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Editorial

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Yes! yet another issue.....It would interest you to know what is in store for you.....now, take a look.....

Mini Review begins with a phrase; Microbiological Media, Simple Complexity. Now for any kind of science there are always the basis, the simple stuff and after there are advances there always comes a complex portion. Well when there is a mention of microbiological media, there are the basics like the water or agar or gelatin and then there are the complex components like extracts, indicators, dyes which give the media different characteristics. Thus microbiological media like Microbiology itself is in every sense of the phrase – **Simple – Complexity.**

Disinfectants, are designed to be toxic, contain chemicals that are corrosive, irritants, and potentially carcinogenic. They are used to kill microbes and achieve an appropriate level of clean. Implementing best management practices will encourage the use of only the amount of disinfectant necessary to do the job, thereby reducing workers and environmental exposure. Ultimately. Incorporating best management practices for use of disinfectants protects patients, employees, and the environment. This is the core of Current Trends....Disinfection Management.

Embroidery, stitching....sounds familiar, usually associated with women, but a man who mastered the art and used the same to save lives and improvise it for effective surgery is Alexis Carrel, a Nobel laureate and is In Profile for this issue.....truly inspiration can come from little things.

Bug of the Month focuses on Streptococcus species, which are capable of playing a myriad roles, they can be a part of the normal flora, opportunistic pathogen and infectious agents. Considering the importance of diagnosis and treatment of these infections is of prime importance.

Did You Know, These abbreviations!. Well now you will. Microbiology encompasses a vast diversity of fields that utilize methods which are specific to the type study. To make notes and observation easier or even coding simpler, the use and implementation of abbreviations becomes indispensable, both for making observations and respective interpretations.

Best Practices tackles the issue of Handling, Storage, and Transportation of Health Care Waste. Health care activities – for instance, immunizations, diagnostic tests, medical treatments, and laboratory examinations – protect and restore health and save lives. But the wastes and by-products generated need to be handled scrupulously in order to prevent spread of infections, and dissemination of toxins and radioactive materials.

Relax Your Mood by helping the mind delve and think of the answers to track your brain, base your actions on thoughts to live by and laugh as you enjoy the humor.

As you immerse yourself into reading, knowing and understanding the different facts, we need your ideas too, tell us what you feel about the journal, what you want to read about and what are the questions that you may have asked yourself about microbiology and disinfection. Till then explore and enjoy yet another Issue.....

Microbiological Media: Simple Complexity

For any kind of science there are always the basis, the simple stuff and after there are advances there always comes a complex portion. Well when there is a mention of microbiological media, there are the basics like the water or agar or gelatin and then there are complex components like extracts, indicators, dyes which give the media different characteristics. Thus microbiological media like Microbiology itself is in every sense of the phrase – **Simple – Complexity.**

Microbiological media refers to any culture media defined as any nutrient liquid or solid that can be used in the laboratory and for the growth of microorganisms. Such a medium may resemble a natural substrate on which the microorganism will usually grow like blood serum for animal pathogens and milk for milk microorganisms. Distilled water is commonly used to dissolve all the media components.

Peptones are used as a nitrogen source and has another function in the medium that is; buffering material, because it is an amphoteric compound and thus it can maintain the medium pH. Carbohydrates, peptides, amino acids and proteins, are used as energy sources. Usually beef extract is used as a growth factor. Gelatin or agar agar is used as a solidifying agent.

Media can be classified based on texture as liquid media, solid media, reversible to liquid , semi solid media. Selective media is used to isolate one species of microorganism and inhibit the growth of another microorganism. Differential media in which there is added natural or chemical compounds to differentiate microbial groups which may be formed in the same culture. Enriched media in which we need to add blood serum or plant tissues to enrich the growth of specific groups of microorganisms like heterophilic bacteria.

The early microbiologists used the foods available for homes and restaurants as foods for their bacteria. They supplemented these with blood, fruit juices and other materials found in the environment where they found the bacteria, yeasts, and fungi they wished to study. When viruses were discovered, living cells became “media”.

Any microbiological medium and environment must provide everything the species under cultivation requires. These requirements include oxygen (or an alternate electron acceptor), water, nitrogen source, carbon source, energy source, minerals, vitamins, and trace biochemicals. Many bacteria can use glucose as energy and carbon source. Some bacteria can use light as energy source and others can oxidize sulfur as energy source. As nitrogen source, most bacteria require protein, peptides, or amino acids, but many can use ammonia, nitrates or nitrogen molecules. Bacteria capable of fermentation can oxidize one molecule and use another as electron acceptor, thus, they are not able to obtain as much energy as would be available were the energy oxidized completely to carbon dioxide and water.

