

Editorial

Contents

■ Editorial	1
■ Mini review	2
■ Encyclopedia	6
■ Current Trends	7
■ In Profile	9
■ Relax Mood	10
■ Bug of the Month	11
■ Did you Know	13
■ Best Practices	14
■ In Focus	16

The Occupational Safety and Health Administration (OSHA) codified universal precautions to protect healthcare workers against the risk of blood borne disease with the Occupational Exposure to Blood borne Pathogens regulations (OSHA, 1991). These regulations prescribe the use of personal protective equipment (PPE) when blood and other potentially infectious materials are handled. The use of personal protective equipment is a key element in protecting both patients and healthcare workers from acquiring infections in ambulatory care settings. Personal Protective Equipment (PPE) can be defined as specialized clothing or equipment worn for protection against a hazard in work place. This issue Mini Review is entitled as “Personal Protective Equipment: A Key Element to Protect Patient and Healthcare Worker”.

The controlled environment is monitored through an appropriate monitoring program. To assure that minimal bioburden is achieved, additional information on the evaluation of the microbiological status of the controlled environment can be obtained by the use of media fill run. This, or an equivalent test, is performed at least annually by each person authorized to compound in a low risk level under conditions that closely simulate the most challenging or stressful conditions encountered during compounding of low-risk level CSPs. According to USP, media fill run is an essential part for compounding sterile preparation in pharmaceuticals. We have given vivid description of media fill run in our Current Trends section. This time our In Profile section is dedicated to Paul Ehrlich, the man behind the discovery of chemotherapy and curing of deadly disease syphilis.

Candida species are confined to human and animal reservoirs. But *Candida albicans* is an opportunistic pathogen. *Candida albicans* causes infection when the defense mechanisms and immunological conditions of the body are suppressed. In Bug of the Month section we have described about the unicellular fungi *Candida albicans*. The principles of Good Laboratory Practices should be applied to the non-clinical safety testing of test items contained in pharmaceutical products, cosmetic products, veterinary drugs as well as food & feed additives and industrial chemicals. 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. We have explained all the detail of good laboratory practices in our Best Practices part. IMViC test is briefed in Did You Know.

September and October are the month of festivals in our country. We wish all our readers a joyful season of celebrations.

Personal Protective Equipment: A Key Element to Protect Patient and Healthcare Worker

Personal Protective Equipment (PPE) can be defined as specialized clothing or equipment worn for protection against a hazard in work place. The use of personal protective equipment is a key element in protecting both patients and healthcare workers from acquiring infections in ambulatory care settings.

Significance of PPE

The purpose of wearing Personal Protective Equipment (PPE) is to wear additional clothing to protect patients from infection and to protect the healthcare worker (HCW) from occupational exposure to blood and other body fluids. PPE is part of a range of standard precautions - including hand washing, decontamination of equipment and the environment and safe disposal of sharps. PPE provides a barrier between susceptible sites (e.g., surgical wound, intravascular site, healthcare workers mucous membranes) and a potential source of microorganisms (HCW's hands, patient's infected wound) and reduces the chance of infection.

Healthcare workers face a well-recognized risk of acquiring blood borne pathogens in their work place. Although a variety of microorganisms may be spread through blood and body fluids, the pathogens of greatest concern are hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV). The risk for HCWs varies among job practices within a healthcare setting and depends on several factors, including immunization status and the frequency of exposure to blood and body fluids. The Occupational Safety and Health Administration (OSHA) rules, the Centers for Disease Control and Prevention's Standard Precautions (CDC) and other guidelines recommend to avoid exposure to blood borne pathogens, including the use of PPE (e.g., gloves, protective clothing and eye/face protection).

Healthcare workers in ambulatory care settings are at risk of infection whenever they have exposure to blood or other potentially infectious materials (OPIM). OPIM refers to the following human body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pericardial fluid, peritoneal fluid, amniotic fluid, and etc.

Factors Influencing PPE Selection

Microorganisms may be present at all moist body sites and in moist body substances. Ambulatory care facilities need to evaluate the tasks performed by staff and then determine the type of PPE is needed to prevent exposures to patients and staffs. The type route and degree of exposure anticipated to blood and OPIM will determine the selection of appropriate PPE. CDC Guidance for the selection and use of personal protective equipment in healthcare settings recommends three criteria in selecting PPE:

- Type of anticipated exposure - This is determined by the type

of anticipated exposure, such as touch, splashes or sprays, or large volumes of blood or body fluids that might penetrate the clothing. PPE selection is determined by the category of isolation precautions a patient is on.

- Durability and appropriateness of the PPE for the task - This will affect for example whether a gown or apron is selected for PPE, or, if a gown is selected, whether it needs to be fluid resistant, fluid proof, or neither.
- Proper fit - PPE must fit the individual user, and it is up to the employer to ensure that all PPE are available in sizes appropriate for the workforce that must be protected.

With these factors in mind, HCWs can evaluate PPE in different categories to make a selection that best suits their needs.

Types of PPE used in Healthcare Settings

Personal Protective Equipment (PPE) is used to protect hands and uniforms/clothing from being contaminated and acting as a possible source of infection to patients and staff. The main items of protective equipment are:

- Gloves
- Gowns or Aprons
- Facial Protection (Masks, Goggles, Face Shields)
- Respiratory Protection

Gloves

Gloves are the most common type of PPE used in healthcare settings. The use of gloves is not meant to be a substitute for hand washing, but rather as an additional protective measure. Gloves are worn for three important reasons in hospitals:

- a) To provide a protective barrier and to prevent gross contamination of the hands when touching blood, body fluids, secretions, excretions, mucous membranes and non-intact skin.
- b) To reduce the likelihood that microorganisms present on the hands of personnel will be transmitted to patients during invasive or other patient care procedures that involve touching a patient's mucous membranes and nonintact skin.
- c) To reduce the chance of transmitting microorganisms to a patient through hands contaminated with microorganisms from another patient. In this situation, gloves must be changed between patient contacts and hands should be washed after gloves are removed.

Different types of gloves are used in healthcare settings namely: disposable non-sterile gloves, disposable sterile gloves and sterile surgeon's gloves. Gloves are also available in many different materials like natural rubber, latex,

powdered, non-powdered, non-allergenic and synthetic rubber materials (e.g. vinyl, neoprene, nitrile). Non-sterile gloves should be used when hands may come into contact with body fluids or equipment contaminated with body fluids. Vinyl is not recommended for tasks where there is a high risk of contact with blood or body fluids. Sterile gloves should be used when hands are likely to come into contact with normally sterile areas or during any surgical procedure. Most patient care activities require the use of a single pair of nonsterile gloves made of latex, nitrile, or vinyl. Polythene gloves must not be used for healthcare activities.

