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Editorial

Contents

We would like to thank all our readers for their precious inputs & encouragement in making this Journal a successful effort. Here's another issue of JHS with loads of valuable information, kindly flip a few pages to believe us…….

Microorganisms capable of causing infection are constantly present in the external environment & on the human body. Sterilization destroys all microorganisms on the surface of an article or in a fluid to prevent disease transmission associated with the use of that item. There are two types of sterilization techniques: Physical & chemical. Our Mini review section this time focuses on Physical sterilization techniques which includes heat, radiation, and filtration. Chemical sterilization includes alcohols, aldehydes, phenolics etc which we will see in our next issue.

Selective and differential media are used to isolate or identify particular organisms. **Selective** media allow certain types of organisms to grow, and inhibit the growth of other organisms. **Differential** media are used to differentiate closely related organisms or groups of organisms. Owing to the presence of certain dyes or chemicals in the media, the organisms will produce characteristic changes or growth patterns that are used for identification or differentiation. MacConkey's Agar is both a selective and differential media; it is selective for Gram negative bacteria and can differentiate those bacteria that have the ability to ferment lactose.

Our In Profile scientist of the month is Paul Ehrilich (born on 14 March 1854), he was a German physician and scientist who worked in the fields of hematology, immunology, and chemotherapy. Ehrlich's chemotherapy research led to his formulating the arsenic compound, Arsphenamine (Salvarsan), which was used in the treatment of syphilis during the first half of this century until it was superseded by penicillin.

Citrobacter freundii - Bug of the month. ***Citrobacter freundii*** are facultative anaerobic Gram-negative bacilli of the Enterobacteriaceae family. It can be found in soil, water, sewage, food and the intestinal tracts of animals and humans. The Citrobacter genus was discovered in 1932 by Werkman and Gillen. Cultures of *C. freundii* were isolated and identified in the same year from soil extracts. As an opportunistic pathogen, *C. freundii* is responsible for a number of significant opportunistic infections. It is known to be the cause of a number of nosocomial infections of the respiratory tract, urinary tract, blood and many other normally sterile sites in patients. *C. freundii* represents about 29% of all opportunistic infections.

We all have heard of Biofilms. Biofilm can be defined as a combination of microorganisms and extracellular products which adhere to a solid support, forming a voluminous and thick layer, with an external structure that is not completely regular and uniform. Its chemical composition, both inorganic and organic, varies according to the substrate composition. Lets see its use in degrading Industrial effluents.

This section provides information regarding the collection & transport of fecal specimens. The media appropriate for the transport of fecal specimens, preserving these specimens etc.

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

So go on, explore the information……..

Physical Sterilization Technologies

Most medical and surgical devices used in healthcare facilities are made of materials that are heat stable and therefore undergo heat, primarily steam, sterilization. However, since 1950, there has been an increase in medical devices and instruments made of materials (e.g., plastics) that require low-temperature sterilization. Ethylene oxide gas has been used since the 1950s for heat- and moisture-sensitive medical devices. Within the past 15 years, a number of new, low-temperature sterilization systems (e.g., hydrogen peroxide gas plasma, peracetic acid immersion, ozone) have been developed and are being used to sterilize medical devices. This section reviews sterilization technologies used in healthcare and makes recommendations for their optimum performance in the processing of medical devices.

Sterilization destroys all microorganisms on the surface of an article or in a fluid to prevent disease transmission associated with the use of that item. The concept of what constitutes "sterile" is measured as a probability of sterility for each item to be sterilized. This probability is commonly referred to as the sterility assurance level (SAL) of the product and is defined as the probability of a single viable microorganism occurring on a product after sterilization. SAL is normally expressed a 10-n. In short, a SAL is an estimate of lethality of the entire sterilization process and is a conservative calculation. Dual SALs (e.g., 10-3 SAL for blood culture tubes, drainage bags; 10-6 SAL for scalpels, implants) have been used in the United States for many years and the choice of a 10-6 SAL was strictly arbitrary and not associated with any adverse outcomes (e.g., patient infections).

Medical devices that have contact with sterile body tissues or fluids are considered critical items. These items should be sterile when used because any microbial contamination could result in disease transmission. Such items include surgical instruments, biopsy forceps, and implanted medical devices. If these items are heat resistant, the recommended sterilization process is steam sterilization, because it has the largest margin of safety due to its reliability, consistency, and lethality. However, reprocessing heat- and moisture-sensitive items requires use of a low-temperature sterilization technology (e.g., ethylene oxide, hydrogen peroxide gas plasma, peracetic acid).

Steam Sterilization

Overview: Of all the methods available for sterilization, moist heat in the form of saturated steam under pressure is the most widely used and the most dependable. Steam sterilization is nontoxic, inexpensive, rapidly microbicidal, sporicidal, and rapidly heats and penetrates fabrics.

The basic principle of steam sterilization, as accomplished in an autoclave, is to expose each item to direct steam contact at the required temperature and pressure for the specified time. Thus, there are four parameters of steam sterilization: steam, pressure, temperature, and time. The ideal steam for sterilization is dry saturated steam and entrained water (dryness fraction ≥97%). Pressure serves as a means to obtain the high temperatures necessary to quickly kill microorganisms. Specific temperatures must be obtained to ensure the microbicidal activity. The two common steam-sterilizing temperatures are 121°C (250°F) and 132°C (270°F). These temperatures (and other high temperatures) must be maintained for a minimal time to kill microorganisms. Recognized minimum exposure periods for sterilization of wrapped healthcare supplies are 30 minutes at 121°C (250°F) in a gravity displacement sterilizer or 4 minutes at 132°C (270°C) in a prevacuum sterilizer. At constant temperatures, sterilization times vary depending on the type of item (e.g., metal versus rubber, plastic, items with lumens), whether the item is wrapped or unwrapped, and the sterilizer type.

The two basic types of steam sterilizers (autoclaves) are the gravity displacement autoclave and the high-speed prevacuum sterilizer. In the former, steam is admitted at the top or the sides of the sterilizing chamber and, because the steam is lighter than air, forces air out the bottom of the chamber through the drain vent. The gravity displacement autoclaves are primarily used to process laboratory media, water, pharmaceutical products, regulated medical waste, and nonporous articles whose surfaces have direct steam contact. **For gravity displacement sterilizers the penetration time into porous items is prolonged because of incomplete air elimination. This point is illustrated with the decontamination of 10 lbs of microbiological waste, which requires at least 45 minutes at 121°C because the entrapped air remaining in a load of waste greatly retards steam permeation and heating efficiency.** The high-speed prevacuum sterilizers are similar to the gravity displacement sterilizers except they are fitted with a vacuum pump (or ejector) to ensure air removal from the sterilizing chamber and load before the steam is admitted. The advantage of using a vacuum pump is that there is nearly instantaneous steam penetration even into porous loads. **The Bowie-Dick test is used to detect air leaks and inadequate air removal and consists of folded 100% cotton surgical towels that are clean and preconditioned. A commercially available Bowie-Dick-type test sheet should be placed in the center of the pack. The test pack should be placed horizontally in the front, bottom section of the sterilizer rack, near the door and over the drain, in an otherwise empty chamber and run at 134°C for 3.5 minutes. The test is used each day the vacuum-type steam sterilizer is used, before the first processed load.** Air that is not removed from the chamber will interfere with steam contact. Smaller disposable test packs (or process challenge devices) have been devised to replace the stack of folded surgical towels for testing the efficacy of the vacuum system in a prevacuum sterilizer. These devices are “designed to simulate product to be sterilized and to constitute a defined challenge to the sterilization process”. They should be representative of the load and simulate the greatest challenge to the load. Sterilizer vacuum performance is acceptable if the sheet inside the test pack shows a uniform color change. Entrapped air will cause a spot to appear on the test sheet, due to the inability of the steam to reach the chemical indicator. **If the sterilizer fails the Bowie-Dick test, do not use the sterilizer until it is inspected by the sterilizer maintenance personnel and passes the Bowie-Dick test.**

Another design in steam sterilization is a steam flush-pressure pulsing process, which removes air rapidly by repeatedly alternating a steam flush and a pressure pulse above atmospheric pressure. Air is rapidly removed from the load as with the prevacuum sterilizer, but air leaks do not affect this process because the steam in the sterilizing chamber is always above atmospheric pressure. Typical sterilization temperatures and times are 132°C to 135°C with 3 to 4 minutes exposure time for porous loads and instruments.

Like other sterilization systems, the steam cycle is monitored by

mechanical, chemical, and biological monitors. Steam sterilizers usually are monitored using a printout (or graphically) by measuring temperature, the time at the temperature, and pressure. Typically, chemical indicators are affixed to the outside and incorporated into the pack to monitor the temperature or time and temperature. The effectiveness of steam sterilization is monitored with a biological indicator containing spores of *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*).

Portable (table-top) steam sterilizers are used in outpatient, dental, and rural clinics. These sterilizers are designed for small instruments, such as hypodermic syringes and needles and dental instruments. The ability of the sterilizer to reach physical parameters necessary to achieve sterilization should be monitored by mechanical, chemical, and biological indicators.

***Uses*:** Steam sterilization should be used whenever possible on all critical and semicritical items that are heat and moisture resistant (e.g., steam sterilizable respiratory therapy and anesthesia equipment), even when not essential to prevent pathogen transmission. Steam sterilizers also are used in healthcare facilities to decontaminate microbiological waste and sharps containers but additional exposure time is required in the gravity displacement sterilizer for these items.

Flash Sterilization

Overview: "Flash" steam sterilization was originally defined by Underwood and Perkins as sterilization of an unwrapped object at 132°C for 3 minutes at 27-28 lbs. of pressure in a gravity displacement sterilizer. Currently, the time required for flash sterilization depends on the type of sterilizer and the type of item (i.e., porous vs non-porous items). Flash sterilization is an effective process for the sterilization of critical medical devices. Flash sterilization is a modification of conventional steam sterilization (either gravity, prevacuum, or steam-flush pressure-pulse) in which the flashed item is placed in an open tray or is placed in a specially designed, covered, rigid container to allow for rapid penetration of steam. Historically, it is not recommended as a routine sterilization method because of the lack of timely biological indicators to monitor performance, absence of protective packaging following sterilization, possibility for contamination of processed items during transportation to the operating rooms, and the sterilization cycle parameters (i.e., time, temperature, pressure) are minimal. To address some of these concerns, many healthcare facilities have done the following: placed equipment for flash sterilization in close proximity to operating rooms to facilitate aseptic delivery to the point of use (usually the sterile field in an ongoing surgical procedure); extended the exposure time to ensure lethality comparable to sterilized wrapped items (e.g., 4 minutes at 132°C) used biological indicators that provide results in 1 hour for flash-sterilized items; and used protective packaging that permits steam penetration.

