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Editorial

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Since the launch of **Journal of Hygiene Science**, it has crossed half the year by creating hygiene awareness worldwide. And it's your support that makes our journey smooth. This time again we have some interesting and informative industrial as well as clinical topics for you.

Quite often, large collections of microbial cultures are used in a laboratory for variety of reasons. We need microbial cultures for further references, comparisons, re-examinations, etc. So it is essential to preserve them properly for further use. Maintenance and preservation of microbial culture is a major task. The basic principle in preserving the cultures is to keep the morphological and physiological characteristics of the organism intact. Mini Review of this issue is totally focused on different techniques of preservation of microbial culture. A recently proposed United States Pharmacopoeia (USP) guidance describes the use, evaluation and control of disinfectants and antiseptics. This pharmacopoeial preview recommends demonstration of the efficacy of the disinfectant used in a manufacturing facility (especially pharmaceutical setups) wherein they have suggested to adequately neutralizing the disinfectant under test. Neutralizing media are used to recover the microorganism by neutralizing biocides. Neutralizing Broth is an employed for neutralizing of antiseptics and disinfectants and detecting organisms remaining after treatment. Here, in Current Trends all we talk about the microbial recovery from biocides by using neutralizing culture media. In Profile is illuminated with the biography of German scientist and Nobel laureate Robert Koch, founded modern medical bacteriology, isolated several diseases causing bacteria, including those of tuberculosis and discovered the animal vectors of a number of major diseases.

Bug of the Month of this issue gives the detail about habitat, cultural characteristics, pathogenicity of *Staphylococcus aureus*, which is the most common cause of nosocomial infections. MRSA (Methicillin Resistant Staphylococcus Aureus) has become prevalent in clinical segment since last two decades. In our Did You Know section we have summarized about triclosan, a potent wide spectrum antibacterial and antifungal agent. Endoscopy procedures are now a routine part of patient diagnosis and treatment in hospitals and surgery centers. The demand for these safe and effective procedures continues to increase. Endoscopies are performed with sophisticated, reusable, flexible instruments that have specific requirements for cleaning, disinfection and sterilization. Because of this, adherence to recommended practices and guidelines for reprocessing is a critical component of infection control and reducing the risk of nosocomial infections. A detailed study of endoscope reprocessing is our topic of choice for Best Practices Section.

Maintenance and Preservation of Microbial Cultures

Microbial culture preservation aims at maintaining a microbial strain alive, uncontaminated, and without variation or mutation, as like original isolate. Many types of work require readily available microorganisms. The delays incurred in acquiring them from other sources or trying to re-isolate them from their natural habitat can be unacceptable. Sometimes it is impossible to obtain the same isolate again. Sometimes repeated attempts of re-isolation of the same organism have been failed.

IMPORTANCE OF MAINTENANCE & PRESERVATION

The efficient practice of microbiology relies on the use of cultures of microorganisms. Authentic reference strains are required for comparison with laboratory isolates, for control cultures in standard methods of analysis, and for use in research and teaching. The great increase in number and size of industrial fermentations has accentuated the value of maintaining collections of microorganisms, especially of production strains, assay organisms and related species.

Considerable work has been devoted to finding methods of maintaining cultures in a vigorous and stable condition. Industrially important microbes are also preserved for use in various industrial processes. The preservation of bacterial stock cultures to maintain viability and biochemical or virulence characteristics is an integral requirement for the continuity of microbiological research.

Easy access to actively growing cultures is a requirement of most microbiological laboratories. Cultures are routinely required generally on a day-to-day basis for quality control, comparative testing, inoculum for bioassays and for various other reasons.

CULTURE COLLECTION CENTRES

Culture collections occupy a fundamental and central position in microbiology and biotechnology. However, apart from their basic role of preserving cultures of past, present and future interests, culture collections offer a number of other services by virtue of the expertise developed in their own infrastructure. Culture collections are important centers of information and offer advice on the availability of cultures, maintenance and preservation, identification, classification and nomenclature, postal regulations and patent culture regulations. A number of culture collections offer other services of particular benefit to industry and biotechnologists.

It takes several years in characterization and development of microbial cultures. Therefore, such important cultures must be deposited at National or International Culture Collection Centres. At these centers the experts properly maintain the cultures. These cultures are governed by stringent rules and regulations so that intellectual property right of the depositors must be protected. On payment basis one can purchase the cultures from these centres. Therefore, for the purpose of commercial exploitation in biotech industries these cultures are very precious.

There are several National and International Culture Collection Centres. Some of them are given below:

- ATCC (American Type Culture Collection Centre, Maryland, U.S.A.)
- NCIB (National Collection of Industrial Bacteria, Britain)
- DSM (Deutsche Sammlung von Mikroorganismen and Zelkulturen, Germany)
- NCTC (National Collection of Type Culture, London)
- MTCC (Microbial Type Culture Collection, Osaka Japan)
- MTCC (Microbial Type Culture Collection and Gene Bank Institute of Microbial Technology, Chandigarh)
- ICIM (Indian Culture of Industrial Microorganisms, National Chemical Laboratory, Pune)

DIFFERENT PRESERVATION TECHNIQUES

Many preservation techniques have been used to preserve microorganisms. The techniques that have been developed and used can be divided into three categories:

- Continuous growth
- Dehydration
- Frozen storage

These categories can be further subdivided. The objective of preservation methods is to maintain the viability and genetic stability of the culture by reducing the organism's metabolic rate thereby extending the period between subcultures. Continuous growth includes all techniques that allow the organism to grow and metabolize during storage.

There are several factors available that increase the time period between subcultures. These include manipulation of growth conditions by limiting carbon, nitrogen and energy sources, lowering the temperature, or preventing dehydration. Other than this, dehydration or drying can be used to preserve organisms: techniques include air-drying, desiccation in or above a desiccant, or drying in a vacuum either from the liquid or frozen state. Frozen storage or cryopreservation is storage at a temperature where the organism is frozen to reduce or completely prevent metabolism and physical change. Success of the preservation depends on the use of the proper medium and cultivation procedure and on the age of the culture at the time of preservation. The method of preservation is mainly of two types: short- term preservation and long-term preservation. Short-term methods include mainly the serial transfer of organisms to fresh medium,

storage at low temperature, maintenance of spores of spore formers in dry sterile soil etc. long term methods are now widely used and use either freeze drying or ultrafreezing in liquid nitrogen (-196ºC).

It is important to recognize that there is no universal method of preservation that is successful for all microorganisms. Taxonomic group of microorganisms respond differently to different preservation methods. The preservation methods used reflect the different biological properties of the various groups of microorganisms such as bacteria, viruses, fungi, algae and protozoa, and their responses to changes in their environment. Most preservation methods achieve a reduction in metabolic rate by withholding nutrients, water and oxygen, by reducing the storage temperature or by a combination of these.

The choice of preservation method depends on the nature of the microorganism, availability of equipment and skilled personnel and on the preservation objective. For example the choice may be influenced by the anticipated period of preservation, the number of cultures and their future use. Other factors may be considered are ease of transportation, frequency of use of cultures and maintenance costs. All preservation methods follow an essentially similar protocol with distinct stages:

- Culture purity check
- Preparation of the ampoules (labeling, sterilizing)
- Growth of the culture
- Suspension of the cells in preservation medium
- Dispensing of cell suspension into ampoules
- Preservation (by method of choice)
- Ampoule stock storage
- Update ampoule stock records
- Ampoule recovery and testing (viability, purity, genetic stability)

Regular Subculture

Periodic transfer on fresh, sterile media can maintain microbial culture. The culture preserved in this way is maintained by alternate cycles of active growth and storage periods obtained by series of subcultures. Subculture is a familiar technique to all practicing microbiologists; it is known as basic skills of aseptic technique without any special equipment. The frequency of transfer varies with the organism. For example, a culture of *E. coli* needs to be transferred at monthly intervals. After growth for 24 hours at 37ºC, the slants can be stored at low temperature for 20-30 days. To keep the cultures viable, it is necessary to use an appropriate growth medium and a proper storage temperature. The frequency of subculture can be reduced if growing it on a medium containing minimal nutrition lowers the metabolism of the organism. Several factors are considered while maintaining a microbial culture by using subculture method.