Gloves should fit the user's hands comfortably they should not be too loose or too tight. They also should not tear or damage easily. Gloves are sometimes worn for several hours and need to stand up to the task. Surgeons and other healthcare personnel who perform invasive patient procedures wear sterile surgical gloves. During some surgical procedures, two pair of gloves may be worn. Environmental services personnel often wear reusable heavy duty gloves made of latex or nitrile to work with caustic disinfectants when cleaning environmental surfaces.

Gloves protect healthcare workers against contact with infectious materials. Contaminated gloves can become a source for spreading infectious materials to HCWs, patients or environmental surfaces. So the usage of gloves can influence the risk of disease transmission in healthcare settings. There are following dos and don'ts of gloves use:

- Work from clean to dirty - This is a basic principle of infection control. In this instance it refers to touching clean body sites or surfaces before you touch dirty or heavily contaminated areas.
- Limit opportunities for "touch contamination" - HCWs should not touch own body parts (nose, face) with contaminated gloves. They should avoid touching unnecessarily environmental surfaces with contaminated gloves. Surfaces such as light switches, door and cabinet knobs can become contaminated if touched by soiled gloves.
- Change gloves as needed - If gloves become torn or heavily soiled during patient care tasks then gloves should be changed before starting the next task. After each patient gloves should be changed and discarded them in the nearest receptacle. Patient care gloves should never be washed and used again. Washing gloves does not necessarily make them safe for reuse; it may not be possible to eliminate all microorganisms and washing can make the gloves more prone to tearing or leaking.

Generally staff members should wear gloves when:

- Handling blood and other potentially infectious materials that are visibly contaminated with blood and or when having contact with mucous membranes.
- Performing wound care.
- Providing care for a patient with active bleeding.
- Obtaining laboratory specimens.

- Providing care for a patient colonized or infected with vancomycin resistant enterococci (VRE) or multi drug resistant *Staphylococcus aureus*.
- Handling or touching contaminated items or surface.

Gowns or Aprons

Gowns are worn to prevent contamination of clothing and the skin. There are three factors that influence the selection of a gown or apron as PPE. First is the purpose of use. Isolation gowns are generally the preferred PPE for clothing but aprons occasionally are used where limited contamination is anticipated. If contamination of the arms can be anticipated, a gown should be selected. Gowns should fully cover the torso, fit comfortably over the body, and have long sleeves that fit snugly at the wrist.

Second is the material properties of the gown. Isolation gowns are made either of cotton or a spun synthetic material that dictate whether they can be laundered and reused or must be disposed. Cotton and spun synthetic isolation gowns vary in their degree of fluid resistance, another factor that must be considered in the selection of this garb. If fluid penetration is likely, a fluid resistant gown should be used. Disposable plastic aprons are recommended if there is a significant risk of splashing of blood and other body fluids.

Aprons are discarded into a biohazard container. The last factor concerns patient risks and whether a clean, rather than sterile gown, can be used. Clean gowns are generally used for isolation. Sterile gowns are only necessary for performing invasive procedures, such as inserting a central line. In this case, a sterile gown would serve purposes of patient and healthcare worker protection.

There is a constant shedding of microorganisms from exposed skin and mucous membrane. Use of barrier clothing in operating rooms is needed to minimize the contamination of the air as well as the wound. Shedding from skin is increased by friction from clothes during activity. Clothing made of ordinary cotton fabric is ineffective in preventing airborne bacterial dispersal from skin to the operating team. Non-woven fabric is more effective.

Facial Protection

Facial barrier protection is used if there is a potential for splashing or spraying of blood or certain body fluids. A combination of PPE types is available to protect all or parts of the face from contact with potentially infectious material. The selection of facial PPE is determined by the isolation precautions required for the patient and/or the nature of the patient contact. Protective glasses, goggles, masks and chin length face shields should be worn whenever splashes, spray, spatter or droplets of blood or other potentially infectious materials may be generated and there is potential for mucous membrane contamination.

Goggles, visors or protective spectacles must be worn to protect the eyes from aerosol or splash contamination of body fluids or chemicals. Prescription spectacles are inadequate protection unless fitted with side protectors. Personal prescription lenses do not provide optimal eye protection and should not be used as a substitute for goggles. Eye protection must fit correctly and be comfortable to wear. Goggles with antifog features help to maintain vision clarity. Some of these items are not disposable. The appropriate method of decontamination must be implemented before reuse.

Masks should fully cover the nose and mouth and prevent fluid penetration. Masks should fit snugly over the nose and mouth. For this reason, masks that have a flexible nosepiece and can be secured to the head with string ties or elastic are preferable. General surgical facemasks must be worn during following procedures:

- Protect HCWs from the potential exposure to microorganisms via splashes of blood and body fluids.
- Protect patients from potential shedding of microorganisms from HCWs.

Masks must only be handled by their strings and should be handled as little as possible. They must not be worn around the neck or be removed from the face except when they are to be discarded. If the mask becomes contaminated with body fluids it should be changed immediately.

When skin protection, in addition to mouth, nose, and eye protection, is needed or desired, for example, when irrigating a wound or suctioning copious secretions, a face shield can be used as a substitute to wearing a mask or goggles. The face shield should cover the forehead, extend below the chin, and wrap around the side of the face.

Respirators

PPE also is used to protect healthcare workers from hazardous or infectious aerosols, such as *Mycobacterium tuberculosis*. Respirators prevent the inhalation of harmful airborne substances and provide fresh air in an oxygen deficient environment. The most commonly used respirators in healthcare settings are the N95, N99, or N100 particulate respirators. The device has a sub-micron filter capable of excluding particles that are less than 5 microns in diameter. The CDC's National Institute approves respirators for Occupational Safety and Health. Like other PPE, the selection of a respirator type must consider the nature of the exposure and risk involved.

Personnel entering the room of a patient with infectious tuberculosis might wear N95 particulate respirators. If a bronchoscopy is performed on the patient, the healthcare provider might wear a higher level of respiratory protection, such as a powered air-purifying respirator or PAPR.

Foot wear

Rubber boots or other boots that are compatible with decontamination solutions are recommended for all employees who work in contaminated areas, and are an essential part of biosecurity protocols. Boots should be left on site. Special footwear should be worn in the operating theatre. All footwear should be cleaned after every use, and procedures should be in place to ensure that this is undertaken at the end of every session. Theatre footwear should not be worn out with the department. Boot covers are not usually durable enough, and do not provide a sufficiently stable walking surface, for most work operations, but may be appropriate for visitors.

Caps

Caps worn to prevent hair from falling into an open wound or sterile surface. Single use disposable caps should be used. Caps should not be worn out of the operation theatre.

Sequence For Donning and Doffing of PPE

Healthcare workers should concern about the correct order in which to don and doff personal protective equipments. Correct sequence of using PPE prevents self- contamination as well as contamination of environment. While using PPE following things should be maintained:

- Don PPE before contact with patient, generally before entering the room.
- Keep gloved hands away from the face and avoid touching and adjusting PPE.
- Remove and discard PPE carefully. This should be done at doorway (just prior to leave patient's room) or immediately outside patient room.
- Immediately perform hand hygiene using high-level disinfectant.

