior is similar to Brownian motion and raises the probability that a particle will be stopped by either of the two mechanisms above; it becomes dominant at lower air flow velocities.

Diffusion predominates below the 0.1 μm diameter particle size. Impaction and interception predominate above 0.4 μm. In between, near the Most Penetrating Particle Size (MPPS) 0.3 μm, both diffusion and interception are comparatively inefficient. Therefore, the HEPA specifications use the retention of these particles to define the filter.

Biomedical applications

Hospital staff modeling a HEPA filter, which can be used if a patient has active tuberculosis. HEPA filters are critical in the prevention of the spread of airborne bacterial and viral organisms and, therefore, infection. Typically, medical-use HEPA filtration systems also incorporate high-energy ultra-violet light units to kill off the live bacteria and viruses trapped by the filter media. Some of the best-rated HEPA units have an efficiency rating of 99.995%, which assures a very high level of protection against airborne disease transmission.

Staff enter and leave through airlocks (sometimes including an air shower stage), and wear protective clothing such as hats, face masks, gloves, boots and coveralls.

Equipment inside the cleanroom is designed to generate minimal air contamination. Even specialized mops and buckets exist. Cleanroom furniture is also designed to produce a minimum of particles and to be easy to clean.

Common materials such as paper, pencils, and fabrics made from natural fibers are often excluded; however, alternatives are available. Cleanrooms are not sterile (i.e., free of uncontrolled microbes) and more attention is given to airborne particles. Particle levels are usually tested using a particle counter.

Some cleanrooms are kept at a positive pressure so that if there are any leaks, air leaks out of the chamber instead of unfiltered air coming in.

Some cleanroom HVAC systems control the humidity to low levels, such that extra equipment ("ionizers") are necessary to prevent electrostatic discharge (ESD) problems.

Low-level cleanrooms may only require special shoes, ones with completely smooth soles that do not track in dust or dirt. However, shoe bottoms must not create slipping hazards (safety always takes precedence). Entering a cleanroom usually requires wearing a cleanroom suit.

In other cleanrooms, in which the standards of air contamination are less rigorous, the entrance to the cleanroom may not have an air shower. There is an anteroom (known as a "gray room"), in which the special suits must be put on, but then a person can walk in directly to the room.

Some manufacturing facilities do not use fully classified cleanrooms, but use some cleanroom practices together to maintain their cleanliness requirements.

Cleanrooms maintain particulate-free air through the use of either HEPA or ULPA filters employing laminar or turbulent air flow principles. Laminar, or unidirectional, air flow systems direct filtered air downward in a constant stream towards filters located on walls near the cleanroom floor or through raised perforated floor panels to be recirculated. Laminar air flow systems are typically employed across 80 percent of a cleanroom ceiling to maintain constant air processing. Stainless steel or other non-shed materials are used to construct laminar air flow filters and hoods to prevent excess particles entering the air. Turbulent, or non-unidirectional, air flow uses both laminar air flow hoods and non-specific velocity filters to keep air in a cleanroom in constant motion, although not all in the same direction. The rough air seeks to trap particles that may be in the air and drive them towards the floor, where they enter filters and leave the cleanroom environment.

Cleanroom classifications

Cleanrooms are classified according to the number and size of particles permitted per volume of air. Large numbers like "class 100" or "class 1000" refer to FED-STD-209E, and denote the number of particles of size 0.5 μm or larger permitted per cubic foot of air. (For e.g. , class 1000; i.e. 1000 particles of size 0.5 μm present in one cubic foot of air).

A discrete-particle-counting, light-scattering instrument is used to determine the concentration of airborne particles, equal to and larger than the specified sizes, at designated sampling locations.

Small numbers refer to ISO 14644-1 standards, which specify the decimal logarithm of the number of particles 0.1 μm or larger permitted per cubic metre of air. So, for example, an ISO class 5 cleanroom has at most $10^5 = 100,000$ particles per m³.

Both FS 209E and ISO 14644-1 assume log-log relationships between particle size and particle concentration. For that reason, there is no such thing as zero particle concentration. The table locations without entries are non-applicable combinations of particle sizes and cleanliness classes, and should not be read as zero.

Class	maximum particles/ft³					ISO equivalent
	≥0.1 µm	≥0.2 µm	≥0.3 µm	≥0.5 µm	≥5 µm	
1	35	7	3	1		ISO 3
10	350	75	30	10		ISO 4
100		750	300	100		ISO 5
1,000				1,000	7	ISO 6
10,000				10,000	70	ISO 7
100,000				100,000	700	ISO 8

Class	maximum particles/m³						FED STD 209E equivalent
	≥0.1 µm	≥0.2 µm	≥0.3 µm	≥0.5 µm	≥1 µm	≥5 µm	
ISO 1	10	2					
ISO 2	100	24	10	4			
ISO 3	1,000	237	102	35	8		Class 1
ISO 4	10,000	2,370	1,020	352	83		Class 10
ISO 5	100,000	23,700	10,200	3,520	832	29	Class 100
ISO 6	1,000,000	237,000	102,000	35,200	8,320	293	Class 1000
ISO 7				352,000	83,200	2,930	Class 10,000
ISO 8				3,520,000	832,000	29,300	Class 100,000
ISO 9				35,200,000	8,320,000	293,000	Room air

Microbial Contamination: Outer layer of human skin can host up to 1 million microorganisms per square cm. Human saliva up to 1 billion per mL. Bacteria is usually primary concern, but foreign organic matter, viruses, fungi, algae are all included here. Cross contamination can be a big issue.

Contamination Measurement: Particulate contamination typically measured with laser particle counter. Microbial contamination can be measured in several ways-Centrifugal sampler, Settle plate method, Contact plate method, Swabbing.

How to Use Measurements: Can use measurements to isolate problem areas. Regular measurements can help to track changes, which can then be tied back to protocol, personnel, or material changes Don't depend upon room to maintain itself. Possible to isolate culture lines responsible.

Cleaning: Critical to remove contaminants that cannot be removed by air handling. Important to follow procedures appropriate to your application. What is appropriate for one industry may not be appropriate for another. Most important thing is to develop standard procedures and FOLLOW THEM.

Dry and wet vacuuming: Dry has low (<25%) efficiency for particles smaller than 10 microns. wet uses liquids which result in greater force on the particles and hence better cleaning.

Wet wiping: Can be very efficient. Liquid breaks some bonds between surface and particles and allows particles to float off. Those adhering on surface can be rubbed off and retained in wiper. Must be careful not to redeposit particles.

Tacky rollers: Efficiency depends of tackiness/sticky ness of roller, cleanliness of tacky surface and softness of roller are also very important.

Protocols to Improve Contamination Control
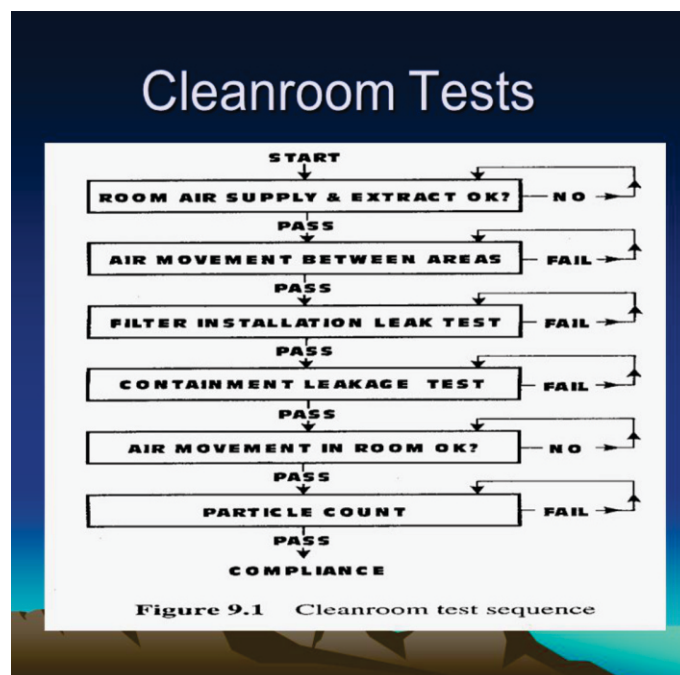
Cleanroom Test Sequence

Things to remember, always while working in a cleanroom area.

You as the User I: Very important to think about each and every action you take: How does this affect cleanliness? Why do we do this the way we do? Is there a better way to do it? What will happen if I do not follow proper protocols? You should know the answers to all of these questions!

You as the User II: Cleanroom environment is very fragile! Your actions have impact on other users. Important to follow procedures EVERY TIME. Make sure fellow workers follow procedures as well; nothing wrong with pointing out mistakes. Be an active participant: keep an eye out for areas that can be improved.

Minimalism is Good: Bring only required materials into cleanroom; if it is not necessary to perform the task, it should not be there. Personnel: only required personnel should be in the clean space. Reduce clutter—do not store materials in clean areas unless they have to be there!

Repetition is Good: Follow exact procedures every time. Wipe down surfaces with cleanroom wipes before and after every usage. Remember: this can seem tedious and unnecessary, but is essential to keeping the cleanroom maintained at its highest levels.

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General Cleaning Strategies for Patient-Care Areas

INTRODUCTION

Microbiologically contaminated surfaces can serve as reservoirs of potential pathogens. The transferral of microorganisms from environmental surfaces to patients is largely via hand contact with the surface. Although hand hygiene is important to minimize the impact of this transfer, cleaning and disinfecting environmental surfaces is fundamental in reducing their potential contribution to the incidence of healthcare-associated infections.

The principles of cleaning and disinfecting environmental surfaces take into account the intended use of the surface or item in patient care. CDC (U.S. Centers for Disease Control and Prevention) retains the Spaulding classification for medical and surgical instruments, which outlines three categories based on the potential for the instrument to transmit infection if the instrument is microbiologically contaminated before use. These categories are "critical," "semicritical," and "noncritical." In 1991, CDC proposed an additional category designated "environmental surfaces" to Spaulding's original classification to represent surfaces that generally do not come into direct contact with patients during care. Environmental surfaces can be further divided into medical equipment surfaces (e.g., knobs or handles on hemodialysis machines, x-ray machines, instrument carts, and dental units) and housekeeping surfaces (e.g., floors, walls, and tabletops).

The following factors influence the choice of disinfection procedure for environmental surfaces: a) the nature of the item to be disinfected, b) the number of microorganisms present, c) the innate resistance of those microorganisms to the inactivating effects of the germicide, d) the amount of organic soil present, e) the type and concentration of germicide used, f) duration and temperature of germicide contact, and g) if using a proprietary product, other specific indications and directions for use.

Spaulding proposed three levels of disinfection for the treatment of devices and surfaces that do not require sterility for safe use. These disinfection levels are "high-level," "intermediate-level," and "low level." The basis for these levels is that microorganisms can usually be grouped according to their innate resistance to a spectrum of physical or chemical germicidal agents.

The process of high-level disinfection, an appropriate standard of treatment for heat-sensitive, semi critical medical instruments (e.g., flexible, fiber optic endoscopes), inactivates all vegetative bacteria, mycobacterium, viruses, fungi, but not all the spores. High-level disinfection is accomplished with powerful, sporicidal chemicals (e.g. glutaraldehyde, peracetic acid etc). These liquid chemicals/high-level disinfectants are highly toxic. Use of these chemicals for applications other than those indicated in their label instructions (i.e., as immersion chemicals for treating heat-sensitive medical instruments) is not appropriate.

Therefore the new generation sterilants have come to the market which are non-toxic & have a wide range of applications. These sterilants are highly effective within one hour of contact time. (E.g. Fourth generation Quaternary ammonium compounds (QAC) – Didecyl dimethyl ammonium chloride (DDAC), polymeric biguanides, Poly Hexa Methylene Biguanide (PHMB), Hydrogen peroxide etc)

Intermediate-level disinfection does not kill bacterial spores, but it does inactivate Mycobacterium tuberculosis var. bovis, which is substantially more resistant to chemical germicides than ordinary vegetative bacteria, fungi, and medium to small viruses (with or without lipid envelopes). Chemical germicides with sufficient potency to achieve intermediate-level disinfection include chlorine-containing compounds (e.g., sodium hypochlorite), alcohols, some phenolics and some iodophors.

Low-level disinfection inactivates vegetative bacteria, fungi, enveloped viruses (e.g., human immunodeficiency virus [HIV], and influenza viruses), and some non-enveloped viruses (e.g., adenoviruses). Low-level disinfectants include first generation quaternary ammonium compounds like cetrimide, some phenolics like chloroxylenol and some iodophors. Sanitizers are agents that reduce the numbers of bacterial contaminants to safe levels as judged by public health requirements, and are used in cleaning operations, particularly in food service and dairy applications. Germicidal chemicals that have been approved by FDA as skin antiseptics are not appropriate for use as environmental surface disinfectants.

The selection and use of chemical germicides are largely matters of judgment, guided by product label instructions, information, and regulations. Liquid sterilant chemicals and high-level disinfectants intended for use on critical and semi-critical medical/dental devices and instruments are regulated exclusively by the FDA as a result of recent memoranda of understanding between FDA and the EPA that delineates agency authority for chemical germicide regulation. Environmental surface germicides (i.e., primarily intermediate- and low-level disinfectants) are regulated by the EPA and labeled with EPA registration numbers.

Strategies for cleaning and disinfecting surfaces in patient-care areas take into account a) potential for direct patient contact, b) degree and frequency of hand contact, and c) potential contamination of the surface with body substances or environmental sources of microorganisms (e.g., soil, dust, and water).

a. Cleaning of Medical Equipment

Manufacturers of medical equipment should provide care and maintenance instructions specific to their equipment. These instructions should include information about a) the equipments' compatibility with chemical germicides, b) whether the equipment is water-resistant or can be safely immersed for cleaning, and c) how the equipment should be decontaminated if servicing is required. In the absence of manufacturers' instructions, non-critical medical equipment (e.g., stethoscopes, blood pressure cuffs, dialysis machines, and equipment knobs and controls) usually only require cleansing followed by low- to intermediate-level disinfection, depending on the nature and degree of contamination. Ethyl alcohol or isopropyl alcohol in concentrations of 60%–90% (v/v) is often used to disinfect small surfaces (e.g., rubber stoppers of multiple-dose medication vials, and thermometers) and occasionally external surfaces of equipment (e.g., stethoscopes and ventilators). However, alcohol evaporates rapidly, which makes extended contact times difficult to achieve unless items are immersed, a factor that precludes its practical use as a large-surface disinfectant. Alcohol may cause discoloration, swelling, hardening, and cracking of rubber and certain plastics after prolonged and repeated use and may damage the shellac mounting of lenses in medical equipment.

b. Cleaning Housekeeping Surfaces

Housekeeping surfaces require regular cleaning and removal of soil and dust. Dry conditions favor the persistence of gram-positive cocci (e.g. coagulase-negative Staphylococcus spp.) in dust and on surfaces, whereas moist, soiled environments favor the growth and persistence of gram-negative bacilli. Fungi are also present on dust and proliferate in moist, fibrous material.

Most, if not all, housekeeping surfaces need to be cleaned only with soap and water or a detergent/disinfectant, depending on the nature of the surface and the type and degree of contamination. Cleaning and disinfection schedules and methods vary according to the area of the health-care facility, type of surface to be cleaned, and the amount and type of soil present.

If using a proprietary detergent/disinfectant, the manufacturers'

instructions for appropriate use of the product should be followed. Consult the products' material safety data sheets (MSDS) to determine appropriate precautions to prevent hazardous conditions during product application. Personal protective equipment (PPE) used during cleaning and housekeeping procedures should be appropriate to the task.

Housekeeping surfaces can be divided into two groups – those with minimal hand-contact (e.g., floors, and ceilings) and those with frequent hand-contact ("high touch surfaces"). The methods, thoroughness, and frequency of cleaning and the products used are determined by health-care facility policy. However, high-touch housekeeping surfaces in patient-care areas (e.g., doorknobs, bedrails, light switches, wall areas around the toilet in the patient's room, and the edges of privacy curtains) should be cleaned and/or disinfected more frequently than surfaces with minimal hand contact. Infection-control practitioners typically use a risk-assessment approach to identify high-touch surfaces and then coordinate an appropriate cleaning and disinfecting strategy and schedule with the housekeeping staff.

Horizontal surfaces with infrequent hand contact (e.g., window sills and hard-surface flooring) in routine patient-care areas require cleaning on a regular basis, when soiling or spills occur, and when a patient is discharged from the facility. Regular cleaning of surfaces and decontamination, as needed, is also advocated to protect potentially exposed workers. Cleaning of walls, blinds, and window curtains is recommended when they are visibly soiled. Fogging with non toxic, environmental friendly disinfectants will be beneficial. Hydrogen peroxide leaves safe residue like water & oxygen & is effective within one hour. Para formaldehyde which was once used in this application is no longer registered by EPA for this purpose. Infection control, industrial hygienists, and environmental services supervisors should assess the cleaning procedures, chemicals used, and the safety issues to determine if a temporary relocation of the patient is needed when cleaning in the room.

Another reservoir for microorganisms in the cleaning process may be dilute solutions of the detergents or disinfectants, especially if the working solution is prepared in a dirty container, stored for long periods of time, or prepared incorrectly. Gram-negative bacilli (e.g., Pseudomonas spp. and Serratia marcescens) have been detected in solutions of some disinfectants (e.g. phenolics and first generation quaternary ammonium compounds). Contemporary EPA registration regulations have helped to minimize this problem by asking manufacturers to provide potency data to support label claims for detergent/disinfectant properties under real- use conditions (e.g., diluting the product with tap water instead of distilled water).

c. Cleaning Special Care Areas

Guidelines have been published regarding cleaning strategies for isolation areas and operating rooms. The basic strategies for areas housing immunosuppressed patients include a) Wet dusting horizontal surfaces daily with cleaning cloths pre-moistened with an EPA-registered hospital disinfectant or disinfectant wipes b) using care when wet dusting equipment and surfaces above the patient to avoid patient contact with the detergent/disinfectant; c) avoiding the use of cleaning equipment that produces mists or aerosols; d) equipping vacuums with HEPA filters, especially for the exhaust, when used in any patient-care area housing immunosuppressed patients and e) regular cleaning and maintenance of equipment to ensure efficient particle removal. When preparing the cleaning cloths for wet-dusting, freshly prepared solutions of detergents or disinfectants should be used rather than cloths that have soaked in such solutions for long periods of time. Doors to immunosuppressed

patients' rooms should be closed when nearby areas are being vacuumed. Bacterial and fungal contamination of filters in cleaning equipment is inevitable, and these filters should be cleaned regularly or replaced as per equipment manufacturer instructions.

d. Cleaning Strategies for Spills of Blood and Body Substances

Studies have demonstrated that HIV is inactivated rapidly after being exposed to commonly used chemical germicides at concentrations that are much lower than those used in practice. HBV is readily inactivated with a variety of germicides, OSHA has revised its regulation for disinfecting spills of blood or other potentially infectious material to include proprietary products whose label includes inactivation claims for HBV and HIV, provided that such surfaces have not become contaminated with agent(s) or volumes of or concentrations of agent(s) for which a higher level of disinfection is recommended. These registered products are listed in EPA's List D – Registered Antimicrobials Effective against Hepatitis B Virus and Human HIV. Additional lists of interest include EPA's List C –Registered Antimicrobials Effective against Human HIV-1 and EPA's List E – Registered Antimicrobials Effective Against *Mycobacterium* spp., Hepatitis B Virus, and Human HIV-1.

Sodium hypochlorite solutions are inexpensive and effective broad-spectrum germicidal solutions. Generic sources of sodium hypochlorite include household chlorine bleach or reagent grade chemical. Concentrations of sodium hypochlorite solutions with a range of 5,000–6,150 ppm to 500–615 ppm free chlorine are effective depending on the amount of organic material (e.g. blood, mucus, and urine) present on the surface to be cleaned and disinfected. EPA-registered chemical germicides may be more compatible with certain materials that could be corroded by repeated exposure to sodium hypochlorite.

Strategies for decontaminating spills of blood and other body fluids differ based on the setting in which they occur and the volume of the spill. In patient-care areas, workers can manage small spills with cleaning and then disinfecting using an intermediate-level germicide or an EPA-registered germicide from the EPA List D or E. For spills containing large amounts of blood or other body substances, workers should first remove visible organic matter with absorbent material (e.g., disposable paper towels discarded into leak-proof, properly labeled containment) and then clean and decontaminate the area. If the surface is nonporous and a generic form of a sodium hypochlorite solution (500ppm) is appropriate for decontamination assuming that a) the worker assigned to clean the spill is wearing gloves and other personal protective equipment appropriate to the task, b) most of the organic matter of the spill has been removed with absorbent material, and c) the surface has been cleaned to remove residual organic matter.

Protocols for cleaning spills should be developed and made available on record as part of good laboratory practice. Workers in laboratories and in patient-care areas of the facility should receive periodic training in environmental surface infection-control strategies and procedures as part of an overall infection-control and safety curriculum.

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Handling, Storage, and Transportation of Health Care Waste

Health care activities – for instance, immunizations, diagnostic tests, medical treatments, and laboratory examinations – protect and restore health and save lives. But the wastes and by products generated need to be tackled scrupulously.

From the total of wastes generated by health care activities, almost 80% are general waste comparable to domestic waste. The remaining approximate 20% of wastes are considered hazardous materials that may be infectious, toxic or radioactive. The wastes and by products cover a diverse range of materials, as the following list illustrates:

Infectious wastes – cultures and stocks of infectious agents, wastes from infected patients, wastes contaminated with blood and its derivatives, discarded diagnostic samples, infected animals from laboratories, and contaminated materials (swabs and bandages) and equipment (disposable – medical devices etc.); and
Anatomic – recognizable body parts and animal carcasses.

Infectious and anatomic wastes together represent the majority of the hazardous waste, up to 15% of the total waste from health care activities.

- Sharps – syringes, disposable scalpels and blades etc.
- Sharps represent about 1% of the total waste from health care activities.
- Chemicals – for example solvents and disinfectants and
- Pharmaceuticals – expired, unused, and contaminated; whether the drugs themselves (sometimes toxic and powerful chemicals) or their metabolites, vaccines and sera.
- Chemicals and pharmaceuticals amount to about 3% of waste from health care activities.
- Genotoxic waste – highly hazardous, mutagenic, teratogenic or carcinogenic, such as cytotoxic drugs used in cancer treatment and their metabolites; and
- Radioactive matter, such as glassware contaminated with radioactive diagnostic material or radiotherapeutic materials;
- Wastes with high heavy metal content, such as broken mercury thermometers.
- Genotoxic waste, radioactive matter and heavy metal content represent about 1% of the total waste from health care activities.

The major sources of health care waste are hospital and other health care establishment, laboratories and research centers, mortuary and autopsy centers, animal research and testing laboratories, blood banks and collection services and nursing homes for the elderly.

Health Impact

Health care waste is a reservoir of potentially harmful microorganisms which can infect hospital patients, health care workers and the general public. Other potential infectious risks include the spread of, sometimes resistant, microorganisms from health care establishments into the environment. These risks have so far been poorly investigated. Wastes and by products can also cause injuries, for instance radiation burns or sharps inflicted injuries; poisoning and pollution, whether through the release of pharmaceutical products, in particular, antibiotics and cytotoxic drugs, through the waste water or by toxic elements or compounds such as mercury or dioxins.

Waste segregation and packaging

The key to minimization and effective management of health care wastes is segregation (separation) and identification of the waste. Appropriate handling, treatment, and disposal of waste by type reduces costs and does much to protect public health. Segregation should always be the responsibility of the waste producer, should take place as close as possible to where the waste is generated, and should be maintained in storage areas and during transport. The same system of segregation should be in force throughout the country.

The most appropriate way of identifying the categories of health care waste is by sorting the waste into color – coded plastic bags or containers.

In addition to the color coding of waste containers, the following practices are recommended:
General health care waste should join the stream of domestic refuse for disposal.

Sharps should all be collected together, regardless of whether or not they are contaminated. Containers should be puncture proof (usually made of metal or high density plastic) and fitted with covers. They should be rigid and impermeable so that they safely retain not only the sharps but also any residual liquids from syringes. Where plastic or metal containers are unavailable or too costly, containers made of dense cardboard are recommended, these fold for ease of transport and may be supplied with a plastic lining.

Bags and containers for infectious waste should be marked with the international infectious substance symbol.

Highly infectious waste should, whenever possible, be sterilized immediately by autoclaving. It therefore needs to be packaged in bags that are compatible with the proposed treatment process: red bags, suitable for autoclaving, are recommended.

Cytotoxic waste, most of which is produced in major hospital or research facilities, should be collected in strong, leak proof containers clearly labeled "Cytotoxic wastes".

Small amounts of chemical or pharmaceutical waste may be collected together with infectious waste.

Large quantities of obsolete or expired pharmaceuticals stored in hospital wards or departments should be returned to the pharmacy for disposal. Other pharmaceutical waste generated at this level, such as spilled or contaminated drugs or packaging containing drug residues should not be returned because of the risk of contaminating the pharmacy; it should be deposited in the correct container at the point of production.

Large quantities of chemical waste should be packed in chemical resistant containers and sent to specialized treatment facilities (if available). The identity of the chemicals should be clearly marked on the containers: hazardous chemical wastes of different types should never be mixed.

Waste with a high content of heavy metals (e.g. cadmium or mercury) should be collected separately.

Aerosol containers may be collected with general health care waste once they are completely empty, provided that the waste is not destined for incineration.

Low level radioactive infectious waste (e.g. swabs, syringes for diagnostic or therapeutic use) may be collected in yellow bags or containers for infectious waste if these are destined for incineration.

Since costs for safe treatment and disposal of hazardous health care waste are typically more than 10 times higher than those for general waste, all general, i.e., non hazardous waste should be handled in the same manner as domestic refuse and collected in black bags. No health care waste other than sharps should be deposited in sharps containers, as these containers are more expensive than the bags used for other infectious waste. Measures of this sort help to minimize the costs of health care waste collection and treatment. When a disposable syringe is used, for example, the packaging should be placed in the general waste bin and the used syringe in the yellow sharps container. In most circumstances, the needle should not be removed from the syringe because of the risk of injury; if removal of the needle is required, special care must be taken.

Appropriate containers or bag holders should be placed in all locations where particular categories of waste may be generated. Instructions on waste separation and identification should be posted at each waste collection point to remind staff of the procedures. Containers should be removed when they are three quarters full.

Staff should never attempt to correct errors of segregation by removing items from a bag or container after disposal or by placing one bag inside another bag of a different color. If general and hazardous health care wastes are accidentally mixed, the mixture should be treated as hazardous health care waste.

Cultural and religious constrains in certain countries make it unacceptable for anatomical waste to be collected in the usual yellow bags; such waste should be disposed of in accordance with local custom, which commonly specifies burial.

On – site collection, transport, and storage of waste
Collection
Nursing and other clinical staff should ensure that waste bags are tightly closed or sealed when they are about three quarters full. Light gauge bags can be closed by tying the neck, but heavier guage bags probably require a plastic sealing tag of the self locking type. Bags should not be closed by stapling. Sealed sharps containers should be placed in a labeled, yellow infectious health care waste bag before removal from the hospital ward or department.

Wastes should not be allowed to accumulate at the point of production. A routine program for their collection should be established as part of the heath care waste management plan.

Certain recommendations should be followed by the ancillary workers in charge of waste collection:
Waste should be collected daily (or as frequently as required) and transported to the designated central storage site.
No bags should be removed unless they are labeled with their point of production (hospital and ward or department) and contents.
The bags or containers should be replaced immediately with new ones of the same type.

A supply of fresh collection bags or containers should be readily available at all locations where waste is produced.

Storage
A storage location for health care waste should be designated inside the health care establishment or research facility. The waste, in bags or containers, should be stored in a separate area, room, or building of a size appropriate to the quantities of waste produced and the frequency of collection.
Unless a refrigerated storage room is available, storage times for health care waste (that is the delay between production and treatment) should not exceed the following:
Temperature climate: 72 hours in winter
                                        48 hours in summer

Warm climate: 48 hours during the cool season
                                        24 hours during the hot season

Cytotoxic waste should be stored separately from other health care waste in a designated secure location.
Radioactive waste should be stored in containers that prevent dispersion, behind lead shielding. Waste that is to be stored during radioactive decay should be labeled with the type of radionuclide, the date, and details of required storage conditions.

Recommendations for storage facilities for health care waste
1. The storage area should have an impermeable, hard standing floor with good drainage; it should be easy to clean and disinfect.
2. There should be a water supply for cleaning purposes.
3. The storage area should afford easy access for staff in charge of handling the waste.
4. It should be possible to lock the store to prevent access by unauthorized persons.
5. Easy access for waste collection vehicles is essential.
6. There should be protection from the sun.
7. The storage area should be inaccessible for animals, insects, and birds.
8. There should be good lighting and at least passive ventilation.
9. The storage area should not be situated in the proximity of fresh food stores or food preparation areas.
10. A supply of cleaning equipment, protective clothing, and waste bags or containers should be located conveniently close to the storage area.

References
WHO: Wastes from Health Care Facilities

Biomedical Waste Management (I)

Definition:
According to Biomedical Waste (Management and Handling) Rules 1998 of India "Any waste which is generated during the diagnosis, treatment or immunization of human beings or animals or in research activities pertaining to or in the production or testing of biological".
Background: Biomedical waste (BMW) has recently emerged as an issue of major concern not only to hospitals and nursing homes, but also to the environmental and law enforcing agencies, media, and the general public. BMW forms approximately 1%–2% of the total municipal solid waste stream.

The human element is found to be far more important than the technology. Almost any system of treatment and disposal of BMW that is operated by well-trained and well-motivated staff can provide greater protection to staff, patients, and the community than an expensive and sophisticated system that is managed by staff who do not understand the risk and the importance of their contribution.

Classification of Bio-Medical Waste
The World Health Organization (WHO) has classified medical waste into eight categories:
- General Waste
- Pathological
- Radioactive
- Chemical
- Infectious to potentially infectious waste
- Sharps
- Pharmaceuticals
- Pressurized containers

Biomedical Waste Management Process
There is a big network of Health Care Institutions in India. The hospital waste like body parts, organs, tissues, blood and body fluids along with soiled linen, cotton, bandage and plaster casts from infected and contaminated areas are very essential to be properly collected, segregated, stored, transported, treated and disposed of in safe manner to prevent nosocomial or hospital acquired infection.

1. Waste collection
2. Segregation
3. Transportation and storage
4. Treatment & Disposal
5. Transport to final disposal site
6. Final disposal

Biomedical Waste Treatment and Disposal
Health care waste is a heterogeneous mixture, which is very difficult to manage as such. But the problem can be simplified and its dimension reduced considerably if a proper management system is planned.

Incineration Technology
This is a high temperature thermal process employing combustion of the waste under controlled condition for converting them into inert material and gases. Incinerators can be oil fired or electrically powered or a combination thereof. Broadly, three types of incinerators are used for hospital waste: multiple hearth type, rotary

Cat. No. 6	Incineration , autoclaving/micro waving	Solid Waste (Items contaminated with blood and body fluids including cotton, dressings, soiled plaster casts, line beddings, other material contaminated with blood)
Cat. No. 7	Disinfections by chemical treatment autoclaving/micro waving& mutilation shredding.	Solid Waste (waste generated from disposable items other than the waste sharps such as tubing, catheters, intravenous sets etc.)
Cat. No. 8	Disinfections by chemical treatment and discharge into drain	Liquid Waste (waste generated from laboratory & washing, cleaning , house-keeping and disinfecting activities)
Cat. No. 9	Disposal in municipal landfill	Incineration Ash (ash from incineration of any bio-medical waste)
Cat. No. 10	Chemical treatment & discharge into drain for liquid & secured landfill for solids	Chemical Waste (chemicals used in production of biological, chemicals, used in disinfection, as insecticides, etc)

(Source- The Bio Medical Waste (Management and Handling) Rules, 1998)

Schedule II: Colour Coding and Type Of Container for Disposal of Bio-Medical Wastes

Schedule III: Label for Bio-Medical Waste Containers/Bags

Recommendations
1. For the use of incinerator Training should be given to some number of persons from staff.
2. Specific fund should be allocated for the use of incinerator.
3. Every hospital should have special boxes to use as dustbin for bio-medical waste.
4. Bio-medical waste should not be mixed with other waste of Municipal Corporation.
5. Private hospitals should also be allowed to use incinerator, which is installed, in govt. hospital. For this purpose a specific fee can be charged from private hospitals.
6. Special vehicle i.e. bio-medical waste vehicle should be started to collect waste from private hospitals and private medical clinics and carry it up to the main incinerator.
7. As provided by bio-medical waste rules, the whole of the waste should be fragmented into colours due to their hazardous nature.
8. Bio-medical waste Management Board can be established in each District.
9. Either judicial powers should be given to the management board or special court should be established in the matters of environment pollution for imposing fines and awarding damages etc.
10. Housekeeping staff wear protective devices such as gloves, face masks, gowned, while handling the waste.
11. There is biomedical waste label on waste carry bags and waste carry trolley and also poster has put on the wall adjacent to the bins (waste) giving details about the type of waste that has to dispose in the baggage as per biomedical waste management rule. Carry bags also have the biohazard symbol on them.

CONCLUSION
Medical wastes should be classified according to their source, typology and risk factors associated with their handling, storage and ultimate disposal. The segregation of waste at source is the key step and reduction, reuse and recycling should be considered in proper perspectives. We need to consider innovative and radical measures to clean up the distressing picture of lack of civic concern on the part of hospitals and slackness in government implementation of bare minimum of rules, as waste generation particularly biomedical waste imposes increasing direct and indirect costs on society. The challenge before us, therefore, is to scientifically manage growing quantities of biomedical waste that go beyond past practices. If we want to protect our environment and health of community we must sensitize ourselves to this important issue not only in the interest of health managers but also in the interest of community.

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Biomedical Waste Management (II)

Let the waste of the "sick"
not contaminate the lives of
"The Healthy"

Disposal Procedures for Infectious Liquid Waste:

Sanitary Sewer Disposal Methods
The sanitary sewer system is designed for the disposal of certain liquid wastes. Use of the sanitary sewer reduces the chance for leaks or spills during transport and thereby reduces disposal costs. Chemical disinfection is done prior to sewer disposal with the aim to eliminate micro-organisms or to reduce the microbial load. Chemical treatment usually involves the use of 1% sodium hypochlorite solution with a minimum contact period of 30 min or other standard disinfectants like, 10-14 gm of bleaching powder in 1 litre water, 70% ethanol, 4% formaldehyde, 70% isopropyl alcohol, 2.5% povidone iodine, or 6% hydrogen peroxide.

Disinfection of culture media differs a little from the usual disinfection process, where due to the high microbial load and the rich protein content of the media plates, rigorous disinfection is required, where inactivation should be done by 5.23% sodium hypochlorite, in a 1:10 dilution and should be left for a minimum of 8 h covered and then finally disposed down the sanitary sewer, followed by flushing with a lot of cold water for a minimum period of 10 min.

Sodium hypochlorite solution, also known as bleach, is a broad-spectrum disinfectant that is effective for enveloped viruses (HIV, HBV, HSV), vegetative bacteria (*Pseudomonas, Staphylococcus*, and *Salmonella*), fungi (e.g., *Candida*), *Mycobacterium* (*M. tuberculosis* and *M. bovis*), and non-enveloped viruses (Adenovirus and Parvovirus), should be stored between 50 and 70°F. Undiluted household bleach has a shelf life of 6 months to 1 year from the date of manufacture, after which it undergoes degradation at a rate of 20% per year until a total degradation to salt and water. Though a 1:10 concentration of bleach solution has a shelf life of 24 h only, some manufacturer prepared 1:10 bleach solutions, contain a stabilizer that increases the shelf life to approximately 18 months.

Hypochlorite (1%) is inefficient in decontaminating blood containing hypodermic needles.
Infectious biomedical waste and sharps have a potential hazard of transmission of pathogens. Among sharps, used needles form a major share and disinfection by 1 % hypochlorite is recommended in biomedical waste management rules of India. The aim of the present study was to evaluate the efficacy of hypochlorite for the decontamination of needles. Needles (16 g) filled with suspensions of standard strains and clinical isolates of gram positive and gram negative bacteria in plain normal saline and in human blood containing anticoagulant, were exposed to 1% hypochlorite and the

surviving bacteria were subjected to viable counts. The observations indicated that 85 - 90 % of the needles filled with bacterial suspensions in saline are disinfected to a level of >5 log bacterial reduction (standard disinfection) on exposure to hypochlorite but only 15 to 30% needles contaminated with the challenge bacteria suspended in blood showed >5 log reduction in viable counts. Thus, hypochlorite treatment is inadequate for disinfecting needles contaminated with pathogenic bacteria in presence of blood and should not be recommended as an option for disinfection of the needles.

Medical waste generated in hospital can be a serious health hazard for the patients in the hospital, hospital staff and if reaches to the community, can become a community health hazard. In order to control this biohazard MOEF (Ministry of Environment and Forests) enacted the Biomedical waste rules (BMW) 1998. The sharps and specifically needles have been given special emphasis since needle stick injuries during handling or medical procedures have the potential to transmit blood borne infections. The diseases that can be transmitted include AIDS, hepatitis B, hepatitis C, malaria and many other bacterial and viral diseases.

The sharps are to be disposed in special coloured puncture proof containers. The treatment options suggested by BMW rules are chemical disinfection with 1% hypochlorite or autoclaving or microwaving. The needles are to be mutilated after treatment. The rules also recommend monitoring of chemical disinfection from time to time. However, there is no data available on monitoring of chemical disinfection of needles.