Uses: Flash sterilization is considered acceptable for processing cleaned patient-care items that cannot be packaged, sterilized, and stored before use. It also is used when there is insufficient time to sterilize an item by the preferred package method. **Flash sterilization should not be used for reasons of convenience, as an alternative to purchasing additional instrument sets, or to save time. Because of the potential for serious infections, flash sterilization is not recommended for implantable devices**. If flash sterilization of an implantable device is unavoidable, record keeping (i.e., load identification, patient's name/hospital identifier, and biological indicator result) is essential for epidemiological tracking (e.g., of surgical site infection, tracing results of biological indicators to patients who received the item to document sterility), and for an assessment of the reliability of the sterilization process (e.g., evaluation of biological monitoring records and sterilization maintenance records noting preventive maintenance and repairs with dates).

Low-Temperature Sterilization Technologies

Ethylene oxide (ETO) has been widely used as a low-temperature sterilant since the 1950s. It has been the most commonly used process for sterilizing temperature and moisture-sensitive medical devices and supplies in healthcare institutions in the United States. Two types of ETO sterilizers are available, mixed gas and 100% ETO. **Until 1995, ethylene oxide sterilizers combined ETO with a chloroflourocarbon (CFC) stabilizing agent, most commonly in a ratio of 12% ETO mixed with 88% CFC.**

For several reasons, healthcare personnel have been exploring the use of new low-temperature sterilization technologies. First, CFCs were phased out in December 1995. CFCs were classified as a Class I substance because of scientific evidence linking them to destruction of the earth's ozone layer. Second, some places require the use of ETO abatement technology to reduce the amount of ETO being released into ambient air from 90 to 99.9% depending on the state. Third, OSHA regulates the acceptable vapor levels of ETO (i.e., 1 ppm averaged over 8 hours) due to concerns that **ETO exposure represents an occupational hazard**. These constraints have led to the development of alternative technologies for low-temperature sterilization in the healthcare setting.

Alternative technologies to ETO with chlorofluorocarbon that are currently available and cleared by the FDA for medical equipment include 100% ETO; ETO with a different stabilizing gas, such as carbon dioxide or hydrochlorofluorocarbons (HCFC); immersion in peracetic acid; hydrogen peroxide gas plasma; and ozone. Technologies under development for use in healthcare facilities, but not cleared by the FDA, include vaporized hydrogen peroxide, vapor phase peracetic acid, gaseous chlorine dioxide, ionizing radiation, or pulsed light. **However, there is no guarantee that these new sterilization technologies will receive FDA clearance for use in healthcare facilities.**

Ethylene Oxide "Gas" Sterilization

***Overview*:** ETO is a colorless gas that is **flammable** and **explosive**. The four essential parameters (operational ranges) are: gas concentration (450 to 1200 mg/l); temperature (37 to 63°C); relative humidity (40 to 80%)(water molecules carry ETO to reactive sites); and **exposure time (1 to 6 hours)**. These influence the effectiveness of ETO sterilization. Within certain limitations, an increase in gas concentration and temperature may shorten the time necessary for achieving sterilization.

The main disadvantages associated with ETO are the lengthy cycle time, the cost, and its potential hazards to patients and staff; the main advantage is that it can sterilize heat- or moisture-sensitive medical equipment without deleterious effects on the material used in the medical devices. Acute exposure to ETO may result in irritation (e.g., to skin, eyes, gastrointestinal or respiratory tracts) and central nervous system depression. **Chronic inhalation has been linked to the formation of cataracts, cognitive impairment, neurologic dysfunction, and disabling polyneuropathies. Occupational exposure in**

healthcare facilities has been linked to hematologic changes and an increased risk of spontaneous abortions and various cancers. ETO should be considered a known human carcinogen.

The basic ETO sterilization cycle consists of five stages (i.e., preconditioning and humidification, gas introduction, exposure, evacuation, and air washes) and takes approximately 2 & a 1/2 hrs excluding aeration time. Mechanical aeration for 8 to 12 hours at 50 to 60°C allows desorption of the toxic ETO residual contained in exposed absorbent materials. Most modern ETO sterilizers combine sterilization and aeration in the same chamber as a continuous process. These ETO models minimize potential ETO exposure during door opening and load transfer to the aerator. Ambient room aeration also will achieve desorption of the toxic ETO but requires 7 days at 20°C.

The use of ETO evolved when few alternatives existed for sterilizing heat and moisture-sensitive medical devices; however, favourable properties account for its continued widespread use. Two ETO gas mixtures are available to replace ETO-chlorofluorocarbon (CFC) mixtures for large capacity, tank-supplied sterilizers. **The ETO-carbon dioxide (CO_2) mixture consists of 8.5% ETO and 91.5% CO_2. This mixture is less expensive than ETO-hydrochlorofluorocarbons (HCFC), but a disadvantage is the need for pressure vessels rated for steam sterilization, because higher pressures (28-psi gauge) are required.** The other mixture, which is a drop-in CFC replacement, is ETO mixed with HCFC. HCFCs are approximately 50-fold less damaging to the earth's ozone layer than are CFCs. The EPA will begin regulation of HCFC in the year 2015 and will terminate production in the year 2030.

ETO is absorbed by many materials. For this reason, following sterilization the item must undergo aeration to remove residual ETO. Guidelines have been promulgated regarding allowable ETO limits for devices that depend on how the device is used, how often, and how long in order to pose a minimal risk to patients in normal product use.

ETO toxicity has been established in a variety of animals. **Exposure to ETO can cause eye pain, sore throat, difficulty breathing and blurred vision.** Exposure can also cause dizziness, nausea, headache, convulsions, blisters and vomiting and coughing. In a variety of *in vitro* and animal studies, ETO has been demonstrated to be carcinogenic. ETO has been linked to spontaneous abortion, genetic damage, nerve damage, peripheral paralysis, muscle weakness, and impaired thinking and memory. Occupational exposure in healthcare facilities has been linked to an increased risk of spontaneous abortions and various cancers. Injuries (e.g., tissue burns) to patients have been associated with ETO residues in implants used in surgical procedures. Residual ETO in capillary flow dialysis membranes has been shown to be neurotoxic in vitro.

Uses: ETO is used in healthcare facilities to sterilize critical items (and sometimes semicritical items) that are moisture or heat sensitive and cannot be sterilized by steam sterilization.

Hydrogen Peroxide Gas Plasma

***Overview*:** New sterilization technology based on plasma was patented in 1987 and marketed in the United States in 1993. Gas plasmas have been referred to as the fourth state of matter (i.e., liquids, solids, gases, and gas plasmas). Gas plasmas are generated in an enclosed chamber under deep vacuum using radio frequency or microwave energy to excite the gas molecules and produce charged particles, many of which are in the form of free radicals. A free radical is an atom with an unpaired electron and is a highly reactive species. The proposed mechanism of action of this device is the production of free radicals within a plasma field that are capable of interacting with essential cell components (e.g., enzymes, nucleic acids) and thereby disrupt the metabolism of microorganisms. The type of seed gas used and the depth of the vacuum are two important variables that can determine the effectiveness of this process.

In the late 1980s the first hydrogen peroxide gas plasma system for sterilization of medical and surgical devices was field-tested. According to the manufacturer, the sterilization chamber is evacuated and hydrogen peroxide solution is injected from a cassette and is vaporized in the sterilization chamber to a concentration of 6 mg/l. The hydrogen peroxide vapor diffuses through the chamber (50 minutes), exposes all surfaces of the load to the sterilant, and initiates the inactivation of microorganisms. An electrical field created by a radio frequency is applied to the chamber to create a gas plasma. Microbicidal free radicals (e.g., hydroxyl and hydroperoxyl) are generated in the plasma. The excess gas is removed and in the final stage (i.e., vent) of the process the sterilization chamber is returned to atmospheric pressure by introduction of high-efficiency filtered air. The by-products of the cycle (e.g., water vapor, oxygen) are nontoxic and eliminate the need for aeration. Thus, the sterilized materials can be handled safely, either for immediate use or storage. **The process operates in the range of 37-44°C and has a cycle time of 75 minutes. If any moisture is present on the objects the vacuum will not be achieved and the cycle aborts.**

A newer version of the unit improves sterilizer efficacy by using two cycles with a hydrogen peroxide diffusion stage and a plasma stage per sterilization cycle. This revision, which is achieved by a software modification, reduces total processing time from 73 to 52 minutes. The manufacturer believes that the enhanced activity obtained with this system is due in part to the pressure changes that occur during the injection and diffusion phases of the process and to the fact that the process consists of two equal and consecutive half cycles, each with a separate injection of hydrogen peroxide. The biological indicator used with this system is *Bacillus atrophaeus* spores.

Another gas plasma system, which differs from the above in several important ways, including the use of **peracetic acid i.e. acetic acid & hydrogen peroxide vapor, was removed from the market place because of reports of corneal destruction to patients when ophthalmic surgery instruments had been processed in the sterilizer.** In this investigation, **exposure of potentially wet ophthalmologic surgical instruments with small bores and brass components to the plasma gas led to degradation of the brass to copper and zinc**. The experimenters showed that when rabbit eyes were exposed to the rinsates of the gas plasma-sterilized instruments, corneal decompensation was documented. This toxicity is highly unlikely with the hydrogen peroxide gas plasma process since a toxic, soluble form of copper would not form.

Uses: Materials and devices that cannot tolerate high temperatures and humidity, such as some plastics, electrical devices, and corrosion-susceptible metal alloys, can be sterilized by hydrogen peroxide gas plasma.

