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Editorial

Contents

Hope you appreciated the contents, format and presentation in the earlier issues of "The Journal of Hygiene Sciences".

Our Mini review section speaks about Sterility Testing. Sterility can be defined as the freedom from the presence of viable microorganisms. In pharmaceutical practice, a container is defined as sterile when the probability is less than one out of one million that it is contaminated with replicating microorganisms. Sterility testing uses methods with broad sensitivity, normally incubation of samples in growth media promoting growth across a wide range of microorganisms where replication can be detected visually.

The Current Trends section highlights guidelines for safe surgery. Surgical care has been an essential component of health care worldwide for over a century. As the incidences of traumatic injuries, cancers and cardiovascular disease continue to raise, the impact of surgical intervention on public health systems will continue to grow. WHO has undertaken a number of global and regional initiatives to address surgical safety. Much of this work has stemmed from the WHO Second Global Patient Safety Challenge "Safe Surgery Saves Lives". Safe Surgery Saves Lives set about to improve the safety of surgical care around the world by de?ning a core set of safety standards that could be applied in all WHO Member States.

Our In Profile scientist for this month is **Sir Ronald Ross**, (13 May 1857 – 16 September 1932), He was an Indian-born British medical doctor who received the Nobel Prize for Physiology or Medicine in 1902 for his work on malaria. His discovery of the malarial parasite in the gastrointestinal tract of mosquito led to the realization that malaria was transmitted by mosquitoes, and laid the foundation for combating the disease.

The Bug for this time is **Nocardia species.** *Nocardia* species are classically gram-positive, strictly aerobic, filamentous, branching, weakly acid-fast bacilli. They may be isolated on routine bacterial, fungal, and mycobacterial media.). It has a total of 85 species. Some species are non-pathogenic while others are responsible for nocardiosis. *Nocardia* are found worldwide in soil that is rich with organic matter.

Lets see the Health effects of overexposure to sunlight in our Did You Know section.

Our Best Practices section flashes light on Sterile Disinfectants. Microbial contamination in pharmaceutical products has massive consequences. Quality Control is an essential function of the Pharmaceutical industry. Drug manufacturers must thoroughly test materials, processes, equipment, techniques, environments and personnel in order to ensure their final products are consistent, safe, effective and predictable.

All work & no play makes Jack a dull boy! We don't forget that ever. Each issue comes with its own bouquet of jokes, so enjoy……

Sterility Testing

A sterility test may be defined as — '**a test that critically assesses whether a sterilized pharmaceutical product is free from contaminating microorganisms**'.

According to Indian Pharmacopoeia (1996) sterility testing is intended for detecting the presence of viable forms of microorganisms in or on the pharmacopoeal preparations.

In actual practice, one invariably comes across certain absolutely important guidelines and vital precautionary measures that must be adhered to strictly so as to accomplish the utmost accuracy and precision of the entire concept of sterility testing for life-saving secondary pharmaceutical products (drugs). A few such cardinal factors, guidelines, and necessary details are as enumerated under:

(a) Sterility testing, due to its inherent nature, is intimately associated with a statistical process wherein the portion of a batch is sampled almost randomly; and, therefore, the chance of the particular batch (lot) duly passed for actual usage (consumption) solely depends upon the 'sample' having passed the stringent sterility test.

(b) Sterility tests should be performed under conditions designed to avoid accidental contamination of the product (under investigation) during the test. Nevertheless, such particular precautions precisely taken for this purpose must not, in any case, adversely affect any microbes that should be revealed in the test ultimately.

(c) Working environment wherein the sterility tests are meticulously carried out must be adequately monitored at regular intervals by sampling the air and the surface of the working area by performing necessary control tests.

(d) Sterility tests are exclusively based upon the principle that in case the bacteria are strategically placed in a specific medium that caters for the requisite nutritive material and water, and maintained duly at a favourable temperature (37 ± 2°C), the microbes have a tendency to grow, and their legitimate presence may be clearly indicated by the appearance of a turbidity in the originally clear medium.

(e) Extent of probability in the detection of viable microorganisms for the tests for sterility usually increases with the actual number supposedly present in a given quantity of the preparation under examination, and is found to vary according to the species of microorganisms present. However, extremely low levels of contamination cannot be detected conveniently on the basis of random sampling of a batch.

(f) In case, observed contamination is not quite uniform throughout the batch, random sampling cannot detect contamination with absolute certainty. Therefore, compliance with the tests for sterility individually cannot certify absolute assurance of freedom from microbial contamination. Nevertheless, greater assurance of sterility should invariably originate from reliable stringent manufacturing procedures vis-a-vis strict compliance with Good Manufacturing Practices (GMPs).

(g) Tests for sterility are adequately designed to reveal the presence of microorganisms in the 'samples' used in the tests. However, the interpretation of results is solely based upon the assumption that the contents of each and every container in the batch, had they been tested actually, would have complied with the tests. As it is not practically possible to test every container, a sufficient number of containers must be examined to give a suitable degree of confidence in the ultimate results obtained of the tests.

(h) It has been duly observed that there exists no definite sampling plan for applying the tests to a specified proportion of discrete units selected carefully from a batch is capable of demonstrating that almost all of the untested units are in fact sterile absolutely. Therefore, it is indeed quite pertinent that while determining the number of units to be tested, the manufacturer must have adequate regar to the environment parameters of manufacture, the volume of preparation per container together with other special considerations specific to the preparation under investigation. However, the interpretation of results is solely based upon the assumption that the contents of each and every container in the batch, had they been tested actually, would have complied with the tests. As it is not practically possible to test every container, a sufficient number of containers must be examined to give a suitable degree of confidence in the ultimate results obtained of the tests.

Sterility testing of pharmaceutical articles is required during the sterilization validation process as well as for routine release testing. United States Pharmacopeia requirements employ sterility testing as an official test to determine suitability of a lot. An understanding of sterility testing is beneficial in terms of designing a validation process. The need to provide adequate and reliable sterility test data is an important quality assurance issue. Sterility testing is a very tedious and artful process that must be performed by trained and qualified laboratory personnel. The investigation of sterility test failures is a process that requires attention to environmental data as well as many other factors including training and sample difficulty.

Sterility testing is an essential part of every sterilization validation. Sterility testing is an extremely difficult process that must be designed and executed so as to eliminate false positive results. False positive results are generally due to laboratory contamination from the testing environment or technician error. The testing environment must be designed to meet the requirements of the United States Pharmacopeia (USP) in terms of viable microbial air and surface counts. Growth media used in sterility testing must be meticulously prepared and tested to ensure its ability to support microbial growth. Procedures for sampling, testing, and follow-up must be defined in the validation procedures.

METHODOLOGIES

The United States Pharmacopeia is a compilation of validated methods and official monographs for pharmaceuticals and medical devices. IT is broken down into the following sections: Monographs, General Informational Chapters, and General Requirements. General Informational Chapters <1000> series are not legal requirements. The Sterility Test (USP Section <71>) is categorized under General Requirements and is therefore a legal requirement.