Solid media should be chosen in preference to liquid as there is a higher chance of contamination in liquid media. Slope cultures are often used for preservation but oxygen sensitive bacteria may benefit from stab culture. After subculturing the tubes should be sealed properly. Cotton wool plugged tubes are not adequate, as media will quickly dry out and cultures will be lost.

Subculturing has many disadvantages, some of them are noted below:

- Change of characteristics- Subculturing can lead to change of characteristics, i.e., characteristics may be lost, reduced, or intensified. Changes probably occur most frequently among strains where intervals between transfers are short.
- Contamination- This occurs frequently, especially when large numbers of cultures are involved and the concentration of the person doing transfer lags.
- Mislabeling- Cultures may be labeled with the wrong name or number. Labels may become distorted and unrecognizable.
- Loss of cultures- This situation occurs from time to time and is probably more common with delicate organisms. Temperature fluctuations in incubation or refrigeration equipment contribute to the possibility of loss.

Paraffin Method

This is a very simple and cost effective method of preserving cultures of bacteria and fungi for longer time at room temperature. In this method sterile liquid paraffin is poured over the slant culture of microbes and stored upright at room temperature. The layer of paraffin prevents dehydration of the medium and ensures anaerobic conditions. It slows the metabolic activity by reduced growth through reduced oxygen tension.

Cultures can also be maintained by covering the agar slants with a layer of sterile mineral oil about half inch above the surface of the slant. The oil must be above the tip of the slanted surface. Mineral oil covered cultures are stored at room temperature or preferably at 0-5ºC. Some microorganisms have been preserved satisfactorily for more than 15-20 years by this method. While preserving the cultures in oil following points should be considered:

- Unless the oil is well above the uppermost level of the medium, the medium tends to dry out, separate from the wall of the tube and float to the surface of the wall, in which the even the organisms are usually found dead.
- The quality of the oil is very important, as any rancidity or toxic substance is harmful to the organisms.
- It is preferable to sterilize the oil in the hot air oven at 150ºC to 170ºC for one hour; for during autoclaving moisture becomes mixed with the oil, giving it a milky appearance.

Storage In Soil

Various fungi such as *Fusarium, Penicillium, Alternaria, Rhizopus, Aspergillus* etc. proved successful for storage in sterile soil. Soil storage involves inoculation of 1 ml of spore suspension into soil (that has been autoclaved twice) and incubating at room temperature for 5-10days. This initial growth period allows the fungus to use the available moisture and gradually to become dormant. The bottles are then stored at refrigerator. Spraying few soil particles on a suitable medium retrieves culture.

Storage In Silica Gel

Both bacteria and yeast can be stored in silica gel powder at low temperature for a period of 1-2 years. In this method, finely powdered, heat sterilized and cooled silica powder is mixed with a thick suspension of cells and stored at low temperature. The basic principle in this technique is quick desiccation at low temperature, which allows the cell to remain viable for a long period.

Storage At Refrigerator Or Cold Room Storage

Live cultures on a culture medium can be successfully stored in refrigerators or cold rooms, when the temperature is maintained at 4ºC. At this temperature range the metabolic activities of microbes slows down greatly but do not altogether stop. As a result, bacterial metabolism will be very slow and only less quantity of nutrients will be utilized. This method cannot be used for a very long time because toxic products get accumulated which can kill the microbes. Refrigerator or cold room storage is of use only for short time preservation of cultures.

Storage By Freezing

Freezing is a common process for storage of bacteria. Thus, thick bacterial suspensions can be frozen at a temperature of - 30ºC. metabolic rates are reduced by lowering the temperature and in the extreme case of storage in liquid nitrogen at -196ºC, are considered to be reduced to nil. Freezing and thawing is a well-known technique for actually disrupting cells. Moreover, as water is removed during freezing as ice, electrolytes become increasingly concentrated in unfrozen water, and this too may be harmful, since electrolyte concentrations outside cells become very different from inside those cells, leading to osmotic stress. Cultures can be preserved very effectively if frozen in the presence of a cryoprotectant, which reduces damage from ice crystals. Glycerol or dimethylsulphoxide (DMSO) are commonly used as cryoprotectants. The simplest way to preserve a culture is to add 15%(v/v) glycerol to the culture and then to store it at -20ºC or -80ºC in a freezer.

Cultures can be preserved for a number of years in glycerol, at a temperature of -40ºC in a freezer. In this method, about 2 ml of glycerol solution is added on to the agar slant culture. Shaking can emulsify the culture. Emulsion is then transferred to ampoules, with each ampoule having 5 ml of the culture. These ampoules are placed in a mixture of industrial methylated spirit and carbon di oxide and frozen rapidly to -70ºC. Ampoules are then removed and placed directly in a deep freeze at -40ºC for utilization of the stock cultures. The ampoules are kept in a water bath at 45ºC for about a few seconds and then used for plate cultures.

The use of cryogenic storage at ultra-low temperature obtained by freezing in liquid nitrogen at -196ºC has proven to be a simple standardized technique for the preservation of a wide range of microorganisms and mammalian cells. Advantages of liquid nitrogen storage include little loss of viability, rapid resuscitation, ready availability as a living suspension and speed of preparation. Disadvantages of the liquid nitrogen storage are the cost of the apparatus and regular supplies of liquid nitrogen, risk of explosion when ampoules are brought into room temperature, loss of large numbers of cultures if careful monitoring of liquid nitrogen levels is not carried out and possible contamination of the liquid nitrogen in the storage container if an ampoule breaks.

Storage By Freeze Drying

Freeze-drying is the most widely used technique for maintaining bacterial cultures. Freeze drying is also called lyophilization. When bacterial cultures or virus suspensions are dried and kept in the dry state under suitable conditions. If such materials are dried from liquid state, a high salt concentration is produced in the later stages of drying; this causes denaturation of proteins, death of organisms and deterioration of serum. Freeze-drying or lyophilization whereby the culture or serum is dried rapidly in vacuo from the frozen state largely avoids the problem. The material is frozen by a suitable method and then dried by sublimation of the ice.

Freeze- drying is a multistage process; it begins with freezing, a temporary stop to metabolic activity, then continues with the removal of water without thawing (sublimation), and ends with a dried product. The dried product is sealed either under vacuum or under an inert gas, can be stored at room temperature with no further metabolic activity until water and nutrients are restored.