Personal protective equipment must be put on (donned), taken off (doffed), and cleaned or disposed of (decontaminated) in a systematic manner. While the specific method of donning and doffing depends on the equipment used. Sequence for donning PPE:

- Gown or apron
- Mask or respirator
- Goggles or face shield
- Gloves

The gown should be donned first. The mask or respirator should be put on next and properly adjusted to fit. Before donning respirator should be checked properly. The goggles or face shield should be donned next and the gloves are donned last.

Donning gowns / aprons

To don a gown first appropriate size has to be selected. The opening of the gown should be in the back and the gown is secured at the neck and waist. The gown should fully cover

torso from neck to knee, arms to end of wrist and wrap around the back.

Doffing (removal) gowns/ aprons

While removing the gown following steps should be followed:

- i. Gowns are tied unfasten first.
- ii. Then gown is peeled away from neck and shoulder.
- iii. Contaminated outside is turned toward the inside.
- iv. Gowns are folded or rolled into a bundle and kept for discarding.

Donning mask or particulate respirator

Some masks are fastened with ties, others with elastic. If the mask has ties, the mask is placed over the mouth, nose and chin. The flexible nosepiece is fit to form the nose bridge. The upper set is tied at the back of head and the lower set is tied at the back of neck. If the mask has elastic headbands, holding the mask in one hand and the bands in the other separates two bands. Placing and holding the masks over nose, mouth and chin, bands are stretched over head and secured them comfortably; one band on the upper back of head and the other below the ears at the base of the neck.

The technique for donning a particulate respirator (e.g. N95) is similar to putting on a pre formed mask with elastic headbands. Manufacturer's instructions should be followed while putting on a respirator. An incorrectly fitted mask will not provide the intended level of protection from airborne infectious diseases.

Doffing of mask or respirator

While removing the mask following steps should be followed:

- i. The bottom band is untied first then the top band is untied.
- ii. Mask is removed from the face and discarded.

Donning eye and face protection

If eye protection is needed, either goggles or a face shield should be worn. Eye or face protections to be worn when attending patients with extensive trauma, gastrointestinal bleed or massive arterial bleeding, bleeding from nose or mouth, dental procedures and during bronchoscopy. Position either device over the face and/or eyes and secure to head using the attached earpieces or headband. Goggles should feel snug but not tight. Face shield is positioned over face and secured on brow with headband. It should be adjusted to fit comfortably.

Doffing of eye and face protection

The following steps should be followed while removal of eye and face protection:

- i. With ungloved hands ear or headpiece is grasped.
- ii. Then they are lifted away from face and placed in designated receptacle for cleaning or disposal.

Donning of gloves

The last item of PPE to be donned is a pair of gloves. While wearing gloves organisms may be picked up from the environment or patients with whom there is contact. Therefore contact with personal or shared equipment while wearing contaminated gloves shall be avoided i.e. telephones, computer keyboards, other equipment or surfaces. Donning of gloves include following steps:

- i. Hands are inserted into gloves and pulled near the wrist towards fingertips until the glove folds over.
- ii. Gown cuffs are adjusted securely under the gloves.

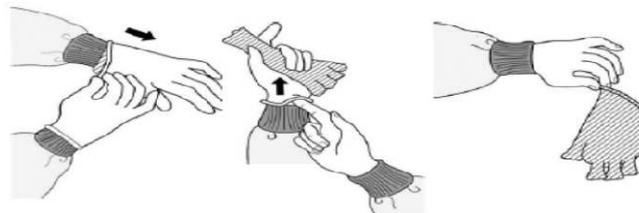


Fig.: Doffing of gloves

Doffing of gloves

Steps followed for doffing the gloves:

- i. Outside of the glove is grasped with opposite gloved hand and peeled off.
- ii. Removed glove is held in gloved hand.
- iii. Fingers of ungloved hands are slide under remaining glove at wrist.
- iv. Another glove is also peeled off and discarded.

PPE should be removed in the following sequence:

- Gloves
- Face shields or goggles
- Gown
- Mask or respirator

It is important to remember that the sequence for removing PPE is intended to limit opportunities for self-contamination and self-inoculation. It is important to identify the clean and contamination sites or areas. This is especially important when identifying the safest sequence for removal.

The use of personal protective equipment (PPE) for prevention of cross infection is very important when considering the mortality and morbidity associated with infection and the cost of treating healthcare associated infections.

References

- Personal Protective Equipment in Healthcare Settings, October 1, 2004. Center for Disease Control and Prevention.
- Healthcare Hazard Control and Safety Management, James T. Tweedy.
- Infection Control in Ambulatory Care. Candace Friedman & Kathleen H. Petersen.

Endocarditis is an inflammation or infection of the inner layer of the heart, endocardium. Endocarditis commonly affects heart valves, but may also involve non-valvular areas and/or mechanical devices that are implanted in the heart such as artificial heart valves, pacemakers or implantable defibrillators. Other structures, which may be involved, include the interventricular septum, the chordae tendinae, and the mural endocardium or even on intracardiac devices. Endocarditis is uncommon in people with a healthy heart. Endocarditis may occur in people who have certain pre-existing heart diseases. Men are twice as likely to be affected by endocarditis as women. Endocarditis can occur at any age, but is more common in people aged 50 years and over.

Endocarditis causes vegetations (clumps of bacteria and cells) to form on the heart valves, making it more difficult for the heart to function properly. It can also cause infection to spread to other parts of the body, such as kidneys, lungs and brain. In some cases endocarditis also causes abscesses to develop in the heart muscle. The bacteria and fungi that cause endocarditis usually enter the body through everyday activities.

Classification

Endocarditis can be classified based on etiology: infective or non infective, depending on whether a microorganism is the source of inflammation. Infective endocarditis has been clinically divided into acute and sub-acute endocarditis. This classifies both the rate of progression and severity of disease. Infective endocarditis may also be classified as culture positive or culture negative.

Symptoms

In most cases endocarditis develop slowly. Symptoms tend to appear gradually, usually over a period of several weeks or months. This is known as sub-acute endocarditis. In some cases, the infection can develop very quickly. This is known as acute endocarditis. The symptoms of acute endocarditis tend to be more severe and can develop after just a few days. The symptoms of 'acute bacterial endocarditis' (ABE) occur within a few weeks of infection. The symptoms of 'subacute bacterial endocarditis' (SBE) may take weeks or months to develop. Some of the general symptoms of endocarditis can include:

- Fever and chills
- Lethargy
- Loss of appetite
- Generalized aching throughout the body
- Abnormal heart rhythms such as a murmur or tachycardia (rapid heart rate)
- Increased breathing
- Persistent cough

Causes Of Endocarditis

Bacteria that enter the blood stream and attach it to heart valves and tissues most commonly cause endocarditis. The most common types of bacteria that cause endocarditis are streptococci or staphylococci bacteria. In rare cases, fungi or other

microorganisms can also cause infection. The infection causes inflammation of the endocardium and can also cause vegetations to develop on the heart valves and muscles. Sometimes the bacteria that cause endocarditis are those that live in mouth, upper respiratory tract or other parts of human normal flora. However, if these organisms make their way into endocardium, they can attack the heart tissue, causing endocarditis to develop.