Hypodermic needles range in length from 1 to 6 inches and thickness of 24 to 16 g are used for a number of clinical procedures in a hospital setup. In case of hypodermic needles which are attached to plastic or rubber tubing, the usual practice in Indian setups is to just cut the needle portion and immerse the needles in 1% sodium hypochlorite. The other needles after use are either directly added to hypochlorite containers or mutilated by cutting part of the needle mechanically in needle cutters or by burning the tip portions in electric needle destroyers before immersing in hypochlorite solutions. The studies with bacterial challenges to test the efficacy of chlorine releasing solutions are numerous but chlorine solutions used have been in the range of (0.0003 to 0.25%). The only study with organic soiling used albumin (1%) and plasma (10 to 50%) which rendered 0.25% hypochlorite totally ineffective. Further, in a study blood was used as organic material using *Staphylococcus aureus* as the challenge organism and quantitative suspension test was performed using 1% hypochlorite. To the best of our knowledge,

studies on efficacy of hypochlorite have been undertaken for conditions simulating spillage but not for needle decontamination. The penetration of hypochlorite into the narrow lumen of needles, especially those filled with blood and body fluids, remains doubtful. Surprisingly scientific data to prove the efficacy of 1% hypochlorite for decontamination of hypodermic needles is not available. Hence, this study was designed to determine the efficacy of 1% sodium hypochlorite for disinfection of contaminated hypodermic needles with or without blood as organic soil.

Materials and methods

Sodium hypochlorite
Sodium hypochlorite having an initial Cl_2 concentration of 10% (checked by chlorinometer, Qualigens, India) was used to prepare in-use solution of 1% sodium hypochlorite.

Bacterial cultures
The standard strains of *S.aureus* NCTC 6538 and *E.coli* NCTC 10418 which were procured from Haffkine Institute for Training, Testing and Research, Mumbai, were used for the experiments. Isolates of *Staphylococcus aureus*, coagulase negative staphylococci, *E.coli*, *Klebsiella* species and *Pseudomonas aeruginosa* were obtained from clinical samples of patients.

Bacterial inoculum
Two sets of inoculum were used for the study:
a) Plain inoculum-Bacterial growth from fresh overnight grown nutrient agar slants were harvested in sterile normal saline to match the turbidity of 0.5 McFarland standard (1 x 108 CFU/mL). Suspension was vortexed to prevent clumping of bacterial cells.
b) Blood inoculum-CPDA (Citrate Phosphate Dextrose and Adenine) anticoagulated blood from blood bank was used for preparing the inoculum. Equal volumes of plain inoculum and blood were mixed to prepare the inoculum.

New sterile hypodermic needles (16 g) from blood donor set were used in the study for the experimental ease of flushing the needles

and sterile 1% sodium thiosulphate solution was used after exposure of needles to neutralize residual chlorine present.

Procedure
Sterile hypodermic needles were filled with inoculum (plain or in blood) by aspiration with syringe. The needles were separated from the syringe and immersed in 1% sodium hypochlorite solution (300 mL) for 30 minutes. The needles were picked up with sterile forceps and 1ml sodium thiosulphate (1%) was passed through the needles with a sterile syringe and collected in sterile tubes. Thereafter, dilutions were made in sterile normal saline. The undiluted and serial dilutions were further subjected to viable bacterial count by plating over nutrient agar in duplicate. The plates were incubated at 37^0C for 48 hours. Ten needles filled with plain inoculum of bacteria were exposed to hypochlorite solution in each experiment and a second set of ten needles filled with inoculum in blood were exposed similarly. As a control, three needles in each experimental set were filled up with plain and blood containing bacterial inoculum and immersed in normal saline and processed like exposed test needles. The experiments were repeated second time using identical number of needles for test and control.

Statistical analysis
Post- hypochlorite exposure reduction in CFU count for the bacterial suspension in plain normal saline versus bacterial suspension in blood were compared by Wilcoxon sign rank test.

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Waterborne Diseases
Prevention and Treatment

Hydrogen and oxygen, arguably, might have never made a better and richer combination on the planet like that of the chemical molecule, H_2O......yes WATER.

Water, is not only a life sustaining drink, but in addition has many other features that makes this fluid indispensable. However it is essential that potable water has to be of the highest quality most specially when the water is used for consumption. When water consumed is not pure, clean, and hygienic, consumption of this life sustaining water can be the primary cause of disease transmission.

Waterborne diseases are any illness caused by drinking water contaminated by human or animal feces, which contains pathogenic microorganisms. Though these diseases are spread either directly or through flies or filth, water is the chief medium for spread of these diseases and hence they are termed as waterborne diseases.

The visualization of water associated diseases is complex for a number of reasons. Over the past decades, the picture of water related human health issues has become increasingly comprehensive, with the emergence of new water related infectious diseases and the re-emergence of ones already known. The burden of several disease groups can only partly be attributed to water determinants. Even where water plays an essential role in the ecology of diseases, it may be hard to pinpoint the relative importance of aquatic components of the local ecosystem.

Facets of the Problem!
In developing countries four – fifths of all the illnesses are caused by water borne diseases, with diarrhea being the leading cause of childhood death.

The global scenario of water and health has a strong local dimension with some billions of people still lacking access to improved drinking water sources and some 2 billion to adequate sanitation. There is a strong evidence that lacs of people die annually after succumbing to water borne diseases.

Most intestinal (enteric) diseases are infectious and are transmitted through fecal waste. Pathogens – which include virus, bacteria, protozoa, and parasitic worms – are diseases producing agents found in the feces of infected persons. These diseases are more prevalent in areas with poor sanitary conditions.

These pathogens travel through water sources and interfuses directly through persons handling food and water. Since these diseases are highly infectious, extreme care and hygiene should be maintained by people looking after an infected patient. Hepatitis, cholera, dysentery, and typhoid are the common waterborne diseases that affect large populations in the tropical regions.

A large number of chemicals that either exist naturally in the land or are added due to human activity dissolve in the water as a result of surface runoff, thereby contaminating it and leading to various diseases.

Pesticides: The organophosphates and the carbonates present in pesticides affect and damage the nervous system and can cause cancer. Some of the pesticides contain carcinogens that exceed recommended levels. They contain chlorides that cause reproductive and endocrinal damage.

Lead: Lead is hazardous to health as it accumulates in the body and affects the central nervous system. Children and pregnant women are most at risk.

Fluoride: Excess fluorides can cause yellowing of the teeth and damage to the spinal cord and other crippling diseases.

Nitrates: Drinking water that gets contaminated with nitrates can prove fatal especially to infants that drink formula milk as it restricts the amount of oxygen that reaches the brain causing the 'blue baby' syndrome. It is also linked to digestive tract cancers. It causes algae to bloom resulting in eutrophication in surface water.

Petrochemicals: Benzene and other petrochemicals can cause cancer even at low exposure levels.

Chlorinated solvents: These are linked to reproduction disorders and to some cancers.

Arsenic: Arsenic poisoning through water can cause liver and nervous system damage, vascular diseases and also skin cancer.

Other Heavy metals: Heavy metals cause damage to the nervous system and the kidney, and other metabolic disruptions.

Salts: It makes fresh water unusable for drinking and irrigation purposes.

Exposure to polluted water can cause diarrhea, skin irritation, respiratory problems, and other diseases, depending on the pollutant that is in the water body. Stagnant water and other untreated water provide a habitat for mosquitoes and a host of other parasites and insects that cause a large number of diseases especially in the tropical regions. Among these, malaria is undoubtedly the most widely distributed and causes most damage to human health.

Transmission
Waterborne diseases spread by contamination of drinking water system with the urine and feces of infected animal or people.
This is likely to occur where public and private drinking water systems, get their water from surface waters (rain, creeks, rivers, lakes etc.), which can be contaminated by infected animals or people. Runoff from landfills, septic fields, sewer pipes, residential or industrial developments can also sometimes contaminate surface water.
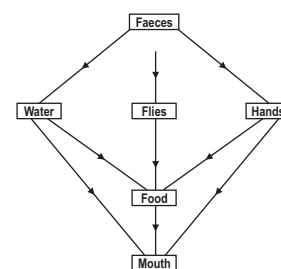
This has been the cause of many dramatic outbreaks of fecal-oral diseases such as cholera and typhoid. However, there are many other ways in which fecal material can reach the mouth, for instance on the hands or on contaminated food. In general, contaminated food is the single most common way in which people become infected.

The germs in the feces can cause the diseases by even slight contact and transfer. This contamination may occur due to floodwaters, water runoff from landfills, septic fields, and sewer pipes.

The picture above shows the fecal oral route of disease transmission

The only way to break the continued transmission is to improve the people's hygienic behavior and to provide them with certain basic needs: drinking water, washing and bathing facilities and sanitation. Malaria transmission is facilitated when large numbers of people sleep outdoor during hot weather, or sleep in houses that have no protection against invading mosquitoes. Malaria mosquitoes, tropical black flies, and bilharzias snails can all be controlled with efficient drainage because they all depend on water to complete their life cycles.

Prevention

Waterborne epidemics and health hazards in the aquatic environment are mainly due to improper management of water resources. Proper management of water resources has become the need of the hour as this would ultimately lead to a cleaner and healthier environment.

In order to prevent the spread of waterborne infectious diseases, people should take adequate precautions. The city water supply should be properly checked and necessary steps taken to disinfect it. Water pipes should be regularly checked for leaks and cracks. At home, the water should be boiled, filtered, or other methods and necessary steps taken to ensure that it is free from infection.

Chlorination: Adding chlorine in liquid or tablet form to drinking water stored in a protected container.

At doses of a few mg/L and contact times of about 30 minutes, free chlorine generally inactivates > 99.99% of enteric bacteria and viruses, provided water is clear. Chlorine can come in a variety of sources, including solid calcium hypochlorite, liquid sodium hypochlorite or Na_2DCC tablets. Household level chlorination has been implemented most commonly in combination with safe storage and behavior change techniques, including social marketing, community mobilization, motivational interviewing, communication and education.

Solar disinfection: Exposing water in disposable clear plastic bottles to sunlight for a day, typically on the roof of a house.

A combination of heat and ultra – violet radiation from the sun are used to inactivate pathogens present in water. One low – cost technique involves exposing water in clear plastic bottles to sunlight for six hours, for example on the roof of a house (or for 2 days if the sun is obscured by clouds). The water should be consumed directly from the bottle or transferred to a clean glass. To be effective, solar disinfection must be applied to relatively clear water.

Filtration: Water filtration is another option to purify water. Higher quality ceramic filters with small pores, often coated with silver to control bacterial growth, have been shown to be effective at removing many microbes and other suspended solids. Filters need to be cleaned regularly to maintain flow rates. If properly maintained, they have a long life. Ceramic filters can be mass produced centrally or manufactured locally in smaller batches. Some commercial systems that combine filtration and disinfection have also been shown to be safe and effective, though their up – front cost may be an obstacle to low – income populations.

Combined flocculation / disinfection systems: Adding powders or tablets to coagulate and flocculate sediments in water followed by a timed release of disinfectant.

These are typically formulated to coagulate and flocculate sediments in water followed by a timed release of chlorine. These typically treat 10 – 15 liters of water, and are particularly useful for treating turbid water. The water is normally stirred for few minutes, strained to separate the flocculation, and then allowed to stand for another half hour for complete disinfection.

Boiling: If practical, households can disinfect their drinking water by bringing it to a rolling boil, which will kill pathogens effectively. In order to be effective, however, the treated water must be protected from re – contamination. Caution must also be exercised to avoid scalding accidents, especially among young children. While boiling is widely practiced, it may be more costly, inconvenient and environmentally unsustainable than other emerging POU water treatment options.

Safe Storage: Research has shown that water that is safe at the point of collection is often subject to fecal contamination during collection, transport and use in the home, mainly by unclean hands. Studies have also shown that vessels with narrow mouths and taps can significantly reduce such contamination and reduce the risk of diarrheal disease. Where possible, safe storage should also be incorporated included in interventions to treat water in the home

Different technologies are better suited for different situations. Solar disinfection, for example, may be especially suited for very poor households in sunny regions that draw relatively clear water. Combined flocculation / disinfection systems are a suitable option for treating turbid water. Filters have higher-up front costs but are straight forward to use, and may not require the same degree of behavior change efforts as other approaches. Household chlorination has achieved widespread use, is appropriate for the very poor, and after boiling is the most common treatment approach.

Treatment

Depending on the type of infection and the infecting organism, the treatment will vary, however for those infection that lead to dehydration the mainstay of treatment lies in Oral rehydration with WHO recommended ORS (Oral Rehydration Solution).

Management of Water Systems in Health-Care Facilities

I. Controlling the Spread of Waterborne Micro-organisms

A. Practice hand hygiene to prevent the hand transfer of waterborne pathogens, and use barrier precautions (e.g., gloves) as defined by other guidelines.

B. Eliminate contaminated water or fluid environmental reservoirs (e.g., in equipment or solutions) wherever possible.

C. Clean and disinfect sinks and wash basins on a regular basis by using product as set by facility policies.

D. Evaluate for possible environmental sources (e.g., potable water) of specimen contamination when waterborne microorganisms (e.g., NTM) of unlikely clinical importance are isolated from clinical cultures (e.g., specimens collected aseptically from sterile sites or, if post-procedural, colonization occurs after use of tap water in patient care).

E. Avoid placing decorative fountains and fish tanks in patient-care areas; ensure disinfection and fountain maintenance if decorative fountains are used in the public areas of the healthcare facility.

II. Routine Prevention of Waterborne Microbial Contamination within the Distribution System

A. Maintain hot water temperature at the return at the highest temperature allowable by state regulations or codes, preferably >124°F (>51°C), and maintain cold water temperature at <68°F (<20°C).

B. If the hot water temperature can be maintained at >124°F (>51°C), explore engineering options (e.g., install preset thermostatic valves in point-of-use fixtures) to help minimize the risk of scalding.

C. When state regulations or codes do not allow hot water temperatures above the range of 105°F - 120°F (40.6°C–49°C) for hospitals or 95°F–110°F (35°C–43.3°C) for nursing care facilities or when buildings cannot be retrofitted for thermostatic mixing valves, follow either of these alternative preventive measures to minimize the growth of Legionella spp. in water systems.

   a. Periodically increase the hot water temperature to >150°F (>66°C) at the point of use.

   b. Alternatively, chlorinate the water and then flush it through the system.

D. Maintain constant recirculation in hot-water distribution systems serving patient-care areas.

III. Remediation Strategies for Distribution System Repair or Emergencies

A. Whenever possible, disconnect the ice machine before planned water disruptions.

B. Prepare a contingency plan to estimate water demands for the entire facility in advance of significant water disruptions (i.e., those expected to result in extensive and heavy microbial or chemical contamination of the potable water), sewage intrusion, or flooding.

C. When a significant water disruption or an emergency occurs, adhere to any advisory to boil water issued by the municipal water utility.

   1. Alert patients, families, staff, and visitors not to consume water from drinking fountains, ice, or drinks made from municipal tap water, while the advisory is in effect, unless the water has been disinfected (e.g., by bringing to a rolling boil for >1 minute).

   2. After the advisory is lifted, run faucets and drinking fountains at full flow for >5 minutes, or use high-temperature water flushing or chlorination.

D. Maintain a high level of surveillance for waterborne disease among patients after a boil water advisory is lifted.

E. Corrective decontamination of the hot water system might be necessary after a disruption in service or a cross-connection with sewer lines has occurred.

   1. Decontaminate the system when the fewest occupants are present in the building (e.g., nights or weekends).

   2. If using high-temperature decontamination, raise the hot-water temperature to 160°F–170°F (71°C–77°C) and maintain that level while progressively flushing each outlet around the system for >5 minutes.

   3. If using chlorination, add enough chlorine, preferably overnight, to achieve a free chlorine residual of >2 mg/L (>2 ppm) throughout the system.

   4. Flush each outlet until chlorine odor is detected.

   5. Maintain the elevated chlorine concentration in the system for >2 hrs (but <24 hrs).

   6. Use a very thorough flushing of the water system instead of chlorination if a highly chlorine-resistant microorganism (e.g., Cryptosporidium spp.) is suspected as the water contaminant.

F. Flush and restart equipment and fixtures according to manufacturers' instructions.

G. Change the pre-treatment filter and disinfect the dialysis water system with an EPA registered product to prevent colonization of the reverse osmosis membrane and downstream microbial contamination.

H. Run water softeners through a regeneration cycle to restore their capacity and function.

I. If the facility has a water-holding reservoir or water-storage tank, consult the facility engineer or local health department to determine whether this equipment needs to be drained, disinfected with an EPA-registered product, and refilled.

J. Implement facility management procedures to manage a sewage system failure or flooding (e.g., arranging with other health-care facilities for temporary transfer of patients or provision of services), and establish communications with the local municipal water utility and the local health department to ensure that advisories are received in a timely manner upon release.

K. Implement infection-control measures during sewage intrusion, flooding, or other waterrelated emergencies.

   1. Relocate patients and clean or sterilize supplies from affected areas.

   2. If hands are not visibly soiled or contaminated with proteinaceous material, include an alcohol-based hand rub in the hand hygiene process 1) before performing invasive procedures; 2) before and after each patient contact; and 3) whenever hand hygiene is indicated.

   3. If hands are visibly soiled or contaminated with proteinaceous material, use soap and bottled water for handwashing.

   4. If the potable water system is not affected by flooding or sewage contamination, process surgical instruments for sterilization according to standard procedures.

   5. Contact the manufacturer of the automated endoscope reprocessor (AER) for specific instructions on the use of this equipment during a water advisory.

L. Remediate the facility after sewage intrusion, flooding, or other water-related emergencies.

   1. Close off affected areas during cleanup procedures.

2. Ensure that the sewage system is fully functional before beginning remediation so contaminated solids and standing water can be removed.

3. If hard-surface equipment, floors, and walls remain in good repair, ensure that these are dry within 72 hours; clean with detergent according to standard cleaning procedures.

4. Clean wood furniture and materials (if still in good repair); allow them to dry thoroughly before restoring varnish or other surface coatings.

5. Contain dust and debris during remediation and repair as outlined in air recommendations.

M. Regardless of the original source of water damage (e.g., flooding versus water leaks from point-of-use fixtures or roofs), remove wet, absorbent structural items (e.g., carpeting, wallboard, and wallpaper) and cloth furnishings if they cannot be easily and thoroughly cleaned and dried within 72 hours (e.g., moisture content <20% as determined by moisture meter readings); replace with new materials as soon as the underlying structure is declared by the facility engineer to be thoroughly dry.

IV. Additional Engineering Measures as Indicated by Epidemiologic Investigation for Controlling Waterborne, Health-Care–Associated Legionnaires Disease.

A. When using a pulse or one-time decontamination method, superheat the water by flushing each outlet for >5 minutes with water at 160°F–170°F (71°C–77°C) or hyperchlorinate the system by flushing all outlets for >5 minutes with water containing >2 mg/L (>2 ppm) free residual chlorine using a chlorine-based product registered by the EPA for water treatment (e.g., sodium hypochlorite [chlorine bleach]).

B. After a pulse treatment, maintain both the heated water temperature at the return and the cold water temperature as per the recommendation wherever practical and permitted by state codes, or chlorinate heated water to achieve 1–2 mg/L (1–2 ppm) free residual chlorine at the tap using a chlorine-based product registered by the EPA for water treatment (e.g., sodium hypochlorite [bleach]).

C. Explore engineering or educational options (e.g., install preset thermostatic mixing valves in point-of-use fixtures or post warning signs at each outlet) to minimize the risk of scalding for patients, visitors, and staff.

D. No recommendation is offered for treating water in the facility's distribution system with chlorine dioxide, heavy-metal ions (e.g., copper or silver), monochloramine, ozone, or UV light.

V. General Infection-Control Strategies for Preventing Legionnaires Disease

A. Conduct an infection-control risk assessment of the facility to determine if patients at risk or severely immunocompromised patients are present.

B. Implement general strategies for detecting and preventing Legionnaires disease in facilities that do not provide care for severely immunocompromised patients (i.e., facilities that do not have HSCT or solid organ transplant programs).

1. Establish a surveillance process to detect health-care–associated Legionnaires disease.

2. Inform health-care personnel (e.g., infection control, physicians, patient-care staff, and engineering) regarding the potential for Legionnaires disease to occur and measures to prevent and control health-care–associated legionellosis.

3. Establish mechanisms to provide clinicians with laboratory tests (e.g., culture, urine antigen, direct fluorescence assay [DFA], and serology) for the diagnosis of Legionnaires disease.

C. Maintain a high index of suspicion for health-care–associated Legionnaires disease, and perform laboratory diagnostic tests for legionellosis on suspected cases, especially in patients at risk who do not require a PE for care (e.g., patients receiving systemic steroids; patients aged >65 years; or patients with chronic underlying disease [e.g., diabetes mellitus, congestive heart failure, or chronic obstructive lung disease]).

D. Periodically review the availability and clinicians' use of laboratory diagnostic tests for Legionnaires disease in the facility; if clinicians' use of the tests on patients with diagnosed or suspected pneumonia is limited, implement measures (e.g., an educational campaign) to enhance clinicians' use of the test(s).

E. If one case of laboratory-confirmed, health-care–associated Legionnaires disease is identified, or if two or more cases of laboratory-suspected, health-care–associated Legionnaires disease occur during a 6-month period, certain activities should be initiated.

1. Report the cases to the state and local health departments where required.

2. If the facility does not treat severely immunocompromised patients, conduct an epidemiologic investigation, including retrospective review of microbiologic, serologic, and postmortem data to look for previously unidentified cases of healthcare– associated Legionnaires disease, and begin intensive prospective surveillance for additional cases.

3. If no evidence of continued health-care–associated transmission exists, continue intensive prospective surveillance for >2 months after the initiation of surveillance.

F. If there is evidence of continued health-care–associated transmission (i.e., an outbreak), conduct an environmental assessment to determine the source of *Legionella* spp.

1. Collect water samples from potential aerosolized water sources.

2. Save and subtype isolates of *Legionella* spp. obtained from patients and the environment.

3. If a source is identified, promptly institute water system decontamination measures per recommendations.

4. If *Legionella* spp. are detected in >1cultures (e.g., conducted at 2-week intervals during 3 months), reassess the control measures, modify them accordingly, and repeat the decontamination procedures; consider intensive use of techniques used for initial decontamination, or a combination of superheating and hyperchlorination.

G. If an environmental source is not identified during a Legionnaires disease outbreak, continue surveillance for new cases for >2 months. Either defer decontamination pending identification of the source of *Legionella* spp., or proceed with decontamination of the hospital's water distribution system, with special attention to areas involved in the outbreak.

H. No recommendation is offered regarding routine culturing of water systems in health-care facilities that do not have patient-care areas (i.e., PE or transplant units) for persons at high risk for *Legionella* spp. infection.

I. No recommendation is offered regarding the removal of faucet aerators in areas for immunocompetent patients.

J. Keep adequate records of all infection-control measures and environmental test results for potable water systems.

References

Guidelines for Environmental Infection Control in Health-Care Facilities ecommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC),U.S. Department of Health and Human Services, Centers for Disease Control and Prevention (CDC), Atlanta, GA 30333.

Best practices in chemical water disinfection

Water purification is the process of removing undesirable chemicals, biological contaminants, suspended solids and gases from contaminated water. The goal is to produce water fit for a specific purpose. Most water is disinfected for human consumption (drinking water), but water purification may also be designed for a variety of other purposes, including fulfilling the requirements of medical, pharmacological, chemical and industrial applications.

The standards for drinking water quality are typically set by governments or by international standards. These standards usually include minimum and maximum concentrations of contaminants, depending on the intended purpose of water use.

Chemical and microbiological analysis, while expensive, are the only way to obtain the information necessary for deciding on the appropriate method of purification.

Disinfection is accomplished both by filtering out harmful micro-organisms and also by adding disinfectant chemicals. Water is disinfected to kill any pathogens which pass through the filters and to provide a residual dose of disinfectant to kill or inactivate potentially harmful micro-organisms in the storage and distribution systems. Possible pathogens include viruses, bacteria, including *Salmonella*, Cholera, *Campylobacter* and *Shigella*, and protozoa, including *Giardia lamblia* and other *cryptosporidia*. Following the introduction of any chemical disinfecting agent, the water is usually held in temporary storage – often called a contact tank or clear well to allow the disinfecting action to complete.

The most common disinfection method involves some form of chlorine. Chlorine is a strong oxidant that rapidly kills many harmful micro-organisms. Because chlorine is a toxic gas, there is a danger of a release associated with its use. This problem is avoided by the use of sodium hypochlorite, which is a relatively inexpensive solution used in household bleach that releases free chlorine when dissolved in water. Chlorine solutions can be generated on site by electrolyzing common salt solutions. A solid form, calcium hypochlorite, releases chlorine on contact with water. Handling the solid, however, requires greater routine human contact through opening bags and pouring than the use of gas cylinders or bleach which are more easily automated. The generation of liquid sodium hypochlorite is both inexpensive and safer than the use of gas or solid chlorine.

All forms of chlorine are widely used, despite their respective drawbacks. One drawback is that chlorine from any source reacts with natural organic compounds in the water to form potentially harmful chemical by-products. These byproducts, trihalomethanes (THMs) and haloacetic acids (HAAs), are both carcinogenic in large quantities and are regulated by the United

States Environmental Protection Agency (EPA) and the Drinking Water Inspectorate in the UK. The formation of THMs and haloacetic acids may be minimized by effective removal of as many organics from the water as possible prior to chlorine addition. Although chlorine is effective in killing bacteria, it has limited effectiveness against protozoa that form cysts in water (*Giardia lamblia* and *Cryptosporidium*, both of which are pathogenic).

Chloro-isocyanurate compounds are smartly used for emergency chlorination of drinking water. For routine treatment of public water supplies, there is little or no use of other disinfectants. Some chemicals, such as chloro-isocyanurate compounds are widely used as a stable source of chlorine for the disinfection of swimming pools and in the food industry, Sodium dichloroisocyanurate is used for temporary emergency disinfection applications as a source of free available chlorine in the form of hypochlorous acid (HOCl) with the attendant residual formation of cyanuric acid from its addition to water. The WHO is currently preparing guideline text on Sodium dichloroisocyanurate for inclusion in their future 4th edition of their Guidelines for Drinking Water Quality. In their background document for development of Guidelines for Drinking-water Quality the WHO advised that "The amounts of sodium dichloroisocyanurate used should be the lowest consistent with adequate disinfection, and the concentrations of cyanuric acid should be managed to be kept as low as is reasonably possible.

Process-Chlorination

Advantages -Well understood disinfectant capability. Established dosing technology. Stable residual in clean networks. Potential for using chlorine for both primary disinfection and distribution, makes for straightforward application.

Limitations -Chlorination by-products and taste and odour issues can affect acceptability. Ineffective against *Cryptosporidium*. By-product formation during distribution. Loss of residual in distribution systems with long residence times.

Chlorination- Chlorine will oxidize both iron and manganese to their insoluble forms. The higher the chlorine residual in the water, the faster the reaction is. For typical iron and manganese removal needs, first you treat the water with an initial chlorine residual of 5 to 10 mg/L, then filter the insoluble iron and manganese formed and then finally dechlorinate the water to an acceptable residual for domestic use. Doses beyond 10 mg/L can causes excess concentrations of total trihalomethanes (TTHMs), which can cause adverse health conditions. The dechlorination uses a reducing agent such as sodium bisulphate to remove the excess chlorine. Usually a reaction basin is added after the dechlorination process.

Requires an additional dechlorination step to the water treatment process.

Always add powder to water and never water to the powder. Sodium hypochlorite deteriorates very rapidly (60 to 90 days), especially when exposed to light, and so it should be stored in a cool, dry, dark area FOR YOUR OWN SAFETY You must wear rubber gloves, a rubber apron, and nose and eye protection when you are working with sodium hypochlorite.

Contact time – The longer the microorganisms are in contact with the free chlorine, the more effective the disinfection.

pH of water – The effectiveness of free chlorine is maximized at pH levels between 6.0 and 7. As chlorine solutions are highly alkaline (i.e. high pH), the dose of chlorine solution increases the pH of the well water and can diminish the effectiveness of the free chlorine. Therefore, it is important to follow the concentration range requirements for free chlorine set out.

Temperature – The effectiveness of free chlorine can change with temperature. Free chlorine is more effective at higher temperatures.

Interfering substances – Available chlorine will be used up by any inorganic and organic compounds in the well water which will reduce the concentration of free chlorine. Biofilm - Biofilm is a slimy substance that attaches to sides of wells and pumping equipment. The slime consists primarily of nuisance microbes (e.g. iron oxidizing bacteria and sulphatereducing bacteria) that can shield pathogens from the oxidizing action of the free chlorine and reduce the amount of available chlorine. The oxidizing action of the chlorine solution kills bacteria, viruses, protozoans and some protozoal cysts.

Household bleaches typically contain 3 to 6 percent available chlorine. Industrial strength commercial bleach and swimming pool products can contain 10 to 12 percent available chlorine. The unstable nature of sodium hypochlorite makes it sensitive to temperature and light and therefore it has a limited shelf life. For example, sodium hypochlorite degrades extremely rapidly in the hot, sunlit cab of a truck.

Requirements and Best Management Practices - All chlorine products utilized for potable water use must be either fresh unscented bleach or must meet the NSF International Standard 60 for Drinking Water Treatment Chemicals – Health Effects, or an equivalent standard ● Avoid using scented bleach or products such as swimming pool chlorine that typically contain additives such as surfactants, thickeners, stabilizers, perfumes, UV inhibitors, algaecides or other additives as they can impair the quality of the water and aquifer after disinfection and are not designed for potable water use ● Always check product labels to verify product contents and manufacturer's suggested usage as well as Material Safety Data Sheets (MSDS) ● Chlorine products should always be stored in a cool, dry and dark environment.

EFFECTIVENESS OF CHLORINE-FREE CHLORINE & RESIDUAL
When a chlorine solution is first added to water the available chlorine will react with substances in the water, and on the surfaces inside. During this reaction, some of the available chlorine is used up by organic and inorganic matter and can no longer kill pathogens and disinfect. The remaining available chlorine is the free chlorine residual that can effectively react to any pathogens. Free chlorine residual consists of two main compounds: hypochlorous acid (HOCl) and hypochlorite ion (OCl-). Hypochlorous acid is much more effective (80 to 200 times better) at killing pathogens than the hypochlorite ion.

Examples of common materials or properties that reduce the free chlorine concentration are: ● Alkalinity ● Hydrogen sulphide (H_2S) ● Methane (CH_4) ● Iron ● Manganese ● Biofilm (iron oxidizing bacteria and sulphate-reducing bacteria) ● Silt ● Clay Therefore, additional cleaning or additional chlorine solution may be needed to meet the required free chlorine residual concentration range.

Best Management Practice – Adjusting the pH of the Chlorine Solution It is important to control the pH to maximize the amount of hypochlorous acid available to kill pathogens. There are several commercial acid products on the market that can lower the pH of water that will be used to make the chlorine solution. Any of the acid products used in the process must not impair the quality of the water in the well or the aquifer and should meet NSF International Standards for potable water or an equivalent standard. Carefully follow the manufacturer's instructions when adding any acids to the water.

Importance of Air Conditioning in Operation Theatres in Healthcare Organizations

As per NABH guidelines, operation theatres have been divided into 2 groups:

1. **Type A (Erstwhile Super speciality OT):** Type A OT means operation threatres for Neurosciences, Orthopedics (Joint Replacement), Cardiothoracic and Transplant Surgery (Renal, Liver, Heart etc.)
2. **Type B (Erstwhile General OT):** Type B OT means operation threatres for Opthalmology, Daycare surgeries, and all other basic surgical disciplines

Requirements – Type A (Erstwhile Super speciality OT)

1. **Air Changes Per Hour:**
- Minimum total air changes should be 20 based on biological load and the location.
- The fresh air component of the air change is required to be minimum 4 air changes out of total minimum 20 air changes
- If Healthcare Organization (HCO) chooses to have 100% fresh air system then appropriate energy saving devices like heat recovery wheel, run around pipes etc. should be installed.

2. **Air Velocity:** The airflow needs to be unidirectional and downwards on the OT table. The air face velocity of 25-35 FPM (feet per minute) from non-aspirating unidirectional laminar flow diffuser/ceiling array is recommended.

3. **Positive Pressure**: The minimum positive pressure recommended is 2.5 Pascal (0.01 inch of water). There is a requirement to maintain positive pressure differential between OT and adjoining areas to prevent outside air entry into OT. Positive pressure will be maintained in OT at all times (operational & non-operational hours).

4. **Air Handling in the OT including air quality**: Air is supplied through terminal HEPA (High Efficiency Particulate Air) filters in the ceiling. The HEPA can be at AHU level if it not feasible at terminal level inside OT. The minimum size of the filtration area should extend one foot on all sides of the OT table.

5. **Air Filtration**: The AHU (air handling unit) must be an air purification unit and air filtration unit. There must be two sets of washable flange type filters of efficiency 90% down to **10 microns** and 99% down to **5 microns** with aluminium / SS304 frame within the AHU. The necessary service panels to be provided for servicing the filters, motors & blowers. HEPA filters of efficiency 99.97% down to **0.3 microns** or high efficiency are to be provided. Air quality at the supply i.e. at grille level should be Class 100/ISO Class 5 (at rest condition). Note: Class 100 means a cubic foot of air should not have more than 0.5 microns or larger.

6. **Temperature & Relative Humidity**: It should be maintained $21^0C \pm 3^0C$ (except for joints replacement where it should be $18^0C \pm 2^0C$) with corresponding relative humidity between 20 to 60%, though the ideal RH is considered to be 55%. Appropriate devices to monitor and display these conditions inside the OT may be installed.

REQUIREMENTS – Type B (Erstwhile General OT)

1. **Air Changes per Hour**: Same as Type A OT requirements above
2. **Air Velocity**: Same as Type A OT requirements above
3. **Positive Pressure**: Same as Type A OT requirements above

4. **Air Filtration**: The AHU (i.e. air handling unit) must be air purification unit and air filtration unit. There must be two sets of washable flange type filters of efficiency 90% down to **10 microns** and 99% down to **5 microns** with aluminium/SS304 frame within the AHU. The necessary service panels to be provided for servicing the filters, motors & blowers. HEPA filters of efficiency 99.97% down to **0.3 microns** or higher efficiency may be provided. The air quality at the supply i.e. at grille level should be Class 1000/ISO Class 6 (at rest condition). Note: Class 1000 means a cubic foot of air must have no more than 1000 particles measuring 0.5 microns or higher.

5. **Temperature & Humidity**: The temperature should be maintained at $21^0C \pm 3^0C$ inside the OT at all times with corresponding relative humidity between 20 to 60%. Appropriate devices to monitor and display these conditions inside the OT may be installed.

Design considerations for Operation Theatres

A. The AHU of each OT should be dedicated one and should note be linked to air conditioning of any other area in the OT and surroundings.
✓   One AHU for multiple OTs is permitted provided there is a back-up/contingency plan to accommodate surgeries in other OTs in the eventuality of failure of infection control in these OTs. Redundancy in terms of multiple fans for return and input air with UPS and DG set supply is provided to such type of common AHU. Direct drive fans will be required in such common AHU. The specific evidence of validation for the above will have to be provided either by the vendor/third party.
B. Outdoor Air Intakes: The location of outdoor air intake for an AHU must not be located near potential contaminated sources like DG exhaust hoods, lab exhaust vents, and vehicle parking area.
C. Window & split A/c **should not** be used in any type of OT because they are pure recirculating units and have pockets for microbial growth, which cannot be sealed.
D. For old constructions and for retrofitting (constructed/renovated prior to 2015)
1. Where space is a constraint, ceiling suspended AHU is permitted provided there is accessibility for maintenance of filters and other parts of AHU.
2. Dx unit with AHU is recommended for OTs where retrofitting solution is possible. It is also recommended as cost effective solution for OTs in **SHCO/Eye care** hospitals.
3. All requirements spelt out for new constructions and Type A and Type B OTs above in terms of air changes, particle count, positive pressure, temperature, humidity and air velocity will have to be met by such OTs in old constructions/HCOs.
E. During the non-functional hours AHU blower will be operational round the clock (may be without temperature control). Variable frequency devices (VFD) may be used to conserve energy. Air changes can be reduced to **25%** during non-operating hours thru VFD provided positive pressure relationship is not disturbed during such period.

Maintenance of the system

Validation of system should be done every 6 months and as per ISO

14644 standards. This should include:
- ✓ Temperature and humidity
- ✓ Air particulate count
- ✓ Air change rate calculation
- ✓ Air velocity at outlet of terminal filtration unit/filters
- ✓ Pressure differential levels of the OT with respect to ambient/adjoining areas
- ✓ Validation of HEPA filters by appropriate tests

Preventive maintenance of the system: It is recommended that periodic preventive maintenance be carried out in terms of cleaning pre filters, micro vee filters at the interval of 30 days. Preventive maintenance of all the parts of AHU is carried out as per manufacturer recommendations.

References

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MICROBIOLOGY

A Handbook of
BEST PRACTICES

Gram Staining

Bacteria are too small and too transparent to be well-described using light microscopy and a wet mount. To make them more visible by imparting contrast, they are stained. Most cellular staining that takes place falls into three categories:

(a) Simple staining : a single stain is used to make them visible under the light microscope.

(b) Differential staining : a differential staining uses more than one dye and stains different kinds of organisms different colors. It is employed to differentiate different group of bacteria. Eg- Gram staining.

(c) Special staining- these are procedures can be used to identify capsules, endospores, flagella, and essentially any molecule made by a microbe using dyes linked to antibodies.

Historical aspects

Gram staining is an empirical method differentiating bacterial species into two large groups (Gram positive and Gram negative) based on the chemical and physical properties of their cell walls. The method is named after its inventor, the Danish scientist Hans Christian Gram (18531938), who developed the technique in 1884 to differentiate between pneumococci and *Klebsiella pneumoniae* bacteria. In the original method of Gram the smear was stained with aniline-gentian violet, treated with Lugol's iodine, decolourized with absolute alcohol and counterstained with Bismarck brown. Later it was modified by Hucker in 1921 and modifications give better result.