Freezing must be very rapid, with the temperature lowered to well below 0ºC (e.g., to -20ºC), since slow freezing would prolong exposure to the denaturing influence of the suspending salt solution. The liquid should be frozen in a shallow layer with a large surface available for evaporation. Freeze drying involves several steps. These are the following:

a) Predrying requirement for cultures: The type of culture media used is an important criterion in the freeze-drying for some microbes, the predrying culture and maintenance may be the same or different. The predrying media should have a rich concentration of microbes. The age of the culture is another important criterion because the cultures that have reached the optimum growth phase survive better than the cultures that are still in the growth phase.

b) Ampoule preparation: The ampoules used in the preservation of the culture should be made of neutral glass and properly disinfected. After plugging with the cotton wool, they should be sterilized with an autoclave for 20 minutes under 20lbs pressure.

c) Harvesting the culture: Cultures grown on agar slants should be harvested for 3-5days after incubation. Suspending fluid like horse serum, glucose, nutrient broth should be used for harvesting culture. The suspensions should be immediately transferred to the ampoules.

d) Primary Drying: Special centrifuges are used for this primary

drying. The primary drying procedure is allowed to proceed between 2.5 to 4 hours during which, more than 90% of free water is removed. Air is then allowed to slowly enter into the vacuum chamber. The centrifuge head is then removed from the machine and the ampoules are plugged again with cotton wool.

e) Secondary drying: The ampoules are then transferred to a secondary dryer, which consists of high grade P_2O_5 contained in a tray, to which is attached a vacuum pump. The ampoules are left on this dryer for 18-20 hours upon which the moisture content is reduced to 1%. The ampoules are checked for maintenance of vacuum, and sealed with flame. The culture in the ampoule is now a light powdery substance. The ampoule can be stored at 4ºC.

Two types of commercial freeze-dryer, the centrifugal and shelf are in common use. In the former freezing is brought about by evaporation that occurs when the vacuum is applied, and the cell suspension is centrifuged during initial freezing to increase the surface area and prevent frothing. For large culture collections, the centrifugal method has advantages in minimizing the likelihood of cross contamination as ampoules may be plugged after filling and sealed under vacuum on a manifold at the end of secondary drying stage. Lyophilized cultures need to be rehydrated according to the manufacturer's directions and maintained viable through frequent transfers or freezing procedures.

One of the major advantages of freeze-drying is that the ampoules are particularly suitable as a means of distributing cultures because the viability and integrity of the ampoules resist the changes in pressure and temperature in the airmail services. A disadvantage of freeze-drying is the relatively high capital cost of commercial equipment.

Storage By Drying Methods

Some strains, which are sensitive to freeze-drying, can be preserved by drying from the liquid state rather than the frozen state. A number of methods for drying suspensions of bacteria for preservation purposes have been developed which are useful in laboratories that cannot afford the expensive equipment used for storing at very low temperatures or for freeze drying, or in which preservation of cultures is performed infrequently.

Some of the following procedures of drying method are mentioned below:

- Paper Disc: A thick suspension of bacteria is placed on sterile discs of thick absorbent paper, which are then dried over phosphorus pentoxide in a desiccator under vacuum.
- Gelatin Disc: A thick suspension of bacteria is prepared and added to nutrient gelatin. Drops of the bacterial suspension in gelatin are placed on sterile waxed paper or on a Plastic Petri dish and then dried off over P_2O_5 under vacuum.
- Pre dried Plugs: Thick suspensions of bacteria are prepared and drops placed on sterile cellophane or on pre dried plugs of peptone, starch or dextran before drying in a desiccator over P_2O_5 in a vacuum.
- L- Drying: Bacteria in small ampoules are dried from the liquid state using a vacuum pump and desiccant and a water-bath to control the temperature. L- drying is also used for vesicular arbuscular mycorrhizal fungi. With this method, suspensions of the organisms are dried under vacuum from the liquid state without freezing taking place. There are several techniques for L- drying:
  i. By using small volumes of suspension spread over a large surface area, the material dries by rapid evaporation before freezing can occur.
  ii. Freezing can be prevented by restricting the water vapour flow from the material being dried, either by inserting cotton plugs into the ampoules or by controlling the vacuum by means of a valve.
  iii. Immersion of the ampoules in a water bath can maintain sufficient heat input to the suspensions to prevent them from freezing under vacuum.

PRESERVATION PROGRAMME

The accession of a culture is not complete until it has been successfully preserved or arrangements are made for its routine maintenance if no adequate long-term preservation method is available. It is desirable to preserve new strains after the least possible subculture to ensure minimum risk of change. Existing stocks of preserved cultures are routinely subjected to viability checks and survival assessment. Initially new cultures are checked after one or two weeks of preservation and then it is preserved for long term. Sometimes unpredicted losses can occur and for this reason it is advisable to preserve cultures by more than one method.

Another important aspect of any preservation program is ampoule stock control. Ampoules are regularly taken from stocks for use or distribution. Numbers of ampoules are adjusted on ampoule stock cards and a new batch of ampoules is prepared when ampoule stocks are reduced to a predetermined minimum number. New ampoules are prepared from oldest batch of ampoules available and not from working stock cultures.

QUALITY CONTROL

Quality control is essential in a culture collection and an effective quality control program must be established to ensure viability, purity and genetic stability are achieved in the preservation program. Industrial collections may collect cultures into broadly based taxonomic groups and place more emphasis on a particular metabolic characteristics or other property. Strains selected for use in a biotechnological process usually have a unique set of characters and productive efficiency. Quality control testing is essential to ensure that these characters, which have been selected or developed, are preserved in as stable and reliable state as possible.

As per USP, cultures of microorganisms should be maintained weekly or monthly transfers to fresh agar slants or stabs, which are kept under refrigeration at 2-8ºC. Transfers must not exceed

five passages from the mother culture. In maintaining the challenged organisms, microbiologists must choose the best method for a particular culture to avoid mutations and minimize variability in the resistance of the microorganism from suspension to suspension.

SHIPMENT OF CULTURES

Culture collections and indeed many microbiologists, are often required to supply cultures on request. Various local, national or international bodies regulate the distribution of cultures and culture collections must build these constraints into their management procedures. Microbiologists who use the local and international postal services should be thoroughly conversant with the postal and quarantine regulations governing the shipment of cultures. This applies particularly to cultures, which may pose a threat to human, animal or plant health if properly not handled.

In general it is desirable to send freeze-dried cultures in ampoules. If it is necessary to send actively growing cultures these should be sent as agar slope cultures in screw capped glass or plastic bottles. Broth cultures should be sent only in screw capped bottles or tubes. Loosely capped tubes of broth cultures or petri dish cultures should not be sent on the post. One should also be concerned with the moral and ethical implications of dispatching cultures, which may have a harmful effect on health, economics, and ecology of the recipient nation.

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Encyclopedia

Antiseptic

An antiseptic is a substance that prevents or arrests the growth or action of microorganisms either by inhibiting their activity or destroying them. The term is used especially for preparations applied topically to living tissue. The wide spread use of antiseptics in surgical methods introduced by Joseph Lister. The extent of killing of the bacteria is governed by three principle factors: (a) concentration of the antiseptic, (b) bacterial cell density and (c) time of contact.

Bacteriophage

Bacteriophages (phage) are obligate intracellular parasites that multiply inside bacteria by making use of some or all of the host biosynthetic machinery. Bacteriophage consists of either DNA or RNA as a genetic material. Genetic material is covered with a protein coat known as capsid. Bacteriophages are much smaller than bacteria, usually between 20 and 200 nm in size. Phages are estimated to be the most widely distributed and diverse entities in the biosphere. Phages are ubiquitous and found in all reservoirs populated by bacterial hosts, such as soil or the intestine of animals. Bacteriophages replicate either by lytic or lysogenic cycle. Phages are used as a therapeutic agent to treat pathogenic bacterial infections.

Example- T4 is a bacteriophage, infects *Escherichia coli*.

Disinfectant

Disinfectants are antimicrobial agents that are applied to non-living objects to destroy microorganisms and the process is known as disinfection. Disinfectants should generally be distinguished from antibiotics that destroy microorganisms within the body and from antiseptics, which destroy microorganisms on living tissue. Disinfectants are frequently used in hospitals, dental surgeries, pharmaceutical and food industries, and households to kill infectious organisms.