Some microbes can make almost everything they need, but others require a long list of vitamins and growth factors. Some metabolize their food down to carbon dioxide and water, while others may have very limited dissimilatory metabolism and leave their food practically untouched. Bacteria used in food processing typically are able to use only a few of the components of the food they used to process. Knowledge of what a bacterium needs helps one to design a medium for growing it.

Microorganisms need nutrients, a source of energy and certain environmental conditions in order to grow and reproduce. In the environment, microbes have adapted to the habitats most suitable for their needs, in the laboratory, however, these requirements must be met by a culture medium. This is basically an aqueous solution to which all the necessary nutrients have been added. Depending on the type and combination of nutrients, different categories of media can be made.

Different Categories of Culture Media

Complex media: are rich in nutrients, they contain water soluble extracts of plant or animal tissue (e.g., enzymatically digested animal proteins such as peptone and tryptone). Usually a sugar often glucose is added to serve as the main carbon and energy source. The combination of extracts and sugar creates a medium which is rich in minerals and organic nutrients, but since the exact composition is unknown, the medium is called 'Complex Medium'.

Defined media: are media composed of pure ingredients in carefully measured concentrations dissolved in double distilled water i.e., the exact chemical composition of the medium is known. Typically, they contain a simple sugar as the carbon and energy source, an inorganic nitrogen source, various mineral salts and if necessary growth factors (purified amino acids, vitamins, purines and pyrimidines). Since all the components and their various concentrations are known, this type of media is appropriately named 'Defined Media'.

Selective / Differential media: are based on either of the two categories above supplemented with growth promoting or growth inhibiting additives. The additives may be specific or organism selective (e.g., a specific substrate, or an inhibitor such as cyclohexamide which inhibits all eucaryotic growth and is typically used to prevent fungal growth in mixed cultures).

Composition of Media (The basic recipe)

Media for the cultivation of microorganisms contain the substances necessary to support the growth of microorganisms. Due to the diversity of microorganisms and their diverse metabolic pathways, there are numerous media. Even slight differences in the composition of a medium can result in dramatically different growth characteristics of microorganisms.

When methods for cultivating microorganisms were first developed in the 19th century, largely by Robert Koch and his

colleagues. Animal and plant tissues were principally used as sources of nutrients used to support microbial growth. One of the major discoveries of Fanny Hesse in Koch's laboratory was that agar could be used to form solidified culture media on which microorganisms would grow. Extracts of plants and animal tissues were prepared as broths or mixed with agar to form a variety of culture media. Virtually any plant, animal, or animal organ was considered for use in preparing media. Infusions were prepared from beef heart, calf brains, and beef liver, as a few examples. These classic infusions still form the primary components of many media that are widely used today, such as Brain heart infusion agar and liver broth.

Media for the cultivation of microorganisms have a source of carbon for incorporation into biomass. For autotrophs, the carbon source most often is carbon dioxide, which may be supplied as bicarbonate within the medium. Carbohydrates, such as glucose, or other organic compounds, such as acetate, various lipids, proteins, hydrocarbons, and other organic compounds, are included in media as sources of carbon for heterotrophs. These carbon sources may also serve as the supply of energy. Other compounds, such as ammonium ions, nitrite ions, elemental sulfur, and reduced iron, may be used as the sources of energy for the cultivation of autotrophs. Nitrogen also is required for microbial growth. It may be supplied as inorganic nitrogen compounds for the cultivation of some microorganisms but more commonly is supplied as proteins, peptones, or amino acids. Phosphates and metals, such as magnesium and iron, are also necessary components of microbiological media. Phosphates may also serve as buffers to maintain the pH of the medium within the growth tolerance limits of the microorganisms being cultivated. Various additional growth factors may also be included in the media.

Agar

Agar is the most common solidifying agent used in microbiological media. Agar is a polysaccharide extract from marine algae. It melts at a 84^{O}C and solidifies at 38^{O}C. Agar concentration of 15.0 – 18.0 grams per liter typically are used for solid media. Lower concentrations of 7.5 – 10.0 g/L are used to produce soft agars or semisolid media.