Principle

Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50-90% of cell wall), which stain purple and Gram-negative bacteria have a thinner layer (10% of cell wall), which stain pink. Gram-negative bacteria also have an additional outer membrane which contains lipids, and is separated from the cell wall by the periplasmic space. There are four basic steps of the Gram stain, which include applying a primary stain (crystal violet) to a heat-fixed smear of a bacterial culture, followed by the addition of a mordant (Gram's iodine), rapid decolorization with alcohol or acetone, and counterstaining with safranin or basic fuchsin. Crystal violet (CV) dissociates in aqueous solutions into CV^+ and chloride (Cl) ions. These ions penetrate through the cell wall and cell membrane of both Gram-positive and Gram-negative cells. The CV^+ ion interacts with negatively charged components of bacterial cells and stains the cells purple. Iodine (I or I_3) interacts with CV^+ and forms large complexes of crystal violet and iodine (CVI) within the inner and outer layers of the cell. When a decolorizer such as alcohol or acetone is added, it interacts with the lipids of the cell membrane. A Gram-negative cell will lose its outer membrane and the peptidoglycan layer is left exposed. The CVI complexes are washed from the Gram-negative cell along with the outer membrane. In contrast, a Gram-positive cell becomes dehydrated from an ethanol treatment. The large CVI complexes become trapped within the Gram-positive cell due to the multilayered nature of its peptidoglycan. The decolorization step is critical and must be timed correctly; the crystal violet stain will be removed from both Gram-positive and negative cells if the decolorizing agent is left on too long (a matter of seconds).After decolorization, the Gram-positive cell remains purple and the Gram-negative cell loses its purple color. Counterstain, which is usually positively-charged safranin or basic fuchsin, is applied last to give decolorized Gram-negative bacteria a pink or red color.

Gram Staining Protocol

Preparation of smear

Before performing gram staining preparation of smear is important. Smear preparation involves the following steps :

- A drop of the suspended culture is transferred to examine on a slide with an inoculation loop. If the culture is to be taken from a Petri dish or a slant culture tube, a drop of water is added on the slide and aseptically transferred a minute amount of a colony from the petri dish. To be visible on a slide, organisms that stain by the Gram method must be present in concentrations of a minimum of 10^4 to 10^5 organisms/ml of unconcentrated staining fluid. At lower concentrations, the Gram stain of a clinical specimen seldom reveals organisms even if the culture is positive.

- The culture is spread with an inoculation loop to an even thin film over a circle of 1.5 cm in diameter, approximately the size of a dime.

- Then it is dried in air and fixed it over a gentle flame, while moving the slide in a circular fashion to avoid localized overheating. The applied heat helps the cell adhesion on the glass slide to make possible the subsequent rinsing of the smear with water without a significant loss of the culture. Heat can also be applied to facilitate drying the smear. However, ring patterns can form if heating is not uniform.

Gram staining
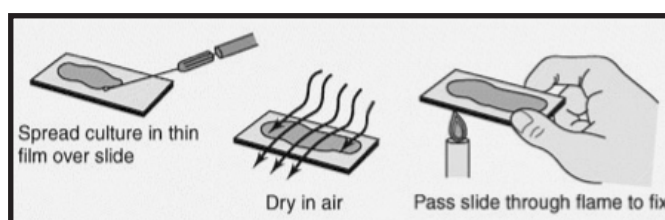
After preparing the smear following steps should be performed for gram staining:

- Crystal violet stain is added over the fixed culture and waited upto 60 seconds. The stain is poured off and gently rinsed the excess stain with a stream of water.

- Gram's iodine solution was added on the smear waited 30- 60 seconds. The iodine solution is poured off and the slide is rinsed with running water. Excess water is shaked off from the surface.

- The slide is decolourized with 95% of ethanol. The exact time to stop is when the solvent is no longer colored as it flows over the slide. Further delay will cause excess decolourization in the gram-positive cells, and the purpose of staining will be defeated.

- Then it is counterstained with safranin (or basic fuchsin) for 40-60 seconds. The solution is washed off with water. Then the slide is blotted dry to remove the excess water.

Interpretation of Gram staining results and precautions

The slides should be observed under microscope to examine the Gram staining results. Organisms that retain the violet-iodine complexes after washing in ethanol stain purple and are termed Gram-positive, those that lose this complex stain red from the safranin counter stain are termed Gram negative. The Gram stain will not detect organisms, which exist within host cells (e.g., *Chlamydia* spp), organisms with no cell wall (e.g.,*Mycoplasma* spp and

Ureaplasma spp), and organisms too small to be seen with light microscopy (e.g., spirochetes). Mycobacteria usually will not stain, and *Legionella* spp stain only when taken directly from culture. Gram-negative bacteria that stain poorly with safranin include *Campylobacter* spp, *Legionella* spp, *Bacteroides* spp, *Fusobacterium* spp, and *Brucella* spp. Certain conditions are known to damage the cell wall, causing gram-positive bacteria to falsely appear gram-negative or gram-variable. These include antibiotic treatment, cultures more than 48 hours old, inflammatory responses in the host, and autolytic enzymes (e.g., *S. pneumoniae*). To minimize ambiguous results, specimens should be collected before the patient begins antibiotic therapy. Also, Gram stains should be performed on colonies taken from culture media that do not contain antibiotics, preferably on colonies that are 18-24 hours old. Finally, correct interpretation of Gram stains requires a theoretical background of bacteria and their morphology, because improper technique or sub optimal reagents can cause unreliable results. Errors in technique that can alter Gram stain results include the following:

- Fixation with excessive heat alters cell morphology and makes organisms more susceptible to over-decolourization.
- Low concentrations of crystal violet make gram-positive organisms more susceptible to over-decolorization.
- Insufficient exposure to iodine and lack of available iodine can prevent crystal violet from bonding firmly with the cell wall, thus making gram-positive organisms more susceptible to over-decolorization. To ensure reliable Gram stain results, only fresh iodine should be used.
- Prolonged decolourization, especially with acetone, can cause gram-positive bacteria to appear gram-negative. Insufficient decolourization can make gram-negative organisms falsely appear gram positive.
- Insufficient counterstaining can fail to stain gram-negative bacteria and background material, whereas excessive counterstaining will leach the crystal violet-iodine complex from gram-positive bacteria and stain them with safranin, thus making them falsely appear gram-negative.
- Prolonged washing between any of the steps can cause over-decolourization.
- Precleaned or degreased glass slides should be used for Gram staining. Storing slides in a jar with 95% ethanol will ensure clean slides.
- The amount of the mordant available is important to the formation of the crystal violet - iodine complex. The lower the concentration, the easier to decolorize (0.33% - 1% commonly used). QC of the reagent is important as exposure to air and elevated temperatures hasten the loss of Gram's iodine from solution.
- As the counter stain is also a basic dye, it is possible to replace the crystal violet- iodine complex in gram- positive cells with an over-exposure to the counter stain. The counter stain should not be left on the slide for more than 30 seconds.

Analytical considerations

- Appearance of the reagents should be checked daily. If crystal violet has precipitated or formed crystal sediment, it should be refiltered before use even when purchased commercially. Some stains, especially basic fuchsin and safranin, can become contaminated. When suspected, either culture or start with fresh material in a clean bottle.
- Evaporation may alter reagent effectiveness; working solutions should be changed regularly if not depleted with normal use.
- Daily when a new lot is used, it should be checked by staining with the *Escherichia coli* (ATCC 25922) or *Staphylococcus aureus* (ATCC 25923). *E. coli* should be appeared as pink gram negative bacilli where as *S. aureus* as purple cocci.

Applications of Gram Staining

Gram staining is a common procedure in the traditional bacteriological laboratory. The technique is used as a tool for the differentiation of Gram-positive and Gram-negative bacteria, as a first step to determine the identity of a particular bacterial sample. Gram stains are performed on body fluid or biopsy when infection is suspected. It yields results much more quickly than culture, and is especially important when infection would make an important difference in the patient's treatment and prognosis; examples are cerebrospinal fluid for meningitis and synovial fluid for septic arthritis.

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Pure Culture Technique

In microbiology, laboratory culture containing a single species of organism is known as pure culture. A pure culture is usually derived from a mixed culture (containing many species) by methods that separate the individual cells so that, when they multiply, each will form an individually distinct colony, which may then be used to establish new cultures with the assurance that only one type of organism will be present. Pure cultures may be more easily isolated if the growth medium of the original mixed culture favours the growth of one organism to the exclusion of others. Microbiologists have developed special techniques and equipment to isolate and grow pure cultures of microorganisms that are free from contaminating forms.

Brief History
A lot of early work on Pure culture technique was done by Brefeld. He introduced the practice of Single cell isolation and carried out a lot of work on Pure culture technique. The earliest method for pure culture isolation of bacteria however was that put forth by Joseph Lister which relied on the use of serial dilutions.

Significance of Pure Culture
Microorganisms usually exist in mixed populations in soil, water and some parts of the human body. It is not feasible to identify or study the characteristics of a particular species and therefore a pure culture must be obtained in preparation of further work. As the name implies pure culture contains only a single species of microorganisms. Pure culture of an organism is also helpful in designing a functional habitat for it.

Different Methods of Pure Culture Technique
Various types of techniques have been designed to achieve this goal. A variety of techniques have been developed whereby isolation into pure culture can be accomplished. Each technique has certain advantages and limitations, and there is no single method that can be used for all bacteria.

Streak Plate Technique
The quadrant streak plate technique is a relatively inexpensive and rapid method for separating bacteria in a mixed population of high cell density. It requires only a single plate of growth medium and it yields excellent distribution of bacterial colonies.

Procedure of quadrant streak plate method is described as follows:
a) A nutrient agar plate is selected for the isolation of pure strain from a mixed culture. The plate is properly labeled before streaking.
b) Aseptically obtain a loopful of the mixed population and lightly streak it several times along one area (quadrant) of the plate. Try not to cut into the agar surface and avoid airborne contamination by lifting the lid of the plate only enough to permit entry of the loop.
c) Replace the lid. Sterilize the loop to destroy any remaining bacteria. To ensure that it is cool, touch the loop to the center of the plate or between the agar and edge of the plate. Pass the loop once across the previous streaks to pick up some bacteria and continue streaking into a second area of the plate. Replace the lid.
d) Sterilize the loop as before, and then cool it. To pick some bacteria pass the loop one time through the second area and continue streaking into third area. And the same procedure should be followed for the fourth area.
e) Invert the streaked (inoculated) plates and incubate them for 24-

48 hours at appropriate temperature. The plates are inverted so that moisture accumulates on the lid rather than on the agar surface, where it may cause colonies to run together. Examine the plates for the well isolated and separated colonies.

The basis behind this technique is to achieve gradual dilution of the biomass so that at some places well-separated single colonies are obtained. Being unicellular each colony is derived by the clonal multiplication of one cell, and being haploid each one of them will be genetically pure.
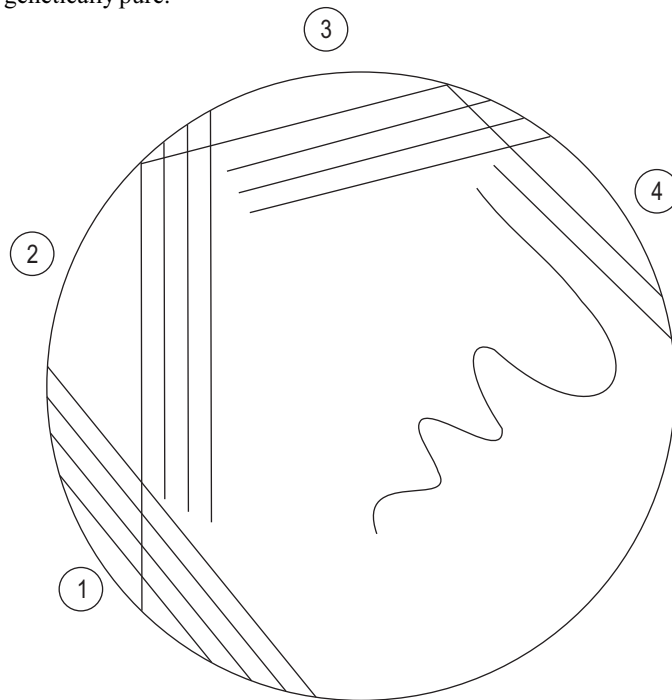
Pour Plate Technique
Pour plate technique was originally developed by Koch, in which the cells were mixed with molten and cooled gelatin (now replaced with agar) then immobilizes cells so that each one of them could produce an isolated colony. The technique has been slightly modified in which a diluted sample is used to ensure the development of isolated colonies.
a) In pour plate method the mixed culture is diluted directly in tubes of liquid agar medium. The medium is maintained in a liquid state at a temperature of 45°C to allow thorough distribution of the inoculum.
b) The inoculated medium is dispensed into Petri dishes, allowed to solidify, inverted and incubated.
c) A series of agar plates give decreasing numbers of colonies resulting from the dilution procedure in the pour plate technique.

The pour plate technique has certain disadvantages. For instance, some of the organisms are trapped beneath the surface of the medium when it gels and therefore both surface and subsurface colonies develop. The subsurface colonies can be transferred to fresh media only by first digging them out of the agar with a sterile instrument.

Another disadvantage is that the organisms being isolated must be able to withstand temporary exposure to the 45°C temperature of the

liquid agar medium. For instance pour plate method would be unsuitable for isolating psychrophilic bacteria.

Spread Plate Technique

The spread plate technique is applied when the microbe to be counted grows best on the surface of the culture medium.

a) In this method the mixed culture is not diluted in the culture medium; instead it is diluted in a series of tubes containing a sterile liquid, usually water or physiological saline.

b) A small sample is then removed from each dilution tube and is placed on the surface of an appropriate solid growth medium. One Petri dish is used for each sample.

c) The inoculum on each plate is then spread as a lawn over the entire surface of the plate using a sterile spreader. The inoculum is spread uniformly by holding the stick at a set angle on the agar and rotating the agar plate or rotating the stick until the inoculum is distributed. Often a sterile bent glass rod is used for this purpose.

d) These inoculated plates are then incubated to allow colonies to grow.

This technique is also used in quantitative enumeration of bacteria. In this, the numbers of colonies are counted from a plate where the colonies are well dispersed. When the same is multiplied with the degree of dilution, total cell count of the original sample can be determined. This technique is also useful in estimating the viable cell counts as colony forming units (CFUs).

When the individual colonies are isolated on a culture medium, the individual colony of interest can further be confirmed as 'pure' by repeatedly streaking a single isolated colony on a fresh medium preferably using a selective medium and observing the growth and other characteristics of the desired organism.

Special Methods for Isolation of Pure Culture

Other than traditional methods, there are some special techniques for isolation of pure culture. Following two methods are in use for isolation of pure culture:

Capillary Pipette Method

Several small drops of a suitably diluted culture medium are put on a sterile glass-coverslip by a sterile pipette drawn to a capillary. One then examines each drop under the microscope until one finds such a drop, which contains only one microorganism. This drop is removed with a sterile capillary pipette to fresh medium. The individual microorganism present in the drop starts multiplying to yield a pure culture.

Micromanipulator Method

Micromanipulators have been built, which permit one to pick out a single cell from a mixed culture. This instrument is used in conjunction with a microscope to pick a single cell (particularly bacterial cell) from a hanging drop preparation. The micro-manipulator has micrometer adjustments by means of which its micropipette can be moved right and left, forward, and backward, and up and down. A series of hanging drops of a diluted culture are placed on a special sterile coverslip by a micropipette.

Now a hanging drop is searched, which contains only a single microorganism cell. This cell is drawn into the micropipette or microprobe by gentle suction and then transferred to a large drop of sterile medium on another sterile coverslip. When the number of cells increases in that drop as a result of multiplication, the drop is transferred to a culture tube having suitable medium. This yields a pure culture of the required microorganism.

The advantages of this method are that one can be reasonably sure that the cultures come from a single cell and one can obtain strains with in the species. The disadvantages are that the equipment is expensive, its manipulation is very tedious, and it requires a skilled operator. This is the reason why this method is reserved for use in highly specialized studies.

Like many aspects of science though Pure Culture Technique has its own limitations for instance the presence of Mycoplasmas and viruses cannot be detected. However the advantages attributed to the technique of Pure culture have outweighed the disadvantages.

Virtually all areas of research on pharmaceuticals, nutrition, ecology, agricultural production and parasitology bear witness to the usefulness of pure culture technique.

Culture Media Preparation and Its Quality Control

A growth medium or culture medium is a liquid or gel designed to support the growth of microorganisms and cells. Microorganisms need nutrients, a source of energy and certain environmental conditions in order to grow and reproduce. In the environment, microbes have adapted to the habitats most suitable for their needs, in the laboratory; however, these requirements must be met by a culture medium.

Culture media are the basis for most microbiological tests. So the quality of the culture media is a critical factor for the success of microbiology laboratory. Media preparation, proper storage and quality control testing can assure a consistent supply of high quality media. As per USP chapter <1117> good laboratory practices in a microbiology laboratory also contains the activities of media preparation and quality control.

Media Preparation

Culture media may originate from following three sources: laboratory prepared from raw materials, commercially manufactured presented either in a dehydrated or ready to use form. The first one is now rarely been used in pharmaceutical industry and microbiological laboratory. Dehydrated culture media and ready to use media is widely used for quality assurance in industrial as well as clinical segment. It is important to choose the correct media or components in making media based on the use of accepted sources or references for formulas. Preparation and evaluation of culture media is a continuous process extending from the raw material, through manufacture to the final product use on the bench.

Storage of incoming raw materials

All incoming raw materials should be purchased from an approved supplier and dated on receipt. Particular care should be given to the sourcing of media used in the production of pharmaceutical materials with respect to the bovine spongiform encephalopathy (BSE) status of herds providing beef extracts. Raw materials should be stored as advised by the manufacturer; humid environment should be avoided.

Water

Water quality can markedly influence the performance of culture media. Purified water is most often used for media preparation, but in certain cases the use of deionized or distilled water is appropriate. Tap water is unsuitable for the preparation of culture media. Water quality should be regularly monitored.

Preparation of Culture Media

Good laboratory practice should be maintained for the preparation of culture media. Media should be prepared according to master formulae and written procedures. Documentation should be completed for each batch of prepared media; a batch number and expiry date is always allocated. Manufacturer's instructions should be closely followed.

Consistent preparation of media requires accurate weighing of dehydrated media or media constituents. A calibrated balance with the appropriate weight range for the ingredients should be used. When dissolving powders in water, the aqueous phase (1/3 volume) should be added first, followed slowly by the powder, dispersed by gentle swirling and finally the remaining liquid can be used to wash down any adhering powder. Balances should be cleaned thoroughly after use; they also require weekly calibration and regular maintenance. Equipment used for dispensing culture should be cleaned properly to prevent the contamination of foreign substances.

Measurement of pH

The properties of a given culture medium are pH dependent. Incorrect pH may result not only in physical changes such as precipitation of components or soft gelling of agar, but also significant chemical changes. Incorrect pH may also affect the recovery of stressed cells and influence cell growth. Since the pH is temperature dependent, measurements are best taken at a standardized temperature i.e. room temperature (25ºC). The pH of media should be in a range of ± 0.2 of the value indicated by the manufacturer. A flat pH probe is recommended for agar surfaces and an immersion probe is recommended for liquids. pH meters should be calibrated weekly by using standardized buffer solutions. During autoclaving the solution that has been adjusted to be a little on the alkaline side of neutrality tend to fall by about 0.1 unit.

Cleaning of glass apparatus

Preparation of media in poorly cleaned glassware can allow inhibitory substances to enter the media. Inhibitory substances can come from detergent residue after cleaning glassware. Cleaning process should remove debris and foreign matter.

Sterilization

The sterilization of culture media is a critical control point in assuring their quality. The media must be sufficiently processed to ensure sterility, but any over processing may affect their nutritive properties and result in the accumulation of toxic substances. Thus, in pharmaceutical production, individual sterilization cycles should be properly validated with thermocouples to ensure that all containers in the load achieve the required temperature.

Commercially prepared media should provide documentation of the sterilization method used. The manufacturer should provide the sterility assurance level (SAL) of the media against a recognized biological indicator. For each batch processed the accompanying temperature time chart recording should indicate the correct conditions in the load have been achieved. Manufacturers recommend an autoclave cycle of 121ºC for 15 minutes using a validated autoclave. The sterilization time will be dependent on the media volume and autoclave load. Storage of the media in the autoclave after the liquid cycle is completed is not recommended after cooling, as it may damage the media. Improper heating or sterilizing conditions for commercially prepared or internally prepared media may result in a difference in colour change, loss of clarity, altered gel strength or pH drift from the manufacturer's recommended range. Membrane filtration is used to sterilize heat sensitive media and components.

Checking of prepared media

Prepared media should be checked by appropriate inspection of plates and tubes for:
- Cracked containers or lids
- Unequal filling of containers
- Dehydration resulting in cracks or dimpled surfaces on solid medium
- Hemolysis
- Excessive darkening or colour change
- Crystal formation from possible freezing
- Excessive number of bubbles

- Microbial contamination
- Status of redox indicators
- Lot number and expiry date checked and recorded
- Sterility of the media

Storage of Media

Media should be stored according to manufacturer's instructions. All media should be used within their given shelf lives. The shelf life of media is determined by a number of factors: the type of media and the container as well as the storage conditions. Agar should not be stored at or below 0ºC, as freezing could damage the gel structure. Agar plates begin to deteriorate from the moment of preparation; correct packaging with low temperature storage slows the rate of deterioration. Before prolonged storage, agar plates should be placed into a sealed package or to container to retard the moisture loss. Plates showing shrinkage or wrinkling of agar should be discarded and wet plates should be dried until visible moisture has disappeared. Using boiling water bath or free steaming in an autoclave should do remelting of solid media. The molten agar medium should be kept in a monitored water bath at a temperature of 45-50ºC for not more than 8 hours. Media should not be re-melted by the direct application of heat.

Quality Control of Culture Media

Quality control in the preparation and evaluation of culture media in the laboratory is essential. The aim of quality control is to ensure that media conform to predetermined standards whereas evaluation implies the determination of their efficacy under the conditions of intended usage. The performance of media prepared in a laboratory or by a manufacturer is highly dependent on preparation. Improper media preparation can cause unsatisfactory conditions for microbial growth or recovery.

Quality control test should be carried out on each batch of culture media, involving a range of physical, chemical and microbiological tests. Physical test should confirm that the powder meets its specification in terms of colour, odour, particle size, homogeneity, flow characteristics and moisture content. Chemical test would include clarity, solubility and pH of the product. Media containing agar must meet the required specification for gel strength.

Microbiological tests are done to check the recovery of the particular organism in that reference media. Expiration dates on media should have supporting growth promotion testing to indicate that the performance of the media still meets acceptance criteria up to and including the expiration date. The length of shelf life of a batch of media will depend on the stability of the ingredients and formulation under specified conditions as well as the type of the container and closure. When a batch of media does not meet the requirements of growth promotion testing, an investigation should be initiated to identify the cause. This investigation should include a corrective action plan to prevent the recurrence of the problem. Special should be taken with media, used in environmental monitoring.

Factors Influence the Quality of Dehydrated Culture Media

Major sources of trouble include errors in weighing and measuring, use of wrong ingredients, incorrect pH adjustment, and presence of inhibiting substances in glasswares. Some of the common faults and their possible cause listed bellow:

- Loss of growth promotion capacity - Over sterilization, Incomplete mixing, Contamination with metallic salts, Incorrect molarity due to careless pH adjustment.
- Darkening of the medium or colour change - Over sterilization, Drift in pH.
- Clumping of Media - Storage of media in humid condition, Improper sealing of container.
- Decreased gel strength - Incorrect proportions of product to volume of water, Incomplete mixing, Repeated remelting.
- Change in pH - Hydrolysis of ingredients, Over sterilization, Impure water or glassware used, Incomplete mixing.
- Precipitation - Chemical incompatibility, Over sterilization, Prolonged holding of melted agar media at high temperature.

Before using media must be at correct temperature. Performance of the media is checked by standard ecometric method. Records should be maintained of all media received and used in the laboratory. Finally, disposal of used culture media should follow local biological hazard safety procedure.

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Best Practices in Blood Culturing

Blood culture specimens are among the most critical specimens processed by the microbiology laboratory. Positive blood cultures reveal the identity of the pathogenic microorganism and help guide therapeutic choices with subsequent antimicrobial susceptibility testing. This has been shown to decrease morbidity and mortality. However, if blood cultures are collected under sub-optimal conditions, positivity rates will be low and patient care will suffer.

When is the best time to draw blood cultures from a patient with suspected sepsis?
Draw blood cultures as close as possible to the episode of chills or fever. Do NOT delay, as recovery of microorganisms diminishes with time after the fever spike.

How many blood culture sets do I need to draw?
In the majority of circumstances, drawing 2 – 3 blood culture sets will detect ~99% of septic episodes. NEVER draw only one blood culture set during the initial evaluation of a septic patient.
In the evaluation of most patients with suspected sepsis, blood culture sets should be obtained within 5 minutes of each other, since the reticuloendothelial system will clear both transient and intermittent bacteremias within 15 – 30 minutes.
In cases of suspected sub acute infective endocarditis, urgent institution of empiric antimicrobial therapy is *usually* not required. It is far more important to establish the identity of the causative agent. Therefore, 3 sets of blood cultures should be drawn one hour apart, which helps document an endovascular source of infection. If those cultures are negative at 24 hours, two additional sets should be obtained. If urgent empiric antimicrobial therapy will be given, then obtain 3 blood culture sets, as above; i.e. within 5 minutes of each other.

How much blood should I draw from the patient?
The volume of blood is the single most critical factor in optimizing the sensitivity of blood culture. The graph below illustrates this point.

Effect of Blood Volume*
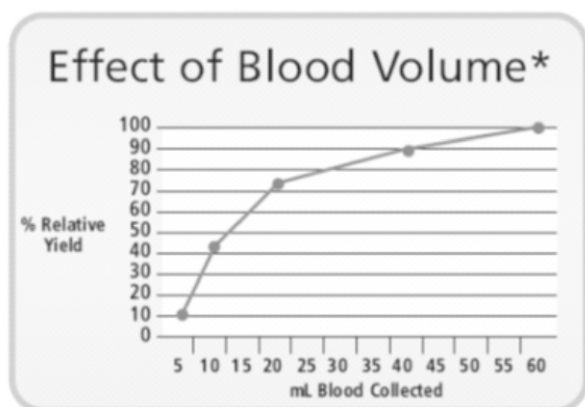
For most 2 bottle sets, at least 10 mL, and preferably 20 mL of blood should be obtained and divided between the 2 bottles of the set. The 2 sets (4 bottles) should therefore have between 20 – 40 mL total blood inoculated.

Recommendations:

A: Take blood for culture when there is a clinical need to do so and not as routine
Blood cultures are taken to identify patients with bacteraemia. There are many signs and symptoms in a patient which may suggest bacteraemia and clinical judgement is required, but the following indicators (which may be subtle in the very young, the elderly, those on steroids or immuno compromised) should be taken into account when assessing a patient for signs of bacteraemia or sepsis:
- Pyrexia > 38ºC
- focal signs of infection
- abnormal heart rate (raised), blood pressure (low or raised) or respiratory rate (raised)
- chills or rigors
- raised or very low white blood cell count
- new or worsening confusion.

Please note: signs of sepsis may be minimal or absent in the very young and the elderly.

Blood cultures should be taken after identification of possible bacteraemia or sepsis and before the administration of antibiotics. If a patient is on antibiotics, blood cultures should ideally be taken immediately before the next dose, with the exception of paediatric patients.
All blood cultures should be documented in the patient's notes, including date, time, site and indications.

B. Competence
Blood cultures should only be collected by members of staff (medical, nursing, healthcare assistant, phlebotomist or technician) who have been trained in the collection procedure and whose competence in blood culture collection has been assessed and maintained.

C. Always make a fresh stab
In patients with suspected bacteraemia, **do not** use existing peripheral lines/cannulae or sites immediately above peripheral lines. If a central line is present, blood **may** be taken from this **and** from a separate peripheral site when investigating potential infection related to the central line; the peripheral vein sample should be collected first. Identify a suitable venepuncture site before disinfecting the skin. Avoid femoral vein puncture because of the difficulty in adequate skin cleansing and disinfection.

D. Thoroughly disinfect the skin before inserting the needle
Thoroughly cleanse the patient's skin before venepuncture. Use soap and water to clean visibly soiled skin and then clean your own hands using the correct hand hygiene technique (use of the World Health Organisation's '5 moments of hand hygiene' or the National Patient Safety Agency (NPSA) 'Clean you hands campaign' is recommended). Use 2% chlorhexidine in 70% isopropyl alcohol to disinfect the patient's skin and allow to dry.

E. Once disinfected, do not touch the skin again

To avoid cross-contamination from the collector's fingers (even when gloved), it is vitally important not to palpate the site again once it has been disinfected.

F. Disinfect the culture bottle cap before transferring the sample

Ideally, remove the plastic cover immediately before collecting the sample; the top of the bottle will be clean but not sterile. Disinfect the tops of the culture bottles with a 2% chlorhexidine in 70% isopropyl alcohol impregnated swab. Allow the alcohol to fully evaporate for 30 seconds before proceeding with bottle inoculation.

Please note: the use of blood collection adapter caps without winged blood collection sets is not recommended. It is not possible to accurately judge sample volume and there is the potential for possible backflow of blood culture media into patient veins.

Procedure for Blood Culture Sampling:

1) Skin preparation
- Clean hands using correct hand hygiene technique (use of the World Health Organisation's '5 moments of hand hygiene' or the NPSA 'Clean you hands campaign' is recommended).
- Clean any visibly soiled skin on the patient with soap and water then dry.
- Apply a disposable tourniquet and palpate to identify vein.
- Clean skin with 2% chlorhexidine in 70% isopropyl alcohol and allow to dry for 30 seconds.
- Do not repalpate skin following cleaning.
- If a culture is being collected from a central venous catheter, disinfect the access port with a 2% chlorhexidine in 70% isopropyl alcohol impregnated swab.

2) Kit preparation
- Have sharps disposal container available in immediate vicinity.
- Clean the tops of culture bottles with a 2% chlorhexidine in 70% isopropyl alcohol impregnated swab and allow to dry for 30 seconds.

3) Sample collection – Use method A as outlined below. (Method B should only be used where method A is not available)

A: WINGED BLOOD COLLECTION SET METHOD
- Clean hands again using correct hand hygiene technique (use of the World Health Organisation's '5 moments of hand hygiene' or the NPSA 'Clean you hands campaign' is recommended) or use alcohol hand rub and apply clean examination gloves (sterile gloves are not necessary).
- Gloves and apron are worn (in line with local policy)
- Personal protective equipment (PPE) is disposed of correctly (in line with local policy) after use.
- Attach winged blood collection set to blood collection adapter cap.
- Insert needle into prepared site. Do not palpate again after cleaning.

- Place adapter cap over blood collection bottle and pierce septum.
- Hold bottle upright and use bottle graduation lines to accurately gauge sample volume and collect sample; inoculate aerobic culture first.
- If blood is being collected for other tests, always collect the blood culture first.
- Cover the site with an appropriate sterile dressing.
- Discard winged blood collection set in a sharps container.
- Clean hands using correct hand hygiene technique (use of the World Health Organisation's '5 moments of hand hygiene' or the NPSA 'Clean you hands campaign' is recommended) after removing gloves.
- Document date, reason for sample, site of venepuncture, operator undertaking procedure and if procedure was high risk with signature.

B: NEEDLE AND SYRINGE METHOD
- Clean hands again using correct hand hygiene technique (use of the World Health Organisation's '5 moments of hand hygiene' or the NPSA 'Clean you hands campaign' is recommended) or use alcohol hand rub and apply clean examination gloves (sterile gloves are not necessary).
- Gloves and apron are worn (in line with local policy).
- Personal protective equipment (PPE) is disposed of correctly (in line with local policy) after use.
- Insert needle. Do not palpate again after cleaning.
- Collect sample and release tourniquet.
- Cover the puncture site with an appropriate dressing.
- If blood is being collected for other tests, always inoculate the blood culture bottles first.
- Inoculate blood into culture bottles; do not change the needle between sample collection and inoculation; inoculate anaerobic culture first.
- Discard needle and syringe in a sharps container.
- Clean hands again using correct hand hygiene technique (use the World Health Organisation's '5 moment of hand hygiene' or the NPSA 'Clean Your Hands Campaign' are recommended).
- Document date, reason for sample, site of venepuncture, operator undertaking procedure and if procedure was high risk with signature.

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Microscopic Techniques

A 'Microscope' the term which literally stands for 'Micro' means small and 'scope' means to 'look', was coined by Giovanni Faber in the year 1625 . The microscope is an optical instrument used to view objects that are too small to be visualized by the naked eye. It was designed to magnify objects so that their structure, morphology and their complexity could be studied.

To know the techniques that are used in microscopes it is also important to know, the essential components that make up a microscope.
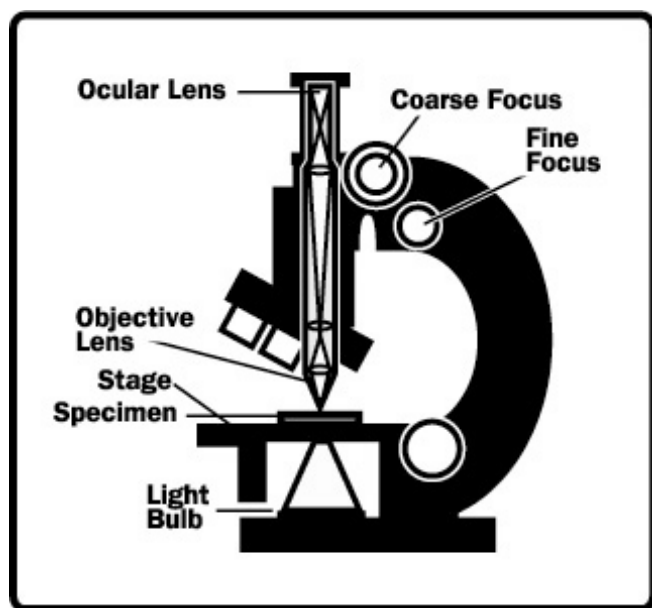
All optical microscopes share the same basic components :

The eyepiece:
A cylinder containing two or more lenses to bring the image to focus for the eye. The eyepiece is inserted into the top end of the body tube. Eyepieces are interchangeable and many different eyepieces can be inserted with different degrees of magnification. Typical magnification values for eyepieces include 5x, 10x and 2x. In some high performance microscopes, the configuration of the objective lenses and the eyepiece are matched to give the best possible optical performance.

The Objective lens:
A cylinder containing one or more lenses, typically made of glass, to collect light from the sample. At the lower end of the microscope tube one or more objective lenses are screwed into a circular nose piece which may be rotated to select the required objective. Typical magnification values of objective lenses are 4x, 5x, 10x, 20x, 40x, 50x and 100x. Some high performance objective lenses may require matched eyepieces to deliver the best optical performance.

The Stage:
A platform below the objective which supports the specimen being viewed. In the center of the stage is a hole through which light passes to illuminate the specimen. The stage usually has arms to hold the slide.

The illumination source:
Below the stage, light is provided and controlled in a variety of ways.

Usually daylight is directed via a mirror. Most microscopes, however, have their own controllable light source that is focused through an optical device called a condenser, with diaphragms and filters available to manage the quality and intensity of light.

Course and Fine adjustment:
The course adjustment helps to focus the image and see the specimen, whereas in order to actually visualize the object in a precise fashion , and to adjust the focus, to one's vision, the fine adjustment of the microscope is a requisite.

On a typical compound optical microscope, there are three objective lenses : a scanning lens (4x), low power lens (10x) and high power lens (ranging from 20x to 100x). Some microscopes have a fourth objective lens, called an oil immersion lens. To use this lens a drop of immersion oil is placed on top of the cover slip, and the lens is very carefully lowered until the front objective immerses in the oil film. Such immersion lenses are designed so that the refractive index of the oil and of the cover slip are closely matched so that the light is transmitted from the specimen to the outer face of the objective lens with minimal refraction. An oil immersion lens usually has a magnification of 50x to 100x.

Though the functioning of the microscope has changed drastically over the years, the general principle of the working of the microscope is the same.

The objective lens is a very high powered magnifying glass i.e a lens with a very short focal length. This is brought very close to the specimen being examined so that the light from the specimen comes to a focus about 160 mm inside the microscope tube. This creates an enlarged image of the subject. This image is inverted and can be seen by removing the eyepiece and placing a piece of tracing paper over the end of the tube. By carefully focusing a brightly lit specimen, a highly enlarged image can be seen. It is this real image that is viewed by the eyepiece lens that provides further enlargement.

In most microscopes the eyepiece is a compound lens, with one component less near the front and one near the back of the eyepiece tube. This forms an – air separated couplet, the virtual image comes to a focus and the second enabling the eye to focus on the virtual image.

A major problem in observing specimens under a microscope is that their images do not have contrast. This is especially true of living things, although natural pigments such as the green in the leaves, can provide good contrast. One way to improve contrast is to treat the specimen with colored pigments or dyes that bind to specific structures within the specimen. The specializations are mainly in the illumination systems and the type of light passed through the specimen.

Various techniques in light microscopy
Brightfield : this is the basic microscope configuration. This technique has very little contrast; much of which is provided by staining the specimen.

Darkfield : this microscope uses a special condenser to block out most of the bright light and illuminate the specimen with oblique light. This optical setup provides a totally dark background and

enhances the contrast of the image to bring out fine details.

Rheinberg illumination :
This set up is similar to darkfield, but uses a series of filters to produce an 'optical staining' of the specimen.

The following techniques use the same basic principle as Rheinfield illumination, achieving different results by using different optical components. The basic idea involves splitting the light beam into two pathways that illuminate the specimen.

Phase contrast :
This technique is best for looking at living specimens, such as cultured cells. In a phase contrast microscope, the annular rings in the objective lens and the condenser separate the light. The light that passes through the central part of the light path is recombined with the light that travels around the periphery of the specimen. The interference produced by these two paths produces images in which the dense structures appear darker than the background.

Differential interference contrast (DIC, or also called Nomarski) :
Uses polarizing filters and prisms to separate and recombine the light paths, giving a three dimensional appearance to the specimen.

Hoffman modulation contrast :
This technique is similar to DIC except that it uses plates with slits in both the axis and the off – axis of the light path to produce two sets of light waves passing through the specimen. In this case like DIC, a three dimensional image is produced.

Polarization :
The polarized light microscope uses two polarizers, one on either side of the specimen, positioned perpendicular to each other so that only light that passes through the specimen reaches the eyepiece. Light is polarized in one plane as it passes through the first filter and reaches the specimen. Regularly – spaced, patterned or crystallized portions of the specimen rotate the light that passes through. Some of this rotated light passes through the second polarizing filter, so these regularly spaced areas show up bright against a black background.

Fluorescence :
This type of microscope uses high energy, short wavelength light (usually ultraviolet) to excite electrons within certain molecules inside a specimen, causing those electrons to shift to higher orbits. When they fall back to their original energy levels, they emit lower energy, longer wavelength light (usually in visible spectrum), which forms the image.