The ISO radiation sterilization microbial methods (11737-2 1998) describes a sterility test which is a modification for the USP method. This test is specific for the detection of aerobic organisms that have been exposed to sub-lethal sterilization

cycles. This ISO sterility test method is recommended for the validation of both gamma and electron beam sterilization processes. ISO recommends that the sterility test be validated by using known sterile products.
The method of choice for EO sterilized products is the official USP <71> procedure.

CULTURE MEDIA AND INCUBATION TEMPERATURES

Media for the test may be prepared as described below or equivalent commercial media may be used provided that they comply with the requirements of the *Growth Promotion Test of Aerobes, Anaerobes, and Fungi*. The following culture media have been found to be suitable for the test for sterility. *Fluid Thioglycollate Medium* is primarily intended for the culture of anaerobic bacteria. However, it will also detect aerobic bacteria. *Soybean–Casein Digest Medium* is suitable for the culture of both fungi and aerobic bacteria.

Fluid Thioglycollate Medium

(Ingredients in grams per liter)	
L-Cystine	0.5
Sodium Chloride	2.5
Dextrose Monohydrate/Anhydrous	5.5/5.0
Agar	0.75
Yeast Extract (water-soluble)	5.0
Pancreatic Digest of Casein	15.0
Sodium Thioglycollate	0.5
or Thioglycolic Acid	0.3 mL
Resazurin Sodium Solution (1 in 1000),	1.0 mL
pH after sterilization: 7.1±0.2.	

Fluid Thioglycollate Medium is to be incubated at 30°–35°C. For products containing a mercurial preservative that cannot be tested by the membrane filtration method, *Fluid Thioglycollate Medium* incubated at 20°–25°C may be used instead of *Soybean–Casein Digest Medium* provided that it has been validated as described in *Growth Promotion Test of Aerobes, Anaerobes, and Fungi*. Where prescribed or justified and authorized, the following alternative thioglycollate medium might be used. Prepare a mixture having the same composition as that of the *Fluid Thioglycollate Medium*, but omitting the agar and the resazurin sodium solution.

Soybean–Casein Digest Medium

(Ingredients in grams per liter)	
Pancreatic Digest of Casein	17.0
Papaic Digest of Soybean Meal	3.0
Sodium Chloride	5.0
Dibasic Potassium Phosphate	2.5
Dextrose Monohydrate/Anhydrous	2.5/2.3
pH after sterilization: 7.3±0.2.	

Soybean–Casein Digest Medium is to be incubated at 22.5 ± 2.5°C.

PROCESSES

Prior to actual sterility testing, it is prudent to send an example sample to the testing laboratory so the laboratory can determine the appropriate testing procedure. Each product should have a unique procedural specification for testing. The procedure should be very specific in terms of which items (or vials/syringes) to test. The procedure must indicate the Sample Item Portion (SIP). The Sample Item Portion is the percentage of the complete product tested. Since medical devices come in all shapes and sizes, it is very difficult to test large and cumbersome medical devices in their entirety. Therefore, the test laboratory will determine a Sample Item Portion which is a portion of the sample expressed in fractional terms (i.e. 0.1 for 10% of the sample).

The USP <71> Sterility Test contains two qualifying assays which must be performed prior to sterility testing. They are the "Suitability Test" (Growth Promotion Test) and the "Validation Test" (Bacteriostasis and Fungistasis Test).

The Suitability Test is used to confirm that each lot of growth media used in the sterility test procedure will support the growth of fewer than 100 viable microorganisms. If the media cannot support the growth of the indicator organisms, then the test fails. Secondly, a portion of each media lot must be incubated and assessed for sterility according to the incubation parameters (time, temperature) established by the method. If the media is found to be non-sterile, then the test fails.

The Validation Test is used to determine if the test sample will inhibit the growth of microorganisms in the test media. Stasis, in terms of microbiology, is defined as the inability of a microorganism to grow and proliferate in microbiological media. Media that is bacteriostatic does not necessarily kill bacteria; it simply may retard bacterial growth and proliferation. The Validation Test must be performed on each product prior to and/or during sterility testing. This test determines if the media volumes are valid for the particular product. Some medical products contain bacteriostatic and fungistatic compounds that may require special procedures and special media for testing. The product sample is placed in the media along with the microorganisms. Microbial growth in the presence of the test samples is compared to controls without test samples. If microbial growth is present in the sample and control containers, then the test is valid. The next step is to proceed to actual sterility testing. Suitability, validation and sterility tests can be performed simultaneously.

The USP describes three general methods for sterility testing:
1) Membrane Filtration
2) Direct Transfer (Product Immersion)
3) Product Flush

Membrane Filtration Sterility Testing

The Membrane Filtration Sterility Test is the method of choice for pharmaceutical products. It is not the method of choice for medical devices; the FDA may question the rationale behind using the membrane filtration test over the direct transfer test for devices. An appropriate use of this test is for devices that contain a preservative and are bacteriostatic and/or fungistatic under the direct transfer method. With membrane filtration, the concept is that the microorganisms will collect onto the surface of a 0.45 micron pore size filter. This filter is segmented and transferred to appropriate media. The test media are fluid thioglycollate medium (FTM) and soybean casein digest medium (SCDM). FTM is selected based upon its ability to support the growth of anaerobic and aerobic microorganisms. SCDM is selected based upon its ability to support a wide range of aerobic bacteria and fungi (i.e. yeasts and molds). The incubation time is 14 days. Since there are many manipulations required for membrane filtration medical device sterility testing, the propensity for laboratory contamination is high. Therefore, in an open system,

more sterility failures are expected when using this method. A closed system is recommended for drugs and small devices or combination products. Most pharmaceutical articles are tested using a closed system. In closed systems, the propensity for extrinsic contamination is very low.

Direct Transfer Sterility Testing

Combination products: This method is the method of choice for medical devices because the device is in direct contact with test media throughout the incubation period. Viable microorganisms that may be in or on a product after faulty/inadequate sterilization have an ideal environment within which to grow and proliferate. This is especially true with damaged microorganisms where the damage is due to a sub-lethal sterilization process. All microorganisms have biological repair mechanisms that can take advantage of environmental conditions conducive to growth. The direct transfer method benefits these damaged microorganisms. The entire product should be immersed in test fluid. With large devices, patient contact areas should be immersed. Large catheters can be syringe filled with test media prior to immersion. Cutting catheter samples to allow for complete immersion is the method of choice.

The USP authors understand that appropriate modifications are required due to the size and shape of the test samples. The method requires that the product be transferred to separate containers of both FTM and SCDM. The product is aseptically cut, or transferred whole, into the media containers. The test article should be completely immersed in the test media. The USP limits the media volume to 2500 ml. After transferring, the samples are incubated for 14 days.