Bacteria can occasionally spread from areas of human body that are already infected. Intestinal disorders such as inflammatory bowel disease may also give bacteria the opportunity to enter bloodstream. Certain medical and dental procedures provide an opportunity for bacteria to enter the bloodstream. For example: professional cleaning or extraction of tooth can allow bacteria in through the gums. Some diagnostic tests also pose a risk, including gastrointestinal procedures used to examine the organs. One of the most common gastrointestinal procedures is colonoscopy. Some procedures on the genitourinary tract (kidney, bladder and urethra) can also lead to infection. Contaminated needles and syringes also pose a threat for intravenous (IV) drug users.

High Risk Groups

Endocarditis rarely occurs in people with a healthy heart. Risk factors that are linked to endocarditis include:

- Congenital heart defects
- Prior surgery to correct heart defect
- Surgical devices such as pace maker, artificial heart valve, and shunt
- Rheumatic heart disease
- Intravenous drug use with no medical history of heart disease

Diagnosis

Following tests should be performed to diagnose endocarditis:

- Physical examination
- Medical history
- Urine tests
- Blood tests
- Diagnostic imaging such as ultrasound

The infection on the valve can cause build up of nodules on the valves called vegetations. These valve vegetations can be detected by echocardiography. The most accurate method of detecting valve vegetations is with a procedure called transesophageal echocardiography.

Treatment

High dose of antibiotics are administered by the intravenous route to maximize the diffusion of antibiotic molecules into vegetation from the blood filling the chambers of the heart. This is necessary because neither the heart valves nor the vegetations adherent to them are supplied by blood vessels. Antibiotics are continued for a long time, typically two to six weeks. Specific drug regimens differ depending on the classification of the endocarditis as acute or sub-acute. Fungal endocarditis requires specific antifungal treatment, such as amphotericin B.

Media Fill Run

An Essential Part of Aseptic Manufacturing in Pharmaceutical Industry

Aseptic processing can be defined as a method by which the drug formulation and the primary packaging components are sterilized separately and assembled in a final filling solution. Before aseptic assembly, different parts of the final product are generally subjected to different sterilization processes, such as dry heat for glass containers, moist heat sterilization for rubber closures and sterile filtration for liquid dosage form. Each of the processes of the aseptic manufacturing operation requires thorough validation and control. The standard for aseptic manufacturing of medicinal drug products have become very high and clearly specified because of the nature of the pharmaceutical form in which they are administered (for e.g. Injections, Infusions, Pharmaceutical forms for the eyes such as eye drops etc.). Parenteral products are intended to be nonpyrogenic too, additionally to the requirement to be sterile. Medicinal drug products that do not meet the requirement to be sterile/nonpyrogenic can otherwise cause severe harm or life threatening health risk to the patient.

Aseptic manufacturing is used in cases, where the drug substance is instable against heat; hence sterilization in the final container closure system is not possible. Aseptic manufacturing means that the used drug substances were sterilized appropriately and all materials, equipments and container closure systems should be used only after sterilization. All working steps should be performed in sterile areas to avoid contamination.

Historical Aspects

Aseptic processing activities are evaluated through process simulations in which a microbiological growth medium is utilized in the process in lieu of the product. The media is incubated after completion of the process to evaluate the procedures utilized. This technique was first applied in the late 1940s by Rhode and incorporated into a World Health Organization guideline in the mid 1970s. In the late 1980s, the PDA (Parenteral Drug Association) developed one of the first guides to the execution of media fills for the evaluation of aseptic processing. USP also recommends aseptic manufacturing for compounded sterile preparations (CSP).

Significance of Media Fill

Simulating production conditions and using a bacterial culture medium as product validate aseptic fill processes. This process simulation test is commonly referred to as a "media fill". According to all guidelines the process simulation with media fill is state of the art for the validation of aseptic manufacturing process.

To evaluate the aseptic processing activities process simulation test has come in trend to take up a wider range of aseptic processing activities in pharmaceutical industry. The purpose of media simulation is to demonstrate that the whole process is capable of reliably producing sterile products from aseptic manufacturing techniques. To do this, it is necessary to simulate

or mimic the entire process. In the last ten years media fills have developed a good impact on regulatory bodies in terms of validation of aseptic manufacturing. Any new aseptic processes require validation by media fill. Any processes (irrespective of the equipment being old or new) beginning in a new clean room requires media fills as a part of validation. A new filling machine in an established clean room requires validation through media fill.

Validation of Media Fill

Media fills or process simulation technique is generally accepted as the procedure to validate aseptic manufacturing processes. Liquid nutrient growth medium, capable of supporting a wide range of microorganisms, is prepared, sterilized, and filled in simulation of a normal manufacturing process that includes compounding, sterile filtration, in-process controls, sterilization of manufacturing process, materials (garments, primary containers, filling equipment), cleaning and sterilization process (e.g., cleaning in place - CIP / sterilization in place - SIP) and filling. The sealed containers of medium thus produced are then incubated under prescribed conditions and subsequently examined for evidence of microbial growth. If the media fill reflects the standard procedure of product filling, the contamination rate or contamination probability may be used as indicator for the safety of the production process. Comprehensive control of production environment, personnel, and installations, influencing the overall hygienic state of manufacturing processes will be performed.

Since, in pharmaceutical production, validated methods have been already used for sterilizing equipment, processing air and water and filtration techniques, media fill validation is very much focused on the aseptic technique of the human operator. Intensive training and education of personnel is required in order to ensure that media fill validation is recognized as a means of checking sterility level of aseptic processing.

Study design

A validation protocol should detail the overall strategy, testing requirements and acceptance criteria for the media fill. The process simulation test should imitate as closely as possible the routine manufacturing process and include all the critical subsequent manufacturing steps. Following steps should be applied for a proper media fill study:

Factors associated with the longest permitted run on the processing line

Ability to produce sterile units when environmental conditions impart a greater risk to the product

Aseptic assembly of equipment

Shift changes, breaks and gown changes (when applicable)

Line speed and configuration

Manual weight checks

Container closure systems (e.g. sizes, type and compatibility with equipment)

Operator fatigue

For each media fill run a batch record should be maintained. The same vigilance should be observed in both media fill and routine production runs.

Frequency and Number of Runs

In a newly started processing line, media fills should be repeated enough times to ensure the results are consistent and meaningful. A minimum of three consecutive separate successful runs should be performed during initial line qualification. Simultaneously, routine semiannual revalidation runs should be conducted for each shift and processing line to evaluate the state of control of the aseptic process. Each change to a product or line change should be evaluated by a written change control system. Any changes or events that have the potential to affect the ability of the aseptic process to exclude contamination from the sterilized product should be assessed through additional media fill run.