Peptone

Many complex media, that is, media in which not all the specific components are known, contain peptone as a source of nitrogen. Peptones are hydrolyzed proteins formed by enzymatic or acidic digestion. Casein most often is used as the protein substrate for forming peptones, but other substances, such as soybean meal, also are commonly employed.

Meat and Plant Extracts

Meat and plant infusions are aqueous extracts that are commonly used as sources of nutrients for the cultivation of microorganisms. Such infusions contain amino acids and low molecular weight peptides, carbohydrates, vitamins, minerals and trace metals. Extracts of chemical tissues contain relatively high concentrations of water soluble protein components and glycogen. Extracts of plant tissues contain relatively high concentrations of carbohydrates.

With regard to infusions, many media list as an ingredient infusion from beef heart or another animal tissue. This ingredient is prepared by boiling a given amount of the animal tissue (eg. 500.0 grams), and then using the liquid or, more commonly, drying the broth and using the solids from the infusion. The actual weight of the dry solids recovered from the infusion and used in the medium is far less. Brain heart infusion is prepared from calf brains and beef heart.

Growth Factors

Many microorganisms have specific growth factor requirements that must be included in media for their successful cultivation. Vitamins, amino acids, fatty acids, trace metals, and blood components often must be added to media. In some cases, specific defined components are used to meet the growth factor requirements. Incorporation of growth factors is used to enrich, that is, to increase the numbers of particular species of microorganisms, most often, mixtures of growth factors are used in microbiological media. Acid hydrolysates of casein commonly are used as sources of amino acids. Extracts of yeast cells also are employed as sources of amino acids and vitamins for the cultivation of microorganisms. Many media, particularly those employed in the clinical laboratory, contain blood or blood components that serve as essential nutrients for fastidious microorganisms. X factor (heme) and V factor (nicotinamide adenine dinucleotide) often are supplied by adding hemoglobin, isovitale X, and / or supplement VX.

Differential Components

The differentiation of many microorganisms is based upon the production of acid from various carbohydrates and other carbon sources or the decarboxylation of amino acids. Some media include indicators, particularly of pH, that permit the visual detection of changes in pH resulting from such metabolic reactions.

pH Buffers

Maintaining the pH of media usually is accomplished by the inclusion of suitable buffers. Because microorganisms grow optimally only within certain limits of a pH range, the pH generally is maintained within a few tenths of a pH unit. For the phosphate buffers, the pH is established by using varying volumes of equimolar concentrations of Na_2HPO_4 and NaH_2PO_4.

Media and Purpose (In brief)

Media	Purpose
Complex	Grow most heterotrophic organisms
Defined	Grow specific heterotrophs and are often mandatory for chemoautotrphs, photoautotrophs and for microbiological assays.
Selective	Suppress unwanted microbes, or encourage desired microbes
Differential	Distinguish colonies of specific microbes from others
Enrichment	Similar to selective media but designed to increase the numbers of desired microorganisms to a detectable level without stimulating the rest of the bacterial population
Reducing	Growth of obligate anaerobes

The mixture of necessary nutrients can be used as a liquid medium, or a solidifying agent can be added. "Agar agar" is a natural polysaccharide produced by marine algae and is the most commonly used as a solidifying agent added to media (end concentration usually 1.5 – 1.8 % w/v). If hydrolysis of the agar is suspected, a silica gel is used as a replacement solidifying agent.

Preparation of Media

The ingredients in a medium are usually dissolved, and the medium is then sterilized. When agar is used as a solidifying agent, the medium must be heated gently; usually to boiling, to dissolve the agar. In some cases, where interactions of components, such as metals, would cause precipitates, solutions must be prepared and occasionally sterilized separately before mixing the various solutions to prepare the complete medium. The pH often is adjusted prior to sterilization, but in some cases sterile acid or base is used to adjust the pH of the medium following sterilization. Many media are sterilized by exposure to elevated temperatures. The most common method is to autoclave the medium. Different sterilization procedures are employed when heat – labile compounds are included in the formulation of the medium.

Aseptic techniques

Before inoculation with the desired microorganisms, microbiological media and all materials coming into contact with it must be sterile, during any subsequent handling of the bacterial cultures, unwanted or contaminant organisms must be excluded employing aseptic techniques.

Sterilization implies the complete destruction of all microorganisms including spores, this is accomplished by the use of heat, chemicals, radiation, and filtration.