Product Flush Sterility Testing

Combination products: The product flush sterility test is reserved for products that have hollow tubes such as transfusion and infusion assemblies where immersion is impractical and where the fluid pathway is labeled as sterile. This method is easy to perform and requires a modification of the FTM media for small lumen devices. The products are flushed with fluid D and the eluate is membrane filtered and placed into FTM and SCDM. This method is not generally used.

BULK DRUG PRODUCTS / BIOLOGICS AND PHARMACEUTICALS

Bulk Pharmaceuticals (APIs) are tested for sterility per USP 71 prior to release to the manufacturing processes. Bulk Biologics are tested according to 21 CFR 610.12 for sterility testing. This method requires one media (FTM).

INTERPRETATION OF STERILITY TEST RESULTS

The technician must be trained in the method of detecting growth during the incubation period. Growth is determined by viewing the media, which is generally clear and transparent, against a light source. Turbid (cloudy) areas in the media are indicative of microbial growth. Once growth is detected, the suspect vessel is tested to confirm that the turbidity present is due to microorganisms and not due to disintegration of the sample; sometimes samples produce turbidity because of particulate shedding or chemical reactions with the media. Once a suspect container has been tested, it should be returned to the incubator for the remainder of the incubation period. Samples that render the media turbid are transferred on Day 14 of the test and incubated for four days. Growth positive samples require further processing such as identification and storage.

STERILITY TEST FAILURE INVESTIGATION

For every positive sterility test (OOS), the laboratory should perform an OOS (Out of specification) investigation to determine the validity of the positive growth. This investigation encompasses the following items:

1. Clean room environmental test (EER - ***Environmental Excursion Report***) data;
2. Media sterilization records;
3. Technician training records;
4. The relative difficulty of the test procedure;
5. Control data (open and closed media controls);
6. Technician sampling data (microbial counts on gloves and/or garments post testing).

The USP allows for a re-test of the product if persuasive evidence exists to show that the cause of the initial sterility failure was induced by the laboratory. Identification and speciation of the isolate(s) is a significant contributing factor to the final decision. If the First Stage sterility test can be invalidated by the laboratory, then the USP allows for Second Stage sterility testing. Second Stage sterility testing requires double the original number of samples tested. The Second Stage test can be repeated if evidence exists invalidating the test due to a laboratory error as above.

A detailed investigation may uncover circumstantial evidence to support a final decision. It is recommended that sterilization cycle data, environmental data, and bioburden data be reviewed prior to making any decision to release product.

It is recommended that medical device manufacturers qualify the test procedure with non-sterile samples.

The probability of a false positive can be calculated using John Lee's formula. The formula is based upon sample container diameter, amount of time container is left open and the room particulate count.

Sterility testing requires high levels of control with regards to GMPs, Good Laboratory Practices, environment (aseptic clean room ISO class 5 or better), and employee practices. It is essential that meticulous technique be employed in the practice of sterility testing. Sterility testing is an integral part of sterilization validation as well as a routine quality control. Generally, false positive results are uncommon in testing drug products using a closed system. Combination products have challenges that should be planned into a robust QA program.

REFERENCES

(1) The United States Pharmacopeia, *Microbiological Tests* / <71> Sterility Tests, 2014. (2) ISO 11137 Sterilization of health care products – Radiation – Part 2 2006: Establishing the sterilization dose. (3) FDA Guidelines 2004 "Guidance for Industry Sterile Drug Products by Aseptic Processing, Current Good Manufacturing Practices," September, 2004. (4) ISO 11737 ANSI/AAMI/ISO 11737-2 1998 – Sterilization of Medical Devices – Microbiological Methods – Part 2, Tests of Sterility Performed in the Validation of a Sterilization Process. (5) ISO 11135 1994 Medical Devices Validation and Routine Control of Ethylene Oxide Sterilization. (6) Code of Federal Regulations Title 21/Chapter I/Part 820, "Quality Systems Requirements: General," 2006. (7) GMPs CFR 201 Title 21 2006. (8) 21 CFR Part 610.12 Bulk Biologics. (9) Lee, John Y. "Investigation Sterility Test Failures" Pharmaceutical Technology, February 1990. (10) Code of Federal Regulations Title 21/Chapter I/Part 58, "Good Laboratory Practice for Nonclinical Laboratory Studies," 2006.

WHO guidelines for safe surgery

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 health-care 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 a 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 ● Thoracic trauma care ● Homodynamic and inotropic treatment ● Basic intensive care unit patient management and monitoring for up to

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		1 week: all types of cases, but possibly with limited provision for: – Multi-organ system failure – Haemodialysis – Complex neurological and – cardiac surgery – Prolonged respiratory failure – Metabolic care or monitoring
Essential procedures	**Essential procedures**	**Essential procedures**
● Normal delivery ● Uterine evacuation ● Circumcision ● Hydrocoele reduction, incision and drainage ● Wound suturing ● Control of haemorrhage with pressure dressings ● Debridement and dressing of wounds ● Temporary reduction of fractures ● Cleaning or stabilization 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 ● 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	● Same as level 1, but also: ● Thiopental 500 mg/g powder or propofol ● Suxamethonium bromide 500 mg powder ● Pancuronium ● Neostigmine 2.5 mg injection ● Ether, halothane or other inhalation anaesthetics ● Lidocaine 5% heavy spinal solution, 2 ml	● Same as level 2 with the following additions: ● Propofol ● Nitrous oxide ● Various modern neuromuscular blocking agents ● Various modern inhalation anaesthetics ● Various inotropic agents ● Various intravenous antiarrhythmic agents

Drugs	Drugs	Drugs
	● 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	● 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 catheters size 16 FG ● 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 catheters size 16 FG ● Sterile 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 additions: ● Ventilator circuits ● Yankauer suckers ● Giving sets for intravenous infusion pumps ● Disposables for suction machines ● Disposables for capnography, oxygen analyser, in accordance with manufacturers' specifications: ● Sampling lines ● Water traps ● Connectors ● Filters and fuel cells

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.
- 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
– post operative in-hospital mortality rate.

- For surgical surveillance at hospital and practitioner levels, the following data should be collected systematically by facilities and clinicians:

– day-of-surgery mortality rate,
– post operative in-hospital mortality rate.