Duration and Size of Runs

The duration of media fill is one of the most important issues. The duration of the run and the overall study design should adequately mimic worst case operating conditions. The duration of the media fill run should be determined by the time it takes to incorporate manipulations and interventions. Interventions that commonly occur should be routinely simulated while those occurring rarely can be simulated periodically. While conventional manufacturing lines are usually automated, operated at relatively high speeds and designed to limit operator intervention. When aseptic manufacturing employs manual involvement the duration of the process simulation should not be less than the length of actual manufacturing process.

Media fill size is an especially important consideration because some batches are produced over multiple shifts. A simulation run sizes should be adequate to mimic commercial production conditions and accurately assess the potential for commercial batch contamination. The number of units filled during the process simulation should be based on contamination risk for a given process and sufficient to accurately simulate activities that are representative of the manufacturing process.

Filling Speed

The extremes of filling speed on the line should be considered in the validation planning. The use of the slowest normal filling speed may increase the potential for contamination ingress via deposition from the surrounding environment. The use of fastest normal speed may increase the potential for human intervention by increasing the number of routine and nonroutine line intervention.

Fill Volume

The volume of media filled into the containers should be sufficient to contact the container-closure seal surfaces and sufficiently enough to allow for easy inspection of the filled units post incubation.

Media

In general, a microbiological growth medium, such as soyabean casein digest medium is used. Use of anaerobic growth medium

(e.g. fluid thioglycollate medium) should be considered in special circumstances. Both media relate to the quality control of pharmaceutical medicinal products because of their use in sterility testing.

The execution of the media fill begins with the sterilization of the liquid media. This can be accomplished using either bulk sterilization of liquid media in a container or filtration. The choice of sterilization method is based on considerations of the volume of media required, growth promotion requirements and filtration rate for the media.

Environmental Conditions

Media fills should be conducted under environmental conditions that simulate normal as well as worst-case conditions of production. An aseptic processing activity is ordinarily supported by monitoring of the environmental air and surfaces in proximity to the process.

Incubation and Examination of Media filled Units

The container filled with media should incubate for 14 days at suitable temperature, which allows the growth of the wide range of microorganisms. Personnel with appropriate education, training and experience in microbiological techniques should examine each media filled unit for contamination. When a firm performs a final product inspection of units immediately following the media fill run, all integral units should proceed to incubation. Units found to have defects not related to integrity (e.g., cosmetic defect) should be incubated; units that lack integrity should be rejected. Erroneously rejected units should be returned promptly for incubation with the media fill lot.

Interpretation of Results

The process simulation run should be observed and contaminated units should be reconcilable with the approximate time and the activity being simulated during the media fill. Any contaminated unit should be objectionable and investigated. In case of a media fill failure; a comprehensive investigation should be conducted, surveying all possible causes of the contamination. Whenever contamination exists in a media fill batch, it should be considered as indication of a potential production problem. Along with the other quality assurance measures, a robust media fill program is a necessary step to validate processes of organizations that prepare compounded sterile preparations (CSPs).

According to United States Pharmacopoeia (USP) chapter <797> Pharmaceutical Compounding Sterile Preparations media fill run is an essential criteria for maintaining sterility in pharmaceutical industry. Media fill testing is just one part of a necessary overall quality assurance program. Alone, it may not provide all data enough data to fully validate compounding, but it is an important step in the overall pharmacy quality assurance process. Organizations should follow the proper guidelines for the validation of media fill test.

References

United States Pharmacopoeia 31- NF 26, 2008.



Paul Ehrlich

Birth: March 14, 1854

Death: August 20, 1915

Nationality: German

Known For: Hematology, Immunology and Chemotherapy

Through his comprehensive study of the effects of the chemicals in the human body, Ehrlich fathered the fields of chemotherapy and hematology. He also made important contributions to the understanding of immunity and discovered Salvarsan, the effective treatment for syphilis.

Ehrlich was born on March 14, 1854, in Strehlen, Silesia, once a part of Germany, but now a part of Poland. He was the fourth child of the prosperous Jewish couple Ismar Ehrlich and Rosa Weigert. Ehrlich attended school in Breslau. Ehrlich's interest in biology and chemistry led him to study medicine. He attended universities in Breslau, Strasbourg, Frieberg-im-Briesgau, and Leipzig, earning his medical degree in 1878.

As a medical student Ehrlich undertook his own investigations into cell staining techniques, observing that dyes could selectively stain different types of cells. Ehrlich noted that different chemical structures of the dyes gave them different cell staining properties, leading him to the hypothesis that there was a very specific chemical attraction between the dye and certain cells or parts of cells. This concept of specific chemical attractions was to guide much of his life's work. Following graduation from medical school in 1878, Ehrlich accepted a position at a Berlin hospital where he employed his knowledge and skill with synthetic dyes to make numerous important contributions to medicine. In Berlin Medical Clinic Ehrlich continued his work with dyes and staining of tissues with them. Ehrlich showed that all the dyes used could be classified as being basic, acid or neutral and his work on the staining of granules in blood cells laid the foundations of future work on hematology and the staining of tissues. He became interested in the selectivity of dyes for specific organs and tissues and cells and he continued his investigations at the Berlin hospital. He was able to use dyes to differentiate several types of red and white blood cells, including leukemia cells, and to assist German bacteriologist Robert Koch in staining and identifying the tuberculosis bacterium. Using aniline dyes Ehrlich investigated white blood cells.

In 1882 Ehrlich became Titular Professor and later he became an Associate Professor there and Senior House Physician to the Charité Hospital in Berlin. In 1890 Robert Koch, Director of the newly established Institute for Infectious Diseases, appointed Ehrlich as one of his assistants and Ehrlich then began the immunological studies with which his name will always be associated.

In 1896 Ehrlich became the director of the newly founded Institute of Serum Research and Examination in Steglitz (Berlin).

There he started work on the chemical nature of immunity, antitoxin sera and the nature of the binding of antigen to antibody. He worked with Emil von Behring and Shibasaburo Kitasato on the study of immunity, or the body's own defense against disease. The group searched for a substance that would give immunity against diphtheria using antitoxins. He also showed that the toxin-antitoxin reaction is, as chemical reactions are, accelerated by heat and retarded by cold. During this work Ehrlich inspired to formulate his famous side chain theory of immunity. Ehrlich also continued his study of blood using staining techniques. Realizing that stains colored bacteria but not surrounding cells, he looked for a way to combine the stain with a substance that could kill the bacteria. This, he reasoned, could be a "magic bullet" in the fight against bacterial diseases. He also identified dyes, such as trypan red, that had the ability to destroy microorganisms on their own.