Phagocytosis

Phagocytosis is the cellular process of engulfing solid particles by the cell membrane to form an internal phagosome. The phagosome is usually delivered to the lysosome, an organelle involved in the breakdown of cellular components, which fuses with the phagosome. The contents are subsequently degraded and either released extracellularly via exocytosis or released intracellularly to undergo further processing. Phagocytosis involves the ingestion and digestion of the following: microorganisms, insoluble particles, damaged or dead host cells, cell debris, activated clotting factors. Many protests are able to phagocytose particles; but in animals specialized cells called phagocytes, which are able to remove foreign bodies, perform phagocytosis. In humans and many other animals, phagocytes include macrophages, monocytes, dendritic cells and granulocytes.

MRSA

Methicillin resistant *Staphylococcus* aureus (MRSA) is a major pathogen responsible for both hospital and community onset disease. MRSA is a resistant variation of the common bacterium *Staphylococcus aureus*. It has evolved an ability to survive treatment with the beta lactam antibiotics. MRSA is especially troublesome in hospital-acquired infections. The mechanism of resistance in methicillin resistant *S. aureus* (MRSA) is alteration of penicillin binding proteins (PBPs). PBPs, the target sites for beta lactam antibiotics are proteins required for maintaining integrity of cell wall. MRSA have an additional PBP. Methicillin cannot bind effectively to the PBPs and inhibit bacterial cell wall synthesis. There are two major ways people become infected with MRSA. The first is physical contact with someone who is either infected or is a carrier of MRSA. The second way is for people to physically contact MRSA on any objects such as door handles, floors, sinks, or towels that have been touched by an MRSA-infected person or carrier. Precautions that are taken to prevent the spread of MRSA include: hand washing after touching the patient and particularly contaminated bodily fluids, secretions, excretions or objects, whether or not gloves are worn. Gloves should be removed immediately after treating patient and before touching any non-contaminated sites and disposed of in special designated clinical waste bins. Gowns should be worn to protect skin and clothing from carrying the infection. Appropriate disposal of contaminated items with MRSA should be done properly.

Microbial Recovery By Using Neutralizing Media

A Trend In Pharmaceutical Industry

A recently proposed United States Pharmacopoeia (USP) guidance describes the use, evaluation and control of disinfectants and antiseptics. This Pharmacopoeial Preview recommends demonstration of the efficacy of the disinfectants used in a manufacturing facility and notes the need to adequately neutralize the disinfectant under test. It is generally understood that if a product possesses antimicrobial properties because of the presence of a specific preservative or because of its formulation, this antimicrobial property must be neutralized to recover viable microorganisms. Complete neutralization of disinfectants is important for the accuracy of a biocidal assay as microbicidal activity is commonly measured as survivors with time, and inhibition of microbial growth by low levels of residual biocide would lead to exaggerated measures of microbicidal activity. This neutralization may be achieved by the use of a specific neutralizer, by dilution, by a combination of washing and dilution, or by any combination of these methods.

Various Methods of Neutralization of Biocide

There are three common methods used to neutralize antimicrobial properties of a product: (1) neutralization by chemical inhibition; (2) neutralization by dilution and (3) neutralization by filtration and washing. Common methods for inhibition of residual biocide include dilution or chemical neutralization of the biocide. Dilution is useful for those biocides with a large concentration exponent and little propensity for binding to the cell. A variation on dilution is filtration of the suspension to remove the biocide.

(1) Chemical Inhibition- Some known neutralizers are mentioned following for a variety of chemical antimicrobial agents:

Neutralizer	Biocide Class
Lecithin	Quaternary Ammonium Compounds, Bisbiguanides
Polysorbate	QACs, Iodine
Thioglycollate	Mercurials
Thiosulfate	Mercurials, Aldehydes
Glycine	Aldehydes
Bisulfate	Glutaraldehyde
Dilution	Phenolics, Alcohol

However, despite potential toxicity, the convenience and quick action of chemical inhibitors encourage their use. Chemical inhibition of bactericides is the preferred method for the antimicobial efficacy test. The potential of chemical inhibitors should be considered in the membrane filtration and the direct transfer sterility tests.

(2) Dilution- A second approach to neutralizing antimicrobial properties of a product is by dilution, as the concentration and antimicrobial effect differs among bactericidal agents but is constant for a particular antimicrobial agent. This relationship is exponential in nature, with the general formula:

$$C\ t = k$$

In which C is the concentration; t is the time required to kill a standard inoculum; k is a constant; and the concentration exponent, , is the slope of the plot of log t versus log C. antimicrobial agents with high values are rapidly neutralized by dilution, while those with low values are not good candidates for neutralization by dilution.

(3) Membrane Filtration- An approach that is often used, especially in sterility testing, is neutralization by membrane filtration. This approach relies upon physical retention of the microorganism on the membrane filter, with the antimicrobial agent passing through the filter into the filtrate. The filter is then incubated for recovery of viable microorganisms. However filtration alone may not remove sufficient quantities of the bactericidal agent to allow growth of surviving microorganisms. Adherence of residual antimicrobial agents to the filter membrane may cause growth inhibition. Filtration through a low binding filter material, such as polyvinylidene difluoride, helps to minimize this growth inhibition. This technique must be approached with caution as the biocide may bind either to the membrane filter or to the cells, inhibiting recovery.

An effective neutralizer should have the following three criteria:

- The neutralizer must effectively inhibit the action of the biocidal solution.
- The neutralizer must not itself be unduly toxic to the challenge organisms.
- Finally, the neutralizer and active agent must not combine to form a toxic compound.

Neutralizer Evaluation Method

Dey and Engley describe a procedure of neutralizer evaluation utilizing *Staphylococcus aureus* as the index organism that measures survival with time. The challenge organism is inoculated directly into the biocide and sampled with time. The relative efficacy of the neutralizer is measured by comparing the relative recovery of the challenge organism among different treatments. A basic procedure was followed for all population examined and the method is modified from earlier procedure. First, a determination of a standard inoculum was made to confirm the presence of 10-100 CFU per plate. This low number

of cells was preferred to enhance the sensitivity of the assay. The second treatment population was exposed to the neutralizing broth without the biocide. The population exposed to neutralizer served as the control population for all statistical analysis. The final treatment population consisted of the neutralizing broth in the presence of the particular disinfectant and referred to as the neutralizer and biocide population.

Test organisms used included *Trichophyton mentagrophytes*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus, Clostridium sporogenes* and *Escherichia coli*. A validated method for neutralizing the antimicrobial properties of a product must meet two criteria: neutralizer efficacy and neutralizer toxicity. The validation study documents that the neutralization method employed is effective in inhibiting the antimicrobial properties of the product (neutralizer efficacy) without impairing the recovery of viable microorganisms (neutralizer toxicity). Evaluation of neutralizer toxicity was performed by comparison between the viability population and the neutralizer-exposed population.

Determination of neutralizer efficacy requires evaluation of the neutralizing broth's ability to neutralize the biocide at a specified dilution. Neutralizer evaluation must be performed for each combination to be tested in any assay involving biocides or preservatives. The first is the test group, where the product is subjected to the neutralization method, and then a low level of challenge microorganism is inoculated for recovery. The second is the peptone control group, where the neutralization method is used with peptone as the test solution. The third is the viability group, where the actual inoculum is used without exposure to the neutralization scheme. Similar recovery between the test group and the peptone group demonstrates adequate neutralizer efficacy; similar recovery between the peptone group and the viability group demonstrates adequate neutralizer toxicity.