Sir Ronald Ross

Born	:	13 May 1857, Almora, India
Died	:	16 September 1932 (aged 75)
Nationality	:	British
Fields	:	Medicine
Known for	:	Discovering that the malaria parasite is transmitted by mosquitoes
Notable awards	:	Nobel Prize in Physiology or Medicine (1902) Albert Medal (1923) Manson Medal (1929)
Spouse	:	Rosa Bessie Bloxam
Children	:	2 sons and 2 daughters

Sir Ronald Ross, (13 May 1857 – 16 September 1932), was an Indian-born British medical doctor who received the Nobel Prize for Physiology or Medicine in 1902 for his work on malaria. His discovery of the malarial parasite in the gastrointestinal tract of mosquito led to the realisation that malaria was transmitted by mosquitoes, and laid the foundation for combating the disease. He was quite a polymath, writing a number of poems, published several novels, and composed songs. He was also an amateur artist and natural mathematician. He worked in the Indian Medical Service for 25 years. It was during his service that he made the groundbreaking medical discovery. After resigning from his service in India, he joined the faculty of Liverpool School of Tropical Medicine, and continued as Professor and Chair of Tropical Medicine of the institute for 10 years. In 1926 he became Director-in-Chief of the Ross Institute and Hospital for Tropical Diseases, which was established in honour of his works. He remained there until his death.

Although he had no predisposition to medicine, at the age of 17 he submitted to his father's wish to see him enter the Indian Medical Service. He began his medical studies at St. Bartholomew's Hospital Medical College, London in 1874 and sat the examinations for the Royal College of Surgeons of England in 1879. He took the post of ship surgeon on a transatlantic steamship while studying for, and gaining the Licentiate of the Society of Apothecaries, which allowed him to enter the Indian Medical Service in 1881, where he held temporary appointments in Madras, Burma, and the Andaman Islands. During a year's leave, from June 1888 to May 1889, he developed his scientific interests and studied for the Diploma in Public Health from the Royal Colleges of Physicians and Surgeons in England and took a course in bacteriology under Professor E. E. Klein. He also married Miss Rosa Bloxam, who accompanied him to Bangalore when he returned for duty as a staff surgeon. In 1892 he became interested in malaria and, having originally doubted the parasites' existence, became an enthusiastic convert to the belief that malaria parasites were in the blood stream when this was demonstrated to him by Patrick Manson during a period of home leave in 1894. (Sir Patrick Manson is considered by many to be the father of tropical medicine. He was the first person to demonstrate, in 1878, that a parasite that causes human disease could infect a mosquito—in this case, the filarial worm that causes elephantiasis).

On his return to India in 1895, Ross began his quest to prove the hypothesis of Alphonse Laveran and Manson that mosquitoes were connected with the propagation of malaria, and regularly corresponded with Manson on his findings. However his progress was hampered by the Indian Medical Service, which ordered him from Madras to a malaria-free environment in Rajputana. Ross threatened to resign but, following representations on his behalf by Manson, the Indian Government put him on special duty for a year to investigate malaria and kala azar (visceral leishmaniasis).

On 20 August 1897, in Secunderabad, Ross made his landmark discovery. While dissecting the stomach tissue of an anopheline mosquito fed four days previously on a malarious patient, he found the malaria parasite and went on to prove the role of Anopheles mosquitoes in the transmission of malaria parasites in humans.

He continued his research into malaria in India, using a more convenient experimental model, malaria in birds. By July 1898, he had demonstrated that mosquitoes could serve as intermediate hosts for bird malaria. After feeding mosquitoes on infected birds, he found that the malaria parasites could develop in the mosquitoes and migrate to the insects' salivary glands, allowing the mosquitoes to infect other birds during subsequent blood meals. In 1899 Ross resigned from the Indian Medical Service and returned to England. He worked for the newly established Liverpool School of Tropical Medicine taking a post as lecturer and later becoming Professor of Tropical Medicine, and accepted a personal chair in Tropical Sanitation at Liverpool University. One of his first roles at the School was to investigate and devise anti-malaria schemes in West Africa. This was the first of many expeditions that Ross undertook to investigate and develop malaria control measures including visits to Ismailia in Egypt at the request of the Suez Canal Company in 1902, Panama in 1904, Greece in 1906, and Mauritius in 1907-1908.

In 1901 Ross was elected a Fellow of the Royal College of Surgeons of England and also a Fellow of the Royal Society, of which he became Vice-President from 1911 to 1913. In 1902 he was awarded the Nobel Prize for Medicine" for his work on malaria, by which he has shown how it enters the organism and thereby has laid the foundation for successful research on this disease and methods of combating it." In 1902 he was appointed a Companion of the Most Honourable Order of Bath by His Majesty the King of Great Britain, and in 1911 he was elevated to the rank of Knight Commander of the same Order. He received an honorary M.D. degree in Stockholm at the centenary celebration of the Karolinska Institute in 1910 and was awarded honorary membership of many learned societies around the world throughout his career. During the First World War (1914-1918), Ross was appointed a consultant physician on tropical diseases to Indian troops and was sent to Alexandria for four months to investigate an outbreak of dysentery that was hampering troops in the Dardanelles. In 1917 he was appointed a consultant physician to the War Office and in 1919 he received an honorary post as consultant to the Ministry of Pensions.

In 1926 the Ross Institute and Hospital for Tropical Diseases was opened on Putney Heath, London by the Prince of Wales as a memorial to and in recognition of Ross' work. The main focus of the Institute was the study of the nature and treatment, propagation, and prevention of tropical diseases. Ross assumed the post of Director in Chief, which he held until his death in 1932. The Institute was incorporated into the London School of Hygiene & Tropical Medicine in 1934. Ross wrote extensively on malaria including his book The Prevention of Malaria in 1911 and on other topics including mathematics. He also wrote a number of novels including The Child of the Ocean, Spirit of the Storm, and The Revels of Orsera. While Ross is remembered for his malaria work, this remarkable man was also a mathematician, epidemiologist, sanitarian, editor, novelist, dramatist, poet, amateur musician, composer, and artist. He died, after a long illness, at the Ross Institute on 16 September 1932.

Funny Quotes

Why Men n Women Don't Understand Each Other?
Bcoz God Gave Good Brains To Men and Good Hearts To Women
But Men Use Their Hearts & Women Use Their Brains

Patient : What are the chances of my recovering doctor?

Doctor : One hundred percent. Medical records show that nine out of ten people die of the disease you have.

Yours is the tenth case I've treated. The others all died.

A man went to hell and asked the Yamraj if he can call his wife.

yamraj said "u can do that"

after man spoke to his wife.. he asked how much to pay yamraj

yamraj said.."hell to hell is free"

The human brain is most outstanding thing..... it functions 24hrs 365 days..... it functions right from the time u r Born.... until you fall in love

Attention plz...!

Don't drink unboiled water..Because..... Fish live in water without pampers..

Seriously..!

GIRL: My heart is like a mobile and you are the sim card

BOY: I m very happy. . .

Gal: dont b too happy. . If I get a new offer I will change the sim card..!

Husband wanted to call the hospital to ask about his pregnant wife, but accidently called the cricket stadium.

He asks, "How's the situation?"

He was shocked & nearly died on hearing the reply.

They said, "It's fine. 3 are out, hope to get another 7 out by lunch, last one was a duck!

On a rainy day, an old man was standing with a book for sale.

A young man came to buy.