Peracetic Acid Sterilization

Overview: Peracetic acid is a highly biocidal oxidizer that maintains its efficacy in the presence of organic soil. Peracetic acid removes surface contaminants (primarily protein) on endoscopic tubing. An automated machine using peracetic acid to

sterilize medical, surgical, and dental instruments chemically (e.g., endoscopes, arthroscopes) was introduced in 1988. The sterilant, 35% peracetic acid is supplied in a single-dose container. The container is punctured at the time of use, immediately prior to closing the lid and initiating the cycle. **The concentrated peracetic acid is diluted to 0.2% with filtered water (0.2 µm) at a temperature of approximately 50°C.** The diluted peracetic acid is circulated within the chamber of the machine and pumped through the channels of the endoscope for 12 minutes, decontaminating exterior surfaces, lumens, and accessories. Interchangeable trays are available to permit the processing of up to three rigid endoscopes or one flexible endoscope. Connectors are available for most types of flexible endoscopes for the irrigation of all channels by directed flow. Rigid endoscopes are placed within a lidded container, and the sterilant fills the lumens either by immersion in the circulating sterilant or by use of channel connectors to direct flow into the lumen(s). The peracetic acid is discarded via the sewer and the instrument rinsed four times with filtered water. **Concern has been raised that filtered water may be inadequate to maintain sterility. Limited data have shown that low-level bacterial contamination may follow the use of filtered water in an AER**. Clean filtered air is passed through the chamber of the machine and endoscope channels to remove excess water. As with any sterilization process, the system can only sterilize surfaces that can be contacted by the sterilant. For example, bronchoscopy-related infections occurred when bronchoscopes were processed using the wrong connector. Investigation of these incidents revealed that bronchoscopes were inadequately reprocessed when inappropriate channel connectors were used and when **there were inconsistencies between the reprocessing instructions provided by the manufacturer of the bronchoscope and the manufacturer of the automatic endoscope reprocessor**. The importance of channel connectors to achieve sterilization was also shown for rigid lumen devices.

***Uses*:** This automated machine is used to chemically sterilize medical (e.g., GI endoscopes) and surgical (e.g., flexible endoscopes) instruments. Lumened endoscopes must be connected to an appropriate channel connector to ensure that the sterilant has direct contact with the contaminated lumen.

Ionizing Radiation: Sterilization by ionizing radiation, primarily by cobalt 60 gamma rays or electron accelerators, is a low-temperature sterilization method that has been used for a number of medical products (e.g., tissue for transplantation, pharmaceuticals, medical devices). **There are no FDA-cleared ionizing radiation sterilization processes for use in healthcare facilities. Because of high sterilization costs**, this method is an unfavourable alternative to ETO and plasma sterilization in healthcare facilities but is suitable for large-scale sterilization. Some deleterious effects on patient-care equipment associated with gamma radiation include **induced oxidation in polyethylene and delamination and cracking in polyethylene knee bearings**.

***Dry-Heat Sterilizers*.** This method should be used only for materials that might be damaged by moist heat or that are impenetrable to moist heat like sharp instruments. The advantages for dry heat include the following: it is nontoxic and does not harm the environment; a dry heat cabinet is easy to install and has relatively low operating costs; it penetrates materials; and it is noncorrosive for metal and sharp instruments. The disadvantages for dry heat are the **slow rate of heat penetration and microbial killing** makes this a time-consuming method. In addition, the high temperatures are not suitable for most materials. The most common time-temperature relationships for sterilization with hot air sterilizers are 170°C (340°F) for 60 minutes, 160°C (320°F) for 120 minutes, and 150°C (300°F) for 150 minutes. ***B. atrophaeus* spores should be used to monitor the sterilization process for dry heat because they are more resistant to dry heat than are *G. stearothermophilus* spores.** The primary lethal process is considered to be oxidation of cell constituents.

There are two types of dry-heat sterilizers: the static-air type and the forced-air type. The static-air type is referred to as the oven-type sterilizer as heating coils in the bottom of the unit cause the hot air to rise inside the chamber via gravity convection. This type of dry-heat sterilizer is much slower in heating, requires longer time to reach sterilizing temperature, and is less uniform in temperature control throughout the chamber than is the forced-air type. The forced-air or mechanical convection sterilizer is equipped with a motor-driven blower that circulates heated air throughout the chamber at a high velocity, permitting a more rapid transfer of energy from the air to the instruments.

Filtration: Although filtration is not a lethality-based process and is not an FDA-cleared sterilization method, this technology is used to remove bacteria from thermolabile pharmaceutical fluids that cannot be purified by any other means. In order to remove bacteria, the membrane pore size (e.g., 0.22 µm) must be smaller than the bacteria and uniform throughout. Some investigators have appropriately questioned whether the removal of microorganisms by filtration really is a sterilization method because of slight bacterial passage through filters, viral passage through filters, and transference of the sterile filtrate into the final container under aseptic conditions entail a risk of contamination.

Microwave: Microwaves are used in medicine for disinfection of soft contact lenses, dental instruments, dentures, milk, and urinary catheters for intermittent self-catheterization. However, **microwaves must only be used with products that are compatible (i.e., do not melt)**. Microwaves are radio-frequency waves, which are usually used at a frequency of 2450 MHz. The microwaves produce friction of water molecules in an alternating electrical field. The intermolecular friction derived from the vibrations generates heat and some authors believe that the effect of microwaves depends on the heat produced while others postulate a nonthermal lethal effect. The initial reports showed microwaves to be an effective microbicide. The microwaves produced by a "home-type" microwave oven (2.45 GHz) completely inactivate bacterial cultures, mycobacteria, viruses, and *G. stearothermophilus* spores within 60 seconds to 5 minutes depending on the challenge organism. Another study confirmed these results but also found that higher power microwaves in the presence of water may be needed for sterilization. Complete destruction of *Mycobacterium bovis* was obtained with 4 minutes of microwave exposure (600W, 2450 MHz). The effectiveness of microwave ovens for different sterilization and disinfection purposes should be tested and demonstrated as test conditions affect the results (e.g., presence of water, microwave power). **Sterilization of metal instruments cannot be accomplished**. Certain precautions are required for compatibility as the home-type microwave ovens may not have even distribution of microwave energy over the entire dry device; hence there may be areas that are not sterilized or disinfected. The use of microwave ovens to disinfect intermittent-use catheters also has been

suggested. Researchers found that test bacteria (e.g., *E. coli*, *Klebsiella pneumoniae*, *Candida albicans*) were eliminated from red rubber catheters within 5 minutes. Microwaves used for sterilization of medical devices have not been FDA cleared.

***Glass Bead "Sterilizer"*:** Glass bead "sterilization" uses small glass beads (1.2-1.5 mm diameter) and high temperature (217°C - 232°C) for brief exposure times (e.g., 45 seconds) to inactivate microorganisms. These devices have been used for several years in the dental profession. FDA believes there is a risk of infection with this device because of potential failure to sterilize dental instruments and their use should be discontinued until the device has received FDA clearance.

Ozone: Ozone has been used for years as a drinking water disinfectant. Ozone is produced when O_2 is energized and split into two monatomic (O_1) molecules. The monatomic oxygen molecules then collide with O_2 molecules to form ozone, which is O_3. Thus, ozone consists of O_2 with a loosely bonded third oxygen atom that is readily available to attach to, and oxidize, other molecules. This additional oxygen atom makes ozone a powerful oxidant that destroys microorganisms but is highly unstable (i.e., half-life of 22 minutes at room temperature).

A new sterilization process, which uses ozone as the sterilant, was cleared by FDA in August 2003 for processing reusable medical devices. The sterilizer creates its own sterilant internally from USP grade oxygen, steam-quality water and electricity; the sterilant is converted back to oxygen and water vapor at the end of the cycle by a passing through a catalyst before being exhausted into the room. **The duration of the sterilization cycle is about 4 h and 15 m, and it occurs at 30-35°C.** Microbial efficacy has been demonstrated by achieving a SAL of 10-6 with a variety of microorganisms to include the most resistant microorganism, *Geobacillus stearothermophilus*.

The ozone process is compatible with a wide range of commonly used materials including stainless steel, titanium, anodized aluminum, ceramic, glass, silica, PVC, Teflon, silicone, polypropylene, polyethylene and acrylic. In addition, rigid lumen devices of the following diameter and length can be processed: internal diameter (ID): > 2 mm, length ≤ 25 cm; ID > 3 mm, length ≤ 47 cm; and ID > 4 mm, length ≤ 60 cm.

The process should be safe for use by the operator because there is no handling of the sterilant, no toxic emissions, no residue to aerate, and low operating temperature means there is no danger of an accidental burn. The cycle is monitored using a self-contained biological indicator and a chemical indicator. The sterilization chamber is small, about 4 ft3.

Formaldehyde Steam: Low-temperature steam with formaldehyde is used as a low-temperature sterilization method in many countries. The process involves the use of formalin, which is vaporized into a formaldehyde gas that is admitted into the sterilization chamber. A formaldehyde concentration of 8-16 mg/l is generated at an operating temperature of 70-75°C. The sterilization cycle consists of a series of stages that include an initial vacuum to remove air from the chamber and load, followed by steam admission to the chamber with the vacuum pump running to purge the chamber of air and to heat the load, followed by a series of pulses of formaldehyde gas, followed by steam. Formaldehyde is removed from the sterilizer and load by repeated alternate evacuations and flushing with steam and air. This system has some advantages, e.g., the cycle time for formaldehyde gas is faster than that for ETO and the cost per cycle is relatively low. However, ETO is more penetrating and operates at lower temperatures than do steam/formaldehyde sterilizers. Low-temperature steam formaldehyde sterilization has been found effective against vegetative bacteria, mycobacteria, *B. Atrophaeus* and *G. stearothermophilus* spores and *Candida albicans*.

Formaldehyde vapor cabinets also may be used in healthcare facilities to sterilize heat-sensitive medical equipment. Commonly, there is no circulation of formaldehyde and no temperature and humidity controls. The release of gas from paraformaldehyde tablets (placed on the lower tray) is slow and produces a low partial pressure of gas. The microbicidal quality of this procedure is unknown. Reliable sterilization using formaldehyde is achieved when performed with a high concentration of gas, at a temperature between 60° and 80°C and with a relative humidity of 75 to 100%.