Some widely used Neutralizing Media

Neutralizing media are used to recover the microorganism by neutralizing biocides. Neutralizing Broth is an employed for neutralizing of antiseptics and disinfectants and detecting organisms remaining after treatment. This broth is especially suited for environmental sampling where neutralization of the chemical is important to determine its bactericidal activity. Engley and Dey formulated neutralizing broth in 1970 for the neutralization and testing of antiseptics and disinfectants. The American Public Health Association (APHA) for environmental sampling recommends it in the Compendium for the Microbiological Examination of Foods. The medium will neutralize a broad spectrum of antiseptic and disinfectant chemicals including mercurials, iodine and chlorine preparations, quaternary ammonium compounds, phenolics, formaldehydes, and glutaraldehydes.

This medium contains casein peptone, yeast extract, dextrose, sodium thioglycollate, sodium thiosulfate, sodium bisulfite, lecithin, polysorbate 80 and bromo cresol purple. Peptone and yeast extract provide amino acids, peptides, nitrogenous compounds, and B-complex vitamins, which are sources of nutrients necessary for growth. Dextrose is a carbon energy source. Lecithin neutralizes quaternary ammonium compounds. Polysorbate 80, a non-ionic surface-active agent, neutralizes phenolics. Thioglycollate neutralizes mercurials, and sodium thiosulfate neutralizes iodine and chlorine preparations. Sodium bisulfite neutralizes formaldehyde and glutaraldehyde. The medium is highly opalescent, and growth is often indicated by a change in the pH indicator from purple to yellow showing utilization of dextrose. This medium is suited for environmental sampling, where neutralization of the chemical is important to determine its bactericidal activity. A bacteriostatic substance may contain bacteria held in bacteriostasis but which may still be able to cause infection.

Tryptone soya agar with lecithin and tween 80 is used for detection of microorganisms on surfaces, sanitized with quaternary ammonium compounds. It is recommended for validation of cleanliness on surface of containers, equipment surfaces and water miscible cosmetics. This medium contains casein, soyabean meal, sodium chloride, polysorbate 80 and lecithin. Casein enzymic hydrolysate and papaic digest of soyabean meal serves as a source of nitrogen. Sodium chloride provides sodium ions for the membrane transport and maintains osmotic equilibrium of the medium. Lecithin and tween 80 inactivates disinfectant. Lecithin neutralizes quaternary ammonium compounds and tween 80 neutralizes substituted phenolic disinfectant.

Significance of Neutralizer

Effective neutralization of a chemical biocide is critically important to the quality of the data derived from any assay of biocidal efficacy. Care must be taken to avoid carry over of active biocide to the recovery media, which may result in biostasis of the organism. This biostasis would lead to an overestimation of the biocide's efficacy. Therefore the experimental design used to establish the efficacy of biocide neutralization has a major impact on the estimation of antimicrobial efficacy. Complete neutralization of disinfectants is important because disinfectant carryover can cause a false no-growth test result. Neutralizing media effectively neutralizes the inhibitory effects of disinfectant carryover, allowing differentiation between bacteriostasis and the true bactericidal action of disinfectants.

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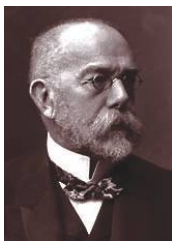

Robert Koch

Birth: December 11, 1843
Death: May 27, 1910
Nationality: German
Known For: Koch's postulates of germ theory of Disease.

Robert Koch, German scientist and Nobel laureate, who founded modern medical bacteriology, isolated several diseases causing bacteria, including those of tuberculosis and discovered the animal vectors of a number of major diseases.

Robert Koch was born on on December 11, 1843, at Clausthal, Germany. He was the son of a mining official. Before starting primary school in 1848, he started learning by himself. He graduated from University of Göttingen with botany, physics and mathematics, 1862. Later on he obtained a medical degree in 1866. Koch finally settled at Rakwitz and started practicing. In 1870 he volunteered for service in the Franco-Prussian war and later became district medical officer in Wollstein.

At that time anthrax was the prevalent disease among the farm animals in Wollstein district and he started investigation about anthrax despite lack of research facilities. He was able to detect the causative agent and found out that the spores of the organism were viable for long time. When conditions were favourable the spores could develop into the rod shaped bacilli of anthrax. In 1880, he accepted an appointment as a government advisor with the Imperial Department of Health in Berlin. His task was to develop methods of isolating and cultivating disease-producing bacteria and to formulate strategies for preventing their spread. In 1881 he published a report advocating the importance of pure cultures in isolating disease-causing organisms. Koch also laid down the conditions, known as Koch's postulates. Four criteria of the Koch's postulates are:

- A specific organism can always be found in association with a given disease.
- The organism can be isolated and grown in pure culture in the laboratory.
- The pure culture will produce the disease when inoculated into a susceptible animal.
- It is possible to recover the organism in pure culture from the experimentally infected animal.

In Berlin Koch discovered the tubercle bacillus and also a method of growing it in pure culture. In 1882 he published his classical work on this bacillus. In 1883 Koch was invited to Egypt to investigate the cause of cholera outbreak. Koch had identified a comma-shaped organism in the intestines of people who had died of cholera. He was able to complete his work on cholera by proceeding to India. His work on cholera, for which a Prize of 100,000 German Marks was awarded to him, also had an important influence on plans for the conservation of water supplies and suggested improve sanitation to prevent the disease. In 1885 Koch was appointed Professor of Hygiene in the University of Berlin and Director of the newly established Institute of Hygiene in the University there. In 1890 he was appointed Surgeon General Class I and Freeman of the City of Berlin. In 1891 he became an Honorary Professor of the Medical Faculty of Berlin and Director of the new Institute for Infectious Diseases. During this period Koch returned to his work on tuberculosis. He sought to arrest the disease by means of a preparation, which he called tuberculin, made from cultures of tubercle bacilli. But the tuberculin was proved as a disappointing curative agent.

In 1896 he went to South Africa. Then followed he worked in India and Africa on malaria, blackwater fever, surra of cattle and horses and plague, and the publication of his observations on these diseases in 1898. Soon after his return to Germany he was sent to Italy and the tropics where he confirmed the work of Sir Ronald Ross in malaria and did useful work on the aetiology of the different forms of malaria and their control with quinine.

Koch discovered that methyl violet dye showed up the septicemia germ under a microscope by staining it. He also photographed the germs so that people outside of his laboratory could see them. Koch perfected the technique of growing pure cultures of germs using a mix of potatoes and gelatin. This was a solid enough substance to allow for the germs to be studied better.

Koch was the recipient of many prizes and medals, honorary doctorates of the Universities of Heidelberg and Bologna, honorary citizenships of Berlin, Wollstein and his native Clausthal, and honorary memberships of learned societies and academies in Berlin, Vienna, Posen, Perugia, Naples and New York. He was awarded the German Order of the Crown, the Grand Cross of the German Order of the Red Eagle (the first time this high distinction was awarded a medical man), and Orders from Russia and Turkey. Long after his death, he was posthumously honoured by memorials and in other ways in several countries. In 1905 he was awarded the Nobel Prize for Physiology or Medicine. In 1906, he returned to Central Africa to work on the control of human trypanosomiasis. He had also made an important observations on pathogenic specis of *Babesia*. Thereafter Koch continued his experimental work on bacteriology and serology.

On April 9, 1910, three days after lecturing on tuberculosis at the Berlin Academy of Sciences, he suffered a heart attack from which he never fully recovered. He died at Baeden on May 27 at the age of 67. He was honored after death by the naming of the Institute after him.

From humble beginnings, and after an ordinary career as a country doctor, Robert Koch rose to the pinnacle of scientific achievement. Along the way, he established the new field of bacteriology. Koch's story is one of the most stirring in modern science and medicine. Thus Robert Koch made many advances and breakthroughs in the world of medicine, and saved many peoples lives by doing so.