Heat

Denatures and coagulates vital proteins. There are various forms of heat sterilization.

Red Heat

Inoculating wires or loops are sterilized by holding them in a Bunsen flame until the loop is red hot.

Moist Heat

Bacteria are more readily destroyed by moist heat (steam) than dry heat. Usually used for the sterilization of culture media, aqueous solutions and the destruction of discarded cultures. Air must first be removed in order to achieve the 121^{O}C necessary for successful sterilization. This is accomplished by the use of an autoclave (the technical version of a pressure cooker), which follows automatic cycles of heating under pressure for the required time.

Dry Heat

Usually employed for materials which could either be corroded by steam or must dry before use. These include metal instruments, glass petri dishes, flasks and pipettes and cotton wool. In practice, dry heat sterilization requires longer time intervals and higher temperatures than steam sterilization, e.g., steam sterilization 121^{O}C for 15 minutes or dry heat sterilization 160^{O}C for 120 minutes.

Chemical

Usually employed for delicate equipment such as optical instruments and electrical devices which would be damaged by heat. Due to the toxicity of the chemicals used, this is not the most popular form of sterilization. Chemicals employed include gaseous oxide, which alkylates amino, sulfhydryl, carboxyl and hydroxyl groups of microbial cell compounds; formaldehyde, used as a fumigant; and hydrogen peroxide vapor used in aseptic packaging.

Radiation

Employed for heat sensitive materials and for environmental samples such as soil and sediment where structural changes caused by heat need to be avoided. Two forms of radiation are used:

UV

Initiates the excitation of atoms which in nucleic acids leads to fatal mutations. UV light cannot penetrate materials so is used mainly for surface treatments e.g., laminar air flow benches, and air and water.

Ionizing Radiation

Can penetrate samples, causing ionization within cells. Gamma radiation generated through a ^{60}Co a source is used to sterilize complex materials such as soil and food stuff. Microorganisms show increased resistance to radiation under anoxic conditions (2–5x) and also from frozen samples.

Filtration

Filtration sterilization operates through the exclusion rather than destruction of microorganisms. It is safe for the user and is employed for sensitive liquids and gases. Three types of filters are currently in use;

Depth Filters

These are made of columns packed with fibrous materials such as glass wool or cotton wool. The twisting and turning fibers entrap particles and so act as filters; they show little resistance to flow and are used mainly for gases or as pre – filters for membrane filters which are easily clogged.

Membrane Filters

Act by screening out particles. Their effectiveness depends on the size of the membrane pores and the electrostatic attractions present. The most commonly used filters in microbiology are usually made of cellulose acetate or cellulose nitrate.

Size of filter pores required to screen out:
Yeast: 0.45 – 1.2 µm
Bacteria: 0.2 µm
Viruses and mycoplasmas: 0.01 – 0.1 µm

Tyndallization

Exposure to steam at 100^{O}C for 30 minutes will kill vegetative bacterial cells but not endospores. Such exposure can be achieved using flowing steam in an Arnold sterilizer. By allowing the medium to cool and incubate under conditions where endospore germination will occur and by repeating the 100^{O}C for 30 minutes

exposure on three successive days, the medium can be sterilized because all the endospores will have germinated and the heat exposure will have killed all the vegetative cells. This process of repititive exposure to 100°C is called tyndallilzation, after its discoverer John Tyndall.

Inspissation

Inspissation is a heat exposure method that is employed with high protein materials, such as egg containing media, that cannot withstand the high temperatures used in autoclaving. This process causes coagulation of the protein without greatly altering its chemical properties. Several different protocols can be followed for inspissation. Using an Arnold sterilizer or a specialized inspissator, the medium is exposed to 75 – 80 °C for 2 hours on each of three successive days. Inspissation using an autoclave employs exposure 85 – 90 °C for 10 minutes achieved by having a mixture of air and steam in the chamber, followed by a 15 minutes exposure during which the temperature is raised to 121°C using only steam under pressure in the chamber; the temperature then is slowly lowered to less than 60 °C.

Autoclaving

Autoclaving uses exposure to steam, generally under pressure, to kill microorganisms. Exposure for 15 minutes to steam at 15 psi – 121 °C is most commonly used. Such exposure kills vegetative bacterial cells and bacterial endospores.