Studies indicate that formaldehyde is a mutagen and a potential human carcinogen. The permissible exposure limit for formaldehyde in work areas is 0.75 ppm measured as a 8-hour TWA. The OSHA standard includes a 2 ppm STEL (i.e., maximum exposure allowed during a 15-minute period). As with the ETO standard, the formaldehyde standard requires that the employer conduct initial monitoring to identify employees who are exposed to formaldehyde at or above the action level or STEL. If this exposure level is maintained, employers may discontinue exposure monitoring until there is a change that could affect exposure levels or an employee reports formaldehyde-related signs and symptoms. The formaldehyde steam sterilization system has not been FDA cleared for use in healthcare facilities.

Gaseous chlorine dioxide: A gaseous chlorine dioxide system for sterilization of healthcare products was developed in the late 1980s. Chlorine dioxide is not mutagenic or carcinogenic in humans. As the chlorine dioxide concentration increases, the time required to achieve sterilization becomes progressively shorter. For example, only 30 minutes were required at 40 mg/l to sterilize the 106 *B. atrophaeus* spores at 30° to 32°C. Currently, no gaseous chlorine dioxide system is FDA cleared.

Infrared radiation: An infrared radiation prototype sterilizer was investigated and found to destroy *B. atrophaeus* spores. Some of the possible advantages of infrared technology include short cycle time, low energy consumption, no cycle residuals, and no toxicologic or environmental effects. This may provide an alternative technology for sterilization of selected heat-resistant instruments but there are no FDA-cleared systems for use in healthcare facilities.

The sterilization technologies mentioned above are for sterilization of critical medical items. Ideally, the microbicidal effectiveness of the technology has to be published in the scientific literature. The selection and use of disinfectants, chemical sterilants and sterilization processes in the healthcare field is dynamic. As newer disinfectants and sterilization processes become available, persons or committees responsible for selecting disinfectants and sterilization processes should be guided as per regulations & as well as information in the scientific literature.

Chemical sterilization technologies will be discussed in the next issue of Journal of Hygiene Sciences.

Differential & Selective Media - MacConkey Agar

Selective and differential media are used to isolate or identify particular organisms. Selective media allow certain types of organisms to grow, and inhibit the growth of other organisms. The selectivity is accomplished in several ways. For example, organisms that can utilize a given sugar are easily screened by making that sugar the only carbon source in the medium. On the other hand, selective inhibition of some types of microorganisms can be achieved by adding dyes, antibiotics, salts or specific inhibitors which affect the metabolism or enzyme systems of the organisms. For example, media containing potassium tellurite, sodium azide or thallium acetate (at concentrations of 0.1 - 0.5 g/l) will inhibit the growth of Gram-negative bacteria. Media supplemented with penicillin (5-50 units/ml) or crystal violet (2 mg/l) will inhibit the growth of Gram-positive bacteria. Tellurite agar, therefore, is used to select **for** Gram-positive organisms, and nutrient agar supplemented with penicillin can be used to select **for** Gram negative organisms.

Differential media are used to differentiate closely related organisms or groups of organisms. Owing to the presence of certain dyes or chemicals in the media, the organisms will produce characteristic changes or growth patterns that are used for identification or differentiation. A variety of selective and differential media are used in medical, diagnostic and water pollution laboratories, and in food and dairy laboratories. Three of the more common selective and differential media are described below and will be used in the laboratory exercise.

MacCONKEY'S AGAR:

MacConkey's Agar is both a **selective and differential media**; it is selective for Gram negative bacteria and can differentiate those bacteria that have the ability to ferment lactose. MacConkey's agar is used as **differential** plating medium in the detection and isolation of all types of dysentery, typhoid and paratyphoid organisms. It is generally used for differentiating strains of *Salmonella typhosa* from members of the coliform group; however, the medium supports the growth of all *Salmonella* and *Shigella* strains and gives good differentiation between these enteric pathogens and the coliform group. When grown on MacConkey.s medium, colonies of coliform bacteria are brick-red in color and are surrounded by a zone of precipitated bile. These reactions are due to the acid produced by the fermentation of lactose. The acid end-products act on bile salts, and neutral red is absorbed by the precipitated salts. Dysentery, typhoid and paratyphoid bacilli do not ferment lactose but give an alkaline reaction when grown on the medium. Colonies of these organisms are noncolored and transparent. The growth of Gram positive organisms is inhibited because of the crystal violet and bile salts in the medium.

Components:

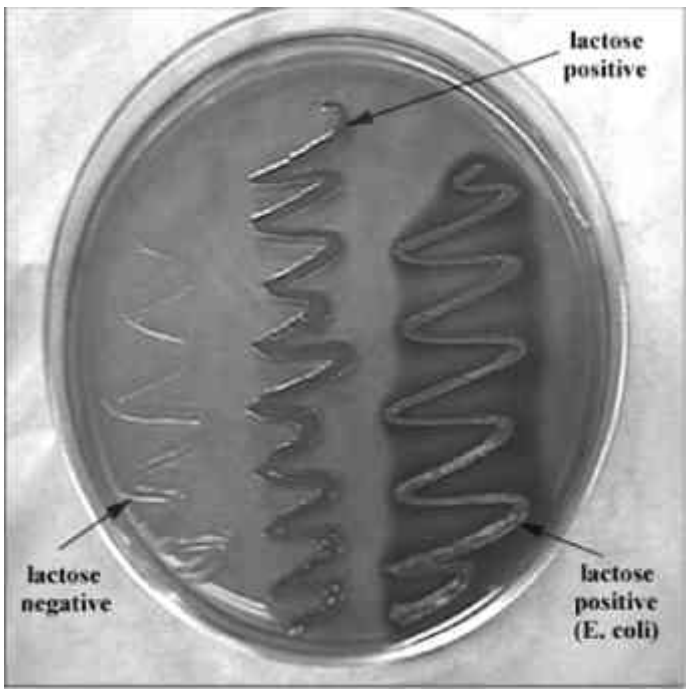
Bile salts - Inhibits most Gram-positive bacteria, except *Enterococcus* and some species of *Staphylococcus* i.e. *Staphylococcus aureus*.

Crystal violet dye- Inhibits certain Gram-positive bacteria thus selecting for Gram negatives.

Lactose- Some bacteria can ferment lactose acid-end products, others cannot.

Neutral pH red indicator - Stains microbes fermenting lactose

* hot pink in acid pH
* rose in neutral pH
* tan in alkaline pH



Peptone - a source of proteins, amino acids for microbial growth.

By utilizing the available lactose in the medium, Lac+ (Lactose positive) bacteria such as *Escherichia coli*, *Enterobacter* and *Klebsiella* will produce acid in the medium, which lowers the pH of the agar below 6.8 and results in the appearance of red or pink colonies. The bile salts in the medium precipitate in the immediate neighborhood of the colony, causing the medium surrounding the colony to become hazy appearance. Non-lactose fermenting bacteria such as, *Proteus* species, *Salmonella, Pseudomonas aeruginosa* and *Shigella* cannot utilize lactose in the medium, and will use peptone instead. This results in the formation of ammonia, which raises the pH of the agar, and leads to the formation of white or colorless colonies in the plate. But, in some cases, they can also look golden to brown with dark centers. They are usually circular colonies and arranged randomly.

MacConkey's Agar Is Selective

MacConkey's is a selective medium that inhibits the growth of Gram-positive bacteria due to the presence of crystal violet and bile salts. Gram-negative bacteria grow well on MAC.

MacConkey's Agar Is Differential

MAC is also a differential, meaning that it differentiates or distinguishes between groups of bacteria on the basis of a color change reaction. MacConkey's contains two additives that make it differential; neutral red (a pH indicator) and lactose (a disaccharide).

Bacteria, known as "lactose fermenters", eat the media's lactose, and, in the process, create an acidic end product that causes the pH indicator, neutral red, to turn pink. With MacConkey's, it is not the media that changes color, but rather the actual colonies of lactose fermenting bacteria that appear pink. Non-lactose fermenting bacteria will be colorless (or, if they have any color, will be their natural color rather than pink).

What does it mean if Bacterial Colonies Grow on MacConkey's?

Whenever bacterial colonies are growing on MacConkey's Agar, they are Gram-negative bacteria (since Gram+ do not grow on

this type of medium). If the colonies are pink, they are Gram-lactose-fermenting bacteria. These pink colonies are typically coliform bacteria in the family *Enterobacteriaceae*, inlcuding the genera Escherichia, Klebsiella, Enterobacter, Hafnia and Citrobacter. Non-lactose fermenting, non-coliform members of Enterboacteriaceae include the genera Proteus, Morganella, Providencia, Edwardsiella, Salmonella, Shigella and Yersenia (plague bacteria).

Types of MacConkey Agar

1. **MacConkey Agar Base**
   A medium for studying carbohydrate fermentation reaction of coliforms by adding the desired carbohydrate.

Ingredients	Grams/Liter
Peptone	17
Proteose Peptone	3.0
Bile Salts	1.5
Sodium Chloride	5.0
Crystal Violet	0.001
Neutral Red	0.03
Agar	13.5
Final pH(at 25 ^{0}C)	7.1±0.2

2. **MacConkey Agar without Crystal Violet and with 0.15% Bile Salts**
   A medium for selective isolation & differentiation of lactose fermenting and non-lactose fermenting enteric bacteria.

Ingredients	Grams/Liter
Peptone	17.0
Proteose Peptone	3.0
Lactose	10.0
Bile Salts	1.5
Sodium Chloride	5.0
Neutral Red	0.03
Agar	15.0
Final pH(at 25 ^{0}C)	7.1±0.2

3. **MacConkey Agar with Crystal Violet, NaCl and 0.15% Bile Salts**
   A slightly selective and differential medium for the detection of coliforms and enteric pathogens. Medium is designed to achieve more differentiation of lactose fermenters & non - lactose fermenters, for the superior growth of enteric pathogens and to improve the inhibition of swarming of *Proteus* species. This medium suppresses a number of gram positive bacteria including *Staphylococcus*.

Ingredients	Grams/Liter
Pancreatic Digest of Gelatin	17.0
Peptone	1.5
Tryptone	1.5
Lactose	10.0
Bile Salts	1.5
Sodium Chloride	5.0
Crystal Violet	0.001
Neutral Red	0.03
Agar	15.0
Final pH(at 25 ^{0}C)	7.1±0.2

4. **MacConkey Agar without CrystalViolet, NaCl and 0.5% Sodium Taurocholate**
   A medium for cultivation and differentiation of enteric bacteria and potentially pathogenic gram-positive organisms while restricting swarming of *Proteus* species. Swarming of Proteus is reduced due to lack of salts in the medium. Sodium taurocholate is a selective agent that inhibits gram-positive bacteria except Staphylococci & Enterococci

Ingredients	Grams/Liter
Peptone	20.0
Lactose	10.0
Sodium Taurocholate	5.0
Neutral Red	0.04
Agar	15.0
Final pH(at 25 ^{0}C)	7.4±0.2

MacConkey agar has a range of media's with their different application.