Though microscopy and viewing a specimen using a microscope may seem very simple there are a lot of details that one has to bear in mind, in order to get the desired results.

Remember........
- Always clean the eyepiece, mirror, diaphragm and most importantly the lens of the microscope with a soft cloth or a paper napkin.
- In cases when the oil immersion is used, make sure that the oil is just enough to dip the lens and should not be in excess.
- Make sure that the slides used for microscopy are clean, grease – free, and have no scratches, the same however holds good for cover slips also.
- Be careful to place the cover slip in a manner that there are no air bubbles in between the slide and the cover slip, to obtain a clear view.
- Take care; to make the smear neither too thick, nor too thin.
- Tissue sections, must not be thick, because thick sections cause a hindrance to the proper viewing of the specimen.

Now try making a Simple Microscope
Requirements
1. 2 magnifying glasses
2. 1 sheet of printed paper
Method
1. Hold one magnifying glass a short distance above the paper. The image of the print will look a little bit large.
2. Place the second magnifying glass between your eye and the first magnifying glass.
3. Move the second magnifying glass up or down until the print comes into sharp focus. You will notice that the print appears larger than it does in the first magnifying glass.

References
www.science.howstuffworks.com.

Antimicrobial Susceptibility Testing

Introduction

Resistance to antimicrobial agents (AMR) has resulted in morbidity and mortality from treatment failures and increased health care costs. Although defining the precise public health risk and estimating the increase in costs is not a simple task, and is beyond doubt that emergent antibiotic resistance is a serious global problem.

The widespread use of antibiotics provide a selective pressure in the development of antibiotic resistance. The association between increased rates of antimicrobial use and resistance has been documented for nosocomial infections as well as for resistant community acquired infections. As resistance develops to "first-line" antibiotics, therapy with new, broader spectrum, more expensive antibiotics increases, but is followed by development of resistance to the new class of drugs as well. Resistance factors, particularly those carried on mobile elements, can spread rapidly within human and animal populations. Multidrug-resistant pathogens travel not only locally but also globally, with newly introduced pathogens spreading rapidly in susceptible hosts. Antibiotic resistance patterns may vary locally and regionally, so surveillance data needs to be collected from selected sentinel sources. Patterns can change rapidly and they need to be monitored closely because of their implications for public health and as an indicator of appropriate or inappropriate antibiotic usage by physicians in that area.

The results of in-vitro antibiotic susceptibility testing, guide clinicians in the appropriate selection of initial empiric regimens and, drugs used for individual patients in specific situations. The selection of an antibiotic panel for susceptibility testing is based on the commonly observed susceptibility patterns, and is revised periodically.

Principle

The principles of determining the effectivity of a noxious agent to a bacterium were well enumerated by Rideal ,Walker and others at the turn of the century, the discovery of antibiotics made these tests (or their modification) too cumbersome for the large numbers of tests necessary to be put up as a routine. The ditch plate method of agar diffusion used by Alexander Fleming was the forerunner of a variety of agar diffusion methods devised by workers in this field .The Oxford group used these methods initially to assay the antibiotic contained in blood by allowing the antibiotics to diffuse out of reservoirs in the medium in containers placed on the surface.

With the introduction of a variety of antimicrobials it became necessary to perform the antimicrobial susceptibility test as a routine. For this, the antimicrobial contained in a reservoir was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. Even now a variety of antimicrobial containing reservoirs are used but the antimicrobial impregnated absorbent paper disc is by far the commonest type used. The disc diffusion method of AST is the most practical method and is still the method of choice for the average laboratory. It is imperative that microbiologists understand the principles of the test well and keep updating the information as and when necessary. All techniques involve either diffusion of antimicrobial agent in agar or dilution of antibiotic in agar or broth. Even automated techniques are variations of the above methods.

Factors Influencing Antimicrobial Susceptibility Testing

i) pH
The pH of each batch of Mueller-Hinton agar should be checked when the medium is prepared. The agar medium should have a pH between 7.2 to 7.4 at room temperature after gelling. If the pH is too low, certain drugs will appear to lose potency (e.g., aminoglycosides, quinolones, and macrolides), while other agents may appear to have excessive activity (e.g., tetracyclines). If the pH is too high, the opposite effects can be expected. The pH can be checked by one of the following means:
* Macerate a sufficient amount of agar to submerge the tip of a pH electrode. * Allow a small amount of agar to solidify around the tip of a pH electrode in a beaker or cup. * Use a properly calibrated surface electrode.

ii) Moisture
If, just before use, excess surface moisture is present, the plates should be placed in an incubator (35°C) or a laminar flow hood at room temperature with lids ajar until excess surface moisture is lost by evaporation (usually 10 to 30 minutes). The surface should be moist, but no droplets of moisture should be apparent on the surface of the medium or on the petri dish covers when the plates are inoculated.

iii) Effects of Thymidine or Thymine
Media containing excessive amounts of thymidine or thymine can reverse the inhibitory effect of sulfonamides and trimethoprim, thus yielding smaller and less distinct zones, or even no zone at all, which may result in false-resistance reports. Mueller-Hinton agar that is as low in thymidine content as possible should be used. Satisfactory media will provide essentially clear, distinct zones of inhibition, 20 mm or greater in diameter. Unsatisfactory media will produce no zone of inhibition, growth within the zone, or a zone of less than 20 mm.

iv) Effects of Variation in Divalent Cations
Variation in divalent cations, principally magnesium and calcium, will affect results of aminoglycoside and tetracycline tests with *P. aeruginosa* strains. Excessive cation content will reduce zone sizes, whereas low cation content may result in unacceptably large zones of inhibition. Excess zinc ions may reduce zone sizes of carbapenems. Performance tests with each lot of Mueller-Hinton agar must conform to the control limits.

v) Testing strains that fail to grow satisfactorily
Only aerobic or facultative bacteria that grow well on unsupplemented Müeller-Hinton agar should be tested on that medium. Certain fastidious bacteria such as *Haemophilus* spp., do not grow sufficiently on unsupplemented Mueller-Hinton agar. These organisms require supplements or different media to grow, and they should be tested on the media prescribed for them.

Methods of Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing methods are divided into types based on the principle applied in each system. They include:

Diffusion	Dilution	Diffusion&Dilution
Stokes method	Minimum Inhibitory Concentration	E-Test method (AB BIODISK)
Kirby-Bauer method	i) Broth dilution ii)Agar Dilution	

In this article we will emphasize on disk diffusion method

Reagents for the Disk Diffusion Test

1. **Müeller-Hinton Agar Medium**

Of the many media available, Müeller-Hinton agar is considered to be the best for routine susceptibility testing of nonfastidious bacteria for the following reasons:

* It shows acceptable batch-to-batch reproducibility for susceptibility testing. * It is low in sulphonamide, trimethoprim, and tetracycline inhibitors. * It gives satisfactory growth of most nonfastidious pathogens. * A large body of data and experience has been collected concerning susceptibility tests performed with this medium.

2. **Preparation of antibiotic stock solutions**

Antibiotics may be received as powders or tablets. It is recommended to obtain pure antibiotics from commercial sources, and not use injectable solutions. Powders must be accurately weighed and dissolved in the appropriate diluents to yield the required concentration, using sterile glassware. Standard strains of stock cultures should be used to evaluate the antibiotic stock solution. If satisfactory, the stock can be aliquoted in 5 ml volumes and frozen at -20ºC or -60ºC.

Preparation of dried filter paper discs

Whatman filter paper no. 1 is used to prepare discs approximately 6 mm in diameter, which are placed in a Petri dish and sterilized in a hot air oven.

The loop used for delivering the antibiotics is made of 20 gauge wire and has a diameter of 2 mm. This delivers 0.005 ml of antibiotics to each disc.

Application of Discs to Inoculated Agar Plates

1. The predetermined battery of antimicrobial discs is dispensed onto the surface of the inoculated agar plate. Each disc must be pressed down to ensure complete contact with the agar surface. Whether the discs are placed individually or with a dispensing apparatus, they must be distributed evenly so that they are no closer than 24 mm from center to center. Ordinarily, no more than 12 discs should be placed on one 150 mm plate or more than 5 discs on a 100 mm plate. Because some of the drug diffuses almost instantaneously, a disc should not be relocated once it has come into contact with the agar surface. Instead, place a new disc in another location on the agar.

2. The plates are inverted and placed in an incubator set to at appropriate conditions.

Reading Plates and Interpreting Results

1. After appropriate incubation, each plate is examined. If the plate was satisfactorily streaked, and the inoculum was correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. If individual colonies are apparent, the inoculum was too light then the test must be repeated. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc. Zones are measured to the nearest whole millimeter, using sliding calipers or a ruler, which is held on the back of the inverted petri plate. The petri plate is held a few inches above a black, nonreflecting background and illuminated with reflected light. If blood was added to the agar base (as with streptococci), the zones are measured from the upper surface of the agar illuminated with reflected light, with the cover removed.

2. The zone margin should be taken as the area showing no obvious, visible growth that can be detected with the unaided eye. Faint growth of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, is ignored. However, discrete colonies growing within a clear zone of inhibition should be subcultured, re-identified, and retested. Strains of *Proteus* spp. may swarm into areas of inhibited growth around certain antimicrobial agents. With *Proteus* spp., the thin veil of swarming growth in an otherwise obvious zone of inhibition should be ignored. When using blood-supplemented medium for testing streptococci, the zone of growth inhibition should be measured, not the zone of inhibition of hemolysis. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth), and measure the more obvious margin to determine the zone diameter.

3. Based on the sizes of the zones of inhibition, the organisms are reported as either susceptible, intermediate, or resistant to the agents that have been tested.

Quality Control in Antibiotic Susceptibility Testing

QC is performed to check the quality of medium, the potency of the antibiotic, to check manual errors. Quality control strains should be included daily with the test. Not more than 1 in 20 results should be outside accuracy limits. No zone should be more than 4 standard deviations away from midpoint between the stated limits.

If, for reasons of expense or manpower constraints, it is not possible to include all strains on a daily basis, then the following guidelines should be followed:

The frequency can be decreased to once weekly if proficiency has been demonstrated by

1. Performing QC daily for 30 days with less than 10% inaccuracy for each drug
2. Proficiency testing is repeated for each new drug included in the testing
3. All documentation is maintained indefinitely
4. Proficiency testing is repeated for each new batch of media or reagents

All tests must be within accuracy limits if QC is done once weekly.

Reference strains for quality control

Escherichia coli ATCC 25922 (beta-lactamase negative)
Escherichia coli ATCC 35218 (beta-lactamase positive)
Staphylococccus aureus ATCC 25923 (beta-lactmase negative, oxacillin susceptible)
Staphylococccus aureus ATCC 38591 (beta-lactmase positive)
Pseudomonas aeruginosa ATCC 27853 (for aminoglycosides)
Enterococcus faecalis ATCC 29212 (for checking of thymidine or thymine level of MHA)
Haemophilus influenzae ATCC 49766 (for cephalosporins)
Haemophilus influenzae ATCC 10211 (for medium control)
Neisseria gonorrheae ATCC 49226
Stock cultures should be kept at -70ºC in Brucella broth with 10% glycerol for up to 3 years. Before use as a QC strain, the strain should be subcultured at least twice and retested for characteristic features. Working cultures are maintained on TSA slants at 2-8ºC for up to 2 weeks.

Reference

Manual on Antimicrobial Susceptibility Testing; Dr. M. K. Lalitha.

Troubleshooting Guide for Disc Diffusion Test in Antimicrobial Susceptibility Testing

ERRONEOUS RESULT	PROBABLE CAUSE	CORRECTIVE ACTION
Tetracycline zone too large and clindamycin zone too small with *E. coli* or *S. aureus* control strains.	pH of medium too low.	Adjust pH 7.2 to 7.4 before pouring media. Commercial media should not have pH problems. Report to manufacturer.
Tetracycline zone too small and clindamycin zone too large with *S. aureus* or *E. coli* control strain.	pH of medium too high.	Get a new lot. (Incubation in CO_2 may alter agar surface pH.)
Aminoglycoside zone too small with *P. aeruginosa,* Acinetobacter control strain.	Calcium ion and/or Magnesium ion too high in medium.	Acquire a new lot of agar medium that will meet QC criteria.
Aminoglycoside zone too large with *P. aeruginosa* control strain.	Calcium ion and/or Magnesium ion too low in medium.	Acquire a new lot of agar medium that will meet QC criteria.
Zones universally too large on control plates.	Inoculum too light.	Adjust inoculum to a McFarland 0.5 turbidity standard.
	Nutritionally poor medium.	Use only Mueller Hinton Agar medium.
	Slow-growing organism. (not seen with controls)	Use minimum inhibtory concentration (MIC) procedure only.
	Improper medium depth. (too thin)	Use 4-5mm depth.
Zones universally too small on control plates.	Inoculum too heavy.	Adjust inoculum to a McFarland 0.5 turbidity standard.
	Agar depth too thick. (minor)	Use 4-5mm depth.
Methicillin zone decreasing over days or weeks with control organisms.	Methicillin degrading during refrigerator storage	Change methicillin discs or use oxacillin or nafcillin as the routine disc.
Methicillin zone indeterminate in disc test.	Methicillin being degraded by strong beta-lactamase producing staphylococci.	Change methicillin discs or use oxacillin or nafcillin as the routine disc.
Carbenicillin zone disappears with *Pseudomonas* control.	Resistant mutant has been selected for testing.	Change *Pseudomonas* control strain every two weeks and whenever resistant mutants appear within the carbenicillin zone.
S.aureus from a patient was resistant to methicillin one day and sensitive the next.	May be two different organisms. Temperature shift from 35-37ºC can dramatically alter the zone size in this case.	Check testing temperature. Test must be performed at 35ºC or 37ºC. for methicillin (oxacillin or nafcillin) and *S.aureus*.
A single disc result above or below the control limit.	Error in reading. Fuzzy zone edge. Transcription error. Bad disc.	Note error. Recheck error and ask for a second opinion.
	Disc may not be pressed firmly onto the agar surface. (Bad discs usually demonstrate a trend toward being out of control.)	Statistically, one may expect an occasional out-of-range result. Values usually fall within range on retesting.

Troubleshooting Guide for Disc Diffusion Test in Antimicrobial Susceptibility Testing

ERRONEOUS RESULT	PROBABLE CAUSE	CORRECTIVE ACTION
Colonies within zone of inhibition.	Mixed culture.	Isolate, identify and retest pure cultures only.
	Resistant mutants within zone.	Gram stain or do another test to rule out contamination.
Very large zones with anaerobes.	–	Do not use disc agar diffusion procedure to test anaerobes.
With colistin, growth seen immediately adjacent to disc, then larger zone at endpoint (Occurs with colistin when testing *Serratia* spp. and some *Enterobacter* spp.).	"Prozone-like" phenomenon.	Confirm with MIC.
The methicillin disc test shows "resistant" but an MIC shows "sensitive" for *S.aureus*.	Mueller Hinton Broth is inadequate in this case. A modified broth used in some commercial MIC systems frequently eliminates this problem.	No action necessary with disc test. To be expected if Mueller Hinton Broth is used in MIC test. Use broth with 2% NaCl if MIC testing is necessary.
	Low methicillin content in disc.	Use new discs
Zones overlap.	Discs too close together.	Use no more than 12 discs on a 150mm plate and 4 to 5 discs on a 100mm plate. Place discs no closer than 15mm from the edge of the plate.
"Zone within a zone"	Swarming / movement of Proteus spp.	Read the wide distinct zone and disregard the growth that swarmed over.
	Feather edges of zones around penicillin or ampicillin discs usually occur with beta-lactamase negative strains of S. aureus.	Take half the distance from the inner zone to outermost zone as measure mark.
	Sulfonamides	Disregard growth from disc margin to the major inner zone.
	Beta-lactamase-positive Haemophilus influenzae with penicillin or ampicillin.	Use inside zone.
Zones indistinct (hazy) with single colonies noted on the plate.	Poorly streaked plate. Inadequate inoculum.	Use properly adjusted inoculum and repeat test.
Indistinct zones with sulfamethoxazole with or without trimethoprim or with trimethoprimalone.	Thymidine in medium inhibits the action of these antimicrobics.	Use commercial thymidine-free plates. Disregard small amount of growth within the zone as with sulfonamides.

Validation of Microbiological Test Methods

Validation of Microbiological Test Methods has become a formalized methodology within the pharmaceutical, biotechnology, and medical device industries, during the past five years as methods have become more company specific and have drifted away from the standard United Stated Pharmacopoeia (USP) assays. Validation of Microbiological Test Methods is designed to assist in the validation of test methods using a step–by–step procedure for manual and compendial methods, automated microbiological systems and rapid methods. Various interactive exercises have been used to assist in familiarization of this process. Other areas include sterile and non sterile product and process requirements, cleaning validation, water, air and surface monitoring and bioburden analysis.

The variety of microbiological tests makes it difficult, if not impossible, to prescribe a single, comprehensive method for validating all types of tests. By their very nature, microbiological tests possess properties that make them different from chemical tests. Consequently, the well-known procedures for validating chemical tests are not appropriate for many microbiological tests. Yet, it is necessary to validate microbiological tests if they are to be useful for controlling the quality of drug products and devices. Test-method validation provides assurance that a method is suitable for its intended use.

Some tests, such as bioburden or viral titer tests, are quantitative in nature while other tests, such as those for the presence of objectionable organisms, are qualitative. As with chemical tests, these differences necessitate different validation approaches. The purpose of a test may also change the procedures for running and validating it. As an example, consider a drug that will be orally administered. Normally, sterility is not a major issue, and the specification allows for a considerable number of organisms. However, if the drug will be administered to immunocompromised cancer or AIDS patients, the bioburden level must be reduced considerably, increasing the test sensitivity required in the validation study.

The nature of the test material itself changes how a test is run and the validation protocol. Consequently, testing for objectionable organisms is different when testing a diuretic for hypertension or an antibiotic for treating pneumonia. Also, a procedure that works perfectly well for checking the bioburden of granulated sugars may fail with sodium chloride. These differences make full coverage of the topic impossible within the context of this primer.

Note also that certain microbiological tests are already associated with well defined validation procedures. For example, the endotoxin test and USP bacterial enumeration tests have clearly defined validation procedures. In addition, individual countries may have specific requirements that modify or change standard procedures. If a test is associated with a compendial or regulatory validation procedure, workers are advised to follow that procedure unless there are clear reasons for not doing so. In such cases, the reasons should be documented and filed with the test procedure.

Media
The suitability of the medium used for cultivating organisms or cells obviously can have a major impact on the test results. Some organisms are extremely fastidious and require a precisely defined medium with several complex nutrients, while others grow in the presence of inorganic salt mixtures and simple carbon sources. It is commonly argued that delicate, fastidious organisms cannot survive manufacturing processes and should not be of concern, but organisms as delicate and fastidious as mycoplasmas can appear in final preparations of biologics.

In addition to the nutrient composition of the media, more general factors such as pH and ionic strength must be validated. While it is commonly believed that media in the range of pH 6.0 – 8.0 are suitable for sterility and bioburden studies, individual organisms may require a more restricted range. The same holds true for ionic strengths and osmolalities outside of the human physiological range. Shifting the pH range from 6.0 – 7.0 to 7.0– 8.0 and raising the ionic strength to 300 mOsm may select for a different set of organisms than those that would be present in the lower pH range at 150 mOsm.

Most validation schemes require the use of five or more "indicator organisms" to demonstrate the medium's ability to support growth. In addition to aerobic bacteria, anaerobic organisms, yeasts, and molds are usually included. This is an important step since a finding of "no growth detected" is meaningless if the medium was incapable of growing any organisms. This leads to two important points.

First, the indicator organisms are supposedly representative of the types of organisms that will be encountered during the testing, but this is not necessarily true. The indicator organisms are a subset of organisms that are known to grow on properly prepared media, but the organisms contaminating a manufacturing process may not belong to that subset. As a result the quality control laboratory may repeatedly face what appears to be a microbial contamination event despite monitoring cultures that show no growth. It is very important to know what organisms are normally present in the working environment and to include these environmental isolates in a validation program. There is little value in proving that a medium will support the growth of indicator organisms if the environment is full of organisms with very different cultivation requirements.

The second issue involves media handling. The qualification or validation study may require autoclaving the medium and then pouring culture plates as the autoclaved material cools. In laboratories with a low testing load, the excess material is often poured into large tubes or culture flasks to cool and solidify and then stored for future use, usually in a refrigerator. However, when future testing is done, the second heating of the medium may not be captured in the qualification or validation check and may not even be mentioned in the test procedure. If the agar is melted under gentle conditions and quickly poured, there may be no problem, but in some cases, technicians have placed the flasks in microwave ovens to heat the medium while taking a short break. With a powerful microwave oven it is easy to boil the medium for an unknown period of time. This can destroy nutrients or produce toxic or inhibitory substances. Consequently, in laboratories where this second heating is a common practice, this procedure must be captured in the validation and described exactly in the test procedures.

When preparing the validation protocol, the analyst should specify the recovery level expected for each of the indicator organisms. Generally, recovery of at least 80% of the inoculum or control is desirable. Recovery of less than 50% is usually unacceptable and should raise questions about the presence of inhibitory substances,

especially when the testing is taking place in the presence of a raw material or product intermediate. It may be necessary to introduce — and validate the performance of — an agent that inactivates the inhibitor. It is important to set the specifications before the study is conducted and to hold to these specifications. If specifications are not pre-set and the test system cannot meet general acceptance specifications, it is very easy to set "acceptable" specifications that would otherwise have been unacceptable. The other problem is the "specification creep" that occurs when a recovery of 78% is found and the specification is 80%. A quality assurance or quality control worker who allows the 78% to pass will soon face the expectation that 75% should pass because it is "only slightly different from the other one." Over the course of a few years, an 80% specification can gradually turn into a 70%, then 65%, specification.

Environment
The incubation temperature can have a major effect on the ability of an organism to grow in a given medium. It is well known that yeasts and molds require a different incubation temperature than bacteria in a sterility test. Similarly, cells in tissue culture are often extremely sensitive to small changes in temperature, not only for their growth but also in their susceptibility to being infected or lysed by viruses. The analyst may need to develop temperature curves to justify the incubation temperatures used for the test. It is also important to verify the incubator's ability to maintain the set temperature within the specified range. If a four-degree temperature variation can cause a significant change in the test results, the incubator's ability to hold a $\pm 1°$ C range at all internal locations is critical. This may not be covered in a validation study, but it should be included in the incubator's qualification studies.

In addition to the usual range from $20 – 40°$ C, it may be necessary to demonstrate the ability to grow organisms at extreme temperatures. If it is necessary to monitor the presence of microbes in a hot or cold room, it will be necessary to demonstrate an ability to cultivate thermophiles or psychrophiles in addition to organisms that grow under more normal conditions. While the significance of these extremophiles may be open to question, their presence and the possibility that they may leave residues such as endotoxins must be considered.

The atmosphere in which the test system is immersed can have a major effect. Anaerobic organisms cannot grow in the presence of oxygen, and tissue cultures may require the presence of 5% CO_2 to grow well. Certain facultative organisms will adjust their metabolic paths to cope with reduced levels of oxygen. This, in turn, can affect their growth rates. When media for general purposes, such as sterility tests, are being considered, it is normal to include one medium that provides anaerobic conditions. The detection of anaerobes is important as they include toxin-producing and other pathogenic bacteria.

Quantitative Issues
One of the problems with quantitative microbiological tests is that as microbe counts become smaller, straight-forward linear behavior is less common than that which follows the Poisson distribution. This is because random distribution is not even distribution. Most quantitative tests for microorganisms require the plating of dilute liquid samples, and it is normal to prepare samples to ensure the dispersion of microbes and a random distribution of bacteria or viruses. When concentrations are high, the lack of even distribution is not a problem; simple linear averaging methods can compensate for the uneven distribution. Problems arise with smaller numbers of microbes.

Consider an example where there are exactly 100,000 organisms per mL. If 0.1 mL is taken and mixed with 0.9 mL of a diluent, it is highly unlikely that the new suspension will contain exactly 10,000 organisms; it would not be surprising to have anywhere from 9,800 – 10,200 organisms. Back-calculating the result produces a range from 98,000 – 102,000 organisms in the original sample, and, if there were enough replicates, the results could be averaged to obtain a number indistinguishable from 100,000. This is the result that would be expected based on linear thinking.

However, if there were only 10 organisms per mL, it is quite possible that a 0.1 mL aliquot would not contain any organisms at all. In fact, in this situation about one third of the aliquots will not contain a single organism. This could lead to the conclusion, on averaging, that the sample only contained 6.7 organisms per mL, which is a significant deviation from the true value.

A transition occurred from a high density that produces a fairly smooth, homogeneous distribution of organisms to a low density that results in organisms that are distributed with significant distances between them. Under these conditions, the suspension behaves according to the Poisson distribution and assumptions related to a normal distribution no longer hold. The Poisson distribution is an exponential function. The problem is that parameters such as the standard deviations may be logarithmic in nature, and when attempts are made to make these numbers "real" by taking the antilogarithms, the results may actually have no "real" meaning. This can cause great difficulties when attempting to validate quantitative microbial test procedures.

When it is necessary to deal with the Poisson distribution, it is wise to consult a statistician who is versed in the use of this distribution. It appears that the transition to the Poisson distribution occurs when approximately 100 colonies or plaques are counted. This is unfortunate because at this level many analysts will declare a colony or plaque count to be "too numerous to count" (TNTC) to avoid the tedium of these measurements. Therefore, most colony or plaque counting procedures actually operate under the Poisson distribution and calculations based on the normal distribution will be incorrect.

Revalidation
The frequency of revalidation is a contentious question. There are many tests, such as the growth promotion test on culture media, that are essentially self-validating and are run frequently. It could be argued that if performance parameters (for example, percent recovery of indicator organisms) are monitored via control charting and no significant changes are seen, revalidation is unnecessary. However, control charting usually does not measure all the parameters included in validation studies. Consequently, it is wise to revalidate tests after any major change in constituents or procedures; in fact, revalidation may be needed to justify the changes. Changes in suppliers (especially of media components) and changes in the composition of test samples have resulted in major changes in microbiological tests. Finally, it is probably wise to re - validate procedures approximately every second year to protect against unseen or unreported changes. A media supplier may change its own suppliers or change its processing procedures without notifying customers. The supplier may have no idea of the impact these changes could have on the end use of their product. In addition, personnel changes in the laboratory and the maturing of analysts' techniques can also have an effect.

Microbiological Testing in Food Industries

1. Traditional Methods for Detection of Food-borne Pathogens

Microbiological examination of foods and food ingredients helps to assess safety to consumers, stability or shelf life under normal storage conditions, and the level of sanitation used during processing. Thus, routine examination of foods to detect selected pathogens is necessary. Most analyses look for indicator organisms, which are more rapidly enumerated. The three basic categories of tests in microbiology include presence–absence tests, enumeration tests, and identification or characterization tests. Pioneers of microbiology developed the fundamental methods traditionally used for many of these tests.

1.1 Presence–Absence Tests

Conventional Plate Count

The most important information used to assess the quality, spoilage, and safety of foods and to determine potential implications of foodborne pathogens is determination of total viable cell counts in food, water, food–contact surfaces, and air in food plants. The Aerobic Plate Count (APC), also known as the Aerobic Colony Count (ACC), Heterotrophic Plate Count (HPC), Total Plate Count (TPC), or standard plate count, estimates the number of total viable aerobic bacteria per gram or milliliter of product. Detailed procedures for determining the APC of foods have been developed by the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA) (Maturin and Peeler, 2001).

Fungi are ubiquitous organisms that often are associated with the spoilage and biodeterioration of a large variety of foods and feedstuffs (Bleve et al., 2003). Prior to analysis, it should be assumed that xerophilic molds and osmophilic yeast are organisms that prefer reduced water for growth. Spread plating is considered superior to the pour-plate method, since surface plating results in more uniform growth and makes colony isolation easier (Tournas et al., 2000). Molds that have been stressed should be enumerated by a surface spread plate technique, which provides maximal exposure of the cells to atmospheric oxygen and avoids heat stress from molten agar. Pour plates may be used when yeast or nonstressed mold cells are being detected (Douey and Wilson, 2004). Osmophilic yeast may be incubated for up to 7 days. Plates are examined on the third day of incubation, and if mold or yeast colonies are numerous, these are counted and then counted again on the fifth day, if possible (Douey and Wilson, 2004).

1.2 Enumeration Tests

Most Probable Number

The most probable number (MPN) technique is a widely used quantification method. An MPN is estimated from responses where results are reported as positive or negative in one or more decimal dilutions of the sample (Peeler et al., 1992). Thus, unlike the aerobic plate count, the MPN does not provide a direct measure of the bacterial count.

Frequently, the composition of many food products makes it difficult to use standard plate procedures, particularly when the microbial concentration of the sample is less than 10 CFU/g. At these low concentrations, the MPN technique gives more accurate counts than the plate count method for bacterial populations.

1.3 Identification or Confirmative Test

For Coliforms: One loop of culture from each positive lauryl tryptose tube is transferred to a tube of Brilliant Green Lactose Bile (BGLB) broth with a fermentation vial. Avoid transferring the pellicle (Christensen et al., 2002). Incubate BGLB tubes at 35°C and examine for gas production at 48 ± 2 h.

For E. coli, gently shake each gas-positive EC broth tube or fluorescing EC-MUG broth tube and streak a loopful of the culture onto an LEMB or Endo agar plate. Plates are incubated at 35°C for 18 to 24 h. Nonmucoid, nucleated, dark-center colonies with or without a metallic sheen are indicative of *E. coli* (Christensen et al., 2002).

For Yeast and Molds microscopic examination after staining with crystal violet may be necessary to distinguish yeast colonies from some bacterial colonies that may resemble yeast. Microscopically, yeast cells are significantly larger than bacteria and some cells may be budding (Douey and Wilson, 2004).

2. Rapid Methods for Food-borne Bacterial Enumeration and Pathogen Detection

Foods are routinely tested with the objectives of establishing the absence of specific pathogens or toxins, to ensure food safety, and to test for total microbial load or for indicators to monitor the sanitary quality of foods. These fundamental objectives have long been achieved with traditional microbiological methods, which are labor, time, and material-intensive. Hence, rapid methods have had a major impact, as evidenced by the vast numbers of papers that describe the use of rapid methods in food testing.

RAPID PATHOGEN TESTING METHODS

Rapid pathogen testing may be divided into identification and detection methods. Bacteria may be identified by many attributes, including fatty acid and carbon oxidation profiles, but most still rely on biochemical analyses, which are labor- and media-intensive procedures. Miniaturized biochemical identification kits, which have been in use for years, have greatly simplified this process, but identification assays are becoming even more user-friendly, as many are now automated.

The use of **specialized substrates** or media for presumptive identification of bacteria is another area that has seen a lot of changes. The use of special substrates became popular with the fluorogenic substrate 4-methylumbelliferone-β-d-glucuronide (MUG) for identifying *E. coli* based on β-glucuronidase activity (Feng and Hartman, 1982). These fluorogenic and chromogenic substrates, however, continue to be used in rapid enumeration tests for indicators.

Antibody-based assays comprise the majority of rapid pathogen detection kits and they use various assay formats. Simple latex agglutination tests that use antibody-bound colored latex beads to serotype pure bacterial cultures have become very popular. Used initially for serotyping *E. coli* O157:H7 isolates from foods, latex assays are now available for many pathogens, including other enterohemorrhagic *E. coli* serotypes that are increasingly causing foodborne illness worldwide (Brooks et al., 2005).

Immunomagnetic separation (IMS) is another antibody-based format that has undergone major advancements, as many are realizing that the selective antibody capture of target by IMS can often improve the sensitivity of other assays (Benoit and Donahue, 2003). Hence, the diversity of antibodies coupled to beads has increased, and automated IMS assays have also become available.

Pathogen detection using the antibody **Enzyme–Linked Immunosorbent Assay (ELISA)** format continues to exist, but few

manual tests are being introduced. Antibodies are also being used increasingly in biosensors, which have biological components (antibodies and ligands) that are coupled with sensitive physicochemical transducer to measure specific biological interactions. Biosensors can simultaneously detect multiple targets (Taitt et al., 2004) and are very fast and sensitive in the detection of bacterial cultures in solutions, but their efficiency in food testing can be variable and is still being explored (Alocilja and Radke, 2003).

DNA-based pathogen detection assay formats consist of DNA probes (Olson, 2002), cloned phages (Favrin et al., 2001), and polymerase chain reaction (PCR). Until recently, most PCR assays, however, used manual gel-based detection that was labor-intensive, hence not very popular. As a result, DNA-based rapid pathogen methods formed only a small percentage of commercially available kits. Advances in genomics (Abee et al., 2004), however, introduced such technologies as real-time PCR (RT-PCR) (Exner, 2005) and DNA chips (microarrays) (Call, 2005), which caused a resurgence in DNA-based assays. As a result, RT-PCR has surpassed other DNA formats, and many new assays for pathogens have been introduced. These assays use fast amplification and a variety of detection technologies (Kubista and Zoric, 2005) for real-time data monitoring that will give results in an hour.

Rapid Enumeration Methods for Foods
A wide range of both direct and indirect rapid enumeration methods have been developed for testing for total and indicator bacteria in foods. They are divided into manual and automated tests.

References

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5. Poulis JA, de Pijper M, Mossel DAA (1993): Assessment of cleaning and disinfection in the food industry with the rapid ATP-bioluminiscence technique combined with the tissue fluid contamination test and a conventional microbiological method. Int J Food Microbiol. 20: 109–116.

Rapid Enumeration Assays for Food Samples

Type	Assay	Principle/Description	Applications
Manual	Petrifilm Sanitakun	Dual-layer film coated with nutrients and gelling agents soluble in cold water	Food, water, environmental
	Iso-Grid	Membrane filtration using hydrophobic grid membranes that are placed on selective agar for enumeration by MPN	Wide range of foods, beverages, ingredients, water
	Easygel	Special petri dish coated with gelling agents and nutrients	Wide range of foods, beverages
	Simplate Quanti-Disc	Multienzyme technology Correlate enzyme activity with the presence and number of viable organisms in foods	Wide variety of foods (Simplate), water (Quanti-Disc)
	ColiTrak, ColiTrak Plus (fecal coliform)	MPN assay utilizing LST and MUG (fecal coliform)	Food, water, environmental
	Millipore's Samplers	Ready-to-use plastic paddle with nutrients, membrane filter for sampling and incubation	Water
Automated	Spiral, Wasp II Spiral Plater	Precise delivery of 1 : 1 to 1 : 10,000 sample dilution in a spiral pattern on each plate	Food, water, environmental
	Tempo	Automated MPN: coliforms, *E. coli,* and Enterobacteriaceae	Poultry, egg, meat, meat products
	Bactometer, Rabbit Impedance Detection System, Bac Trac	Impedance	Food, water, environmental
	Malthus	Conductance	Food, water, environmental
	BacT/Alert Automated Microbial Detection System	Colorimetric sensor and reflected light to monitor cell presence by production of CO_2	Food, beverages
	ChemScan RDI System	Flow cytometry (fluorescent cell labeling and laser scan)	Pharmaceutical, personal care, food, beverages
	D-Count	Flow cytometry (fluorescent cell labeling, laser excitation, and digital processing)	Pharmaceutical, personal care, food, beverages
	Milliflex Rapid Microbiology Detection System	ATP bioluminescence	Water and filterable samples

Collection of specimens/swabs

Samples can arrive at the microbiology laboratory in a variety of formats, often sub-samples of a large production batch or in a clinical setting, samples of body fluids. Swabs however, are unique in their presentation, as the target microorganisms need to have been efficiently collected from the sampling site, carried by an inert vector and then must be recovered from this for subsequent analysis.

During transportation of the swab the numbers and proportions of micro-organisms present should be the same when it arrives at the lab as they were when first sampled. Swab transport systems should be able to keep the more delicate and fastidious bacteria viable whilst preventing the more robust ones from multipyling and obscuring others.

So swabbing techniques, and the swabs themselves, need to provide an efficient collection of sample, its subsequent preservation, and ultimately the release of the target cells. In 2003 the Clinical Laboratory Standards Institute (CLSI) published M40-A, an approved standard for the quality control of microbiological transport systems, this meant that for the first time swab products could be compared and evaluated using set criteria.

Disposable, single-use plastics revolutionized the microbiology laboratory, lending themselves particularly to the testing of surfaces or inaccessible areas of equipment and for clinical specimens.

Traditionally the swab would comprise a flexible shaft terminating in a bud of compacted material, e.g. cotton, viscose, contained within a pre-labelled, tamper-evident tube. The tube may also contain a transport medium designed to maintain the viability of any organisms collected.

There is a wide range of transport media available e.g. Amies with Charcoal Transport Medium, Stuart Transport Medium, and Universal Transport Medium (UTM), these are intended to maintain the viability of any cells present until the swab is returned to the laboratory.

The new nylon flocked swab design has oriented strands of nylon arranged perpendicular to the shaft creating micro-capillaries that not only improve sample collection but also release that sample more efficiently. Sample release efficiencies (>90%) allow truly quantitative results.

General principles:
Successful collection of specimens will depend on the following:
- Collection at the appropriate time
- Use of the correct technique
- Use of the correct equipment
- Safe transportation to the laboratory without delay

Healthcare practitioner's role:
- To identify the requirement of a microbiological investigation
- To initiate the procedure
- To collect the desired material in the correct container
- To arrange prompt delivery to the laboratory

Collection of specimens:
- Samples should be collected before the start of any treatment
- If an unusual specimen is required check any specific

requirements with the laboratory eg skin and mucous membranes, pus, biopsies

Types of investigation:
- Bacterial – culture and sensitivity
- Viral – culture; serology; ideally taken in hospital as viruses do not survive long outside the body
- Serological – antigens and antibodies
- Mycosis – fungal
- Protozoa – malaria

Generic equipment required:
Sterile Water, Spatula, Labelled Specimen Container, Swab in Transport Medium, Disposable gloves as necessary & Laboratory Request Form.