He bought the book for Rs.3000.

Old man advised "DONT OPEN LAST PAGE OF THE BOOK otherwise YOU'll face problem"

Man finished the book with great fear but didn't open the last page.

But, after a week,

Out of curiousity he opend the last page and..

he almost fainted to see..

Retail Price: Rs 30/-

A woman went shopping, At cash counter she opened her purse to pay.

The cashier noticed a TV remote in her purse.

He cud'nt control his curiosity n asked

"Do u always carry ur TV remote with u?"

She replied " No, not always, but my husband refused to accompany me for shopping today..

The story continues....

The shopkeeper laughs and takes back all the items that lady had purchased.

Shocked at this act, she asks the shopkeeper what is he doing.

He said your husband has blocked your credit card.

MORAL : Respect the hobbies of your husband.

Story continues....

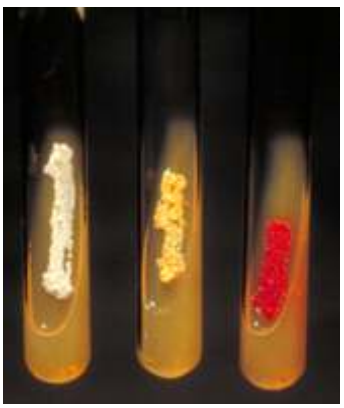
Wife took out her husbands credit card from purse and uses it to clear all the bills.

Unfortunately he didn't block his own card.

Moral:. Don't underestimate the power of a WIFE.

Nocardia

Nocardia asteroides (yellow colonies) see picture below.



Scientific classification
Kingdom : Bacteria
Phylum : Actinobacteria
Class : Actinobacteria
Order : Actinomycetales
Suborder : Corynebacterineae
Family : Nocardiaceae
Genus : ***Nocardia***

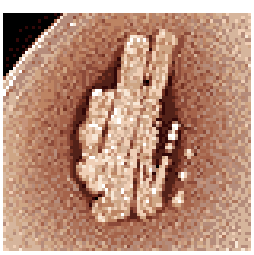
Nocardia is a genus of weakly staining Gram-positive, catalase-positive, rod-shaped bacteria. It forms partially acid-fast beaded branching filaments (acting as fungi, but being truly bacteria). It has a total of 85 species. Some species are non-pathogenic while others are responsible for nocardiosis. *Nocardia* are found worldwide in soil that is rich with organic matter. In addition, Nocardia are oral microflora found in healthy gingiva as well as periodontal pockets. Most *Nocardia* infections are acquired by inhalation of the bacteria or through traumatic introduction.

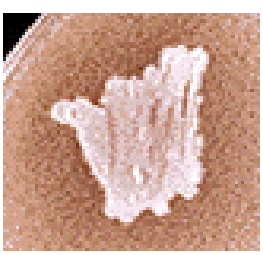
Growth and identification of Nocardia

Nocardia may be difficult to culture in clinical microbiology laboratories

Nocardia species are classically gram-positive, strictly aerobic, filamentous, branching, weakly acid-fast bacilli. They may be isolated on routine bacterial, fungal, and mycobacterial media. Colonies may appear within 4 days, but may require up to 2-4 weeks of culture. Pre-treament of the patient with antibiotics that slow but do not kill *Nocardia* will most often increase the time required to grow *Nocardia* from clinical isolates. If nocardiosis is suspected clinically, the bacteriology laboratory should be informed and cultures should be kept longer than usual. *Nocardia* can also be difficult to isolate by culture because of overgrowth by faster-growing nonpathogenic colonizers that may mask its presence.

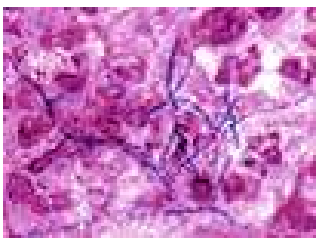
Nocardia colonies may be smooth and moist, or have a "mold-like" verrucous grey-white waxy or powdery appearance from aerial hyphae. They have a very distinct, strong mildew odor that allows experienced microbiologists to suspect their presence. (The Actinomycetes genera including *Nocardia* are responsible for the musty "mildew" odor of the average basement). The colonies of N. brasiliensis (left) and N. caviae (right) pictured below show the wide range of colony appearance, even within the same species:





Nocardia have variable (and sometimes unreliable) staining properties

Nocardia are classically Gram-variable, with a 'beaded' appearance of alternating Gram-positive and Gram-negative segments along a filament:



However, *Nocardia* grown under suboptimal conditions sometimes appear uniformly Gram-negative; therefore, negative Gram-staining cannot be used to rule out *Nocardia*.

Likewise, with a modified Ziehl-Neelsen or Kinyoun acid-fast stain (for cultured organisms) or a Fite-Faraco modified acid-fast stain (for histology sections), *Nocardia* organisms are classically weakly acid-fast. This characteristic may help to distinguish *Nocardia* from negative Actinomyces (which is modified-acid-fast-negative). However, tests based on acid-fastness alone are not reliable for such a distinction. As seen in this case study, *Nocardia* can occasionally appear modified-acid-fast-stain negative.

Nocardia grown under suboptimal conditions (too many passages in culture, lipid-poor media, or presence of inhibitory antibiotics) will have retarded synthesis of mycolic acids in their cell wall that, in turn, compromises the ability of the organisms to retain Gram stain or modified-acid-fast stains.

In this patient, the administration of antibiotics piperacillin and cefuroxime (neither first-line agents for treatment of *Nocardia*) may have generated a "sub-optimal condition" for *Nocardia* growth that led to unexpectedly negative Gram stain and negative modified-acid-fast results.

Differentiation of Nocardia species

Histologically, *Nocardia* has delicate (< 2 microns in thickness) filaments with pronounced branching. Actinomyces in contrast has a similar filamentous appearance, albeit with slightly thicker, straighter, and less-branched filaments. Since the histologic appearance of *Nocardia* is similar to other Actinomycetes family members, culture and biochemical testing is necessary for definitive diagnosis/identification.

The UPMC Presbyterian Mycology Laboratory performs petri-dish cultures with tap water agar (1%) to differentiate the *Nocardia*s and other aerobic Actinomycete genera from the rapid growing mycobacteria. (The trace organic material found in tap water provides sufficient nutrients to support growth). On tap-water agar, *Nocardia* species have recursively branching hyphae with aerial hyphae. Preliminary biochemical tests for differentiation and speciation of *Nocardia* from the other Actinomycetes genera require 14 days, and include the presence of the following biochemical characteristics: (1) hydrolysis of casein, tyrosine, and/or xanthine, (2) presence of urease, (3) utilization of rhamnose, and (4) positive resistance to lysozyme.