In 1897 Ehrlich was appointed Public Health Officer at Frankfurt and when, in 1899, the Royal Institute of Experimental Therapy was established at Frankfurt, Ehrlich became its Director. Here Ehrlich started research on chemotherapy and infectious disease. In 1904 Ehrlich became honorary professor of the University of Göttingen. In 1906 he discovered the structural formula of atoxyl, a chemical compound that had been shown to be able to treat sleeping sickness. Ehrlich and his student Sahachiro Hata developed Salvarsan, an effective drug against syphilis. Later on one more effective drug, Neosalvarsan was discovered. This discovery had a great importance, which led to the development of sulfa drugs, penicillin and other antibiotics. During the later years of his life, Ehrlich was concerned with experimental work on tumours and on his view that sarcoma may develop from carcinoma.

Ehrlich was an ordinary, foreign, corresponding or honorary member of no less than 81 academies and other learned bodies in different countries. He held honorary doctorates of the Universities of Chicago, Göttingen, Oxford, Athens and Breslau, and was also honoured by Orders in Germany, Russia, Japan, Spain, Roumania, Serbia, Venezuela, Denmark (Commander Cross of the Dannebrog Order), and Norway (Commander Cross of the Royal St. Olaf Order). In 1906 the Prize of Honour at the XVth International Congress of Medicine at Lisbon, in 1911 the Liebig Medal of the German Chemical Society, and in 1914 the Cameron Prize of Edinburgh. In 1908 he shared with Metchnikoff the highest scientific distinction, the Nobel Prize. The Prussian Government elected him Privy Medical Counsel in 1897, promoted him to a higher rank of this Counsel in 1907 and, in 1911, raised him to the highest rank, Real Privy Counsel with the title of Excellency.

Ehrlich died of stroke in Bad Homburg in 1915, at the age of 61. His life is portrayed in the movie "The Magic Bullet", which is focused on Salvarsan, his great contribution to cure of syphilis. Paul Ehrlich made notable contributions in several areas of medicine including selective dye staining of cells, immunology, cancer research, and chemical therapy of infectious diseases which have widened the path of modern research.



Candida albicans

The genus *Candida* is yeast like fungi. Clinically, the most significant member of the genus is *Candida albicans*, which can cause numerous infections in humans and especially in immunocompromised patients. The history of candidiasis is very old as the disease was described in the ancient times and gradually its etiological agents, diagnostic procedures and therapeutic measures were established. Bennett isolated the fungus in 1844 from sputum of a patient suffering from tuberculosis. Later on it was isolated from other body sites like vagina, blood and brain by various workers. The nomenclature of the fungus presently known as *Candida albicans* was also designated as *Oidium albicans* and *Monilia albicans*.

Morphology & Cultural Characteristics

The yeasts are spherical, oval or elongated and reproduce by budding. The buds usually separate from the mother, but sometimes some buds remain attached. In such cases, when the cells are elongated, the terminal bud becomes longest and the yeast cells appear elongated. This form is called a *Pseudomycelium*. *Candida albicans* is thin walled, non-capsulated, oval yeast with or without bud, which produces pseudomycelium in the body and in the culture when the aeration is poor. In nutritionally poor media, at temperatures below 26°C it produces thick walled chlamydospores, and it also produces curved elongated germ tube when transferred to mammalian serum from a peptone-containing medium at 37°C. The yeast cell and the pseudomycelium are stained by Gram's method (Gram positive).

Candida albicans grows well on Sabouraud Dextrose Agar with antibacterial antibiotics at 25°C and 37°C incubation. Cream coloured, smooth and pasty colonies are observed after 3-4 days incubation. On CHROMagar candida, the colonies of *C. albicans* appear as light green to bluish green.

Habitat

Candida species are confined to human and animal reservoirs. They are frequently recovered from the hospital environment, including on foods, counter tops, air-conditioning vents, floors, respirators and medical personnel. They are also normal commensals of diseased skin and mucosal membranes of the gastrointestinal tract, genitourinary and respiratory tracts. When the immunological conditions and defense mechanisms are compromised, it causes infections in the sites it is present and also else where in the body.

Pathogenesis

Candida albicans causes infection when the defense mechanisms and immunological conditions of the body are suppressed. So the infection by *Candida albicans* is also known as opportunistic infection. *C. albicans* has several known virulence factors contributing to its pathogenicity that include adherence to the epithelial and endothelial cells, proteinase production, pseudohyphae formation, phenotypic switching, phospholipase production and antigenic modulation as a result of pseudohyphae formation. *Candida* species has the ability to adhere to host cells with their virulence and clearly plays a major role in the pathogenesis of candidiasis. Binding of *Candida* to epithelial and

endothelial cells is controlled by adhesion of the surface of the fungus that interacts with receptors of the host cells. There are three types of aspartyl proteinases and two types of serine proteinases involved as virulence factors in the pathogenicity of *Candida* species. The phenotypic switching denotes the ability of organisms of single strain to switch reversibility at high frequencies among different and/or changing conditions in the host and thereby assist the fungus in evading the host's defense system.

After yeast cells of *Candida* encounter a particular host tissue, colonization takes place at the local site or it invades deeper into the host tissue. The transformation into the hyphal form is observed during an active infection. The hyphae being larger than the yeast form are more resistant to phagocytosis and thus morphological change contributes to the increased pathogenic potential of the fungus. Most of the manifestations are associated with biofilms displaying properties dramatically different from free living cells grown under normal laboratory conditions. *Candida* is present in the healthy and moist areas of the skin. Infections of the mucus membrane are known as thrush. The infections may be of different types.

Clinical Manifestations

The *Candida* species are found commensal on mucosal surfaces of the body but they can cause disease as and when conditions are favourable. This yeast like fungi typically colonizes mucocutaneous surfaces, which can be portals of entry into deeper tissues when the host defenses are compromised. They may cause a simple lesion to even the life threatening systemic infections.

A. Mucocutaneous Manifestations There are various mucosal sites involved in candidiasis which is briefly described below:

- ✓ Oral candidiasis - This is the most common form of disease produced by colonization of *Candida* species and it is also known as oral thrush. Oral candidiasis can occur at any age, but it is most common in infants and aged persons. The oropharyngeal candidiasis occurs in infants, individuals with diabetes mellitus, and those receiving antibacterial antibiotics and in patients infected with HIV-1 and HIV-2. Oral candidiasis may be asymptomatic or have symptoms of burning. The infection may diffuse or may remain confined to buccal mucosa, gums, tongue or palate. The acute infection begins with congestive reddening of the mucous membrane that gives a dry, smooth, shiny varnish like appearance. It is accompanied by thirst, metallic taste and a sensation of dryness and burning at the local site. Oral Candidiasis takes the form of a superficial, curdy, gray to white membrane that can be readily scraped off to reveal an underlying erythematous inflammatory base. In the milder expressions, there is minimal ulceration of the mucosal surface and only a superficial subepithelial inflammatory infiltrate. More severe oral infections may produce mucosal ulceration and a correspondingly greater inflammatory reaction.
- ✓ In oral candidiasis four major types are recognized: a) Pseudomembranous, b) Hyperplastic, c) Erythematous (atrophic) and d) Angular cheilitis.