PRODUCT NAME	USES	MAIN INGREDIENT	APPLICATION
MacConkey Agar Base	To differentiate coliforms based on carbohydrate fermentation reaction.	Peptone Proteose Peptone	Serves as carbohydrate source
MacConkey Agar with Crystal Violet, NaCl & 0.15% Bile Salts	1) To differentiate between lactose fermenters & non lactose fermenters. 2) Promotes growth of enteric pathogens 3) Inhibition of Proteus species	Bile Salts Crystal Violet	Presence of bile salts & crystal violet allows superior growth of enteric gram-negative organisms & inhibits gram-positive organisms
MacConkey Agar without Crystal Violet & with 0.15% Bile Salts	For isolation and cultivation of gram-negative enteric organisms.	Bile Salts	Presence of bile salts allow the growth of enteric gram-negative organisms & inhibits gram-positive organisms
MacConkey Agar without Crystal Violet, NaCl, & with 0.5% Sodium Taurocholate	For isolation & cultivation of gram-negative micro-organisms and gram-positive cocci. Permits the growth of Staphylococcus & Enterococcus	Sodium Taurocholate	Inhibits gram-positive bacteria except Staphylococcus and Enterococcus

Paul Ehrilich

Born	14 March 1854 Strehlen, Lower Silesia, German Kingdom of Prussia
Died	20 August 1915 (aged 61) Bad Homburg, Hesse, Germany
Citizenship	Germany
Fields	Immunology
Known for	Autoimmunity
Notable awards	Nobel Prize in Physiology or Medicine (1908)

Paul Ehrilich (born on 14 March 1854) in Strehlen near Breslau - died 20 August 1915 in Bad Homburg vor der Höhe) was a German physician and scientist who worked in the fields of hematology, immunology, and chemotherapy. He invented the precursor technique to Gram staining bacteria, and the methods he developed for staining tissue made it possible to distinguish between different type of blood cells, which led to the capability to diagnose numerous blood diseases. Ehrlich's chemotherapy research led to his formulating the arsenic compound, Arsphenamine (Salvarsan), which was used in the treatment of syphilis during the first half of this century until it was superseded by penicillin.

He also made a decisive contribution to the development of an antiserum to combat diphtheria and conceived a methodology for standardizing therapeutic serums. In 1908 he received a Nobel Prize in Physiology or Medicine for his contributions to immunology.

He was born in Strzelin, Silesia, Germany, schooled at Breslau College, and went to the University there. He later transferred to the newly founded University of Strasbourg, where he entered the medical faculty and met the great Robert Koch. He completed his studies at Leipzig University with a degree thesis on the value and significance in medicine of staining with aniline dyes.

In 1877 after working in a hospital pathology department, he secured an internship at the Charite Hospital, Berlin, where he set up a primitive laboratory in a disused wing. Showing a colleague a pot containing an excised primary chancre, he said "when the microbe causing syphilis is found, I must be prepared". After graduating, he became interested in staining reactions of tissues and discovered mast cells. He later devised a classification of leukaemias which remains in use to this day, and established the function of bone marrow as a tissue of defence. In 1882, fired with Koch's discovery of the tubercle bacillus, he started experimenting on selected stains which quickly and easily identified the rod shaped organism. This earned him Koch's delight and admiration, and so established a lasting friendship.

Ehrlich married a Prussian lady called Hedwig Pinkus, but shortly afterwards contracted pulmonary tuberculosis. He resigned from his appointment and travelled to Egypt accompanied by his young wife, where he made a complete recovery after two years' treatment with Koch's tuberculin. He had resumed intensive experiments on serum antitoxins, when he saw and heard of Von Behring's antitoxin serum for diphtheria. Ehrlich's standardisation of its unit potency has become the accepted basis for diphtheria serum.

For several years Ehrlich experimented extensively with the organic dye, methylene blue, as a better and cheaper substitute for quinine in treating malaria, but it did not succeed clinically. When quinine supplies were cut off in the second world war, however, Bayer laboratories found Ehrlich's work helpful in producing the successful compounds known as Plasmoquin and Atebrin.

Ehrlich became Head of the Royal Institute of Experimental Therapy, Frankfurt, in 1899, and it was here that his career reached its climax. The following year he gave the Croonian Lecture. He selected a brilliant team of researchers, including C J Browning who was later to become Professor of Bacteriology at the University of Glasgow.

In 1906 Wasserman discovered his reaction for the diagnosis of syphilis, and publicly acknowledged that this was thanks to Ehrlich's pioneer work on haemolysins and the studies of Bordet and Gengou on antibodies. Ehrlich told his workers that, although their past five years' research work on trypanosomiasis had not led to any usable result, they had extended knowledge on protozoa. They now had to make efforts based on Breinl and Thomas' researches at Liverpool University on arsenical organic compounds to find the "silver bullet" (as he called it) against the syphilis spirochaete. These two researchers had produced a pentavalent arsenic compound (Atoxyl) which had been used in treating trypanosomiasis, but had been discontinued as its high arsenic content had caused optic atrophy. The therapeutic problem was how to obtain maximum effect on the parasite with minimum effect on the body tissues. In 1907, Ehrlich produced his six hundred and sixth preparation of an arsenobenzene compound. For some unaccountable reason its potential was overlooked for two years, but later biological work with it on syphilitic rabbits convinced him of its importance. He believed he had found his "silver bullet".

At last the whole world would have a cure for syphilis. When the chemical firm of Hoechst eventually gained his approval to market Salvarsan (healing arsenic), sometimes called "606", a total of 65,000 free samples were sent to doctors in all parts of the world. At a Congress for Internal Medicine in 1910 at Wiesbaden, he announced the promising results he had achieved with Salvarsan. Ehrlich continually warned them that it might be harmful because of its very powerful action, and urged caution with intravenous injections. The chemical structure of the "silver bullet" was based on 32% arsenic, and was thus closely related to the poison generally associated with murder cases.

He had received the greatest academic honours, including the Nobel Prize for Medicine, honorary doctorates from the universities of Oxford, Gottingen, and Chicago, the Prize of Honour of German Chemists, gold medals from eleven countries as far apart as Norway and Japan, the Cameron Prize of the University of Edinburgh, and the Prize of Honour at the Lisbon Congress of Medicine. Salvarsan, and its less toxic derivative Neosalvarsan continued to be widely used in Europe until they were replaced by penicillin in the late 1940s.

Their use against yaws carried on even later in the Far East where penicillin was not easily available and exorbitantly priced.

Honors:

- 1882 Awarded the title of Professor.
- 1890 Appointed Extraordinary Professor at the Friedrich-Wilhelms-Universität (now Humboldt University).
- 1896 Given the nonacademic Prussian title of a Medical Councillor (Geheimer Medizinalrat).
- 1903 Awarded Prussia's highest distinction in science, the Great Golden Medal of Science (which had previously been awarded only to Rudolf Virchow).
- 1904 Honorary professorship in Göttingen honorary doctorate from the University of Chicago.
- 1907 Granted the seldom-awarded title Senior Medical Councillor (Geheimer Obermedizinalrat); granted an honorary doctorate from Oxford University.
- 1908 Awarded The Nobel Prize in Physiology or Medicine for his "work on immunity".
- 1911 Granted Prussia's highest civilian award, Privy Councillor (Wirklicher Geheimer Rat with the predicate "Excellency").
- 1912 Made an honorary citizen of the city of Frankfurt a.M. and of his birthplace Strehlen.
- 1914 Appointed full Professor of Pharmacology at the newly established Frankfurt University.

Enjoy the humour

A Newspaper boy keeps shouting on the street: "Big scam! Big scam! 12 Victims!"
A man decides to buy the newspaper and while browsing it he discovers there is nothing inside it about a scam.
The boy keeps shouting even louder now: "Big scam! Big scam! 13 victims!"

One man caught a thief at the moment who was stealing his wallet
The man ask: don't u feel ashamed of stealing
The thief said: shame to u who wear such an expensive suit
And don't have a single penny in ur wallet.

One man to other: this is the grave of man who created An example of charity.
He gave his whole things to orphange
Second man: Wao! by the way what he gave to the orphange
First man: 6 daughters, one boy & two widows

A short walk is so Difficult
When no one walks with u.
But a long Journey is just like a
few steps when,
...
...
...
A street dog is running behind u!

A very drunk man comes out of the bar-
And sees another very drunk man,
He looks up in the sky and says,
"Is that the sun or the moon?"
The other drunk man answers,
"I don't know. I'm a stranger here myself

Dad to son: when I beat you how do you control your anger?
Son: I start cleaning toilet.
Dad: how does that satisfy you?
Son: I clean with your tooth brush

A successful man is one
Who makes more money
Than
His wife can spend

Great thoughts By Great People

If we cannot love the person whom we see, how can we love God, Whom we cannot see?
-Mother Teresa

Winning doesn't always mean being first, winning means you're doing better than you've done before.
-Bonnie Blair

Everyone thinks of changing the world but no one thinks of changing himself……
-Leo Tolstoy

If someone feels that they had never made a mistake in their life, then it means they had never tried a new thing in their life……
-Einstein

No one can make you feel inferior without your consent.
-Eleanor Roosevelt

Without a sense of urgency, desire loses its value.
-Jim Rohn

There are two ways of meeting difficulties: you alter the difficulties, or you alter yourself to meet them.
-Phyllis Bottome

A man can succeed at almost anything for which he has unlimited enthusiasm.
-Charles Schwab

In the middle of difficulty lies opportunity.
-Albert Einstein

When you know what you want, and you want it badly enough, you'll find a way to get it.
-Jim Rohn

If you are not big enough to lose, you are not big enough to win.
-Walter Reuther

Challenges are what make life interesting; overcoming them is what makes life meaningful.
-Joshua J. Marine

Citrobacter freundii

Scientific Classification:

Kingdom:	Bacteria
Phylum:	Proteobacteria
Order:	Enterobacteriales
Family:	Enterobacteriaceae
Genus:	Citrobacter
Species:	freundii

Citrobacter freundii are facultative anaerobic Gram-negative bacilli. The bacteria are long rod-shaped with a typical length of 1-5 µm. Most *C. freundii* cells are surrounded by several flagella used for locomotion, but a few are non-motile. It can be found in soil, water, sewage, food and the intestinal tracts of animals and humans. It is classified under the family of *Enterobacteriaceae.*

The Citrobacter genus was discovered in 1932 by Werkman and Gillen. Cultures of *C. freundii* were isolated and identified in the same year from soil extracts.