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Enjoy the humour

A new manager spends a week at his new office with the manager he is replacing. On the last day the departing manager tells him, "I have left three numbered envelopes in the desk drawer. Open an envelope if you encounter a crisis you can't solve."

Three months down the track there is a major drama, everything goes wrong - the usual stuff - and the manager feels very threatened by it all. He remembers the parting words of his predecessor and opens the first envelope. The message inside says "Blame your predecessor!" He does this and gets off the hook.

About half a year later, the company is experiencing a dip in sales, combined with serious product problems. The manager quickly opens the second envelope. The message read, "Reorganize!" This he does, and the company quickly rebounds.

Three months later, at his next crisis, he opens the third envelope. The message inside says "Prepare three envelopes".

Thoughts to live by

- Appreciation is like tea or coffee. It refreshes, inspires and spurs you into action.
- When one door closes another opens. But often we look so long so regretfully upon the closed door that we fail to see the one that has opened for us.

(Hellen Keller)

- Accept challenges, so that you may feel the exhilaration of victory.

(George S. Patton)

- To love and be loved is to feel the sun from both sides.

(David Viscott)

- Our lives improve only when we take chances - and the first and most difficult risk we can take is to be honest with ourselves.

(Walter Anderson)

Track your brain

Rearrange the letters to make familiar words. Place one letter in each box or circle. Use the letters in the circles to make words that answer the question below.

SERIES I

(a) S O M L A E T

(b) G P A H E

(c) R I T L E S E

(d) D M E E A

Name the cytoplasmic organelle of algae and higher plants that contains pigments, reserves food and takes part in photosynthesis.

SERIES II

(a) R E P B O

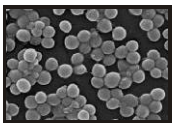
(b) S C T Y

(c) O E O N M R M

(d) I F M O E T

The independent genetic material integrated with chromosome. What is it?

Check your Answers on Page 16



Staphylococcus aureus

Von Recklinghausen first observed staphylococci in human pyogenic lesions in 1871. Sir Alexander Ogston established conclusively the causative role of the coccus in abscesses and other suppurative lesions and named as *Staphylococcus* due to the typical occurrence of the cocci in grape like clusters in pus and in cultures. In 1884, Rosenbach described the two pigmented colony types of staphylococci and proposed the appropriate nomenclature: *Staphylococcus aureus* (yellow) and *Staphylococcus albus* (white).

Bacteriology

Staphylococci are Gram-positive spherical cocci, approximately 1 m in diameter, arranged characteristically in grape like clusters. Cluster formation is due to cell division occurring in three planes, with daughter cells tending to remain in close proximity. They may also be found singly, in pairs and in short chains of three or four cells, especially when examined from liquid culture. Long chains never occur. They are non motile and nonsporing.

They grow readily on ordinary media within a temperature range of 10-42°C, the optimum being 37°C and pH 7.4-7.6. They are aerobes and facultative anaerobes. On nutrient agar, after incubation for 24 hours, the colonies are large, circular, convex, smooth, shiny, opaque and easily emulsifiable. Most strains produce golden yellow pigment, though some may be white, orange or yellow. The pigment does not diffuse into the medium. Pigment production is enhanced when grown aerobically. They produce â hemolytic colonies on blood agar. On MacConkey or CLED agars, it acquires the appropriate colour of the indicator, depending on the ability to ferment lactose of the particular strain. It ferments mannitol sugar anaerbically. They are catalase & coagulase positive and usually hydrolyse urea, reduce nitrates to nitrites, liquefy gelatin and are Methyl red and Voges Proskauer positive but indole negative.

Mode of Transmission

The modes of transmission of *Staphylococcus aureus* may be by direct contact or through fomites, by dust or by airborne droplets. People can get staphylococcal infections from contaminated objects, but Staphylococcus often spread through skin-to-skin contact. The bacteria can be spread from one area of the body to another if someone touches the infected area. Staphylococcal infection can spread through bed linens, towels or clothing. Warm, humid environment contribute for staphylococcal infection. Excessive sweating can increase the chances of developing an infection. Lack of hand washing during food preparation can result in *Staphylococcus aureus* from contaminated hands to food, which may lead to food poisoning.

Epidemiology

Staphylococci are primary parasites of human beings and animals, colonizing the skin, skin glands and mucous membranes. The most common sources of infection are human patients and carriers. Patients with superficial infections and respiratory infections disseminate large numbers of staphylococci into the environment. Staphylococcal carriage starts early in life, colonization of the umbilical stump being very common in new borne. Some carriers disseminate very large numbers of cocci for prolonged periods.

Pathogenicity and Virulence

Staphylococci produce two types of diseases- infections and intoxications. *S. aureus* expresses many potential virulence factors: (1) surface proteins that promote colonization of host tissues, (2) invasins that promote bacterial spread in tissues, (3) surface factor that inhibit phagocytic engulfment , (4) biochemical properties that enhance their survival in phagocytes, (5) immunological disguises, (6) membrane-damaging toxins that lyse eukaryotic cell membranes and (7) exotoxins that damage host tissues or otherwise provoke symptoms of disease.

S. aureus cells express on their surface proteins that promote attachment to host proteins such as laminin and fibronectin that form the extracellular matrix of epithelial and endothelial surfaces. Protein A is a surface protein of *S. aureus*, which binds IgG molecules by their Fc region. In serum, the bacteria will bind IgG molecules in the wrong orientation on their surface, which disrupts opsonization and phagocytosis. Coagulase is an extracellular protein, which binds to prothrombin in the host to form complex called staphylothrombin. Staphylococci produce a number of lipid hydrolases, which help them in infecting the skin and subcutaneous tissues. Hyaluronidase breaks down the connective tissue. Staphylokinase, fatty acid modifying enzymes and proteases help in initiation and spread of infection.

Cytolytic toxins are membrane-active substances, consisting of four hemolysins and a leucocidin. The best-characterized and most potent membrane-damaging toxin of *S. aureus* is a-toxin. It is expressed as a monomer that binds to the membrane of susceptible cells. Subunits then oligomerize to form heptameric rings with a central pore through which cellular contents leak. In humans, platelets and monocytes are particularly sensitive to a-toxin. Susceptible cells have a specific receptor for a-toxin, which allows the toxin to bind causing small pores through which monovalent cations can pass. The mode of action of alpha hemolysin is likely by osmotic lysis. Staphylococci produce enterotoxin, which is responsible for the manifestations of staphylococcal food poisoning. Eight antigenic types of enterotoxin are currently known, named A, B, C_{1-3}, D, E and H. *S. aureus* also produce toxic shock syndrome toxin (TSST) and it is responsible for toxic shock syndrome. The exfoliative toxin, associated with scalded skin syndrome, causes separation within the epidermis, between the living layers and the superficial dead layers. The separation is through the stratum granulosum of the epidermis.

Staphylococcal Diseases

Staphylococcal infections are among the most common of bacterial infections and sometimes causes fatal infections. Common staphylococcal infections are the following:

Skin and soft tissues Most infections caused by *S. aureus* are skin

and soft tissue infections such as folliculitis, furuncle (boil), abscess, wound infection, carbuncle, impetigo and less often cellulitis. These are increasingly severe staphylococcal skin infections. Folliculitis is a tender pustule that involves the hair follicle. A furuncle involves both the skin and the subcutaneous tissues in areas with hair follicles, such as the neck, axillae, and buttocks. They are actually small abscesses characterized by exuding purulent material from a single opening. A carbuncle is an aggregate of connected furuncles and has several pustular openings. Skin infections may be self-limited, but they can also disseminate hematogenously and cause life-threatening septicemia.