- ✓ Angular cheilitis - The patient presents with sore, erythematous, fissured lesions affecting the angles of the mouth and is commonly associated with denture stomatitis. This may be associated with iron deficiency anemia or vitamin B₁₂ deficiency.
- ✓ Gastrointestinal candidiasis - In immunocompromised hosts disease can encompass the mucosal surfaces of the gastrointestinal tract beyond the esophagus. There is abdominal pain and tenderness with or without nausea, vomiting or low-grade fever.
- ✓ Vulvovaginitis, Balanitis, Balanoposthitis - Vaginal candidiasis is one of the most common infections seen in general practice. This affects primarily young and middle aged females, particularly during their active reproductive life. There is curd like vaginal discharge, itching, burning sensation and dyspareunia among these patients. In males balanitis or balanoposthitis are also caused by various *Candida* species. There is pruritus, associated with erythema whitish lesions over the glans penis and sometimes erosions and edema of the prepuce that may lead to phimosis.

B. Cutaneous Manifestations - The cutaneous types of manifestations of candidiasis are also very significant clinical features, which are given below:

- ✓ Intertrigo - This is an inflammatory lesion of the skin folds. The superimposed infection can be accompanied by lymphangitis and enlarged lymphnodes. An intensely red, moist rash characterizes intertrigo with scaling on the edges. Satellite lesions, small areas of the same rash that are close to the main rash, are characteristic of intertrigo.
- ✓ Paronychia and Onychomycosis - Paronychia is an inflammation of the nail fold usually affecting the hands and at times nails of the feet.
- ✓ Diaper dermatitis - Cutaneous candidiasis is a common cause of diaper rash in infants. Maceration and wet diapers predispose infants to diaper dermatitis. It is also known as napkin candidiasis. There are maculopapules and vesicles coalescing into patches with satellite pustules.

C. Systemic Manifestations - In normal persons if candidemia occur, normal host defenses eliminate *Candida*. But *Candida* invades blood stream in persons after surgery, intravenous drug abuse and in indwelling catheter patients. The following systemic infections in candidiasis are most commonly observed:

- ✓ Urinary tract candidiasis - It is commonly seen in females who get the infection from vulva or vagina. The infection may involve the bladder and kidneys.
- ✓ Candiduria - Candiduria is presence of the yeast cells in the urine and it is an increasingly common finding in hospitalized patients.
- ✓ Endocarditis - Candidemia can cause subacute endocarditis, particularly in patients with a previously abnormal native or prosthetic valve.
- ✓ Pulmonary candidiasis- The pulmonary lesions arise from hematogenous seeding, causing diffuse reticulonodular streaking.
- ✓ Meningitis - It is seen in low birth weight neonates with

candidemia. It is also seen in patients with hematologic malignancies, complicated neurological procedures or intracerebral prosthetic devices such as ventriculo-peritoneal shunts.

- ✓ Candidemia and septicemia - The candidal septicemia usually occurs in the immunocompromised patients.
- ✓ Arthritis - Prosthetic or rheumatoid joints are prone to infection by *Candida* species mainly through hematogenous route. It may be due to direct inoculation during joint surgery or intra-articular corticosteroid injections.
- ✓ Nosocomial candidiasis - *Candida* species have been implicated in the nosocomial infections and frequently important cause of fatal consequences among the hospitalized patients. Candidemia is the most extensively studied nosocomial invasive fungal infection.

Diagnosis

The clinical specimens are collected from the patients depending upon the site of involvement i.e. from superficial lesions or deep-seated infections. Whitish patches from the mucous membrane of the mouth, vagina, skin or nails scraping, sputum are collected with the help of sterile swabs. These are examined in the KOH wet mount or normal saline preparation. Gram staining and periodic acid Schiff base are performed to see the presence of yeast and pseudohyphae of *Candida* species. Other than this the laboratory diagnosis of candidiasis includes culture methods, germ tube & chlamydospore formation, biochemical tests (carbohydrate fermentation & carbohydrate assimilation) and antigen-antibody detection.

Treatment

Antifungal agents are used to control candidiasis. For the oral and mucocutaneous lesions of candidiasis, 1% gentian violet is locally applied. Nystatin can be used to the resistant mucosal lesions as suspension containing 200,000 units/ml. The azole creams like clotrimazole, miconazole, ketoconazole and econazole are also given. For the systemic lesions intravenous infusion of amphotericin B is indicated. The drugs like ketoconazole, fluconazole and itraconazole are given orally.

Control Measures

Candida species has become increasingly significant as pathogens in all fields of medicine. *Candida* is an opportunistic pathogen. Fungal infection with *Candida* has become increasingly common in the neonatal intensive care unit and catheter related infections. *Candida* infection causes many deaths and significantly increases health care costs. The most important prevention method for candidiasis is personal hygiene. Healthcare workers should perform proper hand hygiene techniques with high-level disinfectants to reduce nosocomial infections.

References

Candida albicans. Louise Tenney. M.H. Woodland Publication. Fungal Immunology from an organ perspective. Edited by Paul L. Fidel and Gary B. Huffnagle.

IMViC Test

Confirmatory identification of enteric bacilli is highly essential to monitor and control the fecal contamination of food and drinking water supplies. The members of the *Enterobacteriaceae* family are quite common in the intestinal tracts of humans and lower mammals. The different genera of this family can be identified and confirmed by the IMViC series of tests representing Indole, Methyl red, Voges Proskauer and Citrate tests. IMViC mainly differentiates between typical fecal coliform (*Escherichia*) and non-fecal coliform (*Aerobacter*, *Klebsiella*).

Indole Test

Indole test detects the ability of an organism to produce indole from the amino acid tryptophan. Organisms that possess the enzyme tryptophanase can break down the amino acid tryptophan to indole. When indole reacts with para-dimethyl aminobenzaldehyde (Kovac's reagent) a red-colored complex is produced. The test organism is inoculated into tryptone broth, a rich source of the amino acid tryptophan. Indole positive bacteria such as *Escherichia coli* produce tryptophanase, an enzyme that cleaves tryptophan, producing indole and other products. When Kovac's reagent (p-dimethyl aminobenzaldehyde) is added to a broth with indole in it, a cherry red colour layer (ring) develops at the surface of the medium. The indole test must be read by 48 hours of incubation because the indole can be further degraded if prolonged incubation occurs. The acidic pH produced by *Escherichia coli* limits its growth. Kovac's reagent is the mixture of p-dimethyl aminobenzaldehyde, amyl alcohol and concentrated HCl.