The cell structure of *C. freundi* is long and rod-shaped usually 1-5 µm in length. The outside of the cell contains many flagella used for motality. Since *C. freundii* is gram-negative bacteria, it contains two membranes (inner and outer).The periplasmic space lies in between the two membranes. The outer membrane does not contain an energy source; but it does contain many porins embedded within that help the organism acquire important ions. Unlike gram-positive bacteria, *C. freundii* cells do not contain a thick cell wall made up of peptidoglycan.

As an opportunistic pathogen, *C. freundii* is responsible for a number of significant opportunistic infections. It is known to be the cause of a number of nosocomial infections of the respiratory tract, urinary tract, blood and many other normally sterile sites in patients. *C. freundii* represents about 29% of all opportunistic infections.

Surprisingly, this infectious microbe in humans plays a positive role in the environment. *C. freundii* is responsible for reducing nitrate to nitrite in the environment. This conversion is an important and crucial stage in the nitrogen cycle. The bacteria also help in recycling nitrogen.

For metabolism, *C. freundii* has an ability to grow on glycerol as the sole carbon and energy source. Within its cell, a bacterial microcompartment can be found, which is capable of processing propanediol.

***Citrobacter freundii* Infections:**

Urinary tract infections is most commonly caused by *C. freundii*; manifestations include: burning while urinating, increased frequency of urination, nasty smelling urine, occasional blood in the urine, pain in the lower back and / or pelvis and fever.

- *C. freundii* is also known to cause abnormal inflammatory changes in the intestine, sometimes even resulting in necrotic changes.
- *Citrobacter freundii* is known to cause neonatal meningitis. The meninges or coverings of the brain get infected and inflamed due to the bacterial infiltration. *C. freundii* crosses the blood-brain barrier and wreaks havoc within the CNS. It can attack and duplicate in the brain as well. Frequently seen clinical signs and symptoms: high grade fever, projectile vomiting and seizures.
- Peritonitis and tunnel infection due to *C. freundii* have also been reported.

Treatment:

In general, all doctors prescribe anti-biotics to manage *Citrobacter freundii* infections. The doctor will make the diagnosis based on the symptoms of the case, the clinical presentation, X ray images and bacterial culture.

Citrobacter freundii infection is usually treated with anti-biotics such as fluoroquinolones, carbapenems and cephalosporins. The physician decides the treatment plan depending on the susceptibility of the microorganism to the various anti-biotics and the location of the infection and organ system affected. Then again, there is a growing anxiety about the resistance of *Citrobacter freundii* to a host of anti-biotics. The health care provider will suggest supportive and symptomatic treatment to speed up cure.

By and large, the prognosis for *C. freundii* infections is moderate. Cases that have been neglected or not treated at all show particularly poor prognosis and almost always end in death. *Citrobacter freundii* bacteremia frequently occurs in elderly patients and in patients hospitalized for prolonged period of time. Outcome for *C. freundii* urinary tract infection is quite good; while the prognosis for peritonitis is rather moderate to poor. The mortality rate of *Citrobacter freundii* meningitis is exceedingly high, with the death rate ranging from 25 % to 50 %. What's more, severe neurological problems are known to continue in about 75 % of those who have survived the infection.

Application to Biotechnology

In the Biotech industry, *Citrobacter freundii* produces many important enzymes. The first enzymes it produces is phosphatase. Phosphatase activity of *C. freundii* has been postulated to be involved in lead accumulation, which plays an important role in the Biotech industry. The phosphatase activity of *C. freundii* has been also discovered to have resistance to some diagnostic reagents.

The purification and characterization of bacterial selenocysteine beta-lyase, an enzyme which specifically catalyzes the cleavage of L-selenocysteine to L-alanine, has been purified from *Citrobacter freundii*. The enzyme is monomeric with a molecular weight of ca. 64,000 and contains 1 mol of pyridoxal 5'-phosphate as a cofactor per mol of enzyme. The enzyme also catalyzes the alpha, beta elimination of beta-chloro-L-alanine to form NH3, pyruvate.

C. Freundii strains also carry a plasmid that encodes class 1 AmpC cephalosporinase. These enzymes can hydrolzye inactivate new cephamycins and cephalosporins.

Secondly Citrobacter freundii has also been investigated for biodegradation of tannic acid used in tanneries. A bacterial strain capable of utilizing tannic acid as sole carbon source was isolated from the effluent of a tannery and was identified as *Citrobacter freundii*. This organism could grow at concentrations as high as 5% (w/v) of tannic acid and produced extracellular tannase to hydrolyze the same. When grown in minimal medium containing 1% tannic acid (w/v) at 30 degrees C, this strain produced 1.87 U/ml of tannase at 6 h. At that time, tannic acid degradation products, namely glucose and gallic acid, were detectable in the culture filtrate; the other intermediate metabolites formed were pyrogallol (extracellular) and pyruvate (intracellular). 2-hydroxymuconic acid is presumed to form as a result of ortho-cleavage of pyrogallol. The proposed biochemical pathway for the degradation of tannic acid by *Citrobacter freundii* is: Tannic acid--[Glucose + Gallic acid]--Pyrogallol --2-hydroxymuconic acid --Pyruvate.

Current Research

A small scale research concerning certain strains of *C. freundii* was done recently at the University of Tennessee, Knoxville. The importance of certain tetracycline and streptomycin resistance genes and class 1 integrons in *C. freundii* isolated from dairy farm soil and non dairy soils were evaluated. One strain of *C. freundii* extracted from dairy farm soils carried class 1 integrons with different inserted gene cassettes. Results of this small study suggested that the presence of multiple resistance genes and class 1 integrons in *C. freundii* in dairy farm soil may act as a reservoir of antimicrobial resistance genes and could play a role in the dissemination of these antimicrobial resistance genes to other commensal and indigenous microbial communities in soil. However, additional longer-term studies conducted in more locations are needed to support this hypothesis.

A second research concerning *C. freundii* was done in order to devise a polymerase chain reaction (PCR) method that simultaneously uses three pairs of specific primers to detect genes of certain microbes (including *C. freundii*). The method included designing three primer pairs which were: SPVC-1 and SPVC-2, INVA-1 and INVA-2; and VIAB-1 and VIAB-2. PCR was performed using these three primers to identify 14 clinically important bacterial organisms. The following strains were quickly identified using the PCR: (1) *C. freundii*; (2) *S. Typhi*; and *S. Paratyphi* C; (3) S. Dublin (virulence antigen-positive); and (4) *Salmonella* serovars that harbor an spv-type virulence plasmid. Although this PCR method is new, with the advance of technology in the future this method can allow the identification of *C. freundii* in mammals immediately so that appropriate antibiotic treatment can be initiated without delay.

A third study concerning *C.freundii* was done at the University of Barcelona, Spain. The mechanisms of resistance to fluoroquinolones in two *Citrobacter freundii* strains were studied. Both strains were isolated from the same patient. This study allowed partial characterisation of the acrA and acrB genes of this microorganism. Expression of genes in both strains was analysed using DNA microarrays for *Escherichia coli*. Nucleotide similarity between the partially sequenced acrA and acrB genes of *C. freundii* and *E. coli* was 80.7% and 85%, respectively. The acrA and acrB genes of *C. freundii* are similar to those in *E. coli* and their over expression may play an important role in modulating the final minimum inhibitory concentration of fluoroquinolones.

A fourth study concerning *C. freundii* was done in Taiwan. A team of researchers isolated a diabetic patient that developed necrotizing fasciitis which was caused by *C. freundii* from an injury incited by a marine animal. Necrotizing fasciitis is an infection within the deeper layer of the skin and subcutaneous tissues. When treating the patient they took a sample from the fluid in the wound and found *C. freundii*. After three days of starting the antibiotic treatments with cefotaxmine and cefepmine, there was accumulation of subcutaneous abscesses. After 6, 10, 14, and 21 days of tending to the patient, some antibiotics were giving the patient some relief, but no long term recovery was reached. Cefotoaxime, cefepime, ciprofloxamin are among the antibiotics that were no match for *C. freundii*. The patient fully recovered after 42 days of ertapenem treatment. The researchers had isolated two colonies of *C. freundii* within 5 days of each other. As the isolates were grown they became immensely resistant to cefotaxime and cefepime. The reason why entrapenem worked against *C. freundii* is because is active against AmpC producing *Enterobacteriaceae*.

Antibiotic resistance

Citrobacter species are a common cause of nosocomial infections associated with patients that are undergoing prolonged hospital treatments. *C. freundii* has recently been reported to express resistance to broad-spectrum antibiotincs including piperacillin, piperacillintazobactam, vancomycin and cephalosporins. Isolation of ceftriaxone-resistant *Citrobacter freundii* (CRCF) has been associated with the overprescribed broad spectrum antibiotics. The emerging new CRCF strains could suggest induction or depression of resistance genes as well as elimination of competing organisms. CRCF has been mostly isolated from patients with significant comorbidities including AIDS, peripheral vascular disease, and cerebrovascular disease. The usage of fluoroquinolone has also been reported to have no affect against the isolation of CRCF.

Citrobacter freundii is also known to contain in its chromosome a gene coding for cephalosporinase. This enzyme hydrolyses - CO- NH- bond in the lactam ring of cephalosporins and cephamycis thus rendering the bacteria resistant to this type of antibiotics. However when exposed to new third generation cephems and cerbapenems, clinically isolated *C.freundii* showed sensitivity to those substances. A small outbreak of *C.freundii* resistant to third generation cephems has been observed in the surgical ward of Nagoya University Hospital in patients that underwent surgical procedures. The *C. freundii* was isolated from patient's bile, wound gauze, feces, pus, and ascites. It was suggested that these new strains of *C. freundii* contained a plasmid encoding AmpC celphalosporinase but upon failure to transfer the cephems resistance from *C. freundii* to *E.coli* it was concluded that the enzyme must be encoded in the chromosome of *C. freundii*. Since *C. freundi* is associated with nosocomial infections caution to this new strains is recommended.