General techniques for collection of specimens:

Nose swab

Action	Rationale
Moisten swab beforehand with sterile water	To prevent discomfort to the patient
Move swab from the anterior nares and direct upwards into the tip of the nose	To swab the correct site and obtain required sample
Gently rotate the swab once	

Sputum specimen

Action	Rationale
Use a clean, not necessarily sterile container	Sputum is never free from organisms due to passing through the pharynx & mouth
Ensure that the specimen is sputum NOT saliva	To obtain the required sample
Encourage the person to cough deeply or request the help of a physiotherapist	To facilitate expectoration

Throat swab
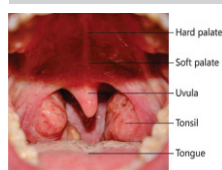
Tonsilar fossa

Action	Rationale
Sit the person facing a strong light and depress the tongue with a spatula	To ensure you can see the area to be swabbed. The procedure is likely to make the individual gag – the tongue moving to the roof of the mouth will contaminate the specimen
Quickly but gently swab the tonsillar fossa or any area with a lesion or visible exudates	To obtain the required sample

Ear swab
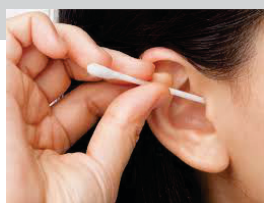
Action	Rationale
No drops should have been used 3 hours prior to taking the swab	To prevent collection of therapeutic material
Place the swab into the outer ear and rotate gently once	To avoid trauma to the ear and collect secretions

Wound swab

Action	Rationale
Take the swab before cleaning the wound	To collect the maximum number of organisms
Rotate the swab gently once	To collect the sample

Urine specimen – early morning

Action	Rationale
Early morning specimen required	The bladder will be full due to overnight accumulation of urine – later specimens may be diluted

Urine specimen – mid-stream

Action	Rationale
Ask the person or assist them to wash around the urethral area	To prevent other organisms contaminating the specimen
Ask the person to discard the first and last part of micturation and collect the middle stream	To avoid contamination with skin organisms

Urine specimen – catheter
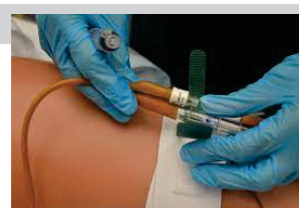
Action	Rationale
Clean the access point on the tubing	To reduce cross infection
If there is no urine, clamp the tubing below the access point	To obtain an adequate specimen
Using a sterile needle and syringe aspirate urine through the access point	To prevent leakage
Re-clean the access point	To reduce contamination

Faeces specimen

Action	Rationale
Person to defaecate into a clean bedpan	To avoid unecessary contamination
Scoop enough material to fill a third of the specimen pot	To obtain a usable amount of specimen
Record colour, consistency and odour	To maintain an accurate baseline record
Segments of tapeworm are easily seen – send to the laboratory	Laboratory confirmation of the head of the tape worm is required to prevent further growth

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Collection, Transport & Field Supplies of Fecal Specimens

Fecal specimens should be collected in the early stages of any enteric illness, when pathogens are usually present in the stool in highest numbers, and before antibiotic therapy has been started. An exception to this rule is when stool is collected from persons with febrile illness: in the case of typhoid fever, the etiologic agent *Salmonella* ser. Typhi may be present in highest numbers in stool in the second and third weeks of the disease.

| Collection and transport of specimens for laboratory diagnosis	
When to collect	When the patient is having diarrhea, as soon after onset of illness as possible (preferably within 4 days of onset) and before antimicrobial treatment is started.
How much to collect	Rectal swab or swab of fresh stool in transport
Transport medium	Cary-Blair or other suitable transport medium (not buffered glycerol saline for *V. cholera*)
Storage after collection	Refrigerate at 4ºc if the specimens will be received by the laboratory within 48 hours. Or freeze at -70ºc. Fecal specimens from patients with suspected cholera can be transported at ambient temperature and held for longer times if necessary; however refrigeration is preferred.
Transportation	Seal tubes/containers to prevent leakage; place in waterproof container to protect from wet or dry ice. Ship in insulated box with ice packs, wet ice or dry ice by overnight delivery.

Collection of stool:
Stools samples should be collected in clean containers without disinfectant or detergent residue and with tight-fitting, leak-proof lids. Specimens should **not** be collected from bedpans, because the bedpans may contain residual disinfectant or other contaminants. U**npreserved stool should be refrigerated, if possible, and processed within a maximum of 2 hours after collection. Specimens that cannot be cultured within 2 hours of collection should be placed in transport medium and refrigerated immediately.**

Transport media for fecal specimens:
This section provides information regarding media appropriate for the transport of fecal specimens that are suspected to contain *Shigella, Vibrio cholerae,* or *Salmonella* (including serotype Typhi) specimens. Once specimens from an outbreak of diarrheal disease have arrived at the laboratory, laboratorians should follow procedures for *Shigella* or *V. cholerae* isolation depending on whether reports from the field indicate the outbreak appears to be dysentery or a cholera-like illness. Because persons suspected of having typhoid will commonly present with fever and not diarrhea, laboratories usually do not receive a surge of fecal specimens in an outbreak of typhoid; however, on occasion fecal specimens may be submitted to a laboratory for diagnosis of infection with S. Typhi.

FIGURE 81: Cary-Blair semisolid transport medium

Leave the swab in the tube after inoculating Cary-Blair medium.
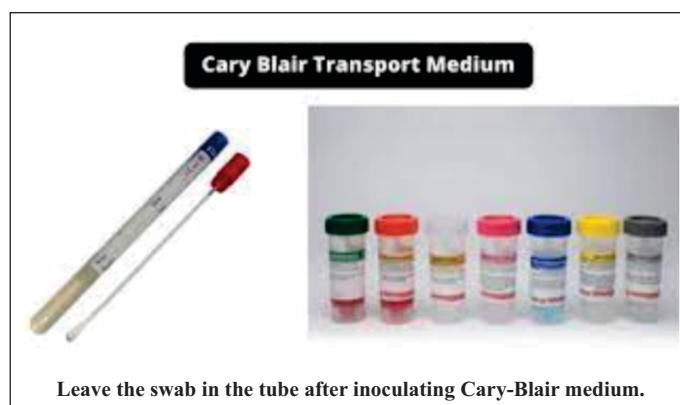
Cary-Blair transport medium:
Cary-Blair transport medium can be used to transport many bacterial enteric pathogens, including *Shigella, Salmonella*, and *Vibrio cholerae* (Figure 81).
Cary-Blair's semisolid consistency provides for ease of transport, and the prepared medium can be stored after preparation at room temperature for up to 1 year. Because of its high pH (8.4), it is the medium of choice for transport and preservation of *V. cholerae*.

Preparation and quality control of Cary-Blair
Prepare according to manufacturer's instructions. [Note: There are several commercially available dehydrated formulations of Cary-Blair. Some require the addition of calcium chloride and some do not. Cary-Blair can also be prepared from individual ingredients.] When Cary-Blair is prepared, it should be dispensed into containers in sufficient volume so that swabs will be covered by at least 4 cm of medium. For example, 5- to 6-ml amounts may be dispensed into 13 x 100- mm screw cap tubes. With the caps loosened, sterilize by steaming (do not autoclave) at 100°C for 15 minutes. Tighten the caps after sterilization.
Cary-Blair is quite stable if stored in tightly sealed containers in a cool dark place so that the medium does not dry out. Cary-Blair may be used for up to 1 year as long as there is no loss of volume, contamination, or color change.

Other transport media
Other transport media that are similar to Cary-Blair are Amies' and Stuart's transport media. Both of these are acceptable for *Shigella* and *Salmonella* (including ser. Typhi), but they are inferior to Cary-Blair for transport of *V. cholerae*. Alkaline peptone water may be used to transport *V. cholerae*, but this medium is inferior to Cary-Blair and should be used only when the latter medium is not available. **Alkaline peptone water should not be used if subculture will be delayed more than 6 hours from the time of collection**, because other organisms will overgrow vibrios after 6 hours. Buffered glycerol saline (BGS), a transport medium that is used for *Shigella*, is unsuitable for transport of *V. cholerae*. Additional disadvantages of buffered glycerol saline are that it can be used for only 1 month after it is made and, because it is a liquid medium, it is more likely to leak or spill during transport.

Placing stool in transport medium:
If possible, chill the transport medium for 1–2 hours in a refrigerator or cold box prior to use. A small amount of stool can be collected by

inserting a sterile cotton or polyester-tipped swab into the stool and rotating it. If mucus and shreds of intestinal epithelium are present, these should be sampled with the swab.

Following sampling of the stool on the swab:

a) Insert the swab containing fecal material into transport medium immediately.

b) Push the swab completely to the bottom of the tube of transport medium.

c) Break off the top portion of the stick touching the fingers and discard it.

d) Replace the screw cap on the tube of transport medium and tighten firmly.

e) Place the tube in a refrigerator or cold box.

Collection of rectal swabs:

Sometimes rectal swabs are collected instead of stool specimens. Rectal swabs may be collected as follows:

a) Moisten the swab in sterile transport medium.

b) Insert the swab through the rectal sphincter 2–3 cm (i.e., 1–1.5 inches) and rotate.

c) Withdraw the swab from the rectal sphincter and examine to make sure there is some fecal material visible on the swab. (If not, repeat the procedure with the same swab.)

d) Immediately insert the swab into cold transport medium (as described in the preceding section).

e) Place the tube in a refrigerator or cold box.

The number of swabs needed will depend on the number of plates to be inoculated. **In general, if specimens will be examined for more than one pathogen, at least two stool swabs or rectal swabs should be collected per patient, and both swabs should be inserted into the same tube of transport medium.** Once the swab is placed in the medium, it should remain in the tube until it is processed in the laboratory.

Storage of specimens in transport medium:

If transport medium has been stored at room temperature, it should be chilled in a refrigerator or cold-box, if possible, for 1–2 hours before use. Specimens preserved in transport medium should be refrigerated until processed. If specimens will be kept more than 2–3 days before being cultured, it is preferable to freeze them immediately at -70°C. It may be possible to recover pathogens from refrigerated specimens up to 7 days after collection; however, the yield decreases after the first 1 or 2 days. Prompt plating, refrigeration, or freezing of specimens in Cary-Blair is particularly important for isolation of *Shigella*, which is more fragile than other enteric organisms. Fecal specimens in transport medium collected from patients with cholera need not be refrigerated unless they are likely to be exposed to elevated temperatures (i.e., >40°C).

Unpreserved specimens:

When transport medium is not available, one option for specimens suspected to contain *V. cholerae* is to soak a piece of filter paper, gauze, or cotton in liquid stool and place it into a plastic bag. The bag must be tightly sealed so that the specimen will remain moist and not dry out. Adding several drops of sterile saline to the bag may help prevent drying of the specimen. Refrigeration during transport is desirable but not necessary. **This method is not suitable for transport of *Shigella* or *Salmonella* specimens and is less effective than transport medium for preserving *V. cholerae* organisms.**

Preparing specimens for shipment:

Specimen tubes should be clearly labeled with the specimen number, and if possible, the patient's name and date of collection. Write the numbers on the frosted portion of the specimen tube using an indelible marker pen. If the tube does not have a frosted area, write the information on a piece of first-aid tape and affix this firmly on the specimen container. Patient information should be recorded on a data sheet; one copy should be sent with the specimens and another kept by the sender. If a package is to be shipped by air, the International Air Transport Association (IATA) regulations presented in the *Dangerous Goods Regulations* (DGR) publication must be followed; these regulations (current as of 2002) are summarized in "Packing and Shipping of Diagnostic Specimens and Infectious Substances." Even if the package will be shipped by other means, these regulations are excellent guidelines for packing all infectious or potentially infectious materials.

Refrigerated specimens:

Refrigerated specimens should be transported to the laboratory in an insulated box with frozen refrigerant packs or ice. If wet ice is used, the tubes or containers should be placed in waterproof containers (e.g., plastic bags) that can be tightly sealed to protect the specimens from the water formed by melting ice.

Frozen specimens:

Frozen specimens should be transported on dry ice. The following precautions should be observed:

- Place tubes in containers or wrap them in paper to protect them from dry ice.
- Direct contact with dry ice can crack glass tubes.
- If the specimens are not in leak-proof containers, protect them from exposure to carbon dioxide by sealing the screwcaps with tape or plastic film or by sealing the tubes in a plastic bag. Carbon dioxide will lower the pH of the transport medium and adversely affect the survival of organisms in the specimen.
- Ensure that the cool box is at least one-third full of dry ice. If the specimens are sent by air and more than 2 kg of dry ice is used, special arrangements may be necessary with the airlines. Airlines accept packages with less than 2 kg of dry ice.
- Address the package clearly; including the sender's name and telephone number as well as the name and telephone number of the receiving laboratory.
- Write in large letters: EMERGENCY MEDICAL SPECIMENS; CALL ADDRESSEE ON ARRIVAL; HOLD REFRIGERATED (or "FROZEN", if applicable).
- Be sure that all applicable labels and forms, such as those required by IATA, are correctly fixed to the outside of the package.

References

1) Manual for Identification and Antimicrobial Susceptibility Testing
2) www.ritm.gov.ph
3) www.who.int

Nasopharyngeal swab specimen collection & culture methodology

Laboratories may receive nasopharyngeal (NP) swabs in the course of prevalence surveys and carriage studies of respiratory organisms. Culture methods for this type of specimen are included below.

Use swabs taken from the upper respiratory tract (*e.g.*, the nasopharynx) to inoculate the primary culture medium; the nasopharyngeal swab should be rolled over one-fourth of the plate (*i.e.*, one quadrant). Because bacteria other than *S. pneumoniae* and *H. influenzae* are generally present, selective media are used. For *S. pneumoniae*, the selective medium is a tryptone soy agar (TSA) plate containing 5% sheep or horse blood and 5 μg/ml of gentamicin sulfate; for *H. influenzae*, a chocolate agar plate containing 300 μg/ml of bacitracin is used. If one swab is being collected for recovery of both *S. pneumoniae* and *H. influenzae*, the blood agar and gentamicin plate should be inoculated first, followed by the inoculation of the chocolate agar and bacitracin plate (because *S. pneumoniae* is more susceptible to the antibacterial activity of the bacitracin than *H. influenzae* is to the antibacterial activity of gentamicin). After direct plating with the swab, use a bacteriological loop to streak the plate.

In areas where overgrowth of contaminants occurs in <10% of cultures, culture media without antibiotics may be used. However, in this case the primary plates must be streaked very carefully to allow separation of individual colonies.

Collection of nasopharyngeal (NP) swabs:
NP swab collection is a clinical procedure and should therefore be performed by trained health-care workers. A specifically designed swab with a flexible wire shaft and a small calcium alginate tip should be used; calcium alginate is inert and non toxic to *Neisseria* and other sensitive bacteria.

Figure 76 depicts the proper method of collecting an NP swab. The patient's head should be tipped slightly backward, as shown, and immobilized. For young infants, a good way to collect NP swabs is for the person taking the specimen to hold his/her hand behind the neck of the infant while the infant is sitting in the lap of the parent or other adult. For children, the adult should lightly hold the child's head against his/her chest with a hand on the child's forehead; the adult's other arm should be used to restrain the child's arms. Sometimes it is
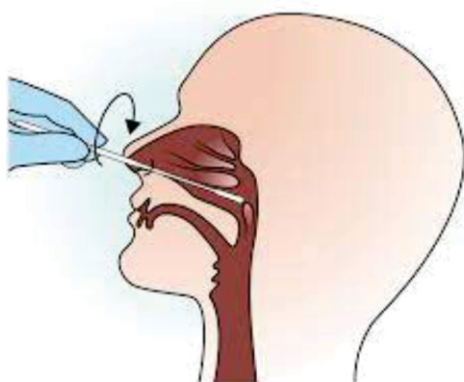
The top figure shows the anatomy of the nasopharynx and the path the swab needs to follow to reach the specimen-collection point at the posterior nares. Note that the swab goes straight back, and is parallel to the ground.

The figure on the right shows the way a child should be held when an NP swab is to be collected. Have an adult sit with the child on his/her lap with one arm around the child's upper body, one arm holding across the forehead to stabilize the head, and the legs and knees stabilizing the child's lower body.

Figure 76

also helpful if the adult's legs are used to stabilize the child's legs; this reduces body movement and kicking during the collection of the NP swab.

When the child's head is immobilized and body is restrained, the NP swab can be collected using the following procedures:
a) Unwrap the swab.
b) Insert the swab into a nostril and pass the swab parallel to the ground, back to the posterior nares. Do not use force. The swab should travel smoothly with minimal resistance; rotating the swab during the insertion will help the swab move. If resistance is encountered, remove the swab and try the other nostril.
c) Once in place, rotate the swab, leave in place approximately five seconds to saturate the tip, and remove slowly.
d) Use the swab to inoculate the appropriate (selective) medium (sheep blood with gentamicin to isolate *S. pneumoniae*; chocolate agar with bacitracin to isolate *H. influenzae;* blood or chocolate with no antimicrobial for *N. meningitidis*) by direct plating, or place the swab in STGG transport medium for transportation to the laboratory.

Skim-milk tryptone glucose glycerol (STGG) transport medium for nasopharyngeal secretions
Skim-milk tryptone glucose glycerol (STGG) transport medium is a tryptone broth with skim (nonfat) milk, glucose, and glycerol that can be used to transport NP swabs to the laboratory when the swabs cannot be plated directly from the patient. (The preparation of STGG medium is described in Appendix 2.) Culturing from the STGG as soon as possible is preferred, though STGG can also be used for storage and transport (for a several hours at room temperature; for up to 8 weeks at -20°C; and, for at least 2 years at -70°C).

Inoculation of STGG with an NP swab
a) Thaw frozen tubes of STGG before use.
b) Label the tube with appropriate patient and specimen information.
c) Using a calcium alginate swab, collect an NP swab from the patient.
d) Insert swab to the bottom of the STGG medium in thawed tube.
e) Raise the swab slightly and cut the wire portion (*i.e.*, the shaft) of the swab at the top level of the container. Allow the bottom portion of the swab (*i.e.*, the tip) containing the calcium alginate material to drop into the tube. Discard the remaining shaft into disinfectant solution or a sharps container.

f) Tighten the screw-cap top securely.
   Optional: If desired, after tightening the cap, vortex on high speed for 10–20 seconds.
g) Freeze specimen immediately in upright position at -70°C, if possible.
In some cases, the inoculated STGG medium has been placed on ice for several hours before placing the STGG medium at -70°C without loss of viable *S. pneumoniae*. Extended storage of inoculated STGG stored at -20°C for 8 weeks results in minimal loss of viability of *S. pneumoniae*, and indications are that *H. influenzae* survive as well as *S. pneumoniae* in STGG.
Short term storage of STGG is best at -70°C although a freezer at -20°C may also be used.

Recovery of bacteria from STGG
a) Remove the inoculated STGG medium from the freezer.
b) Allow the tube to thaw at room temperature.
c) Vortex each tube for a full 10 seconds.
d) Using a sterile loop, aseptically remove a 50–100 μl sample of inoculated STGG to streak onto a plate for culture. (If attempting isolation of *S. pneumoniae*, a 100-μl inoculum is preferable.)
   1) **5% sheep (or horse) blood + 5 μg/ml gentamicin sulfate agar** is the appropriate plated medium for the recovery of *S. pneumoniae* from a nasopharyngeal swab specimen stored in STGG.
      (If a gentamicin-containing medium is not available, attempt recovery from a standard blood agar plate.)
   2) **Chocolate + 300 μg/ml bacitracin agar** is the appropriate plated medium for recovery of *H. influenzae* from a nasopharyngeal swab specimen stored in STGG.
      (If a bacitracin-containing medium is not available, attempt recovery from a standard supplemented chocolate agar plate.)
   3) **5% sheep or chocolate agar** is the appropriate plated medium for recovery of *N. meningitidis*.
e) Re-freeze the specimen (*i.e.*, the STGG) as soon as possible; keep it cool (in an ice water bath if necessary) if the time is extended beyond a few minutes at room temperature.
f) Avoid multiple freeze-thaw cycles whenever possible. One way to decrease risk of freeze-thaw cycles within the freezer is to make sure the cryo tubes are kept in the back of the freezer shelf and not the front or in the door.

Moist Heat Sterilization By Using Autoclave

Sterilization is the freeing of an article from all living organisms, including bacteria and their spores.

Sterilization of culture media, containers and instruments is essential in microbiological work for isolation and maintenance of microbes. Sterilization can be done in a variety of ways, which can be conveniently categorized as follows:

(a) Physical Method- Sterilization can be done by heat, radiation and filtration. Again heat sterilization can be categorized into two:
- Dry Heat
- Moist Heat
(b) Chemical Methods

Moist Heat Sterilization

Moist-heat sterilization is achieved when water vapour at a definite temperature is introduced or generated at the level of the microorganisms to be inactivated and is maintained in such conditions for a definite time. Moist heat kills the organisms by coagulating and denaturing their enzymes and structural protein. Steam contacts a cooler surface, condenses, causing a huge decrease in volume and setting up a negative pressure that draws more steam. Condensation occurs as long as there is a temperature differential. Action of steam ensures: Surface heating, penetration, and protein coagulation. Sterilization by moist heat of the most resistant spores generally requires 121 °C for 15-30 minutes. Moist heat is used for the sterilization of culture media and all other materials through which steam can penetrate. Moist heat is more effective than dry heat. Sterilization can be done at lower temperatures in a given time at a shorter duration at the same temperature. Moist heat can be employed at temperatures below 100ºC, temperatures at 100ºC and temperature above 100ºC. Autoclaving is the most reliable method to do moist heat sterilization above 100ºC.

Autoclave

Autoclave is the method most widely used for sterilization of culture media and surgical supplies. It is a pressurized device designed to heat aqueous solutions above their boiling point to achieve sterilization. Charles Chamberland invented it in 1879. Under ordinary circumstances (at standard pressure), liquid cannot be heated above 100 °C in an open vessel. However, when water is heated in a sealed vessel such as an autoclave, it is possible to heat liquid water to a much higher temperature. As the container is heated the pressure rises due to the constant volume of the container. The boiling point of the water is raised because the amount of energy needed to form steam against the higher pressure is increased. The standard temperature and pressure for sterilization in autoclave is 121ºC at 15 lb/inch^2. This works well on solid objects; when autoclaving hollow objects, it is important to ensure that all of the trapped air inside the hollow compartments is removed. Autoclaves may achieve air removal by downward displacement, super atmospheric, transatmospheric or sub-atmospheric pulses.

Factors Influencing Sterilization by Heat

There are some factors, which influence the sterilization:
- Temperature and time- They are inversely related, shorter time is sufficient at high temperatures.
- Sterilization hold time- This is the time for which the entire load requires to be exposed to pure, dry, saturated steam at the effective temperature in order to ensure sterilization.
- Heat penetration time- Before the sterilization hold time can start, the load needs to be brought up to temperature. This is the

heat penetration time. It varies with the different type of load.
- Number of microorganisms and spores-The number of survivors diminished exponentially with the duration of heating. It also depends on the species, strains and spore forming ability of the microbes.
- Depends on the nature of material- A high content of organic substances generally tends to protect spores and vegetative organisms against heat.

Operation Of Autoclave

Autoclave is used for sterilization as well as decontamination. Basic operating procedures for autoclave are arranging the load, setting of operating control, sterilization and unloading. Packaging is an important step for autoclaving. Some of the following points should be remembered while operating an autoclave:
- Volatile chemicals, radioactive compounds and sharps should not be kept for autoclave.
- Containers and autoclave bags should be utilized for autoclaving.
- Containers should not be overfilled.
- Packaging should be prepared in such a way that steam can penetrate easily.
- Incase of decontamination of biohazardous materials it must be capped at least with aluminum foils.
- The material to be autoclaved should not allow touching the sides or top of the chamber.

Various types of autoclaves are there like: a) Gravity displacement, b) Vacuum assisted, c) Simple transportable, d) Porous load. Gravity refers to the way steam enters from the top of the chamber and forces air out the drain at the bottom. The objective is to get all the air out of the chamber and replace it with steam. But in vacuum assisted model use a vacuum to remove air from the chamber before the steam is allowed to enter. In simple transportable autoclave the air is removed by turbulent displacement. In porous load autoclave the air may be extracted by an oil-seal high vacuum pump or be diluted out by a succession of steam pulses alternating with the drawing of a partial vacuum. Packaged and wrapped goods, linen and instruments with lumens where there may be difficulty in removing the air or allowing sufficient steam penetration, can be effectively processed only in a porous load or multipurpose autoclave.

Safety Instructions

Some of the safety instructions should follow while handling an autoclave:
- Personal protective equipments (PPE) like laboratory coat, globes, eye protecting shield; closed toed shoes should be used during the unloading of autoclave.
- The door (lid) should be closed tightly during the time of loading.
- Chamber pressure should be checked before opening the autoclave.
- The door (lid) should be opened slowly to remove the steam.

Validation Approaches

The validation of moist heat sterilization processes may be performed using any of the three strategies outlined below. The approach selected should be appropriate and adequately supported.
a) Prospective Validation- This approach applies to new or modified processes and new equipment. The studies are conducted, evaluated and the process & equipment system certified prior to initiating routine production.
b) Concurrent Validation- This approach applies to existing

processes and equipment. Concurrent validation studies are conducted during regular production and should only be considered for processes, which have a manufacturing and testing history indicating consistent quality production.

c) Retrospective Validation- This approach can only be applied to existing products, processes and equipment and is based solely on historical information. Normal processing records generally lack sufficient detail to permit retrospective validation.

Each stage of the evaluation of the effectiveness and reproducibility of a sterilization process should be based on a pre-established and approved detailed written protocol. A written change control procedure should be established to prevent unauthorized change to the protocol or process and restrict change during any phase of the studies until all relevant data are evaluated. The protocol should specify the following in detail:

- The process objectives in terms of product type, batch size, container/closure system, and probability of survival desired from the process;
- Pre-established specifications for the process, which include the cycle time, temperature, pressures and loading pattern;
- A description of all of the equipment and support systems in terms of type, model, capacity and operating range;
- The performance characteristics of each system, sub-system or piece of equipment in performance characteristics including pressure gauge sensitivity and response, valve operation, alarm systems functions, timer response and accuracy, steam flow rates and/or pressures, cooling water flow rates, cycle controller functions, door closure gasketing, and air break systems and filters;
- For new equipment: installation requirements and installation check points for each system and sub-system;
- For existing equipment, the necessary upgrading requirements or any compensatory procedures; justification for alternate procedures should be available;
- A description of the following studies like bioburden determination studies, empty chamber heat distribution studies, container mapping studies, loaded chamber heat penetration studies, microbiological challenge studies.

Indicating devices used in the validation studies or used, as part of post-validation monitoring or requalification must be calibrated. Physical and chemical indicators should be tested to demonstrate adequate predetermined response to both time and temperature. Detailed written test procedures and records of test results should be available. The indicators should be used before a written expiry date and stored to protect their quality. Biological indicators should be tested according to detailed written procedures for viability and quantitation of the challenge organism and for the time/temperature exposure response. For commercial indicators, a certificate of testing for each lot indicating the "D" value of the lot should be available. The biological indicator should be used before expiry and adequately stored. Following steps should be followed for validation testing:

- All autoclaves are to be run with cycle log recorders, which ensure that temperature parameters are achieved. Paper copies of the results are to be maintained for a period of 1 year. Any deviations are to be addressed.
- All loads (sterilization and decontamination) are to include items labeled with temperature sensitive tape. Any load where the tape has not changed color will be re-autoclaved with fresh tape.
- All autoclaves will have biological indicator testing performed to confirm efficacy of decontamination cycles. *Bacillus stearothermophilus* is used as biological indicator. Typical biological indicators for combined biological indicator/bioburden based steam sterilization models include: *B. stearothermophilus, C. sporogenes, B. coagulans* and *B. subtilis.*

Applications
Autoclaves are found in many medical settings and other places that need to ensure sterility of an object. Autoclaving is often used to sterilize medical waste prior to disposal in the standard municipal solid waste stream.

Reference
Practical Medical Microbiology, Mackie & McCartney, 13th Edition 1989, Edited by J.G. Collee, J.P. Daguid.

Good Laboratory Practices (GLPs)

Good Laboratory Practice (GLP) embodies a set of principles that provides a framework within which laboratory studies are planned, performed, monitored, recorded, reported and archived. These studies are undertaken to generate data by which the hazards and risks to users, consumers and third parties, including the environment, can be assessed for pharmaceuticals, agrochemicals, veterinary medicines, industrial chemicals, cosmetics, food and feed additives and biocides. GLP helps assure regulatory authorities that the data submitted are a true reflection of the results obtained during the study and can therefore be relied upon when making risk/safety assessments. These test items are frequently synthetic chemicals, but may be of natural or biological origin and, in some circumstances, may be living organisms. The purpose of testing these test items is to obtain data on their properties and/or their safety with respect to human health and/or the environment. Published GLP regulations and guidelines have a significant impact on the daily operation of an analytical laboratory.

Historical Perspective

Earlier days governmental bodies have been faced with problems of unusable or unreliable data. These problems include incomplete reporting of test results, unauthorized deviation from approved protocols, inadequate qualifications and supervision of personnel, poor test system procedures and many more. In 1978 the U.S. Food and Drug Administration (FDA) published a final rule specified for Good Laboratory Practice instructions to all laboratories that intend to develop and submit data to that regulatory agency. In 1979 the U.S. Environmental Protection Agency (EPA) joined with the FDA's efforts and published a good laboratory practice proposal similar to that proposed by FDA.

At the same time with FDA, Organization for Economic Co-operation and Development (OECD) started with the task of an international harmonization of these standards. International harmonization was urgently required for the increasing trade in chemical substances, pharmaceutical products and pesticides. An expert group on GLP first developed the Principles of Good Laboratory Practice of the OECD, in 1978. The expert group of OECD also proceeded to formulate and publish guidelines for the monitoring authorities with regard to the introduction of procedures necessary for the monitoring of industry's compliance with these principles. Many countries with strong interests in chemicals, pesticides and pharmaceuticals and their trade started subsequently to adopt the OECD Principles of Good Laboratory Practice as the basis for safety testing in their industries. Later on OECD member countries decided that there was a need to review and update the Principles of GLP to account for scientific and technical progress in the field of safety testing.

Description of Regulations

The Good Laboratory Practices Regulations are divided into nine subparts, each containing several sections. The major subparts are listed below:

- Subpart A: General Provisions
- Subpart B: Organization and Personnel
- Subpart C: Facilities
- Subpart D: Equipment
- Subpart E: Testing facilities operation
- Subpart F: Test, Control and Reference Substances
- Subpart G: Protocols for and Conduct of Study
- Subpart H and I: Reserved
- Subpart J: Records and Reports
- Subpart K: (FDA only): Disqualification of Testing Facilities

General Provisions

The first subpart in each set is named as "General Provisions". In this subpart, in separate numbered paragraphs, the scope of the regulation is laid out, a number of definitions are listed and the applicability of the regulations to studies performed under grants and contracts is covered.

Organization and Personnel

This subpart state that each individual personnel engaged in the conduct of or responsible for the supervision of a non-clinical laboratory study must have education, training and experience or a combination thereof, to enable that individual to perform the assigned functions. According to this subpart each testing facility must maintain a current summary of training and experience and job description for each individual engaged in or supervising the conduct of a non-clinical laboratory study and a sufficient number of working personnel for the timely and proper conduct of the study according to the protocol. In addition this subpart also mention about the proper health and sanitation of working personnel as well as about their clothing to prevent microbiological, radiological or chemical contamination.

Facilities

The facilities for the study is described in subpart C. According to subpart C key points include: a) each testing facility must be of suitable size and construction to facilitate the proper conduct of non clinical laboratory studies, b) testing facility should be designed such a way that there is degree of separation which prevent any further function or activity from having an adverse effect on the study, c) testing facility for animals must have sufficient number of animal rooms to assure proper separation of species or test systems, isolation of individual projects and routine or specialized housing of animals, d) testing facility must have the proper disposal for all animal waste, e) facilities for handling test articles, control articles and reference substances, including receipt and storage, mixing and storage of prepared mixtures should be organized to prevent mix ups and to preserve the identity, strength, purity and stability of the articles and mixtures, etc.

Equipment

Subpart D represents the regulations for the equipment used in a study. It is mentioned that equipment should be adequately inspected, cleaned and maintained. Formal standard operating procedures must be on file for this and must set forth in sufficient detail the methods, materials and schedules to be used in the inspection, cleaning, maintenance, testing, calibration and standardization of equipment and should specify when appropriate remedial action to be taken in the of failure or malfunction of equipment.

Testing Facility Operations

Subpart E of GLP regulations addresses the operation aspects of the work. This subpart specifies the use and design of standard operating procedure (SOP) and labeling of reagents and solutions. Subpart E

also states following important points: a) the study director should authorize deviations from SOP, b) significant changes should be authorized by management, c) SOPs should be immediately available to personnel, d) historical data of SOPs should be maintained. All reagents and solutions must have proper labeling to indicate identity, titre or concentration, storage requirement and expiration date.

Test, Control and Reference Substances

Subpart F is covered all about test, control and reference substances. Basically this subpart covers all substances under investigation and all known substances used in the investigation in terms of their characterization, handling and mixing. It states that the identity, strength, purity, and composition or other characteristics, which appropriately define the test or control article, should be determined and documented for each batch. Each storage container for a test or control article should be labeled by name, chemical abstract number, or code number, batch number, expiration date, if any, and appropriate storage conditions necessary to maintain the identity, strength, purity, and composition of the test or control article.

In terms of handling the regulations state that procedures must be established to ensure for a) proper storage, b) avoidance of contamination or deterioration during handling, c) maintenance of proper identification throughout the study, d) documentation of receipt and distribution of each batch.

Protocols for and Conduct of a (Non- clinical Laboratory) Study

Subpart G is described the protocols for and Conduct of a (Non-clinical Laboratory) study. The term protocol in GLP is defined as an official written document that clearly indicates the objectives and all methods for the conduct of the study. The protocol should contain the following information: a) a descriptive title and statement of the purpose of the study, b) Identification of the test and control articles by name, chemical abstract number, or code number, c) A description of the experimental design, including the methods for the control bias, d) The date of approval of the protocol by the sponsor and the dated signature of the study director, etc. An approved protocol can be changed or revised, but the changes and revisions must be documented, signed by the director, dated and maintained with the original document. The non-clinical laboratory study should be conducted in accordance with the protocol.

Records and Reports

Subpart J deals with the records and reports generated by the study. A final report of non-clinical laboratory study should include the followings: names, dates, objectives, procedures, statistical methods, laboratory methods, test systems, dosages, data integrity issues, specific data handling procedures, data storage locations and the quality assurance unit statement. All raw data, documentation, protocols, final reports, and specimens generated as a result of a non-clinical laboratory study should be retained.

Disqualification of Testing Facilities

Disqualification of testing facilities is summarized in subpart K. the purposes of disqualification are: a) to permit the exclusion from consideration of completed studies that were conducted by a testing facility which has failed to comply with the requirements of the good laboratory practice regulations until it can be demonstrated that such non-compliance did not occur during or did not affect the validity or acceptability of data generated by, a particular study, b) to exclude from consideration all studies completed after the date of disqualification until the facility can satisfy the Commissioner that will conduct studies in compliance with such regulations. A study may be disqualified for the following reasons: The testing facility failed to comply with one or more of the regulations set forth in this part, The non-compliance adversely affected the validity of the non-clinical laboratory studies, etc.

If it is determined that a study was or would be essential the FDA shall also determine whether the study is acceptable, notwithstanding the disqualification of the facility. A testing facility that has been disqualified may be reinstated as an acceptable source of non-clinical laboratory studies to be submitted to the FDA if the Commissioner determines, upon an evaluation of the submission of the testing facility, that the facility can adequately assure that it will conduct future non-clinical laboratory studies in compliance with the good laboratory practice regulations set forth in this part.

References

OECD Principles Good Laboratory Practice. Directive 87/ 18/ EEC. Directive 88/ 320/ EEC.

Microbiology lab: Health & Safety (I)

A microbiology laboratory is a unique environment that requires special practices and containment facilities in order to properly protect persons working with microorganisms. Safety in the laboratory is the primary concern. The three main elements of safe containment of microorganisms are (1) good laboratory practices and technique, (2) safety equipment, and (3) facility design.

There is a certain element of risk in anything you do, but the potential risks in a microbiology course are greater. Persons who work in a microbiology lab may handle infectious agents in additional to other hazards such as chemicals and radioactive materials. There have been many documented cases of lab personnel acquiring diseases due to their work. About 20% of these cases have been attributed to a specific incident, while the rest have been attributed to work practices in the lab. It is possible that you can be exposed to potentially harmful microbes when you isolate bacteria from environmental materials. So, you should consider environmental samples potentially hazardous and use BSL2 containment practices (see below). If you are immunocompromised or immunosuppressed, then you may be at greater risk of acquiring infections in this class than most students and should carefully consider whether you should enroll in this course.

Microbiology Lab Practices and Safety Rules
1. Wash your hands with disinfectant soap when you arrive at the lab and again before you leave.
2. Absolutely no food, drinks, chewing gum, or smoking is allowed in the laboratory. Do not put anything in your mouth such as pencils, pens, labels, or fingers. Do not store food in areas where microorganisms are stored.
3. Purchase a lab coat and safety glasses, bring them to class, and use them. Alternatively, a long sleeved shirt that buttons or snaps closed is acceptable protective clothing. This garment must cover your arms and be able to be removed without pulling it over your head. Leave protective clothing in the lab and do not wear it to other non-lab areas.
4. Avoid loose fitting items of clothing. Wear appropriate shoes (sandals are not allowed) in the laboratory.
5. Keep your workspace free of all unnecessary materials. Backpacks, purses, and coats should be placed in the cubbyholes by the front door of the lab. Place needed items on the floor near your feet, but not in the aisle.
6. Disinfect work areas before and after use with 70% ethanol or fresh 10% bleach. Laboratory equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, and especially after spills, splashes, or other contamination.
7. Label everything clearly.
8. Replace caps on reagents, solution bottles, and bacterial cultures. Do not open Petri dishes in the lab unless absolutely necessary.
9. Inoculating loops and needles should be flame sterilized in a Bunsen burner before you lay them down.
10. Turn off Bunsen burners when not is use. Long hair must be restrained if Bunsen burners are in use.
11. When you flame sterilize with alcohol, be sure that you do not have any papers under you.
12. Treat all microorganisms as potential pathogens. Use appropriate care and do not take cultures out of the laboratory.
13. Wear disposable gloves when working with potentially infectious microbes or samples (e.g., sewage). If you are working with a sample that may contain a pathogen, then be extremely careful to use good bacteriological technique.
14. Sterilize equipment and materials.
15. Never pipette by mouth. Use a pipetting aid or adjustable volume pipettors. [In the distant past, some lab personnel were taught to mouth pipette. This practice has been known to result in many laboratory-acquired infections. With the availability of mechanical pipetting devices, mouth pipetting is strictly prohibited.]
16. Consider everything a biohazard. Do not pour anything down the sink. Autoclave liquids and broth cultures to sterilize them before discarding.
17. Dispose of all solid waste material in a biohazard bag and autoclave it before discarding in the regular trash.
18. Familiarize yourself with the location of safety equipment in the lab (e.g., eye-wash station, shower, sinks, fire extinguisher, biological safety cabinet, first aid kit, emergency gas valve).
19. Dispose of broken glass in the broken glass container.
20. Dispose of razor blades, syringe needles, and sharp metal objects in the "sharps" container.
21. Report spills and accidents immediately to your instructor. Clean small spills with care (see instructions below). Seek help for large spills.
22. Report all injuries or accidents immediately to the instructor, no matter how small they seem.