Methyl Red Test (MR test)

The methyl red test detects the ability of an organism; growing in a phosphate buffered glucose-peptone medium, produce sufficient acid (from the metabolism of glucose) to reduce the pH of the medium from 7.5 to about 4.4 or below.

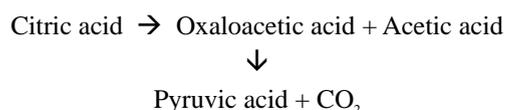
The medium used for the test contains 0.5 % glucose and is buffered with dipotassium phosphate and peptone to give a limiting pH of about 5 when inoculated with typical fecal coliform *Escherichia*. Typical non-fecal coliform *Aerobacter* give a higher final pH. Cultures of *Escherichia* rapidly ferment sugar with the formation of acids until the pH drops about 5. Acidic pH of the medium prevents the further growth. *Aerobacter* produces less amount of acid than *Escherichia* from a given amount of sugar. *Aerobacter* convert some of the sugar to 2:3 butanediol and acetyl methyl carbinol / acetoin. So *Aerobacter* exhausts sugar without producing its limiting pH and continue to grow, utilizing the peptone of the medium for both structure and energy. The medium becomes progressively less acidic in reaction. So the medium containing the inoculum of *Escherichia* turns red colour (acidic pH 5 makes the change in colour of the methyl red indicator) while medium containing the inoculum of *Aerobacter* turns yellow colour (because pH is not in acidic range).

Voges Proskauer Test

VP test detects the ability of an organism to form acetoin (acetyl methyl carbinol). A phosphate buffered glucose-peptone medium is inoculated with the test strain and incubated at 37°C for 48 hours, or at 30°C for at least 5 days. 0.6 ml of an ethanolic solution of 5% -naphthol and 0.2 ml of 40% potassium hydroxide (KOH) solution, are added sequentially to 1 ml of culture. The tube is then shaken vigorously, placed in a sloping position (for maximum exposure of the culture to air). A positive test is indicated by the appearance of a red colour in 2 to 4 hours. *Aerobacter aerogenes* can form 2,3-butanediol (CH₃-CHOH-CHOH-CH₃) and acetyl methyl carbinol (CH₃-CO-CHOH-CH₃) but *E. coli* cannot form these. The acetoin in the presence of KOH and air is further oxidized to diacetyl (CH₃-CO-CO-CH₃), which in the presence of peptone gives rise to red colour.

Citrate Test

This test detects the ability of an organism to use citrate as the sole source of carbon. Media used for the test e.g. Koser's citrate medium (liquid) and Simmon's citrate agar. Media contains citric acid or citrate, ammonium dihydrogen phosphate, sodium chloride and magnesium sulphate. A saline suspension of test organism is made from the growth on a solid medium, using a straight wire. Koser's medium is inoculated from the suspension and is then incubated and examined for signs of growth (turbidity) after one or two days. Koser showed that coliforms could be separated into two groups on the basis of their action on sodium citrate. Typical fecal *Escherichia* was unable to utilize citrate as the only source of carbon; typical non-fecal *Aerobacter* utilized citrate readily. *Aerobacter* uses citrate to acetate, pyruvate and carbon dioxide as follows:



In citrate test, microorganisms are also inoculated into an organic synthetic medium, 'Simmon's citrate agar', where sodium citrate is the only source of carbon and energy. Bromothymol blue is used as an indicator. When the citric acid is metabolized, the CO₂ generated combines with sodium and water to form sodium carbonate an alkaline product, which changes the colour of the indicator from green to blue which indicates positive test.

The growth of *Aerobacter* on citrate as a carbon source required the presence of Na⁺ ion. The optimal concentration of Na⁺ is 0.1 M. the growth of the organism makes the medium alkaline by producing NaOH. Sodium citrate may be impermeable to *E. coli* so unable to utilize it.

References

Fundamental Principles of Bacteriology. 7th Edition By A.J. Salle.

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.

Personal Protective Equipment (PPE) is significantly used in healthcare as well as in industrial segment as we discussed in our Mini Review. So linens used in hospitals, hotels and industries are recommended to disinfectant properly for further usage.

BioShields offers an aldehyde free disinfectant cleaner to serve for the above purpose.

- **Linofsafe™**

It is an aldehyde free disinfectant cleaner for linen recommended for use in hospitals, hotels and industries with good cleansing power and a rapid bactericidal and fungicidal action. Linofsafe™ removes all stains and odours, leaving behind a pleasant smell. It contains 5% w/v Didecyl Dimethyl Ammonium Chloride.

In our earlier issue we have discussed about chlorhexidine gluconate and triclosan as an effective germicidal agent. Sterimax™, the product from BioShields Hand care range, contains chlorhexidine gluconate and triclosan.

- **Sterimax™**

Sterimax™ is a clear pale blue pleasantly perfumed alcoholic hand rub with a powerful triple action, suitable for Surgical Hand Disinfection and Hygienic Hand Disinfection. It contains 2.5% v/v Chlorhexidine Gluconate Solution IP, 0.5% w/v Triclosan USP, 50% v/v Isopropyl Alcohol IP, 25% v/v N-Propanol BP, Skin emollients, perfume, Brilliant Blue FCF as colour. Sterimax™ has following special features:

- Potent, Synergistic formulation
- HIV, HBV, TB cidal
- Combined instant and residual action
- Skin safe
- Non sticky, Soft feel

According to our Current Trends Topic “Media Fill Run: An Essential Part of Aseptic Manufacturing in Pharmaceutical Industry” **Microexpress** recommends the following media for media fill run.

- **Soyabean Casein Digest Medium (Tryptone Soya Broth)**

A general-purpose medium for the isolation and cultivation of a wide variety of fastidious and non-fastidious microorganisms.

- **Fluid Thioglycollate Medium**

A medium for sterility testing of biological and cultivation of aerobes, anaerobes and microaerophiles.

In this issue Did You Know states about the IMViC test for water quality testing and Microexpress offers the following media and reagents for that:

- **MR-VP Medium**

A medium for the differentiation of *coli-aerogenes* group by means of the Methyl Red and Voges Proskauer reactions.

- **Simmons Citrate Agar**

A medium for the differentiation of Gram negative bacteria on the basis of citrate utilization.

- **Kovac's Reagent**

For indole test, particularly useful in identification of *E. coli*.

- **Methyl Red Indicator**

To detect the ability of an organism to produce and maintain stable acid end products formed from glucose fermentation.

- **Barritt Reagent A, Barritt Reagent B, Creatine.**

For Voges Proskauer (VP) Test.

Biochemical tests are important in the identification of *Candida* species and Microexpress offers the following Biochemical Identification Test Kit:

- **Candida Identification Kit**

A biochemical identification kit contains 12-miniature test panel.

Highlights of the coming issue



SERIES I

H E M O L Y S I S

E N D O S P O R E

K O C H

F L A G E L L A

SERIES II

A N T I B I O T I C

N O S O C O M I A L

D I S I N F E C T I O N