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Applications of Biofilms in degradation of contaminants in Industrial Effluents

The formation of biofilms is a natural phenomenon through which microorganisms adhere to solid surfaces whenever they are in contact with water. The biofilm can be defined as a combination of microorganisms and extracellular products which adhere to a solid support, forming a voluminous and thick layer, with an external structure that is not completely regular and uniform. Its chemical composition, both inorganic and organic, varies according to the substrate composition.

Biofilms are typically comprised of water, microorganisms, extracellular polymeric substances (EPS), retained particles and dissolved and adsorbed substances. Water is the most significant fraction of the total mass of the biofilm, and it can vary from 70 to 95%. Polymeric substances represent around 70 to 95% of the organic material of the dry biofilm mass. The composition determines important properties of the biofilm, such as the adhesion force, elasticity, and adsorption capacity.

In general, the biomass, when adhered, has greater activity, that is, it has higher rates of growth and substrate usage, in relation to the free biomass. Studies have shown that adhered cultures are less affected than suspended cultures by environmental changes: temperature, pH, nutrient concentration, metabolic products and toxic substances.

Another important aspect is that the biofilm is an ecosystem, in which many species of microorganisms coinhabit and which are subject to interactions such as symbiosis or competition for space and nutrients. This is due to the constant environmental variation within the biofilm, for example, the concentration of the substrates, electron acceptors and intermediary products.

The development of the biofilm can be described in stages:

1. *Latency or activation phase:* Corresponds, in the reversible phase, to phenomena related to the adsorption of soluble materials (organic and inorganic nutrients) and particulates (microorganisms) at the surface of the support, which occurs through different forces: electrostatic forces, the Van der Waals attractive forces and weak forces of chemical and hydrophobic interactions; and, in the irreversible phase, the fixation, in which extracellular polymers play a fundamental role since it appears that these polymers act as ligands between the microorganisms and the support.
2. *Exponential or dynamic phase:* Is the phase in which the colonization of the support surface occurs and the growth rate is at the maximum. In this phase there is a considerable increase in the rate of polysaccharide and protein production and high consumption of the substrate.
3. *Linear accumulation phase:* Corresponds to a constant rate of biomass accumulation on the support.
4. *Stabilization phase:* In this phase the physical phenomena, such as the shearing and attrition forces, originating from the system fluid dynamics, begin to have an effect, causing the detachment of cells and hindering additional accumulation.
5. *Stationary phase:* In this phase, equilibrium between the detachment and growth of the biofilm cells can be observed, characterizing a permanent regime in relation to the solid phase.
6. *Detachment phase:* The detachment of the biofilm is a random phenomenon which is dependent on the behavior of the microorganisms directly adhered to the support.

The fixation of the microorganisms on the surface is the result of physical, chemical and biological phenomena, and the main factors affecting the biofilm formation and maintenance are:

1. *Characteristics of the support:* The properties of the solid surface are important in terms of the formation of the biofilm, in particular, the surface charge, roughness and hydrophobicity. Some authors consider the roughness as the most important factor since it increases the fixation surface and protects it from the detachment caused by shearing, thus maintaining the microorganisms on the surface long enough for irreversible adhesion to occur and thereby allowing the formation of the biofilm.
2. *Microorganism species:* The formation of the biofilm varies according to the types of microorganisms present, due to their cell surface properties and their capacity to produce extracellular polysaccharides, which are responsible for maintaining cell aggregates.
3. *Characteristics of the liquid phase:* The characteristics of the liquid phase, which affect the formation of the biofilm, may be related to both its composition/concentration (organic and inorganic compounds) and its environmental conditions of pH and temperature, since both influence the microbial growth and the production of extracellular polysaccharides.
4. *Fluid dynamics conditions:* The formation and maintenance of the biofilm are influenced by the balance between the adhesion, growth and detachment of the cells. The detachment process is closely related to the shearing and attrition forces (resulting from collision between particles) which are a function of the fluid dynamics of the process.

Ulson de Souza *et al.* (2011) have presented three case studies relate to: the treatment of real effluent (Case study 1) from a textile plant using a mixture of sludge from an industrial and domestic effluent treatment station, with the inoculation carried out in a fed-batch regime; and the treatment of a synthetic effluent containing a textile dye (Case study 2) and containing BTX compounds (Case study 3), using sludge from a domestic effluent treatment station (anaerobic and activated sludge) in a fed-batch system for the textile dye and batch system for the BTX compounds.

The BTX compounds in the mixture are biodegraded by the previously adapted biofilm, with a more accentuated drop at the beginning of the column. o-xylene is the compound which is the most difficult for the microorganisms to biodegrade, followed by benzene and toluene, when the feed concentration of the BTX compounds is the same. It can also be observed that for the input concentration used (40 mg/L), the flow is high, since the concentration at the column output is higher than the discharge level permitted by the environmental bodies. Thus, for a high input flow, the biodegradation is not complete, requiring a longer bioreactor, and the reverse situation occurs when the feed flow is decreased.

Collection, Transport & Field Supplies of Fecal Specimens

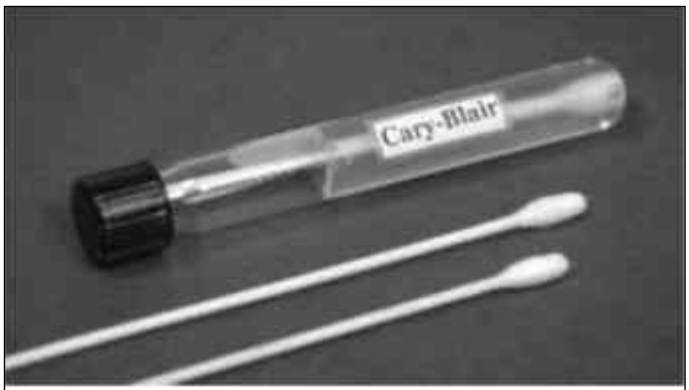
Fecal specimens should be collected in the early stages of any enteric illness, when pathogens are usually present in the stool in highest numbers, and before antibiotic therapy has been started. An exception to this rule is when stool is collected from persons with febrile illness: in the case of typhoid fever, the etiologic agent *Salmonella* ser. Typhi may be present in highest numbers in stool in the second and third weeks of the disease.

Collection and transport of specimens for laboratory diagnosis	
When to collect	When the patient is having diarrhea, as soon after onset of illness as possible (preferably within 4 days of onset) and before antimicrobial treatment is started.
How much to collect	Rectal swab or swab of fresh stool in transport
Transport medium	Cary-Blair or other suitable transport medium (not buffered glycerol saline for V. cholera)
Storage after collection	Refrigerate at 4ºc if the specimens will be received by the laboratory within 48 hours. Or freeze at -70ºc. Fecal specimens from patients with suspected cholera can be transported at ambient temperature and held for longer times if necessary; however refrigeration is preferred.
Transpotation	Seal tubes/containers to prevent leakage; place in waterproof container to protect from wet or dry ice. Ship in insulated box with ice packs, wet ice or dry ice by overnight delivery.

Collection of stool:
Stools samples should be collected in clean containers without disinfectant or detergent residue and with tight-fitting, leak-proof lids. Specimens should **not** be collected from bedpans, because the bedpans may contain residual disinfectant or other contaminants. U**npreserved stool should be refrigerated, if possible, and processed within a maximum of 2 hours after collection. Specimens that cannot be cultured within 2 hours of collection should be placed in transport medium and refrigerated immediately.**

Transport media for fecal specimens:
This section provides information regarding media appropriate for the transport of fecal specimens that are suspected to contain *Shigella, Vibrio cholerae,* or *Salmonella* (including serotype Typhi) specimens. Once specimens from an outbreak of diarrheal disease have arrived at the laboratory, laboratorians should follow procedures for *Shigella* or *V. cholerae* isolation depending on whether reports from the field indicate the outbreak appears to be dysentery or a cholera-like illness. Because persons suspected of having typhoid will commonly present with fever and not diarrhea, laboratories usually do not receive a surge of fecal specimens in an outbreak of typhoid; however, on occasion fecal specimens may be submitted to a laboratory for diagnosis of infection with S. Typhi.

FIGURE 81: Cary-Blair semisolid transport medium



Leave the swab in the tube after inoculating Cary-Blair medium.

Cary-Blair transport medium:
Cary-Blair transport medium can be used to transport many bacterial enteric pathogens, including *Shigella, Salmonella*, and *Vibrio cholerae* (Figure 81).
Cary-Blair's semisolid consistency provides for ease of transport, and the prepared medium can be stored after preparation at room temperature for up to 1 year. Because of its high pH (8.4), it is the medium of choice for transport and preservation of *V. cholerae*.

Preparation and quality control of Cary-Blair
Prepare according to manufacturer's instructions. [Note: There are several commercially available dehydrated formulations of Cary-Blair. Some require the addition of calcium chloride and some do not. Cary-Blair can also be prepared from individual ingredients.] When Cary-Blair is prepared, it should be dispensed into containers in sufficient volume so that swabs will be covered by at least 4 cm of medium. For example, 5- to 6-ml amounts may be dispensed into 13 x 100- mm screw cap tubes. With the caps loosened, sterilize by steaming (do not autoclave) at 100°C for 15 minutes. Tighten the caps after sterilization.
Cary-Blair is quite stable if stored in tightly sealed containers in a cool dark place so that the medium does not dry out. Cary-Blair may be used for up to 1 year as long as there is no loss of volume, contamination, or color change.

Other transport media
Other transport media that are similar to Cary-Blair are Amies' and Stuart's transport media. Both of these are acceptable for *Shigella* and *Salmonella* (including ser. Typhi), but they are inferior to Cary-Blair for transport of *V. cholerae*. Alkaline peptone water may be used to transport *V. cholerae*, but this medium is inferior to Cary-Blair and should be used only when the latter medium is not available. **Alkaline peptone water should not be used if subculture will be delayed more than 6 hours from the time of collection**, because other organisms will overgrow vibrios after 6 hours. Buffered glycerol saline (BGS), a transport medium that is used for *Shigella*, is unsuitable for transport of *V. cholerae*. Additional disadvantages of buffered glycerol saline are that it can be used for only 1 month after it is

made and, because it is a liquid medium, it is more likely to leak or spill during transport.