Table 1: Hydrolysis Tests for differentiating *Nocardia* strains

	Casein hydrolysis	L-tyrosine hydrolysis	Xanthine hydrolysis
N. asteroides complex, N. farcinica, or N. nova	-	-	-
N. brasiliensis	+	+	-
N. otitidis	-	+	-
N. caviae	-	-	+
Streptomyces or Nocardiopsis	+	+	+

Hydrolysis test agar is made by adding casein, crystalline L-tyrosine, or crystalline xanthine to plate media. If a *Nocardia* species can hydrolyze the substrate, the substrate disappears as seen by a zone of clearance around the colony.
In the pictures below, observe the hydrolysis patterns of the yellowish *N. brasiliensis* (left petri dish, below) versus *N. caviae* (right petri dish, below). In each petri dish, casein media is in the lower left quadrant, followed clockwise by L-tyrosine (upper left quadrant) and xanthine (upper right quadrant).

If the organism is determined by hydrolysis pattern to be one of the members of the *N. asteroides* group (*N. asteroides* complex; *N. farcinica*; or *N. nova*), then an antibiotic susceptibility test may be performed for a more precise identification. The test uses the antibiotic disc diffusion method and requires 72 hours to complete:

Table 2: Antibiotic Resistance of *N. asteroides* group members

	Tobramycin	Cefamandole or Cefotaxime	Erythromycin
N. asteroides complex	Variable Resistance	Sensitive	Resistant
N. farcinica	Resistant	Resistant	Resistant
N. nova	Resistant	Sensitive	Sensitive

Virulence

The various species of *Nocardia* are pathogenic bacteria with low virulence; therefore clinically significant disease most frequently occurs as an opportunistic infection in those with a weak immune system, such as small children, the elderly, and the immunocompromised (most typically, HIV). Nocardial virulence factors are the enzymes catalase and superoxide dismutase (which inactivate reactive oxygen species that would otherwise prove toxic to the bacteria), as well as a "cord factor" (which interferes with phagocytosis by macrophages by preventing the fusion of the phagosome with the lysosome).

Clinical disease and microbiological diagnosis

Nocardia asteroides is the species of *Nocardia* most frequently infecting humans, and most cases occur as an opportunistic infection in immunocompromised patients. Other species of medical interest are *N. brasiliensis* and *N. caviae*. Because it is acid-fast to some degree, it stains only weakly gram positive.

The most common form of human nocardial disease is a slowly progressive pneumonia, whose common symptoms include cough, dyspnea (shortness of breath), and fever. It is not uncommon for this infection to spread to the pleura or chest wall. Pre-existing pulmonary disease, especially pulmonary alveolar proteinosis, increases the risk of contracting a *Nocardia* pneumonia. Every organ can be affected if a systemic spread takes place. Nocardia spp are deeply involved in the process of endocarditis as one of its main pathogenic effects.
In about 25–33% of people *Nocardia* infection will take the form of encephalitis and/or brain abscess formation.
Nocardia may also cause a variety of cutaneous infections such as actinomycetoma (especially *Nocardia brasiliensis*), lymphocutaneous disease, cellulitis and subcutaneous abscesses.
Nocardia isolation from biological specimens can be performed using agar medium enriched with yeast extract and activated charcoal (BCYE), the same utilized for *Legionella* spp. Selective media for *Mycobacteria* or fungi can also be inoculated. The most suitable specimens are the sputum or, when clinically necessary, bronchoalveolar lavage or biopsy. Further biochemical tests for species identification are not routinely performed. Serological or cutaneous tests are not available.

Treatment

Antibiotic therapy with a sulfonamide, most commonly trimethoprim-sulfamethoxazole, is the treatment of choice. People who take trimethoprim-sulfamethoxazole for other reasons, such as prevention of *Pneumocystis jirovecii* infection, appear to have fewer *Nocardia* infections, although this protective effect has been considered unreliable and some studies have disputed it altogether. Minocycline is usually substituted when a sulfa cannot be given; high-dose imipenem and amikacin have also been used in severe or refractory cases. Linezolid appears to be highly effective against *Nocardia*, but it is very expensive and may cause severe adverse effects.[6] Antibiotic therapy is continued for six months (in immunocompetent people) to a year (in immunosuppression), and may need to be continued indefinitely. Proper wound care is also critical.

Genetics

Although *Nocardia* has interesting and important features such as production of antibiotics and aromatic compound-degrading or converting enzymes, the genetic study of this organism has been hampered by the lack of genetic tools. However, practical *Nocardia–E. coli* shuttle vectors have been developed recently.
It has also recently been found that the genera *Nocardia* and *Rhodococcus* are closely related. A close relationship between them is supported by two conserved signature indels consisting of a 1 amino acid deletion in the alpha subunit of acetyl coenzyme A carboxylase (ACC), as well as a 3 amino acid insertion in a conserved region of an ATP-binding protein that are specifically shared by species from these two genera. In addition, 14 hypothetical conserved signature proteins have been identified which are unique to the genera *Nocardia* and *Rhodococcus*.

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Health Effects of Overexposure to the Sun

Ozone layer depletion decreases our atmosphere's natural protection from the sun's harmful ultraviolet (UV) rays. This fact sheet provides a quick overview of the major health problems linked to overexposure to UV radiation:

- Skin cancer (melanoma and non-melanoma)
- Premature aging of the skin and other skin problems
- Cataracts and other eye damage
- Immune system suppression

Understanding these risks and taking a few sensible precautions will help you enjoy the sun while lowering your chances of sun-related health problems.

Skin Cancer

Each year, more new cases of skin cancer are diagnosed in the U.S. than new cases of breast, prostate, lung, and colon cancer combined. One in five Americans will develop skin cancer in their lifetime. One American dies from skin cancer every hour.

Unprotected exposure to UV radiation is the most preventable risk factor for skin cancer.

Melanoma

Melanoma, the most serious form of skin cancer, is now one of the most common cancers among adolescents and young adults ages 15-29. While melanoma accounts for about three percent of skin cancer cases, it causes more than 75 percent of skin cancer deaths. UV exposure and sunburns, particularly during childhood, are risk factors for the disease. Not all melanomas are exclusively sun-related—other possible influences include genetic factors and immune system deficiencies.

Non-melanoma Skin Cancers

Non-melanoma skin cancers are less deadly than melanomas. Nevertheless, they can spread if left untreated, causing disfigurement and more serious health problems.

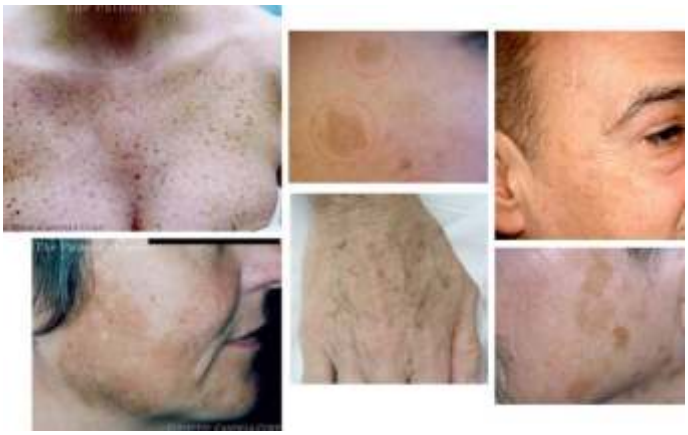
There are two primary types of non-melanoma skin cancers: basal cell and squamous cell carcinomas. If caught and treated early, these two cancers are rarely fatal.