Laboratory Safety Equipment
Biological Safety Cabinet
A biological safety cabinet (BSC) is used as a primary barrier against exposure to infectious biological agents. A BSC has High Efficiency Particulate Air (HEPA) filters. The airflow in a BSC is laminar, i.e. the air moves with uniform velocity in one direction along parallel flow lines.

Depending on the design, a BSC may be vented to the outside or the air may be exhausted into the room. BSCs are not chemical fume hoods. A percentage of the air is recirculated in most types of BSCs. HEPA filters only trap particulates, allowing any contaminant in non-particulate form to pass through the filter.

Proper Use of BSCs:
1. Operate the cabinet for five minutes before and after performing any work in it in order to purge airborne contaminants.

2. Before and after use, wipe the surface of the BSC with a suitable disinfectant, e.g., 70% alcohol or a recommended disinfection solution.

3. Place everything you will need inside the cabinet before beginning work, including a waste container. You should not have to penetrate the air barrier of the cabinet once work has begun.

4. Do not place anything on the air intake grills, as this will block the air supply.

5. You should prevent unnecessary opening and closing of door because this will disrupt the airflow of the cabinet.

6. Always wear a lab coat while using the cabinet and conduct your work at least four inches inside the cabinet.

7. Place burners to the rear of the cabinet to reduce air turbulence.

8. Do not work in the BSC while the ultraviolet light is on. Ultraviolet light can quickly injure the eye.

9. When finished with your work procedure, decontaminate the surfaces of any equipment.

10. Remove the equipment from the cabinet and decontaminate the work surface.

11. Thoroughly wash your hands and arms.
    Eyewash and shower
    Fire Extinguisher
    First Aid Kit
    Emergency Gas Valve

Cleaning Small Spills

First, contact your instructor or the Biology Department Safety Officer. If it is a small spill of a low hazard microorganism or sample, then you should clean the spill yourself.

The proper procedures for cleaning small spills of microorganisms or samples (BSL1 and BSL2 levels):

1. Wear a lab coat, disposable gloves, safety glasses or a face shield, and if needed, approved respiratory equipment.

2. Soak a paper towel(s) in an appropriate disinfectant (70% ethanol or a recommended disinfection solution) and place around the spill area.

3. Working from the outer edges into the centre, clean the spill area with fresh towels soaked in the disinfectant. Be sure to decontaminate any areas or surfaces that you suspect may have been affected by the spill. Allow 10 minutes contact time.

4. Place the paper towels and gloves into a biohazard bag and autoclave these materials to sterilize them.

5. Dispose of any contaminated clothing properly.

6. Wash your hands with a disinfectant soap.

Each lab is equipped with a spill response kit.

Biosafety Levels and Practices

Biosafety levels are selected to provide the end-user with a description of the minimum containment required for handling different microorganisms safely in a laboratory setting and reduce or eliminate exposure to potentially hazardous agents. Containment refers to safe methods for managing infectious material in the laboratory environment. These biosafety levels are applicable to facilities such as diagnostic, research, clinical, teaching, and production facilities that are working at a laboratory scale. The four biosafety levels are described as:

Biosafety Level 1 (BSL1)

Examples of BSL1 Agents: *Bacillus subtilus*, *Naegleria gruberi*, many *Escherichia coli*, *Infectious Canine Hepatitis Virus*

BSL1 containment is suitable for work involving well-characterized agents not known to cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment.

A BSL1 lab requires no special design features beyond those suitable for a well-designed and functional laboratory. Biological safety cabinets (BSCs) are not required. Work may be done on an open bench top, and containment is achieved through the use of practices normally employed in a basic microbiology laboratory.

Biosafety Level 2 (BSL2)

Examples of BSL2 Agents: *Bacillus anthracis*, *Bordetella pertussis, Brucella* spp., *Cryptococcus neoformans*, *Clostridium botulinum, Clostridium tetani, Helicobacter pylori,* most *Salmonella* spp., *Yersinia pestis, Mycobacterium leprae*, *Shigella* spp., *Human Immunodeficiency Virus*, *Human blood*

The primary exposure hazards associated with organisms requiring BSL2 are through the ingestion, inoculation and mucous membrane route. Agents requiring BSL2 facilities are not generally transmitted by airborne routes, but care must be taken to avoid the generation of aerosols (aerosols can settle on bench tops and become an ingestion hazard through contamination of the hands) or splashes. Primary containment devices such as BSCs and centrifuges with sealed rotors or safety cups are to be used as well as appropriate personal protective equipment (i.e., gloves, laboratory coats, protective eyewear). As well, environmental contamination must be minimized by the use of hand washing sinks and decontamination facilities (autoclaves).

Biosafety Level 3 (BSL3)

Examples of BSL3 Agents: *Myobacterium tuberculosis, Salmonella typhi, Vesicular Stomatitis Virus*, *Yellow Fever Virus*, *Francisella tularensis*, *Coxiella burnetti*

Laboratory personnel have specific training in handling these pathogenic and potentially lethal agents and are supervised by scientists who are experienced in working with these agents. These agents may be transmitted by the airborne route, often have a low infectious dose to produce effects and can cause serious or life-threatening disease. BSL3 emphasizes additional primary and secondary barriers to minimize the release of infectious organisms into the immediate laboratory and the environment. Additional features to prevent transmission of BSL3 organisms are appropriate respiratory protection, HEPA filtration of exhausted laboratory air and strictly controlled laboratory access.

Biosafety Level 4 (BSL4)

Examples of BSL4 Agents: smallpox virus, Ebola virus, hemorrhagic fever viruses

This is the maximum containment available and is suitable for facilities manipulating agents that are dangerous/exotic agents, which post a risk of life threatening disease. These agents have the potential for aerosol transmission, often have a low infectious dose and produce very serious and often fatal disease; there is generally no treatment or vaccine available. This level of containment represents an isolated unit, functionally and, when necessary, structurally independent of other areas.

BSL4 emphasizes maximum containment of the infectious agent by complete sealing of the facility perimeter with confirmation by pressure decay testing; isolation of the researcher from the pathogen by his or her containment in a positive pressure suit or containment of the pathogen in a Class III BSC line; and decontamination of air and other effluents produced in the facility.

TO BE CONTINUED...

Microbiology lab: Health & Safety (II)

Safety checklist

This checklist is intended to assist in assessments of microbiological laboratory safety and security status of biomedical laboratories.

Laboratory premises

1. Have guidelines for commissioning and certification been considered for facility construction or post-construction evaluations?
2. Do the premises meet national and local building requirements, including those relating to natural disaster precautions if necessary?
3. Are the premises generally uncluttered and free from obstructions?
4. Are the premises clean?
5. Are there any structural defects in floors?
6. Are floors and stairs uniform and slip-resistant?
7. Is the working space adequate for safe operation?
8. Are the circulation spaces and corridors adequate for the movement of people and large equipment?
9. Are the benches, furniture and fittings in good condition?
10. Are bench surfaces resistant to solvents and corrosive chemicals?
11. Is there a hand-washing sink in each laboratory room?
12. Are the premises constructed and maintained to prevent entry and harbourage of rodents and arthropods?
13. Are all exposed steam and hot water pipes insulated or guarded to protect personnel?
14. Is an independent power support unit provided in case of power breakdown?
15. Can access to laboratory areas be restricted to authorized personnel?
16. Has a risk assessment been performed to ensure that appropriate equipment and facilities are available to support the work being considered?

Storage facilities

1. Are storage facilities, shelves, etc. arranged so that stores are secure against sliding, collapse or falls?
2. Are storage facilities kept free from accumulations of rubbish, unwanted materials and objects that present hazards from tripping, fire, explosion and harbourage of pests?
3. Are freezers and storage areas lockable?

Sanitation and staff facilities

1. Are the premises maintained in a clean, orderly and sanitary condition?
2. Is drinking-water available?
3. Are clean and adequate toilet (WC) and washing facilities provided separately for male and female staff?
4. Are hot and cold water, soap and towels provided?
5. Are separate changing rooms provided for male and female staff?
6. Is there accommodation (e.g. lockers) for street clothing for individual members of the staff?
7. Is there a staff room for lunch, etc.?
8. Are noise levels acceptable?
9. Is there an adequate organization for the collection and disposal of general household rubbish?

Heating and ventilation

1. Is there a comfortable working temperature?
2. Are blinds fitted to windows that are exposed to full sunlight?
3. Is the ventilation adequate, e.g. at least six changes of air per hour, especially in rooms that have mechanical ventilation?
4. Are there HEPA filters in the ventilation system?
5. Does mechanical ventilation compromise airflows in and around biological safety cabinets and fume cupboards?

Lighting

1. Is the general illumination adequate (e.g. 300–400 lx)?
2. Is task (local) lighting provided at work benches?
3. Are all areas well-lit, with no dark or ill-lit corners in rooms and corridors?
4. Are fluorescent lights parallel to the benches?
5. Are fluorescent lights colour-balanced?

Services

1. Is each laboratory room provided with enough sinks, water, electricity and gas outlets for safe working?
2. Is there an adequate inspection and maintenance programme for fuses, lights, cables, pipes, etc.?
3. Are faults corrected within a reasonable time?
4. Are internal engineering and maintenance services available, with skilled engineers and craftsmen who also have some knowledge of the nature of the work of the laboratory?
5. Is the access of engineering and maintenance personnel to various laboratory areas controlled and documented?
6. If no internal engineering and maintenance services are available, have local engineers and builders been contacted and familiarized with the equipment and work of the laboratory?
7. Are cleaning services available?
8. Is the access of cleaning personnel to various laboratory areas controlled and documented?
9. Are information technology services available and secured?

Laboratory biosecurity

1. Has a qualitative risk assessment been performed to define risks that a security system should protect against?
2. Have acceptable risks and incidence response planning parameters been defined?
3. Is the whole building securely locked when unoccupied?
4. Are doors and windows break-proof?
5. Are rooms containing hazardous materials and expensive equipment locked when unoccupied?
6. Is access to such rooms, equipment and materials appropriately controlled and documented?

Fire prevention and fire protection

1. Is there a fire alarm system?
2. Are the fire doors in good order?
3. Is the fire detection system in good working order and regularly tested?
4. Are fire alarm stations accessible?
5. Are all exits marked by proper, illuminated signs?
6. Is access to exits marked where the routes to them are not immediately visible?
7. Are all exits unobstructed by decorations, furniture and equipment, and unlocked when the building is occupied?
8. Is access to exits arranged so that it is not necessary to pass through a high-hazard area to escape?
9. Do all exits lead to an open space?
10. Are corridors, aisles and circulation areas clear and unobstructed for movement of staff and fire-fighting equipment?
11. Is all fire-fighting equipment and apparatus easily identified by

an appropriate colour code?

12. Are portable fire extinguishers maintained fully charged and in working order, and kept in designated places at all times?

13. Are laboratory rooms with potential fire hazards equipped with appropriate extinguishers and/or fire blankets for emergency use?

14. If flammable liquids and gases are used in any room, is the mechanical ventilation sufficient to remove vapours before they reach a hazardous concentration?

15. Are personnel trained to respond to fire emergencies?

Flammable liquid storage

1. Is the storage facility for bulk flammable liquids separated from the main building?

2. Is it clearly labelled as a fire-risk area?

3. Does it have a gravity or mechanical exhaust ventilation system that is separate from the main building system?

4. Are the switches for lighting sealed or placed outside the building?

5. Are the light fittings inside sealed to protect against ignition of vapours by sparking?

6. Are flammable liquids stored in proper, ventilated containers that are made of non-combustible materials?

7. Are the contents of all containers correctly described on the labels?

8. Are appropriate fire extinguishers and/or fire blankets placed outside but near to the flammable liquid store?

9. Are "No smoking" signs clearly displayed inside and outside the flammable liquid store?

10. Are only minimum amounts of flammable substances stored in laboratory rooms?

11. Are they stored in properly constructed flammable storage cabinets?

12. Are these cabinets adequately labelled with "Flammable liquid – Fire hazard" signs?

13. Are personnel trained to properly use and transport flammable liquids?

Compressed and liquefied gases

1. Is each portable gas container legibly marked with its contents and correctly colour coded?

2. Are compressed-gas cylinders and their high-pressure and reduction valves regularly inspected?

3. Are reduction valves regularly maintained?

4. Is a pressure-relief device connected when a cylinder is in use?

5. Are protection caps in place when cylinders are not in use or are being transported?

6. Are all compressed gas cylinders secured so that they cannot fall, especially in the event of natural disaster?

7. Are cylinders and liquid petroleum gas tanks kept away from sources of heat?

8. Are personnel trained to properly use and transport compressed and liquefied gases?

Electrical hazards

1. Are all new electrical installations and all replacements, modifications or repairs made and maintained in accordance with a national electrical safety code?

2. Does the interior wiring have an earthed/grounded conductor (i.e. a three-wire system)?

3. Are circuit-breakers and earth-fault interrupters fitted to all laboratory circuits?

4. Do all electrical appliances have testing laboratory approval?

5. Are the flexible connecting cables of all equipment as short as practicable, in good condition, and not frayed, damaged or

spliced?

6. Is each electric socket outlet used for only one appliance (no adapters to be used)?

Personal protection

1. Is protective clothing of approved design and fabric provided for all staff for normal work, e.g. gowns, coveralls, aprons, gloves?

2. Is additional protective clothing provided for work with hazardous chemicals and radioactive and carcinogenic substances, e.g. rubber aprons and gloves for chemicals and for dealing with spillages; heat-resistant gloves for unloading autoclaves and ovens?

3. Are safety glasses, goggles and shields (visors) provided?

4. Are there eye-wash stations?

5. Are there emergency showers (drench facilities)?

6. Is radiation protection in accordance with national and international standards, including provision of dosimeters?

7. Are respirators available, regularly cleaned, disinfected, inspected and stored in a clean and sanitary condition?

8. Are appropriate filters provided for the correct types of respirators, e.g. HEPA filters for microorganisms, appropriate filters for gases or particulates?

9. Are respirators fit-tested?

Health and safety of staff

1. Is there an occupational health service?

2. Are first-aid boxes provided at strategic locations?

3. Are qualified first-aiders available?

4. Are such first-aiders trained to deal with emergencies peculiar to the laboratory, e.g. contact with corrosive chemicals, accidental ingestion of poisons and infectious materials?

5. Are non-laboratory workers, e.g. domestic and clerical staff, instructed on the potential hazards of the laboratory and the material it handles?

6. Are notices prominently posted giving clear information about the location of first-aiders, telephone numbers of emergency services, etc.?

7. Are women of childbearing age warned of the consequences of work with certain microorganisms, carcinogens, mutagens and teratogens?

8. Are women of childbearing age told that if they are, or suspect that they are, pregnant they should inform the appropriate member of the medical/scientific staff so that alternative working arrangements may be made for them if necessary?

9. Is there an immunization programme relevant to the work of the laboratory?

10. Are skin tests and/or radiological facilities available for staff who work with tuberculous materials or other materials requiring such measures?

11. Are proper records maintained of illnesses and accidents?

12. Are warning and accident prevention signs used to minimize work hazards?

13. Are personnel trained to follow appropriate biosafety practices?

14. Are laboratory staff encouraged to report potential exposures?

Laboratory equipment

1. Is all equipment certified safe for use?

2. Are procedures available for decontaminating equipment prior to maintenance?

3. Are biological safety cabinets and fume cupboards regularly tested and serviced?

4. Are autoclaves and other pressure vessels regularly inspected?

5. Are centrifuge buckets and rotors regularly inspected?

6. Are HEPA filters regularly changed?

7. Are pipettes used instead of hypodermic needles?

8. Is cracked and chipped glassware always discarded and not reused?
9. Are there safe receptacles for broken glass?
10. Are plastics used instead of glass where feasible?
11. Are sharps disposal containers available and being used?

Infectious materials
1. Are specimens received in a safe condition?
2. Are records kept of incoming materials?
3. Are specimens unpacked in biological safety cabinets with care and attention to possible breakage and leakage?
4. Are gloves and other protective clothing worn for unpacking specimens?
5. Are personnel trained to ship infectious substances according to current national and/or international regulations?
6. Are work benches kept clean and tidy?
7. Are discarded infectious materials removed daily or more often and disposed of safely?
8. Are all members of the staff aware of procedures for dealing with breakage and spillage of cultures and infectious materials?
9. Is the performance of sterilizers checked by the appropriate chemical, physical and biological indicators?
10. Is there a procedure for decontaminating centrifuges regularly?
11. Are sealed buckets provided for centrifuges?
12. Are appropriate disinfectants being used? Are they used correctly?
13. Is there special training for staff who work in containment laboratories – Biosafety Level 3 and maximum containment laboratories – Biosafety Level 4?

Chemicals and radioactive substances
1. Are incompatible chemicals effectively separated when stored or handled?
2. Are all chemicals correctly labelled with names and warnings?
3. Are chemical hazard warning charts prominently displayed?
4. Are spill kits provided?
5. Are staff trained to deal with spills?
6. Are flammable substances correctly and safely stored in minimal amounts in approved cabinets?
7. Are bottle carriers provided?
8. Is a radiation protection officer or appropriate reference manual available for consultation?
9. Are staff appropriately trained to safely work with radioactive materials?
10. Are proper records of stocks and use of radioactive substances maintained?
11. Are radioactivity screens provided?
12. Are personal radiation exposures monitored?

References:
1. *Biosafety in Microbiological and Biomedical Laboratories, by* Centers for Disease Control (CDC) and the National Institutes of Health (NIH)
2. Laboratory biosafety manual: Third edition; World Health Organization, 2004

Isolation and Presumptive Identification of Bacterial Agents from Normally Sterile Sites

In general laboratories commonly receive blood samples or cerebrospinal fluid from patients with pneumonia, meningitis, or unexplained febrile illness. Laboratories may also receive urine, joint fluid, pleural fluid, or other sterile site specimens from these patients. This section of the laboratory manual provides methods for the isolation and presumptive identification of agents from these normally sterile sites. Pathogens included in this laboratory manual that could be isolated from normally sterile sites are *Haemophilus influenzae, Neisseria meningitidis, Salmonella* serotype Typhi, and *Streptococcus pneumoniae*. Personnel who are at risk for the routine exposure to aerosolized *N. meningitides* should strongly consider vaccination. The risk of infection when working in the laboratory with *H. influenzae* and *S. pneumoniae* is very low and it is not required that laboratorians receive vaccination against these organisms. However, least two good vaccines (oral and injection) are available for S. typhi, and laboratorians should ensure that their vaccination status remains current. After bacteria are recovered from normally sterile sites, the isolates require confirmatory identification; isolates received by a reference laboratory (*e.g.*, for antimicrobial susceptibility testing) must also undergo confirmatory testing.

Blood cultures
Laboratory personnel handling blood culture specimens must be able to identify culture bottles that may have bacterial growth, isolate bacteria on solid media, and subculture isolates. Provisional identification of an isolate will often be possible on the basis of colony morphology and the microscopic appearance of a Gram stained specimen.

Several variables affect the sensitivity of blood cultures: the number of collections, the volume of each collection, and the steps taken to inhibit or neutralize bactericidal properties of blood may vary with the age of the patient. As stated in the section on specimen collection, blood cultures from young children should be diluted to 1–2 ml of blood in 20 ml of broth (1:10 to 1:20), whereas blood cultures from adults should be diluted to 5–10 ml of blood in 50 ml of broth (1:5 to 1:10). Ideally, the blood samples should be processed in a bacteriology laboratory as soon as possible after collection (*i.e.*, within 2 hours).

Inoculation of primary culture media
Blood should be cultured in a tryptone-based soy broth (commonly referred to as "Trypticase" or "tryptic" soy broth [TSB]) or brain heart infusion with a supplement, such as haematin or sodium polyanetholesulfonate (SPS). If only one blood-culture bottle is used, it should contain TSB. Neutralization of normal bactericidal properties of blood and potential antimicrobial agents is accomplished by adding chemical inhibitors such as 0.025% SPS to culture media and by diluting the blood. SPS, which has anticoagulant, antiphagocytic, anti complementary, and antilysozymal activity, **may be inhibitory if used in higher concentrations**, but it is important to use. The blood-culture bottles should be inoculated directly with blood and should be vented before incubation at 35°– 37°C. Venting is accomplished by inserting a sterile cotton-plugged needle into the diaphragm (*i.e.*, rubber part) of the blood-culture bottle. Adding growth supplements, such as

IsoVitaleX or Vitox, to blood culture bottles to help support the growth of *H. influenzae* is appropriate; however, if resources are limited, a laboratory would benefit more by using this costly resource to supplement chocolate agar medium.

Identifying positive blood culture bottles
Blood-culture bottles should be examined at 14–17 hours and then every day for up to 7 days. Any turbidity or lysis of erythrocytes may be indicative of growth, and subcultures should be made immediately. Because *H. influenzae, N. meningitidis,* and *S. pneumoniae* are fragile organisms, **subcultures should be routinely performed after 14–17 hours of incubation, again at 48 hours, and again at day 7, regardless of the appearance of the blood-culture bottles** because the absence of turbidity does not always correlate with the absence of bacterial growth. Before sub culturing, swirls the bottle to mix the contents.

Subculture
Subcultures are made by first disinfecting the surface of the blood-culture bottle diaphragm with alcohol and a povidone-iodine swab, and then aspirating a small volume (*i.e.*, 0.5 ml) with a syringe and needle from the blood-culture bottle and inoculating the agar media with the fluid. If the bottle has a screw-cap, open the bottle and take the fluid using sterile technique (*i.e.*, flaming the bottle mouth upon opening and closing the cap). Ordinarily, both chocolate agar plates and blood agar plates are used for subculture. **When only one agar plate is used, it should be chocolate agar, because chocolate agar contains the X and V growth factors needed for *H.influenzae,* whereas blood agar does not.** If a blood specimen is received from a patient with a primary diagnosis of fever of unknown origin, if typhoid is suspected symptomatically, or if a Gram stain of blood-culture broth reveals gram negative bacilli, add a total of 3–4 loopfuls of the blood culture onto MacConkey agar (MAC) in addition to chocolate agar and/or blood agar. Incubate the media with suspect pathogens at 35°–37°C in a 5% CO_2 atmosphere (incubator or candle-extinction jar). Because *N. meningitidis* grows well in a humid atmosphere, if an infection with *N. meningitidis* is suspected, laboratories may choose to add a shallow pan of water to the bottom of the incubator or add a dampened paper towel to the candle-extinction jar; the moisture source should be changed regularly (*e.g.*, daily) to prevent contamination with molds. If the laboratory has the resources to support the use of a third plate for subculture, MacConkey agar should be used, particularly when the specimen was obtained from a patient with fever of unknown origin (when typhoid fever [*S. typhi*] or blood stream infection by gram-negative rods of other species [*e.g.*, *E. coli, Klebsiella,* etc.] may be suspected). Chocolate agar should be periodically confirmed to support growth of *H. influenzae*. The agar plates should be streaked, and incubated for up to 48 hours. The MAC and blood plates for *S. typhi* should be incubated for 18–24 hours at 35°–37°C. When bacterial growth has been confirmed by subculture of the blood-culture bottle, the bottle no longer requires incubation. The bottle should be disposed of according to safety procedures.

Presumptive identification of isolates from sterile-site specimens
Because the primary purpose of this section of the manual is to aid in the identification of *N. meningitidis, S. pneumoniae, H. influenzae,*

and *S. typhi* from sterile-site specimens, the methods described here will not apply to the identification of other bacterial agents (of pneumonia and meningitis) of clinical importance that are more rarely encountered. Microbiologists should refer to clinical microbiology manuals (*e.g.*, the American Society for Microbiology's *Manual of Clinical Microbiology,* the WHO's *Manual for the Laboratory Investigations of Acute Enteric Infections,* the *Clinical Microbiology Procedures Manual, Basic Laboratory Procedures in Clinical Microbiology* [WHO 2001] or a medical microbiology manual or textbook for procedures to identify other bacteria. Presumptive identification of *N. meningitidis, H. influenzae,* and *S. pneumoniae* can be made on the basis of the growth on blood agar and chocolate agar and on the basis of the microscopic morphology of the organisms.

N. meningitidis grows on blood agar, whereas *H. influenzae* will not grow without supplements (found in chocolate agar). When grown on chocolate agar, *H. influenzae* and *N. meningitidis* look similar; the two organisms can be distinguished on the agar plate by the pungent smell of indole from *H. influenzae.*

The following procedures should be followed to prepare a dried smear for Gram stain of pure culture.

a) Place one drop of physiological saline or distilled water on an alcohol-rinsed and dried slide.

b) With a flamed and cooled, sterile inoculating needle or loop, touch the center of the bacterial colony.

c) Prepare a smear from the colony by adding the bacteria from the inoculating loop to the physiological saline or distilled water drop with a gentle tap. Use the loop to mix the organisms into suspension.

d) Spread the suspension and allow it to dry, either by air (approximately ten minutes) or incubator.

Upon microscopic examination, organisms that are gram-positive will appear violet, while gram-negative organisms will appear pink. The staining further enables the laboratorian to see morphology of the bacteria.

Presumptive identification of *H. influenzae*
H. influenzae appears as large, flat, colorless-to-grey opaque colonies on chocolate agar. No hemolysis or discoloration of the medium is apparent. Encapsulated strains appear more mucoidal than non-encapsulated strains, which appear as compact greyish colonies. Gram staining will yield small, gram-negative bacilli or coccobacilli.

Presumptive identification of *N. meningitidis*
On blood agar plates, young colonies of *N. meningitidis* are round, smooth, moist, glistening and convex, with a clearly defined edge. Some colonies appear to coalesce with other nearby colonies. Growth of *N. meningitidis* on blood agar is greyish and unpigmented; older cultures become more opaquely grey and sometimes cause the underlying agar to turn dark. Well-separated colonies can grow from about 1 mm in diameter in 18 hours to as large as 4 mm, with a somewhat undulating edge, after several days. Gram staining will yield a gram negative, coffee-bean-shaped diplococcus.

Presumptive identification of *S. pneumoniae*
S. pneumoniae appears as small, greyish, moist (sometimes mucoid), watery colonies with a greenish zone of hemolysis surrounding them on blood agar and chocolate agar. The degree of mucoidness of *S. pneumonia* colonies is dependent on the freshness of the medium and the incubation atmosphere. Some serotypes appear more mucoid than others, and the fresher the medium, the more mucoid the cultures appear.

Young pneumococcal colonies appear raised, similar to viridans streptococci. Differentiating pneumococci from viridans streptococci on chocolate agar is difficult. However, a hand lens or microscope (30X-50X) is a useful aid in differentiating pneumococci from hemolytic viridans streptococci, which also produce a greenish zone of hemolysis on a blood- or chocolate agar plate. However, as the culture ages 24-48 hours, the colonies become flattened and the central part of each colony becomes depressed. This does not occur with the viridans streptococci.

Another type of colony that might appear on the culture plate along with *S. pneumoniae* is *Staphylococcus aureus* (or another *Staphylococcus* species). Two types of colonies will grow on the 5% sheep blood trypticase soy agar medium: the dull gray flat colony surrounded by a greenish zone of hemolysis will be *S. pneumoniae* and the yellowish colony with no hemolytic action will be *S. aureus.* Gram staining of *S. pneumoniae* will reveal a gram positive diplococci or chain of cocci.

Presumptive identification of *Salmonella* ser. Typhi
Salmonella ser. Typhi grows on both blood agar and chocolate agar; on these media, *S. typhi* colonies are grayish, transparent to opaque, glistening (shiny) and usually >1 mm in diameter. On MacConkey agar (MAC), *S. typhi* colonies appear as colorless non fermenters. (Colonies of S. Paratyphi A, S. Paratyphi B, and S. Paratyphi C and most other *Salmonella* serotypes look similar to those of *S. typhi* on these media.) Gram staining of *Salmonella* serotypes will reveal gram-negative bacilli.

Presumptive identification of *Haemophilus influenzae, Neisseria meningitidis,* and *Streptococcus pneumonia*

Growth on		Gram stain morphology	Presumptive identification
Chocolate agar	Sheep blood agar		
+	+	gram-negative diplococci	*N. meningitidis*
+	+	gram-positive cocci or diplococci	*S. pneumoniae*
+	-	small, gram-negative pleomorphic coccobacilli	*H. influenzae*

References
(1) www.who.ntm/drugresistance/Publications   (2) www.ncbi.nlm.nih.gov

Preventing MRSA infection

What is MRSA? When *Staphylococcus aureus* develops reduced susceptibility to the Beta-lactam class of antibiotics including methicillin and other more common antibiotics such as oxacillin, penicillin and amoxicillin it is known as methicillin-resistant *Staphylococcus aureus* (MRSA).

The number of deaths from MRSA in the U.S. are more than from fatalities of those who die every year from AIDS.

When not treated properly, MRSA infections can wind up to be fatal.

WHAT MRSA LOOKS LIKE

What does a staph or MRSA infection look like?

Staph bacteria, including MRSA, can cause skin infections that may look like a pimple or boil and can be red, swollen, painful, or have pus or other drainage. More serious infections may cause pneumonia, bloodstream infections, or surgical wound infections. Most staph infections, including MRSA, will grow as a bump or infected area on the skin. You should look for skin that is:

Red
Swollen
Painful
Warm to the touch
Full of pus or other drainage
Accompanied by a fever

How MRSA Spreads?

The single most important mode of transmission of MRSA in a health care setting is via transiently colonized hands of health care workers who acquire it from contact with colonized or infected clients/patients/residents, or after handling contaminated material or equipment. The unrecognized colonized client/patient/resident presents a particular risk for transmission to other clients/patients/residents. The number of colonized clients/patients/residents ("colonization pressure") will also influence the likelihood of acquiring MRSA

The best defense against spreading MRSA is to practice good hygiene, as follows:

- Keep your hands clean by washing thoroughly with soap and water. Scrub them briskly for at least 15 seconds, then dry them with a disposable towel and use another towel to turn off the faucet. When you don't have access to soap and water, carry a small bottle of hand sanitizer containing at least 62 percent alcohol.
- Always shower promptly after exercising.
- Keep cuts and scrapes clean and covered with a bandage until healed. Keep wounds that are draining or have pus covered with clean, dry bandages. Follow your healthcare provider's instructions on proper care of the wound. Pus from infected wounds can contain S. aureus and MRSA, so keeping the infection covered will help prevent the spread to others. Bandages or tape can be discarded with regular trash.
- Avoid contact with other people's wounds or bandages.
- Avoid sharing personal items, such as towels, washcloths, razors, clothes, or uniforms.
- Wash sheets, towels, and clothes that become soiled with water and laundry detergent; use bleach and hot water if possible. Drying clothes in a hot dryer, rather than air-drying, also helps kill bacteria in clothes.
- Healthcare providers are fighting back against MRSA infection by tracking bacterial outbreaks and by investing in products, such as antibiotic-coated catheters and gloves that release disinfectants.
- Follow the clothing label's instructions for washing and drying. Drying clothes completely in a dryer is preferred.
- Hands should be cleaned before and after playing sports and activities such as using shared weight-training equipment, when caring for wounds including changing bandages, and after using the toilet.
- Both plain and antimicrobial soap are effective for hand washing, but liquid soap is preferred over bar soap in these settings to limit sharing.
- If hands are not visibly dirty and sinks are not available for hand washing, for example, while on the field of play or in the weight-room, alcohol based hand rubs and sanitizers can be used.
- Do not share ointments that are applied by placing your hands into an open-container.
- Use a barrier (such as clothing or a towel) between your skin and shared equipment like weight-training, sauna and steam-room benches.
- Cleaning with detergent-based cleaners or Environmental Protection Agency (EPA) -registered detergents/disinfectants will remove MRSA from surfaces.
- Athletes with active infections or open wounds should not use whirlpools or therapy pools not cleaned between athletes and other common-use water facilities like swimming pools until infections and wounds are healed
- Keep your fingernails short and clean, because bacteria can grow under long nails.
- Don't share any products that come into contact with your skin, such as soaps, lotions, creams and cosmetics.
- Clean high touch areas (e.g., taps, light switches, doorknobs) at least daily and when soiled. Sufficient quantity of detergent-disinfectant in the correct concentration, applied with

a clean cloth, is essential for an effective cleaning process. Comply with contact time on manufacturer's label and workplace safety requirements. Facilities should establish standards and cleaning frequencies. The home environment

should be regularly (when visibly soiled) cleaned with a standard household detergent. Deposit laundry into hamper – avoid touching outside areas. Garbage contained and not leaking. Regular dishes and utensils may be used – wash dishes in hot soapy water or correctly functioning dishwasher.

Hospital staff

Hospital staff who come into contact with patients should maintain high standards of hygiene and take extra care when treating patients with MRSA.

- Staff should thoroughly wash their hands before and after caring for a patient, before and after touching any potentially contaminated equipment or dressings, after bed making and before handling food.
- Hands can be washed with soap and water or, if they are not visibly dirty, a fast-acting antiseptic solution like a hand wipe or hand gel.
- Disposable gloves should be worn when staff have physical contact with open wounds – for example, when changing dressings, handling needles or inserting an intravenous drip. Hands should be washed after gloves are removed.
- The hospital environment, including floors, toilets and beds, should be kept as clean and dry as possible.
- Patients with a known or suspected MRSA infection should be isolated.

Which disinfectants should I use against MRSA?

Read the label first. Each cleaner and disinfectant has instructions on the label that tell you important facts.

Disinfectants effective against Staphylococcus aureus or staph are most likely also effective against MRSA. These products are readily available from grocery stores and other retail stores. Check the disinfectant product's label on the back of the container. Most, if not all, disinfectant manufacturers will provide a list of germs on their label that their product can destroy. Use disinfectants that are registered by the EPA (check for an EPA registration number on the product's label to confirm that it is registered).

How should cleaners and disinfectants be used?

Read the label first. Each cleaner and disinfectant has instructions on the label that tell you important facts:

- How to apply the product to a surface.
- How long you need to leave it on the surface to be effective (contact time).
- If the surface needs to be cleaned first and rinsed after using.
- If the disinfectant is safe for the surface.
- Whether the product requires dilution with water before use.
- Precautions you should take when applying the product, such as wearing gloves or aprons or making sure you have good ventilation during application.

Laundry

- Routine laundry procedures, detergents, and laundry additives will all help to make clothes, towels, and linens safe to wear or touch. If items have been contaminated by infectious material, these may be laundered separately
- Facility Cleaning & Disinfection after a MRSA Infection
- When MRSA skin infections occur, cleaning and disinfection should be performed on surfaces that are likely to contact uncovered or poorly covered infections.
- Cleaning surfaces with detergent-based cleaners or Environmental Protection Agency (EPA)-registered disinfectants is effective at removing MRSA from the environment.
- It is important to read the instruction labels on all cleaners to make sure they are used safely and appropriately.
- Environmental cleaners and disinfectants should not be used to treat infections.

Surfaces to Clean

- Focus on surfaces that touch people's bare skin each day and any surfaces that could come into contact with uncovered infections. For example, surfaces such as benches in a weight room or locker room.
- Large surfaces such as floors and walls have not been directly associated in the spread of staph and MRSA.
- There is no evidence that spraying or fogging rooms or surfaces with disinfectants will prevent MRSA infections more effectively than the targeted approach of cleaning frequently touched surfaces and any surfaces that have been exposed to infections.

Shared Equipment

Shared equipment that comes into direct skin contact should be cleaned after each use and allowed to dry. Equipment, such as helmets and protective gear, should be cleaned according to the equipment manufacturers' instructions to make sure the cleaner will not harm the item.

Cleaning Keyboards and other Difficult Surfaces

Many items such as computer keyboards or handheld electronic devices may be difficult to clean or disinfect or they could be damaged if they became wet. If these items are touched by many people during the course of the day, a cleanable cover/skin could be used on the item to allow for cleaning while protecting the item. Always check to see if the manufacturer has instructions for cleaning.

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Food, Hygiene, and Microbiology

Food microbiology is an important field in sciences which requires a lot of attention, since what one consumes should not only be healthy and nutritious but also hygienic and free from pathogens.

Food Hygiene

Food contamination projects a major health risk to communities and is a leading cause of disease outbreaks and transmission. Food that is kept too long can go bad and often contains toxic chemicals or pathogens, and food-stuffs that are eaten raw, such as fruits or vegetables, can become contaminated by dirty hands, unclean water or flies and other such vectors. Improperly prepared food can also cause chemical poisoning. Half cooked or over cooked food for example hard boiled eggs and over cooked meat are bad for health.

To promote good health, therefore, food should be properly prepared and stored.