Placing stool in transport medium:
If possible, chill the transport medium for 1–2 hours in a refrigerator or cold box prior to use. A small amount of stool can be collected by inserting a sterile cotton or polyester-tipped swab into the stool and rotating it. If mucus and shreds of intestinal epithelium are present, these should be sampled with the swab.
Following sampling of the stool on the swab:

a) Insert the swab containing fecal material into transport medium immediately.
b) Push the swab completely to the bottom of the tube of transport medium.
c) Break off the top portion of the stick touching the fingers and discard it.
d) Replace the screw cap on the tube of transport medium and tighten firmly.
e) Place the tube in a refrigerator or cold box.

Collection of rectal swabs:
Sometimes rectal swabs are collected instead of stool specimens. Rectal swabs may be collected as follows:

a) Moisten the swab in sterile transport medium.
b) Insert the swab through the rectal sphincter 2–3 cm (i.e., 1–1.5 inches) and rotate.
c) Withdraw the swab from the rectal sphincter and examine to make sure there is some fecal material visible on the swab. (If not, repeat the procedure with the same swab.)
d) Immediately insert the swab into cold transport medium (as described in the preceding section).
e) Place the tube in a refrigerator or cold box.

The number of swabs needed will depend on the number of plates to be inoculated. **In general, if specimens will be examined for more than one pathogen, at least two stool swabs or rectal swabs should be collected per patient, and both swabs should be inserted into the same tube of transport medium.** Once the swab is placed in the medium, it should remain in the tube until it is processed in the laboratory.

Storage of specimens in transport medium:
If transport medium has been stored at room temperature, it should be chilled in a refrigerator or cold-box, if possible, for 1–2 hours before use. Specimens preserved in transport medium should be refrigerated until processed. If specimens will be kept more than 2–3 days before being cultured, it is preferable to freeze them immediately at -70°C. It may be possible to recover pathogens from refrigerated specimens up to 7 days after collection; however, the yield decreases after the first 1 or 2 days. Prompt plating, refrigeration, or freezing of specimens in Cary-Blair is particularly important for isolation of *Shigella*, which is more fragile than other enteric organisms. Fecal specimens in transport medium collected from patients with cholera need not be refrigerated unless they are likely to be exposed to elevated temperatures (i.e., >40°C).

Unpreserved specimens:
When transport medium is not available, one option for specimens suspected to contain *V. cholerae* is to soak a piece of filter paper, gauze, or cotton in liquid stool and place it into a plastic bag. The bag must be tightly sealed so that the specimen will remain moist and not dry out. Adding several drops of sterile saline to the bag may help prevent drying of the specimen. Refrigeration during transport is desirable but not necessary. **This method is not suitable for transport of *Shigella* or *Salmonella* specimens and is less effective than transport medium for preserving *V. cholerae* organisms.**

Preparing specimens for shipment:
Specimen tubes should be clearly labeled with the specimen number, and if possible, the patient's name and date of collection. Write the numbers on the frosted portion of the specimen tube using an indelible marker pen. If the tube does not have a frosted area, write the information on a piece of first-aid tape and affix this firmly on the specimen container. Patient information should be recorded on a data sheet; one copy should be sent with the specimens and another kept by the sender. If a package is to be shipped by air, the International Air Transport Association (IATA) regulations presented in the *Dangerous Goods Regulations* (DGR) publication must be followed; these regulations (current as of 2002) are summarized in "Packing and Shipping of Diagnostic Specimens and Infectious Substances." Even if the package will be shipped by other means, these regulations are excellent guidelines for packing all infectious or potentially infectious materials.

Refrigerated specimens:
Refrigerated specimens should be transported to the laboratory in an insulated box with frozen refrigerant packs or ice. If wet ice is used, the tubes or containers should be placed in waterproof containers (e.g., plastic bags) that can be tightly sealed to protect the specimens from the water formed by melting ice.

Frozen specimens:
Frozen specimens should be transported on dry ice. The following precautions should be observed:

- Place tubes in containers or wrap them in paper to protect them from dry ice.
- Direct contact with dry ice can crack glass tubes.
- If the specimens are not in leak-proof containers, protect them from exposure to carbon dioxide by sealing the screwcaps with tape or plastic film or by sealing the tubes in a plastic bag. Carbon dioxide will lower the pH of the transport medium and adversely affect the survival of organisms in the specimen.
- Ensure that the cool box is at least one-third full of dry ice. If the specimens are sent by air and more than 2 kg of dry ice is used, special arrangements may be necessary with the airlines. Airlines accept packages with less than 2 kg of dry ice.
- Address the package clearly; including the sender's name and telephone number as well as the name and telephone number of the receiving laboratory.
- Write in large letters: EMERGENCY MEDICAL SPECIMENS; CALL ADDRESSEE ON ARRIVAL; HOLD REFRIGERATED (or "FROZEN", if applicable).
- Be sure that all applicable labels and forms, such as those required by IATA, are correctly fixed to the outside of the package.

References:
1) Manual for Identification and Antimicrobial Susceptibility Testing
2) www.ritm.gov.ph
3) www.who.int

Microxpress Presents Chromogenic Media's……

Chromogenic Medium

Chromogenic media's are designed for quick detection of bacteria from water samples, food samples and clinical & non-clinical specimens.

The chromogenic mixture consists of chromogenic substrates, which release differently coloured compounds upon degradation by specific enzymes. This helps in differentiation of certain species with minimum confirmatory tests.

Features	Benefits
Quick & fast identification of organisms	Saves time
Clear differentiation	Superior performance
Improved colours aid interpretation	Easy to identify
Minimises Confirmatory testing	Reduces costs

Product Range

Cat No.	Product Name	Application
AM10251 AM50251	Chromogenic Coliform Agar	For detection of *Escherichia coli* and total coliforms in water & food samples.
AM10252 AM50252	Chromogenic E.coli Agar	For detection & enumeration of *Escherichia coli* in foods without further confirmation on membrane filter or by indole reagent.
AM10253 AM50253	Chromogenic Enterococci Broth	For differentiation and identification of *Enterococci* from water samples
AM10254 AM50254	Chromogenic UTI Agar	For presumptive identification of microorganisms causing urinary Tract Infection.
AM10255 AM50255	Chromogenic Improved Salmonella Agar	For differentiation and identification of *Salmonella* from water samples

Pack Size Available- 100gms & 500gms

BioShields Presents Nusept

Composition - 1% w/v Poly (hexamethylene biguanide) hydrochloride, Perfume, Fast green FCF as color.

Description: NUSEPT™ is a new generation, powerful, non stinging, safe, highly effective and resistance-free microbicidal antiseptic solution. NUSEPT™ is an ideal antiseptic for use in medical settings. The main active ingredient of NUSEPT™ is poly (hexamethylenebiguanide) hydrochloride (PHMB). PHMB is a polymeric biguanide. There is no evidence that PHMB susceptibility is affected by the induction or hyper expression of multi-drug efflux pumps, neither there have been any reports of acquired resistance towards this agent.

ACTIVITY : Broad spectrum: Bactericidal, Fungicidal & Virucidal.

CONTACT TIME : 1 min (undiluted & 10% v/v solution), 5 min (5% v/v solution), 10 min (2.5% v/v solution).

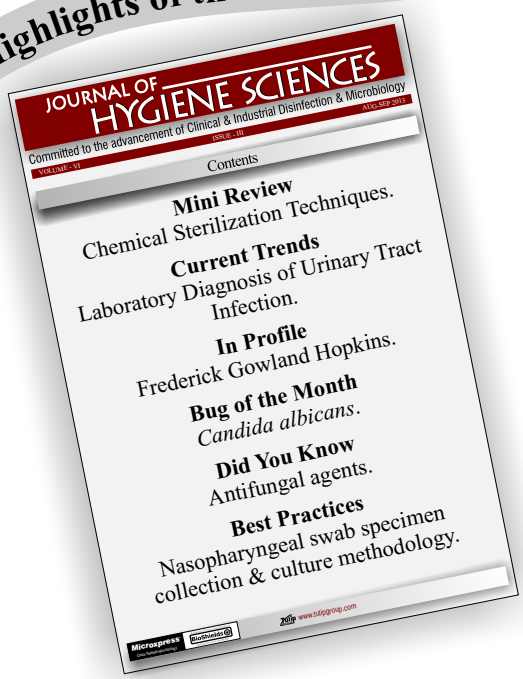
APPLICATIONS :

Medical: In Hospitals, Nursing homes, Medical colleges, Pathological laboratories for Inter-operative irrigation. Pre & post surgery skin and mucous membrane disinfection. Post-operative dressings. Surgical & non-surgical wound dressings. Surgical Bath/Sitz bath. Routine antisepsis during minor incisions, catheterization, scopy etc. First aid. Surface disinfection.

Industrial: In Pharmaceutical industry, Food & beverage industry, Hotel industry etc. General surface disinfection. Eliminating biofilms.

USAGE	DOSAGE AND ADMINISTRATION
Pre & post-surgery skin cleaning & disinfection	Use undiluted
Surgical, post operative, non surgical dressing	Use undiluted, once a day/alternate day
Surgical bath/Sitz bath	Add 50 mL of NUSEPT™ in 1 L of water & use
Antisepsis during minor incisions, Scopy, Catheterization, first aid, cuts, bites, stings etc	Use undiluted
Chronic wound management (diabetic foot, pressure and venous leg ulcers)	Use undiluted
Burn wound management (Only for 1st and 2nd Degree burns, chemical burns)	Use 100 mL NUSEPT™ in 1 L sterile water for both washing (with 1 minute contact time) and dressing of burn wound (Dressing must be changed everyday/ alternate days or as directed)
Midwifery, nursery & sickroom	Use undiluted
Intra-operative irrigation	Use 50 mL NUSEPT™ in 1 L sterile water
General hard surface disinfection	Add 100 mL of NUSEPT™ in 1 L of water and gently mop the floor or surfaces

Highlights of the coming issue



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