Musculoskeletal - It causes osteomyelitis, arthritis, bursitis and pyomyositis. Children often present with sudden onset of fever and bony tenderness or a limp. The pain may be throbbing and severe. Rupture of a focus of osteomyelitis into joint space can result in septic arthritis.

Respiratory Illness - It also causes some respiratory illness like tonsillitis, pharyngitis, sinusitis, otitis, lung abscess and pneumonia. Staphylococcal pneumonia most commonly occurs in infants, young children, and patients who are debilitated.

Staphylococcal food poisoning - It is caused by the enterotoxins of some strains of *S. aureus*. The onset of symptoms in staphylococcal food poisoning is usually rapid and in many cases acute, depending on individual susceptibility to the toxin, the amount of contaminated food eaten, the amount of toxin in the food ingested, and the general health of the victim. The most common symptoms are nausea, vomiting, retching, abdominal cramping, and prostration. In more severe cases, headache, muscle cramping, and transient changes in blood pressure and pulse rate may occur.

Endocarditis - *S. aureus* endocarditis is rare in pediatric patients. These patients usually present with right-sided disease with evidence of pulmonary disease, such as pulmonary abscesses or shifting infiltrates. In children with preexisting heart disease, endocarditis is often temporally related to cardiac surgery or catheterization. Endocarditis can lead to heart failure.

Urinary tract infections - Stahylococci are uncommon in routine urinary tract infections, though they do cause infection in association with local instrumentation, implants or diabetes.

Diagnosis

Depending upon the type of infection present, an appropriate specimen is obtained accordingly and sent to the laboratory for definitive identification The mainstay of diagnosis of *S. aureus* infections is isolation of the organism from appropriate specimens. The specimens to be collected depend on the type of lesion (e.g. pus from suppurative lesions, sputum from respiratory infections). In cases of food poisoning feces and the remains of suspected food should be collected. Gram staining is the primary step of diagnosis the specimen. Diagnosis may readily be made by culture. Blood agar culture is mostly preferred and observed for hemolytic colonies. Mannitol salt agar, a selective medium is used to detect *S. aureus*. Furthermore it is tested for catalase, coagulase, lipase etc. Bacteriphage typing may be done if the information is desired for epidemiological purposes. Other typing methods include antibiogram pattern, plasmid profile, DNA finger printing, ribotyping and PCR based analysis for genetic pleomophism.

Treatment

As drug resistance is so common among staphylococci, the appropriate antibiotic should be chosen based on antibiotic sensitivity tests. Penicillin was the most effective antibiotic. Staphylococcal resistance to penicillin is mediated by penicillinase production, an enzyme that breaks down the -lactam ring of the penicillin molecule. Methicillin was the first compound developed to combat resistance due to penicillinase (-lactamase) production by staphylococci. But methicillin resistant strains of *Staphylococcus aureus* (MRSA) became common. MRSA is especially troublesome in hospital-acquired infections. In hospitals, patients with open wounds, invasive devices and immunocompromised patients are at higher risk of MRSA infection than general public. Hospital acquired infection is often caused by antibiotic resistant strains (MRSA) and can only be treated with vancomycin or an alternative. Topical application of drugs not used systemically, as bacitracin, chlorhexidine or mupirocin may be sufficient. The treatment of carriers is by local application of suitable antibiotics such as bacitracin and antiseptics such as chlorhexidine. In resistant cases posing major problems, rifampicin along with another oral antibiotic may be effective in long-term suppression or elimination of the carrier state.

Control Measures

Spread of *S. aureus* (including MRSA) is through human-to-human contact. Following measures should be taken to control the spread of the infection:

- Good hand washing technique should be followed to prevent the infection. Quaternary ammonium compounds can be used as an effective sanitizing agent in preventing the transmission of *S. aureus*.
- The use of disposable aprons and gloves by staff reduces skin to skin contact and therefore effective in preventing the transmission of *S. aureus*.
- Strict aseptic techniques should be maintained in OTs and ICUs.
- Patients with open staphylococcal infection should be isolated in hospitals.
- The prevention of nosocomial infections involves routine and terminal cleaning. In health care environments, MRSA can survive on surfaces and fabrics, including garments worn by care providers. Complete surface sanitation is necessary to eliminate MRSA in areas where patients are recovering from invasive procedures.
- Antibiotics should be used properly to combat the resistance.

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Triclosan

Triclosan is a potent wide spectrum antibacterial and antifungal agent. The molecular formula of triclosan is $C_{12}H_7Cl_3O_2$ and chemically it is known as 5-chloro-2- (2,4-dichlorophenoxy) phenol or 2,4,4'-trichloro-2'-hydroxydiphenyl ether. This organic compound is a white powdered solid with a slight aromatic or phenolic odour. It is a chlorinated aromatic compound which has functional groups representative of both ethers and phenols. Phenols often show antibacterial properties. Triclosan is sparingly soluble in water. But it is readily soluble in a range of solvents (e.g. ethanol, diethyl ether) and in alkaline solution. Triclosan can be made from the partial oxidation of benzene or benzoic acid. It can also be found as a product of coal oxidation. Although triclosan is a strictly phenoxy phenol but it has structural similarity with bis phenol compounds.

Mode of Action

Earlier studies suggested that the bacterial cytoplasmic membrane was the major target for triclosan action. However, it was later demonstrated in *Escherichia coli*, *S. aureus* and other triclosan susceptible organisms that the growth-inhibitory activities of the phenylether resulted from blocking lipid synthesis by specifically inhibiting an NADH-dependent enoyl-acyl carrier protein (ACP) reductase. The enoyl reductase, InhA, in *Mycobacterium smegmatis* was also found to be a target for triclosan action.

Triclosan works by blocking the active site of the enoyl-acyl carrier protein reductase enzyme (ENR), which is an essential enzyme in fatty acid synthesis in bacteria. Enoyl-ACP reductase (ENR; EC 1.3.1.9), encoded by the *fabI* gene, is a key enzyme of the Type II fatty-acid biosynthetic system in prokaryotes. It uses NADH or NADPH as the cofactor to reduce the double bond between C2 and C3 positions of a fatty acyl chain bound to the acyl carrier protein in the terminal rate-limiting step of the fatty acid chain elongation cycle. This binding increases the enzyme's affinity for nicotinamide adenine dinucleotide (NAD^+). This results in the formation of a stable ternary complex of ENR-NAD^+-triclosan, which is unable to participate in fatty acid synthesis. Fatty acid is necessary for reproducing and building cell membranes. Humans do not have an ENR enzyme, and thus are not affected.

In terms of its antimicrobial activity, triclosan is principally bacteriostatic with some fungistatic activity. Unlike the classic bisphenols, this activity is reasonably broad, being equally effective against Gram-positive and most Gram-negative bacteria. It is also active against some fungi. Its spectrum includes high activity against staphylococci, some streptococci, some mycobacteria, *Escherichia coli* and *Proteus spp*. Enterococci are much less susceptible than staphylococci. Triclosan is bacteriostatic at low concentrations but higher levels are bactericidal.

Application

Triclosan is an antibacterial agent added to a wide range of consumer products to offer long lasting protection against bacteria, moulds and yeasts. The majority of its usage is associated with household and personal care products. For example it is used in toothpastes, mouthwashes, soaps and deodorants. Other specialized applications utilize triclosan in fibers to control odour and in plastics like cutting boards and conveyor belts for food processing.