Operational Safety

However, certain media components such as Basic fuchsin and acid fuchsin are carcinogens, and caution must be used in handling media with these compounds to avoid dangerous exposure that could lead to the development of malignancies. Thallium salts, sodium azide, sodium biselenite, and cyanide are among the toxic components found in some media. These compounds are poisonous, and steps must be taken to avoid ingestion, inhalation, or skin contact. Azides also react with many metals, especially copper, to form explosive metal azides. The disposal of azides must avoid contact with copper or achieve sufficient dilution to avoid the formation of such hazardous explosive compounds which may result in the formation of hydrogen sulfide, which is a toxic gas.

Care must be taken to ensure proper ventilation. Media with human blood or human blood components must be handled with great caution to avoid exposure to human immunodeficiency virus and other pathogens that contaminate some blood supplies. Proper handling and disposal procedures must be followed with blood containing media as well as other media that are used to cultivate microorganisms.

Quality Control of Microbiological Culture Media

Control of Media Preparation

QC laboratories acquire media in one of two ways, either purchasing the media pre-made from a manufacturer, or making the media (either in whole or in part) in-house. These preparation schemes must be considered separately.

Clearly, if the media is purchased from the vendor there is little opportunity to control the preparation beyond having confidence in the supplier. However, agar acquired in large aliquots for pour-plates must be carefully melted prior to use – this melting must be under controlled conditions to avoid damaging the media. Of course, all media used is expected to be checked for physical and chemical parameters and growth promotion, and prepared media is no exception to this expectation.

Media prepared in-house offers several opportunities for quality control. The raw materials (either the dehydrated complete media or the components) must be stored under appropriate and controlled conditions and used within established expiry dates. The compounding of the media must be controlled to ensure the media is prepared correctly. Agar media must be pre-warmed to dissolve the agar prior to sterilization, but not heated so extensively as to damage any heat-labile components. The sterilization procedure also must be under control. Normally this means using a validated autoclave cycle (and load configuration) shown to hold the media at 121°C for 15 minutes (note this is not the same as a 15 minute cycle with a maximum temperature of 121°C). Each batch of media should be clearly labeled to allow for unambiguous audit of each stage of preparation.

Control of Physical and Chemical Parameters

The goal of this testing is to provide a gate-keeping function before investing the time in growth-promotion testing. pH of the finished media (pH measurement must be conducted at room temperature unless specific allowance is made for the temperature) is a critical attribute to confirm. The color of the media should be examined and a decision made as to its correctness, as well as an examination for any crystal formations or variations in color (for agars). The containers of media should be thoroughly examined for cracks or defects, and all defective units discarded. There are additional checks that can be performed (HPLC of major components, determination of sugar concentration, etc. Curtis 1985), but these are not normally conducted in the pharmaceutical QC lab.

Growth Promotion Testing

There are some significant concerns as to the need for GP testing of standard media. It can be argued that since all preparation conditions are under control and the physical parameters of the finished media is checked, there is little additional information gathered by the labor-intensive and time-consuming procedure of checking the growth promoting capabilities of the media. This topic has been debated not only among workers in QC laboratories, but also in the clinical microbiological industry.

The next concern is test design. There are two types of media commonly used in the microbiological lab – broth and agar. These two types must be considered separately as they show growth by completely different means. The fundamental question of GP testing can be expressed as: Is the new batch of media as good as a previously qualified batch? This question cannot be answered adequately except by statistical comparison, given the variability of microbiological data.

Control of Storage Conditions

Media quarantine and release

The laboratory must have some procedures in place to prevent unqualified media from entering the testing process. This ideally would be a separate storage room from that used to store qualified

media, but may also be accomplished through tagging the quarantined material and placing it in a clearly identified area within the same room. All quality control checks on the quarantined media should be completed before its documented release for general use. Storage conditions of the quarantined media should match those of the released media.

Media Storage and Expiry

Media should always be stored under controlled conditions to ensure its quality through to the expiry date. Factors to be evaluated in these controlled conditions include:

Temperature
Container (glass, plastic, container closure system, etc)
Humidity
Light

Though the whole process of making media, inoculating it, obtaining results, and analyzing data with the help of deduction is indeed a herculean task, but the truth is that it is tough but rewarding and satisfying. Therefore go ahead and experiment Simple Complexity!!