- Basal Cell Carcinomas- are the most common type of skin cancer tumors. They usually appear as small, fleshy bumps or nodules on the head and neck, but can occur on other skin areas. Basal cell carcinoma grows slowly, and it rarely spreads to other parts of the body. It can, however, penetrate to the bone and cause considerable damage.

- Squamous Cell Carcinomas - are tumors that may appear as nodules or as red, scaly patches. This cancer can develop into large masses, and unlike basal cell carcinoma, it can spread to other parts of the body.

Other Skin Damage

Other UV-related skin disorders include actinic keratoses and premature aging of the skin. Actinic keratoses are skin growths that occur on body areas exposed to the sun. The face, hands, forearms, and the “V” of the neck are especially susceptible to this type of lesion. Although premalignant, actinic keratoses are a



risk factor for squamous cell carcinoma. Look for raised, reddish, rough-textured growths and seek prompt medical attention if you discover them.

Chronic exposure to the sun also causes premature aging, which over time can make the skin become thick, wrinkled, and leathery. Since it occurs gradually, often manifesting itself many years after the majority of a person's sun exposure, premature aging is often regarded as an unavoidable, normal part of growing older.

However, up to 90 percent of the visible skin changes commonly attributed to aging are caused by the sun. With proper protection from UV radiation, most premature aging of the skin can be avoided.

Immune Suppression

Scientists have found that overexposure to UV radiation may suppress proper functioning of the body's immune system and the skin's natural defenses. For example, the skin normally mounts a defense against foreign invaders such as cancers and infections. But overexposure to UV radiation can weaken the immune system, reducing the skin's ability to protect against these invaders.

Cataracts and Other Eye Damage

Cataracts are a form of eye damage in which a loss of transparency in the lens of the eye clouds vision.

If left untreated, cataracts can lead to blindness. Research has shown that UV radiation increases the likelihood of certain cataracts. Although curable with modern eye surgery, cataracts diminish the eyesight of millions of Americans and cost billions of dollars in medical care each year.

Other kinds of eye damage include pterygium (tissue growth that can block vision), skin cancer around the eyes, and degeneration of the macula (the part of the retina where visual perception is most acute). All of these problems can be lessened with proper eye protection. Look for sunglasses, glasses or contact lenses if you wear them that offer 99 to 100 percent UV protection.

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www.epa.gov/ozone/ strathome.html.

Sterile Disinfectants

Microbial contamination in pharmaceutical products has massive consequences. In recent years, the number of major drug recalls within the Pharmaceutical industry has skyrocketed from 426 in 2008 to 1,742 in 2009 according to CNN Money. Even more alarming is the increase in drug recalls due to microbial contamination, which are among the most dangerous as they can result in serious illness or death.

Undoubtedly, a company suffers enormous damage when a drug product is recalled. The direct hit will include loss of product sales, decreased customer confidence, damage to the brand and company name, and in many cases, legal proceedings. The overall costs associated with a major drug recall are almost immeasurable.

Quality Control is an essential function of the Pharmaceutical industry. Drug manufacturers must thoroughly test materials, processes, equipment, techniques, environments and personnel in order to ensure their final products are consistent, safe, effective and predictable.

PHARMACOPEIAS

In addition to their local government authorities, many pharmaceutical manufacturers look to a Pharmacopeia for guidance on ensuring the quality, safety and benefit of the medicines they produce. A Pharmacopeia is an organization that develops and publishes standards for manufacturing prescription and over-the-counter medicines as well as other healthcare products.

Three major Pharmacopeias throughout the world include: the United States Pharmacopeia (USP), the European Pharmacopoeia (Ph. Eur.) and the Japanese Pharmacopoeia (JP). Each of these organizations has their own set of standards; however the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) is working to facilitate international harmonization in the interpretation and application of technical guidelines and requirements for the pharmaceutical industry.

Pharmaceutical manufacturing takes place within a series of specially controlled environments – cleanrooms. On one level, a cleanroom or clean zone is simply an area that is clean in terms of both particle counts (as defined in the international cleanroom standard ISO14644) and microbial counts (as defined in a second cleanroom standard for bio-contamination control, ISO14698. In addition, regulatory requirements for cleanrooms are detailed by EU GMP or the FDA guidelines.

One important step towards achieving microbial control within a cleanroom is the use of defined cleaning techniques, together with the application of detergents and disinfectants. The detergents and disinfectants used in pharmaceutical grade cleanrooms need to be of a high quality and effective at killing micro-organisms. Both correct product selection and cleaning techiques are important, particularly in relation to some of the newer cleanroom technologies.

Cleaning agent selection: The types of detergents and disinfectants used represent an important decision for the pharmaceutical manufacturer. There are various different types of disinfectant with different spectrums of activity and modes of action.

Disinfectants have differing efficacies. Some are bacteriostatic, where the ability of the bacterial population to grow is halted. Here, the disinfectant can cause selective and reversible changes to cells by interacting with nucleic acids, inhibiting enzymes or permeating into the cell wall. Once the disinfectant is removed from contact with bacteria cells, the surviving bacterial population could potentially grow.

Other disinfectants are bactericidal in that they destroy bacterial cells through different mechanisms, including: causing structural damage to the cell; autolysis; cell lysis and the leakage or coagulation of cytoplasm. Within these groupings the spectrum of activity varies, with some disinfectants being effective against vegetative Gram positive and Gram negative micro-organisms only, while others are effective against fungi. Some disinfectants are sporicidal in that they can cause the destruction of endospore forming bacteria. Pharmaceutical manufacturers are expected to use at least two disinfectants of different modes of activity in order to conform to current Good Manufacturing Practices.