Food Preparation at Home

Families must understand the principles of basic hygiene and know how to prepare food safely:

- Before preparing food, hands should be washed with soap or ash.
- Raw fruit and vegetables should not be eaten unless they are first peeled or washed with clean water.
- It is also important to cook food properly, particularly meat. Both cattle and pigs host tapeworms that can be transferred to humans through improperly cooked meat; for this reason, raw meat should never be eaten.
- Eggs, too, must be cooked properly before eating, since they may contain salmonella, a virulent pathogen.
- The kitchen itself should be kept clean and waste food disposed off carefully to avoid attracting vermin, such as rats and mice that may transmit diseases.
- Keeping food preparation surfaces clean is critical, because harmful organisms can grow on these surfaces and contaminate food.
- Fresh meat should be cooked and eaten on the same day, unless it can be stored in a refrigerator; if not, it should be thrown away immediately.
- Cooked food should be eaten while it is still hot and should not be left to stand at room temperature for long periods of time, since this provides a good environment for pathogens to grow.
- Food that is ready to eat should be covered to keep off flies and should be thrown away if not eaten within 12 – 16 hours.
- If food must be stored after cooking, it should be kept covered and in a cool place, such as a refrigerator and if a refrigerator is not available, food can be stored on ice blocks or in a preservative such as pickling vinegar or salt.
- Food that is already prepared, or food that is to be eaten raw, must not come into contact with raw meat as this may contain pathogens that can contaminate the other foods (particularly if slaughtering was not carried out hygienically).

Hotels and Restaurant

In many urban centers food is bought and consumed at eating-houses (cafes, restaurants or canteens). If basic health and safety rules for storing, preparing and handling food are not followed in the eating-houses; these places will represent a health hazard for the customers and may cause serious disease outbreaks. The most important aspects of food hygiene in these establishments relate to sanitation, water supply and personal cleanliness:

- Eating-houses should have clean water for washing and drinking, and separate sanitation facilities, away from the kitchen area, for customers, cooks and food-handlers.
- The staff should have clean uniforms each day and have regular medical check-ups.
- The cooks, chefs and waiters should be the most particular in their own personal hygiene so as to prevent any contamination of food handled by them.
- Food to be served should be prepared fresh always and any that is spilled or not used should be disposed off.
- The kitchens and eating areas must be kept clean and free of vermin and insects.
- Eating-houses should also be well-ventilated, with adequate lighting.

Street Food Vendors

Street food-vendors are common in urban and peri-urban areas, but they also operate in rural areas, particularly if there is a market or community fair. Although people enjoy food from these vendors, in many cases the food is of poor quality and it represents a serious health risk. In part, this is because the street vendors have little or no access to safe water supplies or sanitation facilities, and they commonly cook and handle food with dirty hands. Raw foodstuffs, too, cannot be kept in safe storage places and are easily contaminated by vermin and insects. Moreover, the street vendors often keep cooked food at ambient (environmental) temperatures for prolonged periods of time and may heat the food only slightly before serving. All these factors may make the food from street vendors dangerous.

What causes food poisoning?

Food poisoning is usually caused by micro-organisms (germs), including bacteria, viruses and molds. The spread of these germs can be prevented by practicing good food hygiene.

The most serious types of food poisoning are caused by bacteria. Bacteria multiply best in a moist environment between 5°C and 63°C. Just a single bacterium on an item of food, left out of the fridge overnight, could generate many millions of bacteria by the morning, enough to make you ill if eaten. Storing food below 5°C prevents bacteria from multiplying, and cooking food at temperatures over 70°C will kill off any existing bacteria.

Food poisoning and other food borne hazards

The term 'food poisoning' is commonly used to cover a wide variety of illnesses or clinical conditions affecting the gastrointestinal tract. The very large majority of such illnesses found in developed countries result from the consumption of contaminated food or drink, and because they are caused by infection with or the presence of bacteria, these organisms will receive the greatest attention here. However, it is necessary to consider, albeit more briefly, other forms of food poisoning and food-borne hazards since these may sometimes be of concern and pose serious health hazards in other parts of the world.

Bacterial food poisoning

It may be helpful to distinguish between bacterial food poisoning and food-borne bacterial infections. In the former the causative organism multiplies in the food and by its heavy growth induces illness by one mess or another after ingestion of the contaminated food. In food-borne infections the food merely acts as a carrier for the causative organism which does not require to multiply in the food.

Food poisoning is characterized by an acute gastroenteritis (inflammation of the lining of the alimentary canal) following ingestion of food in which multiplication of bacteria has taken place,

the ingested viable bacteria continuing to grow within the host's body to produce the typical symptoms. Salmonellas are principally responsible for this type of food poisoning in which toxins are released as the bacterial cells disintegrate. This toxin type (often termed an intoxication) is a genuine toxin having been produced by the bacteria which have grown in the food prior to consumption. The toxin also causes an acute gastroenteritis but ingestion of viable bacteria is usually not a prerequisite of the induction of the disease. Bacteria causing toxin food poisoning include *Clostridium perfringens* and *Staphylococcus aureus*.

Improving Hygiene

- Maintaining high levels of personal and kitchen hygiene are important and effective ways to stop germs from spreading.
- Wash your hands and nails with hot, soapy water before handling food, between handling cooked and uncooked foods, and after going to the toilet.
- Rinse your hands well and dry them on a clean hand towel, a disposable paper towel, or under a hand dryer. Wet hands transfer germs more effectively than dry hands.
- Use different cloths for different jobs (eg washing up and cleaning surfaces). Wash them regularly on the hot cycle or soak in a dilute solution of bleach.
- Wipe down and disinfect surfaces and utensils regularly, using a detergent or dilute solution of bleach - always read the safety instructions first.
- Wash up using hot, soapy water - use rubber gloves if necessary.
- Don't handle food if you have stomach problems such as diarrhea and vomiting, or if you're sneezing or coughing frequently.
- Cover up cuts and sores with waterproof plasters.
- If possible, remove rings, watches and bracelets before handling food. Germs can hide under these.

Bacteria can spread from raw food, in particular meat, to food that has already been cooked or is eaten raw, such as salads.

- Use separate chopping boards for preparing raw meat, poultry and seafood and for fresh produce such as salads, fruits and vegetables.
- Never use a marinade that has already been used on raw meat for cooked food, unless it has been boiled thoroughly.
- Always use a clean plate to serve food.
- After using a knife or other utensil on raw meat, clean it thoroughly before using it on other foods.

Storing food correctly

- It's very important that food is stored in the right place (eg fridge or freezer) and at the correct temperature.
- Always check labels for guidance on where and how long to store food, in particular, fresh or frozen food.
- Store fresh or frozen food in the fridge or freezer within two hours of purchase - sooner if the weather is hot.
- Allow meal leftovers to cool to room temperature before storing them in the fridge, ideally within two hours of preparation. If necessary, divide leftovers into smaller portions to help food cool more quickly.
- Use up leftovers within two days. Cooked rice should only be kept for one day.
- Store raw food such as meat in airtight containers at the bottom of the fridge to prevent juices or blood from dripping onto other food.
- Defrost frozen foods in the fridge. Place them on a plate or in a container as they defrost so they don't drip on or contaminate other foods.
- Don't overfill the fridge - food may not cool properly.
- Keep the fridge at less than 5°C and the freezer at less than -18°C - consider getting a thermometer.

- Don't store opened tins of food in the fridge - transfer the contents to a suitable airtight container instead.

Cooking Food Safely

If food isn't cooked at a high enough temperature, bacteria can still survive. The following advice will help you to cook safely.

- Follow the recipe or packet instructions for cooking time and temperature, ensuring the oven is pre-heated properly.
- Food should be piping hot (steaming) before serving.
- Take special care that pork, sausages, burgers and poultry are cooked thoroughly and aren't pink in the middle. Using a clean skewer, pierce the meat. When cooked properly, the juices run clear. Lamb and beef joints and steaks can be cooked rare, but must be thoroughly sealed (browned) on the outside.
- When microwaving, stir food well from time to time to ensure even cooking.
- Only reheat food once and serve piping hot.
- Eggs contain harmful bacteria which can be dangerous to pregnant women, older people and babies. Don't serve eggs with runny yolks, or egg-containing foods that won't be cooked, for example homemade mayonnaise.

Eating Out

When eating out, it's also important to consider food hygiene. You can't usually inspect the kitchens in restaurants, cafés or pubs, but there are certain warning signs of poor hygiene standards that you can look out for:

- Unhygienic personnel, dirty dining areas, toilets, cutlery or crockery
- Rubbish and overflowing bins outside - these could attract vermin
- Hair or insects in food
- Raw food and ready to eat food displayed together
- Hot food that isn't cooked properly and cold food that is served lukewarm

Promoting nutrition

A healthy and well-balanced diet is essential for good health. Undernourishment and malnourishment can lower resistance and make individuals/kids more likely to suffer from infectious diseases. It is important to make children's food less spicy than adult food, because their stomachs are small, children can eat only small portions and need to be fed more frequently than healthy adults. It is also important that children are fed not just foods high in starch or carbohydrate (for instance rice). Although these foods can quickly make a child feel full, he or she may become malnourished if other key foodstuffs are not eaten.

A well-balanced diet usually has a mixture of food:

- Proteins (for example beans, peas, meat, fish or eggs).
- Carbohydrates (such as maize, potatoes, cassava, rice and many other staple foods).
- Vitamins (such as vegetables, fish, fruits or milk).
- Fats or oils (such as cooking oil).

Sometimes not all these foods are available and it is important that community members ask health workers how to make best use of available foods for a balanced diet. In many situations, nutrition can be improved by changing agricultural or gardening practices. Often, even small plots of land can provide nutritious food provided that the right crops are grown. Health workers or agricultural extension workers can be asked for advice about which crops to grow to provide community members with well-balanced diets.

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Blood Agar, Hemolysis, and Hemolytic Reactions

Blood agar is a nutrient culture medium that is enriched with whole blood and used for the growth of certain strains of bacteria.

In 1919, Brown experimented with blood agar formulations for the effects of colony formation and hemolysis. Blood Agar Base media are specified in standard method procedures for food testing.

Blood agar consists of a basal medium such as TSA (Tryptone Soya Agar) enriched with 5% defibrinated sheep blood or in some locations, horse blood. This is the most commonly used medium, and supports the growth of most of the common fastidious organisms, as well as, all of the less fastidious organisms.

Blood agar is a solid growth medium that contains red blood cells. The medium is used to detect bacteria that produce enzymes to break apart the blood cells. This process is also termed hemolysis. The degree to which the blood cells are hemolyzed is used to distinguish bacteria from one another.

The blood agar medium is prepared in a two-step process. First, a number of ingredients are added to water, including heart infusion, peptone, and sodium chloride. This solution is sterilized. Following sterilization , a known amount of sterile blood is added. The blood can be from rabbit or sheep. Rabbit blood is preferred if the target bacterium is from the group known as group A *Streptococcus*. Sheep blood is preferred if the target bacterium is *Haemophilus parahaemolyticus*.

Blood agar is a rich food source for bacteria. So, it can be used for primary culturing, that is, as a means of obtaining as wide a range of bacterial growth from a sample as possible. It is typically not used for this purpose, however, due to the expense of the medium. Other, less expensive agars will do the same thing. What blood agar is uniquely suited for is the determination of hemolysis.

Hemolysis is the break down of the membrane of red blood cells by a bacterial protein known as hemolysin, which causes the release of hemoglobin from the red blood cell. Many types of bacterial posses hemolytic proteins. These proteins are thought to act by integrating into the membrane of the red blood cell and either punching a hole through the membrane or disrupting the structure of the membrane in some other way. The exact molecular details of hemolysin action is still unresolved.

The blood used in the agar is also treated beforehand to remove a molecule called fibrin, which participates in the clotting of blood. The absence of fibrin ensures that clotting of the blood does not occur in the agar, which could interfere with the visual detection of the hemolytic reactions.

When streaked on Blood Agar, many species of bacteria cause hemolysis – i.e., destruction of the erythrocytes (and hemoglobin) in the medium. Hemolytic reactions are generally classified as alpha, beta or gamma according to the appearance of zones around isolated colonies growing on or in the medium:

Alpha hemolysis: The colony is surrounded by a zone of intact but discolored erythrocytes that have a greenish color. This appearance is generally due to the action of peroxide produced by the bacteria. This type of hemolysis represents a partial decomposition of the hemoglobin of the red blood cells. Alpha hemolysis is characteristic of *Streptococcus pneumonia* and so can be used as a diagnostic feature in the identification of the bacterial strain.

When Alpha hemolysis (α-hemolysis) is present the agar under the colonies is dark and greenish. *Streptococcus pneumoniae and a group of oral streptococci (Streptococcus or viridans streptococci) display alpha hemolysis. This is sometimes called green hemolysis* because of the color change in the agar. Other synonymous terms are *incomplete hemolysis* and *partial hemolysis*. Alpha hemolysis is caused by hydrogen peroxide produced by the bacterium, oxidizing hemoglobin to green methemoglobin .

Streptococcus is a genus of spherical Gram-positive bacteria belonging to the phylum Firmicutes and the lactic acid bacteria group. Most *streptococci* are oxidase- and catalase-negative, and many are facultative anaerobes. In the medical setting, the most important groups are the alpha-hemolytic streptococci, *S. pneumoniae* and *Streptococcus Viridans*-group.

Beta hemolysis: The colony is surrounded by a white or clear zone in which few or no intact erythrocytes are found. This reaction is best seen when the organism is growing under anaerobic conditions. Beta hemolysis is caused by one or more erythrocyte-lysing enzymes called hemolysins.

Beta hemolysis represents a complete breakdown of the hemoglobin of the red blood cells in the vicinity of a bacterial colony. There is a clearing of the agar around a colony. Beta hemolysis is characteristic of *Streptococcus pyogenes* and some strains of *Staphylococcus aureus*.

Beta hemolysis (β-hemolysis), sometimes called *complete hemolysis*, is a complete lysis of red cells in the media around and under the colonies: the area appears lightened (yellow) and transparent. Streptolysin, an exotoxin, is the enzyme produced by the bacteria which causes the complete lysis of red blood cells. There are two types of streptolysin: Streptolysin O (SLO) and streptolysis S (SLS). Streptolysin O is an oxygen-sensitive cytotoxin, secreted by most Group A streptococcus (GAS), and interacts with cholesterol in the membrane of eukaryotic cells (mainly red and white blood cells, macrophages, and platelets), and usually results in β-hemolysis under the surface of blood agar. Streptolysin S is an oxygen-stable cytotoxin also produced by most GAS strains which results in clearing on the surface of blood agar. SLS affects immune cells, including polymorphonuclear leukocytes and lymphocytes, and is thought to prevent the host immune system from clearing infection. *Streptococcus pyogenes , or Group A beta-hemolytic Strep (GAS), displays beta hemolysis.*

Some weakly beta-hemolytic species cause intense beta hemolysis when grown together with a strain of Staphylococcus . *This is called the CAMP test . Streptococcus agalactiae displays this property. Clostridium perfringens can be identified presumptively with this test.*

The most important groups of beta-hemolytic streptococci are Lancefield groups A and B (also known as "Group A strep" and "Group B strep"). Beta-hemolytic streptococci are further characterized via the Lancefield serotyping – based on specific carbohydrates in the bacterial cell wall.

Group A

S. pyogenes, also known as Group A Streptococcus (GAS), is the causative agent in Group A streptococcal infections, including streptococcal pharyngitis ("strep throat" AmE), acute rheumatic fever, scarlet fever, acute glomerulonephritis and necrotizing

fasciitis. Other *Streptococcus* species may also possess the Group A antigen, but human infections by non-S. pyogenes GAS strains (some S. dysgalactiae subsp. equisimilis and S. anginosus Group strains) appear to be uncommon.

Group A Streptococcus infection is generally diagnosed with a Rapid Strep Test (AmE) or by culture. Rheumatic fever, a disease that affects the joints and heart valves, is a consequence of untreated strep A infection caused not by the bacterium itself. Rheumatic fever is caused by the antibodies created by the immune system to fight off the infection cross-reacting with other proteins in the body. This cross-reaction causes the body to essentially attack itself and leads to the damage above.

Group B

S. agalactiae, or GBS, causes pneumonia and meningitis in neonates and the elderly, with occasional systemic bacteremia. They can also colonize the intestines and the female reproductive tract, increasing the risk for premature rupture of membranes and transmission to the infant. The American College of Obstetricians and Gynecologists, American Academy of Pediatrics and the Centers for Disease Control recommend all pregnant women between 35 and 37 weeks gestation should be tested for GBS. Women who test positive should be given prophylactic antibiotics during labor, which will usually prevent transmission to the infant.

Group C

Includes *S.equi*, which causes strangles in horses, and *S.zooepidemicus* - *S.equi* is a clonal descendent or biovar of the ancestral *S.zooepidemicus* - which causes infections in several species of mammals including cattle and horses.

Group F

Group F streptococci were first described in 1934 by Long and Bliss amongst the "minute haemolytic streptococci". They are also known as Streptococcus anginosus (according to the Lancefield classification system) or as members of the *S.milleri* group (according to the European system).

Group G

These streptococci are usually but not exclusively beta hemolytic. *Streptococcus canis* is an example of a GGS which is typically found on animals but can cause infection in humans.

Gamma hemolysis is simply a synonym for negative hemolysis in which there is no change in the medium surrounding the colony. A blood agar plate displaying gamma hemolysis actually appears brownish. This is a normal reaction of the blood to the growth conditions used (37° C in the presence of carbon dioxide). Gamma hemolysis is a characteristic of *Enterococcus faecalis*.

If an organism does *not* induce hemolysis, it is said to display gamma hemolysis (γ-hemolysis): the agar under and around the colony is unchanged (this is also called *non-hemolytic*). *Enterococcus faecalis (formerly called Group D Strep) displays gamma hemolysis.*

Hemolytic reactions can also display some synergy. That is, the combination of reactions produces a reaction that is stronger than either reaction alone. Certain species of bacteria, such as group B Strep (n example is *Streptococcus agalactiae*) are weakly beta-hemolytic. However, if the bacteria are in close proximity with a strain of *Staphylococcus* the betahemolysins of the two organisms can combine to produce an intense beta hemolytic reaction. This forms the basis of a test called the CAMP test (after the initials of its inventors).

The determination of hemolysis and of the hemolytic reactions is useful in distinguishing different types of bacteria. Subsequent biochemical testing can narrow down the identification even further. For example, a beta hemolytic reaction is indicative of a Streptococcus. Testing of the Streptococcus organisms with bacitracin is often the next step. Bacitracin is an antimicrobial that is produced by the bacterium *Bacillus subtilis*. *Streptococcus pyogenes* strains are almost uniformly sensitive to bacitracin. But other antigenic groups of Streptococcus are not bacitracin sensitive.

Applications:
- Blood agar is used to support the growth of fastidious organisms and to determine the type of hemolysis (destruction of red blood cell walls) an organism produces.

- Chocolate agar (CHOC), type of blood agar plate in which the blood cells have been lysed by heating the cells to 56 °C. Chocolate agar is used for growing fastidious (fussy) respiratory bacteria, such as *Haemophilus influenzae*.

- Tryptose Agar is used for cultivating a wide variety of fastidious microorganisms, particularly for isolating Brucella. Tryptose Agar with 5% bovine serum, with or without antibiotics, remains a standard plating medium for the isolation of brucellae. For isolation of Brucella stains from contaminated milk, crystal violet (gentian violet) can be added to Tryptose Agar to suppress gram-positive organisms. Tryptose media can be supplemented with thiamine or citrate for the cultivation and maintenance of fastidious aerobic and facultative microorganisms.

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Dairy Microbiology

Dairy is the term given to a facility for the extraction and processing of animal milk, mostly from goats or cows and sometimes also from buffaloes, sheep, horses or camels primarily for human consumption.

Milk is the secretion of the mammary gland of female mammals and is often the sole source of food for the very young mammal. The role of milk is to nourish and provide immunological protection. It is a complex biological fluid containing different components including water, lactose, fat, proteins, and minerals. Milk in its natural state is a highly perishable material because it is susceptible to rapid spoilage by the action of naturally occurring enzymes and contaminating microorganisms. However, it is desirably converted into a wide variety of milk products using a range of advanced processing technologies. The products include a variety of cheeses, yogurts, butter and spreads, ice cream, and dairy desserts.

In order to assess the quality of the final product; milk or processed milk, it is essential to know:

Initial Microflora of Raw Milk

The numbers and types of microorganisms in milk immediately after production (i.e., the initial microflora) directly reflect microbial contamination during production, collection, and handling. The microflora in the milk when it leaves the farm is influenced significantly by the storage temperature and the elapsed time after collection. Where milk is stored at $=4^0$C, this low temperature normally will delay bacterial multiplication for at least 24 hours. The microflora, therefore, is similar to that present initially. However, if unsanitary conditions exist with the milking equipment or storage tank, the low temperature could mask these conditions.

A useful indicator for monitoring the sanitary conditions present during the production, collection and handling of raw milk is the "total" bacterial count or standard plate count (SPC). The SPC is determined by plating (or using equivalent procedures) on a standardized plate count agar followed by aerobic incubation for 2 or 3 days at 32^0C or 30^0C, respectively. Microorganisms failing to form colonies, of course, will not be counted. The SPC does not indicate the source/s of bacterial contamination or the identity of production deficiencies leading to high counts. Its sole value is to indicate changes in the production, collection, handling and storage environment.

Follow-up microbial assessments for psychrotrophs or thermoduric bacteria, spore-forming bacteria, streptococci, and coliforms can assist in determining sanitary deficiencies.

Certain groups can be enumerated selectively. For instance, psychrotrophs can be counted either by incubating SPC plates for 10 days at $5 – 7^0$C or by using a preliminary incubation of the raw milk at 13^0C for 16 hours followed by performing the SPC procedure. Thermoduric bacteria can be determined by laboratory pasteurization of milk before plating. Selective or diagnostic media can be used for coliforms, lactic acid bacteria, mastitis pathogens, Gram-negative rods, lipolytic, proteolytic and caseinolytic microbial types, and so on. An increased number of automated methods are now being employed for plating and enumerating bacteria. Also, rapid quantifying techniques are being used, such as the direct epifluorescent filtration technique, adenosine triphosphate method and impedance measurements.

Microbiology of Milk and Dairy
Types of microorganisms present in raw milk
Pathogens for Humans in Raw Milk
Raw milk may contain microorganisms that are pathogenic to humans, and their source may lie either within or outside the udder. Historically, the most serious human diseases disseminated by the consumption of contaminated raw milk are tuberculosis and brucellosis. In both the diseases, the causative organisms that may be excreted in milk from infected animals are *Mycobacterium bovis* or *M. tuberculosis* and *Brucella abortus, B.melitensis,* or *B. suis*. Often with Brucella infections, there is little change in the milk or udder (i.e., mastitis is not present), but in the case of tuberculosis mastitis, a pronounced and characteristic change in the milk and udder is observed.

Pathogenic bacteria also may be present in raw milk as a direct consequence of udder disease. Among the organisms commonly producing mastitis, *Streptococcus agalactiae, Staphylococcus aureus* and *Escherichia coli* are pathogens known to humans. *Streptococcus agalactiae* can initiate a variety of clinical conditions, the most serious of which are bacteremia and meningitis in newborns, which are potentially fatal to infected infants.

However, for humans the pathogenicity of bovine strains of *Streptococcus agalactiae* is uncertain and is carried by a large proportion of the human population. While it seems likely that the consumption of contaminated raw milk may play a part in infections of the population at large, some researchers have reported higher rates of *S. agalactiae* among consumers of raw milk who do not experience symptoms of a milk-borne illness.

Staphylococcal mastitis of the cow poses a more direct threat to public health because some bovine strains produce enterotoxin. Consumption of food containing enterotoxin leads to asymptomatic illness, usually of approximately 24-hour duration, characterized by nausea, diarrhea, and abdominal pain. The production of enterotoxin usually associated with the multiplication of staphylococci under favorable growth conditions during the storage of the milk. Because enterotoxin is relatively heat stable, subsequent pasteurization of the contaminated milk will not make it safe for consumption.

Further biohazards stem from the adventitious contamination of raw milk by pathogenic bacteria from sources external to the udder. Salmonellae and thermoduric Campylobacter strains fall into this category and have produced many outbreaks of enteritis. Human carriers also may be sources of infection in milk-borne outbreaks. This has been reported for Salmonella infections and for cases of scarlet fever or septic sore throat attributed to *Streptococcus pyogenes*.

All of these pathogens are destroyed by pasteurization, except *Clostridium perfringens* and *Bacillus cereus*, which can survive the pasteurization process because of their ability to sporulate. It is improbable, however, that *C. perfringens* will germinate and multiply under the modern-day conditions of milk storage.

Contamination of milk

Contamination of milk can vary widely depending on milk handling practices ranging from milking a few cows by hand in the out of doors to milking 3000 cows by a complex, automated system in a well-equipped parlor. There are however three basic sources of microbial contamination of milk: (1) from within the udder, (2) from the exterior of the teats and udder, and (3) from the milk handling and storage equipment.

Milk is produced at ambient temperatures ranging from sub zero centigrade, where it is necessary to protect milk from freezing, to above 25°C, where refrigeration is needed. Furthermore, the duration of milk storage time on the farm can vary widely. Therefore depending on the duration of milk storage on the farm before leaving can differ, often unpredictably, even under similar conditions.

In most dairying areas, milk production methods, equipment, and on-farm storage have improved over time. However, udder disease remains widespread because of the presence of mastitis-associated microorganisms. Refrigeration on the farm all too often masks the effects of unsanitary practices, including the use of inadequately cleaned and sanitized milking equipment. As a result, the microbiological quality of raw milk supplies produced under apparently good sanitary conditions and stored under adequate refrigeration may produce off-flavors, yield poor product, and present a risk of food-borne infections to the consumer.

Environmental sources

While the lactating animal, the production environment, and the milk handling equipment remain the principal sources for microbial contamination of raw milk, other environmental sources include the following:

Air

Air is not considered a significant source for microbial contamination in raw milk. Through its movement, air transfers soil and dust particles from a microbial-laden source into exposed milk surface such as soil and microbes. The main sources of airborne microbes may include the activity of factory personnel, ventilation and air-conditioning systems, the inflow of outdoor air and packaging materials. The potential contamination from bacterial biofilms is also of major concern because microbial cells may attach, grow and colonize on open exposed wet surfaces.

Outdoor Environment

The control of airborne microorganisms in the immediate surroundings of dairy premises is more difficult than in closed, indoor environments where more controlled measures can be taken. Natural agents such as UV light, humidity, temperature, wind direction, and speed have a significant influence on the total number of airborne microorganisms in the outdoor atmosphere.

The Milk Handler

When cows are hand-milked, the milk handler can contribute to an increased microbial load in the raw milk by dislodging dirt particles from the udder, increasing aerial contamination through accelerated air movement, and contacting the milk with infected hands. Risks of contamination from the milk handler are much less with machine milking.

The Water Supply

Water used in the milk production process should be of potable quality. This means that the water supply must be from an approved source free from pathogens and fecal contamination.

One must recognize that a potable water supply can become contaminated within the dairy production environment, such as in a farm storage tank that is not properly protected from rodents, birds, insects, and dust. Bacteria also may be introduced into the water supply through dirty wash troughs, buckets, and hoses. If untreated water gains access into milk or is used for rinsing equipment and containers, the microbes present in the water eventually will contaminate the milk.

The reasons why and how milk gets contaminated are numerous, therefore it is also necessary to know the means that are adopted for the treatment of milk so that dairy products are free from pathogens and other contaminating microbes.

Many processes have been developed over the years to enhance its utilization and safety. These processes can be grouped and analyzed in a variety of ways.
- Fractionation
- Concentration
- Preservation

Fractionation: The term is used to describe the fractionation or disassembly of the components of milk, utilizing their various properties of the individual components.
- Centrifugal separation, utilizing the density difference of the components. The most common equipment used is the disc bowl separator, which allows the separation of light and heavy phases and also allows removal of any sediment.
- Membrane separation, utilizing size or charge difference. This is normally a pressure – motivated, flow – dependent process, involving the use of a selective membrane, with a wide range of fractionation possible, from simple water removal to separation of different proteins.
- Ion exchange, utilizing charge difference. In this process, tiny resin beads exchange charged ions on their surface with charged ions or larger charged molecules in solution, removing them for subsequent recovery.
- Precipitation and Crystallization, utilizing differences insolubility and suspension stability.
- Filtration, utilizing size difference, the principle is similar to that already mentioned with membrane separation but involves the separation of larger components.
- Homogenization is a process of size reduction of the fat globules to prevent fractionation of the cream and skim milk by density difference. A combination of a high-pressure pump and special valves provides high shear.

Concentration Process: This grouping involves the removal of one or more components resulting in a concentration of the remaining components. Many of these processes also involve fractionation. The processes include the following:
- Evaporation, utilizing phase change of the aqueous component. An evaporator is a specialized heat exchanger operating under vacuum, facilitating efficient water vapour generation and removal from a liquid with minimal thermal damage to the remaining liquid.
- Freeze concentration, also utilizing phase change. This involves freezing and crystallization of the aqueous component of a liquid by refrigeration followed by crystal removal. It is not widely used in dairy processing.
- Here the permeate, or material passing through the membrane, includes water, enabling concentration of the retentate or material retained.

- Drying, utilizing phase change. This is a very important process, particularly in the production of milk powder, casein, and whey products. It involves water removal from a liquid concentrate or solid by heating with hot air.

Preservation Process: This category is primarily concerned with reducing microbiological and chemical change. They include the following:

- Pasteurization, thermalization, and sterilization utilizing heat to kill microorganisms. All these processes involve the transfer of heat into the product in order to raise the temperature to achieve a closely controlled time–temperature process (e.g., 72^0C, 15 sec.) for pasteurization.
- Chilling and freezing, to slow microbial growth and chemical change and is widely used both during or prior to processing or for final product storage. Heat exchangers of the type can be used for liquid products, with cool stores and freezing chambers for finished goods.
- Reduction of pH, to inhibit microbial growth. This may be achieved by addition of acids or by bacterial fermentation of lactose.
- Dehydration (drying), to inhibit microbial growth and chemical change. The water content of dairy products influences the microbial changes and hence drying also serves the purpose of storage and to prolong the shelf life of the product.
- Salting, to reduce water activity and inhibit microbial growth. Salt may be added as dry granular salt or by means of a brine solution, with the product being immersed for a period in a tank of concentrated brine.
- Packaging, to contain the product, protect it, and reduce microbiological and chemical changes.

Heat Treatment of Milk

Pasteurization
This process is done to eliminate all non-spore forming pathogens commonly associated with milk. This process also effectively destroys spoilage organisms and thus contributes to product keeping quality under required refrigeration storage. The process of heating every particle of milk to at least 143^0F(61.7^0C) and holding at such temperature for at least 30 minutes, or to at least 160^0F (71.1^0C) and holding at such temperature for at least 15 seconds.

Ultra-Pasteurization (Extended Shelf-Life Milk)
In this process, milk is 'thermally processed' at or above 138^0C for at least 2 seconds, so as to produce a product that has an extended shelf life under refrigerated conditions.

Ultra-High-Temperature Sterilization
Ultra-High-Temperature (UHT) sterilization is a process that combines rapid heating of milk to very high temperatures followed by aseptic handling and packaging to produce a shelf-stable, commercially sterile product. Though heat treatments for UHT vary indifferent countries, temperatures of $130 – 150^0$C with holding time of 1 second or more are prescribed, with holding times of $2 – 8$ seconds commonly applied.

In-container Sterilization
Conditions specified for in-container sterilization of milk include temperatures from 105^0C to 120^0C for 20–40 minutes. With this strategy, milk is prefilled into cans or bottles that are hermetically sealed, and then the milk is heated in an autoclave or a batch or continuous retort. In-container sterilized milk products have expected shelf lives of a year or more with no refrigeration required.

Hazard Analysis Critical Control Points (HACCP)
The 'Hazard Analysis Critical Control Points' system offers a structured approach to the control of hazard in food processing and, when properly applied, identifies areas of concern and appropriate control measures before product failure is experienced. It represents a shift from retrospective quality control through end-product testing to a preventative quality assurance approach.

The HACCP procedure is generally targeted at food safety management (pathogenic microorganisms and their toxins), but, as an approach in the context of broader quality management, it can be effectively applied to microbiological spoilage, foreign-body contaminations or pesticide contamination. It is preferable to conduct a HACCP program with a narrow scope (a single pathogen or possibly pathogens) rather than attempt to cover an extended list of hazard areas then documentation tends to become complex.

Processed Dairy Products
Here there is a brief description of the various processed products that are made from milk.

Fluid Milk Products
This group of products falls into the 'consumer products' family, competing in the beverage sector of the grocery business. Their manufacture is relatively simple, involving *fractionation* processes such as centrifugal separation to produce cream, skim milk, or reduced-fat milk, *concentration* processes such as membrane separation (ultra filtration) to produce high calcium milk, and *preservation* processes such as pasteurization, ultra high temperature (UHT), and refrigeration to extend the safety and shelf life of the product range. Homogenization is used to prevent separation of the fat in the liquid product.

Fermented Milk Products
There are two groups in this family: (a) cheese products in which part of the original liquid is removed during manufacture as whey and (b) products in which there is no whey drainage, such as yogurts.

Both groups have a very long history of preparation and were probably developed by accident as a means of preserving milk. All or some of the standard food preservation tools of *moisture* removal, *acid* development, *salt* addition, and *temperature* adjustment may be used. The first letters of the italic words spell the conveniently remembered acronym MAST.

Cheese is an example of a fermented processed milk and its manufacture is a highly complex process. The composition of milk is adjusted or standardized (fractionation) by centrifugal separation and possibly also ultra filtration. For most cheese types, the milk will then be pasteurized (72^0C, 15 sec) to reduce the risk from pathogenic organisms, adjusted to the desired fermentation temperature, and then pumped into a cheese vat. Starter culture consisting of a carefully selected species of lactic acid bacteria and a coagulate (e.g., calfrennet) are then added and the milk is allowed to coagulate. This is by destabilization of the case in micelle. This permits the beginning of the fractionation and selective concentration processes that form the basis of cheese making. Once the coagulum is of sufficient strength, it is cut into small particles and, by a process of controlled heating and fermentation, syneresis or expulsion of moisture and minerals (whey) occurs. Separation of the curd from the whey over a screen (filtration) follows. Depending on the cheese type, the curd may be allowed to fuse together (e.g., cheddar) or may be kept in granules (e.g., Colby). Salt may then be incorporated into

the curd for preservation, as dry granules or by immersion in brine. The curd is pressed into blocks by either gravity or mechanical compression, and the cheese then goes into controlled storage conditions for final fermentation and maturation.

For fermented milk products, the process of manufacture is some what simpler. For example, in yogurt manufacture, the milk to be used is fortified with additional protein (skim milk powder or concentrated milk), severely heated (e.g., 95^0C, 5 minutes) to reduce the microbial load and to encourage whey protein/casein interaction, cooled to fermentation temperature (e.g.,36^0C), and transferred to a fermentation vessel. Selected cultures are then added, and fermentation is continued until the desired pH of around 4.5 is reached. This causes the coagulation of the vessel contents, and the plain yogurt is then cooled prior to possible in corporation of fruit and flavoring agents, followed by packaging.

When all the raw materials and the necessary equipment that comes in contact with milk is properly treated and adequate measures are taken for safety, there is a drastic drop in the number of organisms in milk. Some processes, however, ensure that milk supplied has no microbes at all, which serves as an excellent starting material for any process and also making the milk safe for consumption without prior heating.

Quality control in the Dairy Industry

The quality of food, such as milk and dairy products, may be defined as that sum of characteristics, which enables the food to satisfy definite requirements and which determine its fitness for consumption. In this sense, quality can be judged by means of sensory evaluation, its nutritive value, and according to its chemical, physical, and microbiological characteristics.

Cases of food-borne disease and food poisoning are becoming more and more common throughout the world. Both of these public health problems and the microbiological spoilage of foods can be minimized by the careful choice of raw materials and correct manufacturing and storage procedures. Achievement of such objectives requires, in many cases, monitoring at various stages to assess microbiological load or to look for particular microbial types.

Monitoring procedures commonly used include the air and water supplies in a factory environment, the hygiene of packaging material, and the sampling and testing of raw materials and end products.

The analytical procedures will include standard and rapid methods for assessing microbiological load and for enumerating and detecting specific microbial genera or groups.

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Potential Impact of Increased Use of Biocides in Consumer Products on Prevalence of Antibiotic Resistance

Introduction

Problems associated with the development and spread of antibiotic resistance in the clinic have been increasing since the early 1960s and are currently viewed as a major threat to clinical practice. It is generally accepted that the main cause of this problem has been and still is widespread inappropriate use and overprescribing of antibiotics in clinical medicine, animal husbandry, and veterinary practice. Concern about bacterial resistance has led to calls for increased education of both the public and professionals on the correct use of antibiotics and more stringent infection control measures to reduce the transmission of infection.

In recent years, a number of scientists have expressed concern that the use of antimicrobial chemicals (biocides, preservatives) in general practice and in domestic and industrial settings may be a contributory factor to the development and selection of antibiotic-resistant strains. This has been particularly the case with regard to the recent trend towards inclusion of antibacterial agents within a multitude of otherwise traditional consumer products and apparent increases in the environmental impact of many active ingredients used in personal care and consumer products, together with pharmaceuticals. The general concerns are (i) that commonality of target site between biocide and antibiotic might lead to selection of mutants altered in such targets by either agent and the emergence of cross-resistance, (ii) that subtle differences in the biocide and antiseptic susceptibility of antibiotic-resistant strains might facilitate their selection and maintenance in the environment by low, sub-effective concentrations of biocides and antiseptics as well as the primary antibiotic, and (iii) that indiscriminate biocide application might cause the evolution and selection of multidrug-resistant strains through polygamous mechanisms such as efflux pumps.

The current indications are that if the concerns that the widespread deployment of biocidal molecules impacts antibiotic efficacy are genuine, then its contribution is likely to be relatively minor. Conversely, the tremendous contributions of disinfection and acceptance of hygienic measures towards advances in public health over the last century cannot be denied. Indeed, if reductions in the number of infections requiring antibiotic treatment can be achieved through effective hygiene, including the use of biocidal products, then this is likely to decrease rather than increase the incidence of antibiotic resistance. Accordingly, it is important to ensure that biocide use, as an integral part of good hygiene practice, is not discouraged when there is real benefit in terms of preventing infection transmission. This means that it is also necessary to assess the possibility that the indiscriminate use of biocides and antibacterial products might compromise the in-use effectiveness of such biocides in truly hygienic applications. Use of such products must be associated with appropriate analyses of added value to the consumer, particularly when there is no apparent gain in public health.