Encyclopedia

Sterility Assurance Level (SAL): Invasive medical devices are never fully "sterilized", however one can control the level of sterility based on one's product's bioburden and sterilization.
When a product is sterilized, either by ethylene oxide or gamma radiation cycles (or other methods), the level of sterilization never guarantees that the product will be completely sterile. One reason for this is that the sterilization methods work by reducing the number of viable microorganisms logarithmically. Several factors result in the ultimate level of living organisms on medical devices following sterilization. The two factors that are usually at work are the number of organisms that present on the product (known as bioburden) and the amount of sterilization that is applied.

Bioburden

When a product is manufactured a number of microorganisms are introduced, depending on the circumstances, such as whether or not a clean room is used, the level or degree of contamination will vary.

It is highly recommended that a medical device manufacturer of invasive or mucus or blood contacting devices, adopt a bioburden program early in development. Controlling the types and quantity of organisms coming into contact with the device results in controlling and demonstrating the level of sterility of that device. It is for this reason many manufacturers have put in place clean rooms with varying classifications for the manufacturing of their devices.

A good bioburden program can be demonstrated using metrics. Some manufacturers have adopted Statistical Process Control (SPC) techniques for quantifying the number of microorganisms introduced to their products during the manufacturing process. Over time those SPC can show if their process is in control and whether any anomalies have been introduced into that process. Anomalies or spikes in the process can be an indication that something has gone wrong. An unusual spike in the amount of bioburden should result in a nonconformance and subsequent investigation.

In addition to the numbers of microorganisms it is equally important to determine the types of microorganisms that are present. One can submit a number of unprocessed units to a good laboratory to quantify and determine the types of microorganisms present on pre – sterilized products. The frequency of determining bioburden is based on the method of sterilization that one decides to use however it is recommended that if its done at least monthly or more often, especially if an out – of – control situation (unusual spike) occurs.

Sterilization

Sterilization can occur by various methods. Depending on the nature of the design, material and / or packaging some methods have advantages over others. In all cases the level of sterilization is based on the pre – sterilization bioburden. In some cases the bioburden should influence the sterilization method. For instance there is some cotton that should be sterilized by steam due to the presence of a fungus known as *Pyronema domesticum*.

When the sterilization method is chosen the method requires validation. The validation method is based on determining the Sterility Assurance Level (SAL). The sterility assaurance level can vary depending on the application of the device and is set by various standards and requirements. For invasive products the level is usually 10^{-6} or 1 in a million. The number 10^{-6} denotes the number of products that will have a living microorganism following the sterilization process. In other words, once the sterilization is completed the odds of finding an unsterile product will be 1 in a million. As indicated some products are adequate with having a SAL of only 1 in a thousand.

How is SAL determined?

For instance when ethylene oxide sterilization is the method employed then the method for validating the sterilization process can follow ISO 13485 or EN 550. These standards require that a number of Biological Indicators (BI) be placed within the products in an orientation that is deemed "worst case scenario". Many manufacturers will validate using product that has been seeded with the microorganism that is used in the Biological indicators. For ethylene oxide sterilization the bacterium used is *Bacillus subtilis*. Since it is most resistant to the toxic effects of ethylene oxide and therefore if the test organism is affected therefore the sterilization test agent is effective for the devices to be tested.

The amount of *Bacillus subtilis* on each biological indicator will be in the range of 10^{-6} but never less than one million. The actual number of organisms on each lot of biological indicators should be validated by sending them to the laboratory. The lab will enumerate them and send a certificate. There are a number of reasons one should not count on the certificate that is issued by the biological indicator manufacturer alone. One is that of control. Biological indicators should be controlled and stored in temperature / humidity environment that is recommended by the manufacturer. This environment should be monitored to ensure compliance. Out of specification changes in environment should result in a new enumeration by an accredited laboratory.

Disinfection Management

In Health care facilities, dental clinics, veterinary homes, public building, schools and institutions, all use some form of disinfection. Incorporating disinfection best management practices protects building occupants and the environment.

Disinfectants, are designed to be toxic, they contain chemicals that are corrosive, irritants, and potentially carcinogenic. They are used to kill microbes and achieve an appropriate level of clean. Implementing best management practices will encourage the use of only the amount of disinfectant necessary to do the job, thereby reducing workers and environmental exposure. Ultimately. Incorporating best management practices for use of disinfectants protects patients, employees, and the environment at large.

Right Level of Clean

Different levels of cleanliness are needed for different activities. Use lowest cleaning level that meets their respective needs.