- Plain soap and water are not always adequate; they do not provide long lasting antibacterial effects. Hand wash containing triclosan should be used after person comes into contact with potentially harmful bacteria such as using bathroom, handling raw food, touching pets, caring for the sick, etc.
- Triclosan based shower products either in liquid form helps to control bacteria that can cause problems on the skin.
- Dental diseases are chronic infections caused by oral bacteria harboring the dental biofilm. Triclosan is an agent that was found to have an antibacterial effect against oral bacteria.
- Triclosan acts against skin inflammation and reduces the irritant effect from aggressive surfactant.
- Bacteria are responsible body odours in armpits and on feet. triclosan controls the growth of odour forming bacteria caused by perspiration.

Triclosan is a widely accepted antimicrobial ingredient because of its safety and antimicrobial efficacy. Triclosan is a unique antimicrobial well suited for use in the health care industry in which mildness is a necessity to protect the health care worker during repeated use and antimicrobial activity is a necessity to protect public health. Triclosan has demonstrated immediate, persistent, broad-spectrum antimicrobial effectiveness and utility in clinical health care settings. Thus, triclosan provides an added layer of hygiene and protection which can be life saving in unhealthy condition.

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Endoscope Reprocessing

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

HISTORICAL ASPECTS

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

TYPES OF ENDOSCOPY

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

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

SIGNIFICANCE OF ENDOSCOPE REPROCESSING

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

STEPS OF MANUAL ENDOSCOPE REPROCESSING

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

a) Pre-cleaning- Pre-cleaning is an essential reprocessing step that removes patient biomaterial and microorganisms from the endoscope. Following an endoscopy, biomaterial from the patient is present on the insertion tube and within the internal channels of the endoscope. All channels must be cleaned, even if unused, due to fluid and debris entering these channels at the distal tip. Patient biomaterial provides a nutrient source that will promote the

growth of potentially pathogenic microorganisms. Also, when this biomaterial is not removed immediately after a procedure, it will dry and harden. The surface of the hardened material functions as a barrier that prevents the penetration of disinfecting and sterilizing agents that kill microorganisms. Preliminary cleaning should be started before the endoscope is detached from the light source/videoprocessor. Immediately after removing the endoscope from the patient, wipe the insertion tube with the wet clothe or sponge soaked in the freshly prepared enzymatic detergent solution.

A reprocessing delay may occur when a patient has both upper and lower procedures performed during the same visit. The endoscope from the first procedure is kept in the procedure room until the second procedure is completed. If pre-cleaning is not initiated within an hour, the endoscope should be soaked in an appropriate enzymatic detergent according to the manufacturer's recommendations, before continuing with mechanical cleaning and then terminal reprocessing. This process will allow for any dried debris to be loosened and ensure its removal during cleaning.

b) Leak testing - Followed by cleaning leak testing should be done. Manufacturer's instructions should be followed for leak testing. The leak tester is attached and pressurized the endoscope before submerging it in water. With the pressurized insertion tube completely submerged, the distal portion of the endoscope is flexed in all directions, observing for bubbles. The entire endoscope is submerged and observed the head of it, the insertion tube, distal bending section and the universal cord for bubbles coming from the interior of the endoscope. The leak test will detect damage to the interior or exterior of the endoscope. If a leak is detected or the endoscope appears damaged manufacturer's instruction should be followed.

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

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

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

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

QUALITY ASSURANCE & SAFETY INSTRUCTIONS

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

References

D. B. Nelson & L. F. Muscarella. 2006. Current Issues in endoscope reprocessing and infection control during gastrointestinal endoscopy. *World J. Gastroenterol*. **12(25):** 3953-3964.

As you have gone through the article on Current Trends related to Neutralizing Media **Microxpress** recommends following media for used in sanitizer efficacy testing:

- Dey-Engley Neutralizing Agar & Broth (D/E Agar&Broth Disinfection Testing)
  A medium used in disinfectant testing where the neutralization of antiseptics and disinfectant is important for determining its bactericidal activity.
- Inactivator Broth (Twin Pack)
  A medium for detection and isolation of bacterial contamination from clean surfaces and accidently contaminated raw material samoles of pharmaceutical formulations.
- Tryptone Soya Agar with Lecithin and Tween 80
  A medium recommended for validation of cleanliness on surfaces of containers, equipment surfaces and water miscible cosmetics.

In this issue we have discussed about *Staphylococcus aureus*, **Microxpress** offers following Kit and dehydrated culture media for the isolation and identification of *Staphylococcus* species:

- Staph Identification Kit
  A biochemical identification kit contains 12-miniature test panel.
- Coagulase Mannitol Agar Base
  A basal medium for preparation of blood and chocolate agar and for various selective and identification media in isolating and cultivating fastidious organisms.
- Mannitol Salt Agar
  A selective medium for isolation and identification of Staphylococcus aureus from clinical and non-clinical specimens. Mannitol Agar also conforms to the specification of USP and IP.
- Vogel Johnson Agar Base w/o Tellurite
  A medium with the addition of potassium tellurite for isolation of *Staphylococcus aureus* from clinical and non-clinical specimens.

Endoscopy procedures are now a routine part of patient diagnosis and treatment in hospitals and surgery centers. Endoscopes are notorious to nosocomial infections. To reduce the bioburden proper reprocessing of endoscope should be done. **BioShields** Instrument Disinfectant range has following high technology disinfectant for endoscope reprocessing.

- Endomax™
  It is a 14 day stable, fluoresecent green coloured liquid upon activation with a characteristic odour. Endomax™ contains 2.45% w/v glutaraldehyde with powdered activator and corrosion inhibitors.
- Endomax Kit™
  This concentrated solution when prepared is a fluorescent green coloured liquid upon activation with a characteristic odour. Endomax Kit™ contains w/v glutaraldehyde and activator with 7.5% w/v buffer salts.
- Endomax 2.0™
  It is a 28 days stable, colourless fluorescent green coloured liquid upon activation with a characteristic odour. Endomax 2.0™ is composed of 2% w/v glutaraldehyde with powdered activator with corrosion inhibitors.

Main features of Endomax™, Endomax Kit™, Endomax 2.0™ and Endomax Plus™:

- HIV, HBV, TB & Sporicidal Potent, Proven formulation
- Excellent Material Compatibility
- With Corrosion Inhibitors
- Consistent Performance

- Clenzyme™
  It is a multi-enzyme cleaner for Surgical, Medical and Dental instruments including flexible and rigid endoscopes. Clenzyme™ is composed of 12% v/v enzymes(combination of proteases, lipases and amylases).

SERIES I

MALTOSE

PHAGE

STERILE

EDEMA

PLASTID

SERIES II

PROBE

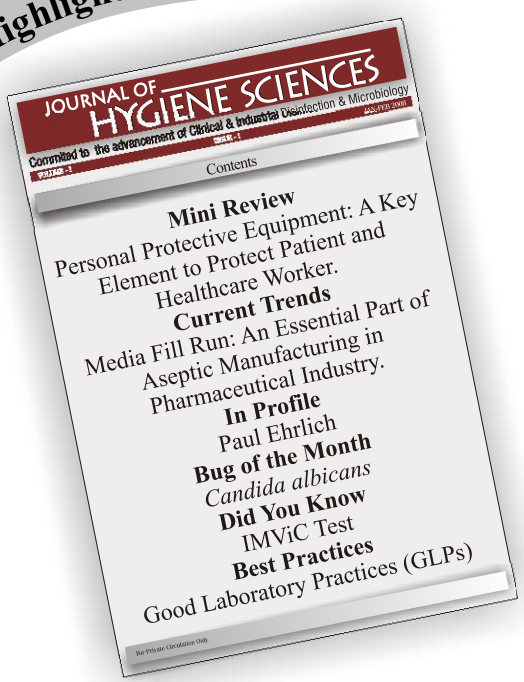
CYST

MONOMER

FOMITE

EPISOME

Highlights of the coming issue



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