I has to consider the mechanisms by which bacteria may become less sensitive to biocide action and then to look at the potential links between antibiotic and biocide resistance and their implications for the inclusion of antibacterial agents within consumer products. The relevance of laboratory monoculture experiments in particular, where competitive selection pressures are absent, will be viewed in the context of field studies and complex ecologies. First, however, it is necessary to consider the precise meaning of some of the terms used and misused by various opinion-forming groups.

Possible associations between biocide use and resistance—field studies

Association between chronic sub lethal exposure of bacterial monocultures to biocides and changes in susceptibility to both the biocides themselves and third-party antibiotics has been demonstrated unequivocally in the laboratory. Such phenomena have not yet been demonstrated to have any relevance to the real world. In such situations, individual species of bacteria are in fierce competition with other forms of bacteria, and their competitive fitness determines their survival. Arguably, the clinic represents an environment where biocide use has been and still is extreme. If the increasing use of antibacterial agents within consumer products is likely to impact antibiotic resistance within the home, similar effects should already be apparent in clinical and hospital settings. Accordingly, a large number of studies have been carried out to evaluate whether clinical and environmental isolates taken from such settings show any evidence of significant reductions in their susceptibility to biocides and whether this might be linked with antibiotic resistance.

The results of such studies have been largely ambiguous. Thus, no differences were found in the MICs of hospital and laboratory gram-negative isolates for cationic antiseptics and two organo mercurial compounds. Three separate studies by Stickler's group assessed the MICs of a range of antiseptics, disinfectants, and antibiotics for gram-negative bacteria isolated from a hospital environment and found that approximately 10% of the isolates (mainly *Pseudomonas, Proteus,* and *Providencia* spp.) exhibited some level of reduced susceptibility to chlorhexidine and cetrimide and were also generally more resistant to multiple antibiotics. More recently, Block and Furman isolated 251 strains of staphylococci*, Klebsiella, Pseudomonas, Acinetobacter,* and *Candida* spp. from a hospital environment and detected an inverse correlation between chlorhexidine use and susceptibility. It was noteworthy that when individual taxa were analyzed separately, no significant correlation was noted. This indicates a clonal expansion of existing less-susceptible strains rather than adaptation of individual species, as has been noted in other recent studies of hospital isolates.

Similar results showed that 12.8% of 148 clinical *E. coli* isolates selected for their elevated chlorhexidine MICs were no less susceptible to use concentrations. Such changes, in the case of the *Providencia* isolates, were thought to affect binding of the biguanides to the cell surface and therefore reflected envelope modification. Freney et al. found no evidence of decreased susceptibility within 169 novel *Enterobacteriaceae* isolated from the general environment relative to clinical isolates. Arguably, such studies support the view that antiseptic use in hospitals does not contribute to the biocide susceptibilities of enterococcal isolates. Equally, Lear et al. examined over 100 factory isolates and compared the MICs of triclosan and chloroxylenol for these to those of the equivalent culture collection strains. They concluded that there was no evidence suggesting that the residual levels of biocides in the

factory environment had led to changes in susceptibility. Equally, Braid and Wale showed that triclosan-impregnated storage boxes were effective at reducing the numbers of various challenge inocula and that the susceptibility of the strains was unaffected after repeated exposure on these treated items.

By way of contrast, Reverdy et al. showed that antibiotic-sensitive *S. aureus,* and other staphylococci, for which the MICs of various antiseptics were elevated, were nevertheless less sensitive to a wide variety of antibiotics. Increased MICs for methicillin-resistant *S. aureus* strains have been reported for some biocides, including chlorhexidine, cetrimide, benzalkonium chloride, hypochlorite, triclosan, parahydroxybenzoates, and betadine. Thus, while the MIC of chlorhexidine was higher against methicillin-resistant *S. aureus* clinical isolates (4 to 8 µg/ml) than for susecptible ones (0.37 to 21 µg/ml), there was no significant difference in the efficacy of this agent when these strains were tested on the arms of volunteers with a bactericidal assay. No significant differences were noted in the chlorhexidine susceptibility of 33 clinical methicillin-resistant and -susceptible *S. aureus* isolates, and there was no loss of sensitivity to the bactericidal effects of triclosan when a clinical methicillin-resistant *S. aureus* isolate showing an elevated MIC (2 to 4 µg/ml) was challenged.

Bamber and Neal found that of 16 methicillin-resistant *S. aureus* that exhibited low-level mupirocin resistance, none had increased MICs of triclosan, but Suller and Russell found clinical methicillin-resistant *S. aureus* isolates to have slightly decreased susceptibility, relative to susceptible isolates, to a range of biocides that included chlorhexidine, cetylpyridinium chloride, benzalkonium chloride, and triclosan. Most of the strains described in the above studies remained equally susceptible to bactericidal concentrations of the biocidal agents, an observation that was repeated recently for vancomycin-resistant *Staphylococcus aureus* (L. M. Sehulster and R. L. Anderson, Abstr. 98th Annu. Meet. Am. Soc. Microbiol., 1998, abstr. Y3). Four antiseptic formulations (Savlon, Dettol, Dettol hospital concentrate, and Betadine) retained their bactericidal activity in a European suspension test against a variety of antibiotic-resistant strains, including methicillin-resistant *S. aureus* and vancomycin-resistant enterococci. These data bear testimony to the multiplicity of target sites implicated in the bactericidal action of biocides.

Many other studies failed to observe any change whatsoever in MIC. Thus, Stecchini et al. showed that, despite widespread antibiotic resistance in 100 strains of *Enterobacteriaceae* isolated from minced meat, these were not resistant to the bactericidal activity of an amphoteric Tego disinfectant. Similarly, among 330 psychrotrophic non-fermenting gram-negative strains isolated from vegetables, those antibiotic-resistant strains were demonstrated to be susceptible to the bactericidal action of quaternary ammonium compounds and hypochlorite disinfectants.

Baillie et al. evaluated the chlorhexidine sensitivity of 33 clinical isolates of *Enterococcus faecium* that were sensitive to both vancomycin and gentamicin with vancomycin-resistant and gentamicin-resistant strains. The results showed no increase in resistance to chlorhexidine as indicated by MIC. lnterestingly, a study of 67 ciprofloxacin-resistant isolates of *P. aeruginosa* yielded four which were hypersensitive to chlorhexidine (MIC, 5 mg/liter), while none were found among 179 ciprofloxacin-sensitive isolates.

Marshall et al. (P. J. Marshall, P. Rumma, and E. Reiss-Levy, presentation at the 11th National Conference of the Australian Infection Control Association, 7-9 May 1997, Melbourne, Australia) reported that during an intensive policy of antiseptic handwashing involving a triclosan-based medicated soap, aimed at combating a methicillin-resistant *S. aureus* infection episode, not only did the incidence of methicillin-resistant *S. aureus* decrease significantly, but the percentage of ciprofloxacin-sensitive isolates increased from 8.1% to 22.5% within the trial. In a study of *Streptococcus mutants* isolated from the mouths of 114 schoolchildren and students from families in which about 70% used oral preparations containing chlorhexidine on a regular basis, there was no evidence of decreased susceptibility either to chlorhexidine or to a range of antibiotics, as tested with MICs.

Anderson et al. determined the susceptibilities of vancomycin-resistant and vancomycin-sensitive enterococci to various concentrations of commonly used hospital disinfectants, including quaternary ammonium compounds, phenolics, and a iodophore, at recommended use dilutions and extended dilutions with suspension tests. They concluded that there was no relationship between levels of vancomycin resistance and their susceptibility to disinfectants at the use dilution. Such findings have been confirmed by showing that a series of vancomycin-resistant and vancomycin-resistant enterococcal clinical isolates had no significant differences in their growth-inhibitory or bactericidal sensitivities to chlorhexidine, cetylpyridinium chloride, or triclosan.

Published data for triclosan state that the expected MIC for staphylococci should be between 0.01 ppm and 0.1 ppm. Bamber and Neal determined the MIC for 186 isolates of methicillin-resistant and methicillin-sensitive *S. aureus* and found 14 isolates (7.5%) with MICs greater than 1.0 ppm. These were, however, equally distributed between the methicillin-resistant and methicillin-sensitive *S. aureus* strains.

A series of antibiotic-resistant clinical and environmental isolates that included *P. aeruginosa*, *Klebsiella* species, *E. coli, S. aureus,* and *S. epidermidis* were found to be no less susceptible to the bactericidal activity of phenolic and quaternary ammonium disinfectants, chloroxylenol, cetrimide, and povidone iodine. Similarly, some variation in the vancomycin susceptibility and biocide (chlorine, alcohol, aldehyde) susceptibility of enterococci has been noted, but the two did not correlate.

The food processing industry represents an environment other than the clinic where the use of biocidal products is high. In this respect, Heir et al. reported that 13% of staphylococcal isolates from a food manufacturing environment had MICs of benzalkonium chloride that were between 4 and 11 mg/liter, compared with 70% of remaining isolates, which had MICs of less than 2 mg/liter. This resistance probably related to the presence of *qac* efflux mechanisms and encoded only small changes in susceptibility. Accordingly, suspension tests showed that recommended use concentrations of the agent produced the desired 5-log reduction in viable count in 5 min. In an examination of poultry carcasses, two strains of *Pseudomonas* were isolated that were deemed resistant to benzalkonium chloride by virtue of possessing a MIC greater than 200 µg/ml. Only one of these organisms failed the suspension test. A more recent study showed that *S. aureus* cells that expressed *qacG* efflux suffered reduced killing in environments that contained low concentrations of benzalkonium chloride but 5-log reductions in viable counts at higher concentrations. The latter were nevertheless still well below the recommended use concentrations.

Latterly, Heir et al. found a new member of the *qac* family of genes in *Staphylococcus saprophyticus* (*qacH*) isolated from a poultry processing plant. The same authors, however, conceded that quaternary ammonium compound use in the production facilities might have led to a selection for staphylococci bearing the *qacAB*

genes. Bass et al. demonstrated that approximately one third of diseased poultry carried plasmids that encoded multiple antibiotic resistance; 63% of these contained markers for the class 1 integrons *intI* and *qacE*and were part of transposon Tn*21*. The selection pressure for Tn*21*, which also encodes mercury resistance, could not be determined.

The field studies discussed so far suggest strongly that the variable nature of the observable links between biocide and antibiotic susceptibility have no single underlying cause and that worries and concerns raised through laboratory monoculture experiments cannot be echoed in the environment. There are, however, a few published studies that indicate the contrary and show reductions in susceptibility to various oxidizing biocides that are sufficient to compromise their in-use effectiveness. In most instances, such studies make no distinction between phenotypic and hence reversible changes in susceptibility and that which may be acquired. In other instances, data were collected from large numbers of isolates taken from environments where biocide use is widespread but without reference to control habitats. The extent to which the data reflect adaptation to the biocides or the natural selection and clonal proliferation of existing strains is therefore often unknown. These studies are discussed below.

Several reports have described isolates, especially among gram-negative species, from various food processing environments that possess a reduced susceptibility to chlorine and quaternary biocides that relates to practical usage. Thus, an early report noted that after changing the sterilization practices from steam to chlorine-based disinfectant compounds, there was a higher occurrence of dairy isolates that were resistant to hypochlorite. Similarly, Mead and Adams and Bolton et al. found that chlorine concentrations of 1 mg/liter produced a 4-log reduction in viability of *S. aureus* strains isolated from turkeys and turkey products, but only a 2-log reduction when tested against endemic strains that had colonized the processing equipment. All three reports could be related to growth of the resistant isolates as coaggregates within extracellular slime. This was also the explanation for the apparent resistance of lactobacillus strains isolated from packed meat that could survive exposure to 200 mg of benzalkonium chloride per liter. The resistance in all of these instances was therefore phenotypic in nature.

Pseudomonads are not generally noted for their susceptibility to quaternary ammonium compounds, a property that is generally attributed to the unique properties of the *Pseudomonas* cell envelope. Approximately 30% of *Pseudomonas* isolates taken from poultry carcasses were able to grow at concentrations of 200 µg/ml. While it was recognized that clonal selection of existing resistant strains, through a constant usage regimen involving benzalkonium chloride as the disinfectant, might have been the cause, these workers later reported (S. Langsrud and G. Sundheim, 1997, Pseudomonas ′97, p. 102) that the resistance was lost within 4 to 8 h of removal from the quaternary ammonium compound and was developed in batch culture only during the lag phase. These observations therefore more probably reflect a regulated process involving efflux genes, and the resistance shown for these cells could not be replicated in a bactericidal assay.

In a similar study, the susceptibility of 350 isolates collected from commercial chicken hatcheries to commercial preparations of quaternary ammonium compounds, phenolics, and glutaraldehyde was examined. Nineteen isolates (ca. 6%, including *Serratia marcescens*, *Bacillus* species, *Enterococcus* species, and *P. stutzeri*) from two of three hatcheries were resistant to disinfectant at and above the recommended use concentrations and exposure times. Some isolates were multi resistant, but only three showed resistance to quaternary ammonium compounds compared with 7 to phenol and 15 to glutaraldehyde. The authors suggested that this might be correlated with the usage of glutaraldehyde in U.S. hatcheries over many years. No investigations were carried out to determine whether the resistance was reversible, although all isolates had been grown once through tryptone soy medium.

In a study of the effects of repeated antiseptic use on the bacterial flora of the urethral meatus in patients undergoing intermittent bladder catheterization, the bacterial flora was examined from the date of injury to the time at which urinary tract infection developed after daily washing with aqueous chlorhexidine (600 µg/ml). Prior to the regular application of chlorhexidine, the predominant flora comprised gram-positive, chlorhexidine-sensitive bacteria. These were superseded by a gram-negative flora that included some resistant strains (mainly*Proteus mirabilis*, *P. aeruginosa*, *Providencia stuartii*, and *Klebsiella* species) less sensitive to chlorhexidine, with MICs of 200 to 800 µg/ml. These were well above the levels of 10 to 50 µg/ml usually reported for gram-negative species.

In a subsequent study, the susceptibility to an array of antiseptics and disinfectants that included chlorhexidine, cetrimide, glutaraldehyde, and a phenolic formulation was assessed against a large collection of gram-negative isolates taken from a variety of clinical and hospital settings. The general conclusion drawn was that antiseptic and disinfectant resistance was not a widespread phenomenon in species responsible for urinary tract infections. They found that approximately 10% of the isolates (mainly *Pseudomonas, Proteus,* and *Providencia*) exhibited some resistance to chlorhexidine, but these came from situations where there was extensive use of chlorhexidine.

It would appear therefore that in the earlier study, the routine application of chlorhexidine had eliminated the natural colonization resistance provided by the sensitive autochthonous flora and had enabled innately resistant environmental strains to infect. The innate recalcitrance of environmental gram-negative bacteria to antiseptics has been demonstrated by Nagai and Ogas. They isolated strains of *Achromobacter xylosoxidans* from a 0.4% chlorhexidine solution handwashing reservoir for which minimum bactericidal concentrations were more than 10-fold higher than the chlorhexidine solution in the reservoir. Two separate investigations with *Providencia stuartii,* and an antibiotic-resistant clinical strain of *P. mirabilis that* was resistant to the growth-inhibitory action of chlorhexidine at 800 mg/liter failed to show any evidence of a plasmid link. Both sets of authors concluded that the resistance was most likely an intrinsic property induced by persistent exposure to the biocide.

More recently, strains of *P. stutzeri* and *P. aeruginosa* have been shown to become much less susceptible to chlorhexidine and cetylpyridinium chloride when passaged through gradually increasing concentrations of each. Such decreased susceptibility was stable for *P. stutzeri* but not for *P. aeruginosa* and could not be transferred by conjugation. The authors concluded that resistance resulted from a nonspecific decreases in cell permeability such as might arise from deletion or depression of a porin protein. In this context, passage with increasing concentrations of isothiazolone biocides has been shown to repress the synthesis of an outer membrane porin protein (OmpT) that appears to facilitate the entry of this group of thiol-interactive biocides into the cell.

Overall, there is good evidence to suggest that good standards of hygiene in the domestic setting, which includes not only day-to-day cleaning of the home but food hygiene, hand hygiene, and hygiene

related to the protection of vulnerable groups, can have a significant impact in reducing the number of infections arising in the home. Indeed, a number of recent studies have reported increased incidence of critical pathogens such as methicillin-resistant *S. aureus* into the home environment, often associated with household pets like dogs and cats, and their transfer to humans. Such work highlights the need for targeted hygiene within the home. A variety of different procedures can be used to achieve hygiene in the home, and in some cases this may require the use of a disinfectant or antiseptic. This being the case, it can be seen that responsible use of biocides and antimicrobial cleaning products could contribute to reducing the impact of antibiotic resistance. Thus, if reducing the number of infections through effective hygiene is important, then it is also important to ensure that biocide use is not discouraged in situations where there is real benefit.

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Quality of Potable Water

Essential to the survival of all organisms, water has always been an important and life – sustaining drink to humans. Excluding fat, water composes approximately 70 % of the human body by mass. It is a crucial component of metabolic processes and serves as a solvent for many bodily solutes.

Drinking water or Potable water is water of sufficiently high quality that it can be consumed for drinking as well as cooking purposes without risk of immediate or long term harm.

Water Contamination & Parameters For Drinking Water
Throughout most of the world, the most common contamination of raw water sources is from human sewage and in particular human fecal pathogens and parasites.

Parameters for drinking water quality typically fall under two categories:
- Chemical parameters: Include heavy metals, trace organic compounds, total suspended solids (TSS), and turbidity. These parameters tend to pose more of a chronic health risk through buildup of heavy metals although some components like nitrates / nitrites and arsenic may have a more immediate impact.
- Physical parameters: Affect the aesthetics and taste of the drinking water and may complicate the removal of microbial pathogens.
- Microbiological parameters: Include coliform bacteria, *E. coli*, and specific pathogenic species of bacteria (such as cholera – causing *Vibrio cholerae*), viruses and protozoan parasites, serves as an indication of contamination by sewage. Microbial pathogens are however of greater concern since these agents are highly infectious.

Indicators of safe drinking water
Access to safe drinking water is indicated by the number of people using proper sanitary sources. These improved drinking water sources include household connections, public standpipe, borehole condition, protected dug well, protected spring and rain water collection. Sources that don't encourage improved drinking water include: unprotected well, unprotected spring, rivers or ponds, vender-provided water, bottled water (consequential of limitations in quantity, not quality of water), and tanker truck water. Access to sanitary water comes hand in hand with access to improved sanitation facilities for excreta. These facilities include connection to public sewer, connection to septic system, pour – flush latrine, and ventilated improved pit latrine. Unimproved sanitation facilities are: public or shared latrine, open pit latrine, or bucket latrine.

Improving the availability of drinking water
Water which is contaminated can be treated to turn it into potable water. One of the easiest ways to treat water is boiling. Boiling water may not remove heavy contaminants, but it can neutralize most bacteria and viruses which may be present. Water can also be treated with chemicals such as bleach, which sometimes come in the form of tablets for field and camping use. In addition, water can be pumped through a filter to remove particulates.

Water Treatment & Testing
Most water requires some type of treatment before use. Even water from deep wells or springs. The extent of treatment depends on the source of the water. Appropriate technology options in water treatment include both community – scale and household – scale point – on – use (POU) designs.

The most reliable way to kill microbial pathogenic agents is to heat water to a rolling boil but this requires abundant sources of fuel and is very onerous on the households, especially where it is difficult to store boiled water in sterile conditions. Other techniques, such as filtration, chemical disinfection, and exposure to ultraviolet radiation (including solar UV) have been demonstrated.

Solar water disinfection: Is a low-cost method of purifying water that can often be implemented with locally available materials. Unlike methods that rely on firewood, it has low impact on the environment.

Gross Alpha : The gross alpha test is used to determine if the water has any dissolved radionuclides that emit alpha particles. Generally, this will be an indication of whether or not the water has uranium or radium dissolved in it. This test does not indicate the presence of radon.

Test for organics and pesticides: Testing for organics or pesticides is usually only done if there is some suspicion of contamination. Close proximity to a waste site, fuel tanks or farm may be enough to warrant this type of test.

Lead Testing: Although there is much concern about lead in drinking water, the techniques and philosophies for testing and control are less than perfect. In almost all cases, lead found in drinking water is deposited there by the corrosion of lead in the distribution system. The source of the lead is usually lead street connections, old soldered joints (solder used today does not contain lead) or brass fixtures.

Testing Public Water Supplies
Generally public water supplies are not tested. Though there are agencies to test safety of drinking water, this does not, however, mean there is no complaint or there is no problem with the supply. It may need one of the secondary (aesthetic not health) standards of the supply treated, especially, if the supply is from a well. Other concerns such as those about lead, taste, sediment, staining, cyst etc...; may motivate someone to have their water treated.

Follow-up testing after the installation of equipment for health related parameters should always be done. Use two tests. One of the tests will be of treated water and one will be raw or untreated water. Two tests are needed to show if performance standards are met.

Microbiological testing of water
The most common and widespread health risk associated with drinking water is contamination; whether directly or indirectly, by human or animal excreta, particularly faeces. If such contamination is recent, and if those responsible for it include carriers of communicable enteric disease, some of the pathogenic microorganisms that cause these diseases may be present in the water. Drinking the water, or using it in food preparation, may then result in new cases of infection. The pathogenic agents involved include bacteria, viruses, and protozoa, which may cause diseases that vary in severity from mild gastroenteritis to severe and sometimes fatal diarrhea, dysentery, hepatitis, or typhoid fever, most of them are widely distributed throughout the world. Faecal contamination of drinking water is only one of several faeco oral

mechanisms by which they can be transmitted from one person to another or, in some cases, from animals to people.

Other pathogens cause infection when water containing them is used for bathing of for recreation involving water contact, rather than by the oral route. Some may also cause infection by inhalation when they are present in large numbers in water droplets, such as those produced by showers and some air-conditioning systems or in the irrigation of agricultural land. Ideally, all samples taken from the distribution system including consumers' premises should be free from coliforms organisms. In practice, this is not always attainable. To control purity of water the following microbiological parameters for water collected in the distribution system is therefore recommended.

Indian standard IS 1622 : 1981

a. Throughout any year, 95 % of samples should not contain any coliform organisms in 100 ml.
b. No sample should contain *E. coli* in 100 ml.
c. No sample should contain more than 10 coliform organisms per 100 ml.
d. Coliform organisms should not be detectable in 100 ml of any two consecutive samples.

E. coli

E. coli is a Gram-negative, non-spore forming, rod-shaped bacterium which can be either motile or nonmotile (motile cells are peritrichous); growth is aerobic or facultatively anaerobic. Metabolism is both respiratory and fermentative; acid is produced by the fermentation of glucose and lactose.

1. *E. coli* is found in large numbers in the feces of humans and of nearly all warmblooded animals; as such it serves as a reliable index of recent fecal contamination of water.
2. *E. coli* is abundant in human and animal feces, in fresh feces it may attain concentrations of 109 per gram. It is found is sewage, treated effluents, and all natural waters and soils subject to recent fecal contamination; whether from humans, wild animals, or agricultural activity.
3. *E. coli* may be present or even multiply in tropical waters not subject to human fecal pollution. However, even in the remotest regions, fecal contamination by wild animals, including birds, can never be excluded, because animals can transmit pathogens that are infective in humans, the presence of *E. coli* must not be ignored.

Total Coliform

The term "coliform organisms (total coliforms)" refers to Gram negative, rod shaped bacteria capable of growth in the presence of bile salts or other surface-active agents with similar growth-inhibiting properties, and able to ferment lactose at $35 - 37^{o}C$ with the production of acid, gas, and aldehyde within $24 - 48$ hours.

They are also oxidase-negative and non-spore-forming. These definitions have recently been extended by the development of rapid and direct enzymatic methods for enumerating and confirming members of the coliform group.

The existence both of non-fecal bacteria that fit the definitions of coliform bacteria and of lactose negative coliform bacteria limits the applicability of this group as an indicator of fecal pollution.

The coliform test can therefore be used as an indicator both of treatment efficiency and of the integrity of the distribution system.

Microbiological water quality testing methodology:

Sample collection procedure for Bacteriological analysis of drinking water.

1. Remove any attachment from the tap.
2. Using a clean cloth outlet of the tap wipe to remove any dirt.
3. Turn on the tap for maximum flow and the water may run for two minutes.
4. Outlet of the tap is sterilized by means of flame from cigarette lighter.
5. Tap again opened to flow for 1 to 2 minutes at medium flow rate.
6. Sterile 250 ml plastic bottle is taken for sample collection.
7. Carefully unscrew the cap and immediately hold the bottle under the water jet and fill.
8. Water filled up to 200 ml and a small air space is left to make shaking before analysis.
9. Collected sample delivered to laboratory within 20 to 30 minutes and inoculated immediately.

Method for testing Total Coliform & *E. coli*

Name of the method: Multitube fermentation technique/MPN method

Presumption test:

1. Inoculated in 10 ml tubes containing Mac Conkey broth and Durham tubes.
2. Tubes are kept in the incubator $37^{o}C$ for 24 to 48 hours.
3. Any presence of bacteria will show gas production or color change of the broth from violet to yellow.

Confirmation test for total coliform:

1. Inoculate from the positive tube from presumption test in Brilliant green broth which contains Durham tubes.
2. The temperature is $37 + 0.5^{o}C$.
3. Presence of gas production confirm the presence of bacteria.

Confirmational test for *E. coli*

1. Inoculate from the positive tube from presumption test in EC broth which contains Durham tubes.
2. The temperature is $44 + 0.5^{o}C$.
3. Presence of gas production confirm the presence of bacteria.

Completed test for *E. coli*

1. Inoculate from the positive tube from conformation test for *E. coli* in EMB agar.
2. Presence of metallic sheen confirm the presence of *E. coli*.

Considering the important aspects of the need to test the potability of water, it is essential that water quality is continuously monitored and adequate steps are taken to ensure the same.

Vancomycin-resistant Enterococci (VRE)

What is VRE?

Vancomycin-resistant enterococci (VRE) are strains of Enterococcus faecium and Enterococcus faecalis that have become resistant to vancomycin. Enterococci are germs that live in the gastrointestinal tract (bowels) of most individuals and, generally, do not cause harm (this is termed "colonization").If a person has an infection caused by VRE, such as a urinary tract infection or blood infection, it may be more difficult to treat.

Out of more than a dozen forms of enterococci bacteria, two are the primary concern for human disease: *E. faecium* and *E. faecalis*. *E. faecium* is the most frequent species of VRE found in hospitals.

How is VRE Acquired and Spread? Risk factors for VRE acquisition include severity of underlying illness, presence of invasive devices, prior colonization with VRE, antibiotic use, and length of hospital stay. VRE is most commonly spread via the transiently colonized hands of healthcare workers who acquire it from contact with colonized or infected clients/patients/ residents, or after handling contaminated material or equipment. VRE can survive well on hands and can survive for weeks on inanimate objects such as toilet seats, taps, door handles, bedrails, furniture, and bedpans. Hospitalized clients/patients/residents with gastrointestinal carriage of VRE are the major reservoirs. VRE transmission via environmental sources includes: Most items in the healthcare environment including blood pressure cuffs, electronic thermometers, monitoring devices, stethoscopes, call bells, and bed rails, touching articles soiled by feces. Contamination of the environment with VRE is more likely when a client/patient/resident has diarrhea. The number of colonized clients/patients/residents ("colonization pressure") will also influence the likelihood of acquiring VRE. VRE is not transmitted through the air.

Where does VRE come from?

VRE occurs from the bowels of some people who have taken antibiotics, often at very low or undetectable levels. When people receive specific antibiotics such as vancomycin, VRE may be selected for and become detectable. Excessive use of antibiotics for minor infections, such as the common cold, where antibiotics are not required, is likely to be a major contributor to the emergence of VRE. In some parts of the world, the emergence of VRE has also been linked to use of antibiotics in animal husbandry.

When is hospital patients tested?

As the VRE germ lives in the bowel, testing for VRE involves taking a faeces sample or a rectal swab. Unfortunately, the VRE germ is difficult to detect and for this reason it may be necessary to take several samples. Pending the outcome of results, which will take several days to finalise, nursing staff will take special precautions such as the wearing of a gown and gloves. A card alerting the staff to take these precautions may be placed on the door.

BEST PRACTICES

Consistent use of Routine Practices with all clients/patients/residents is critical to preventing transmission of microorganisms from client/patient/resident to client/patient/resident and to staff.
Four elements include: hand hygiene, risk assessment, risk reduction and education.

RP is the base upon which additional precautions are applied as indicated by the nature of the microorganism or syndrome encountered. These practices describe prevention and control strategies to be used with all clients/patients/residents during all care, and include:

Hand hygiene with an alcohol-based hand rub (ABHR) or with antimicrobial soap and water before and after physical contact with a client/patient/resident, or with a contaminated environment. Personal protective equipment (PPE) to be worn to prevent healthcare worker contact with blood, body fluids, secretions, excretions, non-intact skin, or mucous membranes includes: gloves when there is a risk of hand contact with blood, body fluids, secretions, excretions, non-intact skin, or mucous membranes; gloves shall be used as an additional measure, not as a substitute for hand hygiene. A long-sleeved gown if contamination of uniform/clothing or skin is anticipated. A mask and eye protection or a face shield where appropriate to protect the mucous membranes of the eyes, nose, and mouth during procedures and care activities likely to generate splashes or sprays of blood, body fluids, secretions, or excretions.

Clients/patients/residents who visibly soil the environment, or for whom appropriate hygiene cannot be maintained, shall be placed in single rooms with dedicated toileting facilities. This includes mobile clients/patients/residents with faecal incontinence if stools cannot be contained in diapers and clients/patients/residents with draining wounds who do not keep their dressings in place. Preventing injuries from needles, scalpels, and other sharp devices; never recap used needles. Place sharps in approved sharps containers. Careful handling of soiled linen and waste to prevent personal contamination and transfer to other clients/patients/residents. Cleaning and disinfecting all equipment that is being used by more than one client/patient/resident between uses.

Contact Precautions is the term used to describe additional practices to reduce the risk of transmitting infectious agents that are normally spread via contact with an infectious person. Contact Precautions are used in addition to Routine Practices. Contact Precautions include: Hand hygiene as described in Routine Practices. Appropriate patient placement as described in Routine Practices (e.g. single room). Gloves for entering the patient"s room or bed space. Long-sleeved gown for contact with patient, bed space, frequently touched environmental surfaces or objects. In acute care, putting on a gown on room entry may be advisable. Dedicated use of equipment or adequate cleaning and disinfecting of shared equipment. Visitor Contact Precautions for VRE include: If a visitor is in contact with other patients or is providing direct patient care, they shall wear the same PPE as healthcare workers. Visitors shall receive education regarding hand hygiene and the appropriate use of PPE

Role of the Laboratory Infection Prevention and Control programs must have an established working relationship with a microbiology laboratory.

Screening for other multidrug resistant organisms is based on culture methods. Vancomycin resistant enterococci may be detected in the course of screening programs, and isolates of enterococci from specimens are screened for vancomycin resistance routinely. Isolates that are suspected of being resistant are referred for confirmatory testing.

Notification/Flagging Tracking clients/patients/residents who are colonized or infected with VRE (e.g. by flagging their chart or electronic file) and their contacts has been shown to improve identification and appropriate management of such clients/patients/residents on re-admission. VRE colonized/infected clients/patients/residents should be educated to notify healthcare providers of their positive status. A process should be in place to ensure that patients discharged from hospital receive communication regarding positive culture results. The receiving healthcare setting, family physician, or the physician most responsible for their care should be notified of the screening results. Electronic flagging is the responsibility of the hospital generating the specimen report. VRE have been isolated from various healthcare surfaces including door handles, hydrotherapy tubs, gowns and linens, hospital furnishings, client/patient/resident charts, tourniquets, call bells, telephones, computer keyboards, faucets, and medical equipment such as glucose meters, blood pressure cuffs, electronic thermometers, and intravenous fluid pumps. Widespread contamination of VRE is likely to occur in the rooms of clients/patients/residents who have diarrhea, and VRE may survive on surfaces for days or weeks.

Incontinent faeces or diarrhoea: In addition, wipe over all surfaces with a solution containing 500 ppm of sodium hypochlorite, leave for 10 minutes, rinse the surfaces with clean warm water and leave to dry.

Hospital grade disinfectants are effective against VRE and general routine cleaning and disinfection methods are adequate for dealing. However, routine cleaning may not be adequate to remove VRE from contaminated surfaces. Studies have shown that surface cultures for VRE remain positive when a cloth is dipped back into a cleaning solution after use and re-used on another surface; when supplies in the room are re-used after discharge; when there is insufficient contact time between the disinfectant solution and the surface being cleaned; and when surfaces are sprayed and wiped, rather than actively scrubbed. There has also been reported success in ending an outbreak of VRE using intensive environmental disinfection with twice-daily cleaning. Current disinfecting protocols will be effective if they are diligently carried out and properly performed using friction (scrubbing) and conscientious cleaning of patient-care surfaces, such as bed rails, and frequently touched surfaces, such as hallway handrails, at least once daily. Processes for cleaning and disinfection should include sufficient contact time for disinfectants, appropriate strength of solutions used, use of damp dusting, working from clean to dirty areas and eliminating the practice of dipping a cloth into the cleaning solution after use and reusing it on another surface .

Patient Care Equipment where ever possible, will not be shared between patients, it is recommended for patients known to be colonized or infected. Reusable equipment that has been in direct contact with one patient should be appropriately reprocessed before use by another patient. Items that are routinely shared should be cleaned between patients. Equipment that is visibly soiled should be cleaned immediately, Commodes, like toilets, should be cleaned regularly and when soiled. Bedpans should be reserved for use by a single patient and labeled appropriately. Procedures should be established for assigning responsibility and accountability for routine cleaning of all patient care equipment. Soiled patient care equipment should be handled in a manner that prevents exposure of skin and mucous membranes and contamination of clothing and the environment. Personal care supplies (e.g. lotions, creams, soaps) should not be shared between patients. Nail polish, cuticle conditioner used in hand/foot care should not be shared.

VANCOMYCIN RESISTANT ENTEROCOCCUS (VRE) Information Sheet for Clients/Patients/Residents and Visitors should be prepared.

Always tell your physician, paramedics, nurses, or other care providers that you have VRE. This helps prevent spread to others.

Risk Factors for VRE People at risk for colonization or infection with VRE are usually hospitalized and have an underlying medical condition, making them susceptible to infection. These conditions include clients/patients/residents with: Recent hospitalization in healthcare facilities. Critical illness(es) in intensive care units. Severe underlying disease or weakened immune systems. Urinary catheters.

Admission screening for VRE shall be completed: Check for previous history of VRE, or high risk for VRE, using the admission screening tool. If the client/patient/resident has been a contact of a VRE case in the past, screening specimens shall be obtained. If the client/patient/resident is considered to be at risk for VRE based on the results of the screening tool, screening specimens shall be

obtained. Notify the Infection Control Professional (ICP) or delegate to discuss the infection control management of client/patient/resident activities.

Treatment of infection

Cephalosporin use is a risk factor for colonization and infection by VRE, and restriction of cephalosporin usage has been associated with decreased VRE infection and transmission in hospitals. *Lactobacillus rhamnosus* GG (LGG), a strain of *L. rhamnosus*, was used successfully for the first time to treat gastrointestinal carriage of VRE. In the US, linezolid is commonly used to treat VRE.

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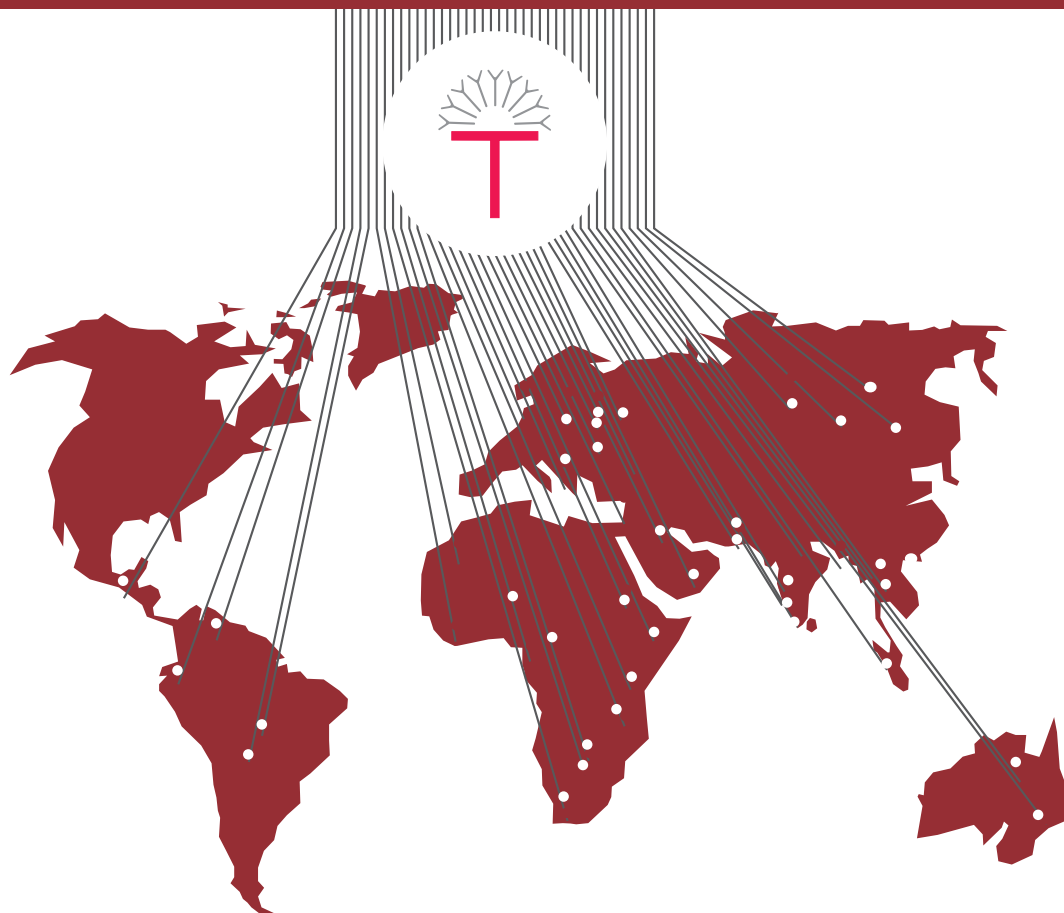
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