Antimicrobial Agent	Possible Target(s)
Alcohols	Cytoplasmic membrane permeability
Bronopol	Enzymes with thiol groups
Chlorhexidine	Cytoplasmic membrane permeability; general cytoplasm coagulation
Chlorine, chlorine releasers	Bacterial cell wall structure; enzyme thiol groups; amino groups of proteins
Ethylene oxide	Enzyme thiol groups; amino and other groups of proteins
Formaldehyde, formaldehyde releasers	Bacterial cell wall (low concentrations); amino and thiol groups
Glutaraldehyde	Bacterial cell wall; thiol, amino and other reactive groups; general cytoplasm coagulation
Heat ● Dry ● Moist Enzyme	Oxidation denaturation and cytoplasm coagulation
Hydrogen peroxide	Enzyme thiol groups
Irradiation	DNA
Mercury (II) salts, organic mercurials	Bacterial cell wall (low concentrations); enzyme thiol groups; general cytoplasm coagulation
Parabens (p-hydroxybenzoic acid esters)	Cytoplasmic membrane permeability; DNA and RNA synthesis
Phenols, halogenated phenols	Bacterial cell wall (low concentrations); cytoplasmic membrane permeability
Quaternary ammonium compounds	Cytoplasmic membrane permeability
Sorbic acid, other lipophilic weak acids	Cytoplasmic membrane permeability
Sulfur dioxide, sulphites	Enzymes with thiol groups; amino groups of proteins

The use of a sporicidal disinfectant is recommended for sterile areas on an occasional basis, even where such a disinfectant does not form part of the standard set. When selecting disinfectants, it is prudent to opt for manufacturers who offer a range of disinfectants

of different modes of activity (which target and kill microbial cells in different ways). For a disinfectant to work effectively, "soil" (such as grease and dust particles) must be removed first using a suitable cleanroom grade detergent. Detergents are typically either sterile, neutral solutions or have added cleaning chemicals, such as premium sterile non-ionic surfactants.

When selecting a detergent a check should be made to determine that it is compatible with the disinfectant because some detergents, where there are residues, can neutralise the active ingredient in certain disinfectants. This can be overcome where detergents are purchased from the same manufacturer as the disinfectant.

It is important to understand the manufacturer of the disinfectant and to be assured that the cleaning agents purchased are of an appropriate quality. High quality disinfectants and detergents are manufactured under clean conditions, with the quality assessed through appropriate manufacturing techniques and for the product to be held in appropriate packaging. With disinfectants, it is also important that the chemicals have been tested to the appropriate European standards. Disinfectant should ideally be sprayed onto the surface rather than the wipe and left for the required contact time before wiping.

Cleaning products presentation: As cleanroom technology has advanced, the types and ranges of cleanrooms and clean devices have expanded. The pharmaceutical manufacturer, when selecting cleaning agents, should aim for products that are supplied in different presentations. For smaller spillages and the cleaning of process area surfaces and laboratory benches, sterile disinfectants in trigger sprays are the most effective design.

With slightly larger areas, ready-to-use disinfectant solutions in bigger volumes are desirable. For cleaning larger areas, such as process area floors, disinfectant concentrates (where the disinfectant is prepared by cleanroom operators by dilution) are the most appropriate presentation. Several manufacturers offer ready-to-use products in different volumes and concentrate solutions which are of great advantage for cleanroom use.

Cleaning techniques for cleanrooms: The cleaning technique used for cleanrooms should be defined and standardised. It does not matter how effective the cleaning agents selected are if the cleaning technique practised by cleanroom operators is poor.

Traditionally, microbiological control has been associated particularly with the manufacture of sterile products. However, experience has shown that microbiological contamination can also cause problems with a wide range of non-sterile products, such as tablets, creams, ointments, and oral liquids. The traditional response to this problem has been the addition of preservatives, but there is now increasing consumer and medical pressure to remove or reduce the levels of preservatives in products. In turn, the pharmaceutical manufacturer has had to respond by increasing the level of microbiological monitoring and control. One of the main consequences of this has been the introduction of microbiological control of existing premises and practices in a piecemeal fashion that is often expensive and ineffective. An alternative and preferred course of action involves a thorough review of all aspects of the operation with a team consisting of representatives from the microbiology, engineering, manufacturing, and training departments. This should ensure that there is a consistent approach throughout the factory and that no important aspects are missed.

GMP has been interpreted in many different ways. The most succinct definition is "getting it right first time, every time." One of the best ways of achieving this is to prevent mistakes from occurring at the source. Most companies are good at identifying possible hazards for which they then introduce checks or tests at subsequent manufacturing stages to determine whether the hazard has occurred. This is not good manufacturing practice. The correct approach is to identify the hazard and prevent it from occurring in the first place. In the long term, this is also probably the most cost effective approach, but it may take time and effort on the part of the people involved in solving the problem. It is recognized that going from one crisis to another is a common problem in manufacturing industries and that lack of time and resources often.

Good Manufacturing Practice in the Control of Contamination prevents the ideal problem-solving approach from being taken. Indeed, there can be considerable commercial pressure to maintain the status quo rather than develop better, more secure systems or working practices. In such a situation, a cost-benefit analysis may be necessary to demonstrate that the benefits from an initial investment of staff and resources can often be significant in terms of longer shelf life (especially in warmer climates), lower rejection rate, and fewer customer complaints. It is important, therefore, that scientific and manufacturing personnel are able to present their requirements in such a way that they are readily understandable to the commercial arm of the company. GMP must evolve from, and involve, senior management. Without their commitment, resources may be unduly limited.

A cleaning and disinfection policy should be prepared that covers the whole factory or hospital manufacturing suite. This should be drawn up in conjunction with a microbiologist and manufacturing and janitorial staff. The cleaning and disinfection policy should cover written cleaning schedules and procedures including the name of cleaning or disinfection agent, concentration of agent, quality of water to be used in preparation of use-dilutions, and shelf life of the diluted agent. The procedures should also include details of how the disinfectant is measured and diluted. For instance, it is not uncommon to find small volumes being measured in large measuring cylinders, a bad practice that arises particularly when disinfectants are changed, especially to ones that are used at concentrations of 0.5% or below. Both above and below certain concentrations, biocidal activity may decrease dramatically. Validation of disinfectant activity is an area that has been somewhat ignored in the past. Retrospective validation can no longer be accepted, especially where products that are susceptible to microbial contamination are involved. A validation protocol should be prepared and used whenever a new disinfectant is proposed; this If the results of this initial survey and prospective validation are satisfactory, and then the next stage is to consider the current operating procedures. Procedures may need to be rewritten to incorporate such changes as the name of the new disinfectant, its concentration and frequency of use, rotation with other agents, and so forth. Dilutions may well be different, requiring new measures or containers for diluting should include an initial assessment of the disinfectant's properties. Disinfectants can become contaminated with microorganisms and special precautions need to be taken to prevent this problem arising (Anonymous 1958, Burdon and Whitby 1967, Ayliffe et al. 1969, Bassett et al. 1970, Berkelman et al. 1984). Disinfectants and cleaning agents should be monitored for microbial contamination. Dilutions should be kept in previously cleaned containers and should not be stored unless sterilized. Part-empty containers should not be refilled (Whyte and Donaldson 1989).

Alternative to the risk of disinfectant contamination is use of sterile disinfectants. Sterile disinfectants help to control the contamination of clean room due to possible use of non-sterile disinfectants. It also helps to prevent possible chances of cross contamination and resistance development in clean room areas.

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Highlights of the coming issue

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