Surface Cleaning

General surface cleaning physically removes all visible dirt, organic matter, and bacteria. It is normally accomplished with water, mechanical action like scrubbing, and detergents. Surface cleaning should always precede disinfecting and sterilizing. If organic matter is not first removed it can inactivate disinfectants. In many cases, including the health care industry, general surface cleaning requires the highest level of cleaning necessary.

Disinfecting

Disinfection reduces the risk of infection from microbial contamination. It is done to reduce the chance of infecting patients and others. Disinfecting is necessary for surfaces or equipment that may contact broken skin or mucus membranes. High level disinfection is required for semi invasive medical procedures like endoscopy. Lower levels of disinfection are used on high touch surfaces in surgery wards and kennels.

Sterilizing

Sterilizing virtually eliminates or destroys bacteria and viruses. Objects are sterilized if they will enter a sterile area, such as a body cavity. Sterilization is accomplished with host steam and pressure, toxic gases such as ethylene oxide, or hydrogen peroxide plasma.

Read the Label

Review the labels of your current disinfectant. Do these solutions match the profile of the microbes you need to kill? The labels of concentrated disinfectants also state the proper level of dilution for maximum effectiveness.

Antibacterial soaps

Increasingly antibacterial chemicals such as triclosan and triclocarban are added to soaps, cleaners, and other products. Limit the use of antibacterials. Non foaming alcohol hand rubs are just effective. Widespread use of antibacterials has created concern about increasing bacterial resistance and pollution of drinking water.

Disinfectant Wipes

Often disinfectant wipes dry before adequate contact time is achieved. They are also often used in applications where they are not needed adding expense and pollution. A re – usable cloth wet with the appropriate disinfectant applied for the recommended contact time is less expensive, more efficacious option. If disinfectant wipes are not dry prior to disposal they may be considered hazardous waste.

Procedures

Health care facilities, dental offices, veterinary clinics, schools, day care centers, and public buildings all use some form of disinfecting. Often formal cleaning procedures are passed on verbally or guessed at based on experience with other cleaning chemicals and potentially mistaken assumptions.

Many certification programs now require written procedures to ensure best management practices.

Establish procedures based on current needs, equipment, and disinfectants.

Writing a Procedure

Procedures need to include information on why cleaning is done, what products and tools should be used, and how to use them. Consider the following information when writing a disinfection procedure.

Pre – clean

Conduct general surface cleaning to remove dirt and debris. This will remove many microorganisms and increase the effectiveness of the disinfectant.

Many products are one step cleaner / disinfectants. These products are intended for use on relatively clean surfaces. If a surface only requires cleaning then use of cleaner / disinfectant is not necessary. General surface cleaners are often less costly than disinfectants and can be better for the environment. If a surface is visibly dirty it should be pre – cleaned before a disinfectant is used. When using other disinfectants always pre – clean even if a surface looks clean.

Evaluate the Need to Disinfectant

Determine if a lower level of clean is adequate. Does an item need to be disinfected or is surface cleaning sufficient? If the item only touches intact skin, then surface cleaning is appropriate.

Assess the Level of Disinfection

Know the target microbes you need to kill. Make a list of the specific targets like Mycobacterium of Parvo virus, and more general targets like bacilli, spores, or viruses. Items used in a semi – sterile area require different levels of disinfection than hard surfaces in public areas or patient rooms.

Select an ideal Disinfectant

Choose a disinfectant that is highly effective and is the least toxic to employees and the environment. Disinfectants that act by oxidizing, such as hydrogen peroxide or peracetic acid, create fewer by – products than quaternary compounds or chlorine bleach. This means fewer toxins reach the sewer. Hydrogen peroxide and peracetic acid are also generally more effective against all types of microbes and are also easily inactivated by organic matter.

Ensure that the disinfectant used is compatible with the surface being cleaned. Improper use of chemicals and scrubbing can damage surfaces. For example bleach can be corrosive to metal surfaces and scrubbing can remove some coatings. A cleaner or disinfectant with a pH that is too high or too low can strip the finish off the floor. Chemical damage is not reversible and can be costly to repair. In most cases floors do not need to be disinfected.

Ensure Proper Dispensing

To protect workers from concentrated solutions, determine what equipment is necessary to ensure proper dilutions and easy use. Do not mix different disinfectants together or mix bleach with a disinfectant. Together they may create toxic gases, such as chlorine.

For employees protection use personal protective equipment like goggles and gloves. To reduce waste, use washable towels or applicators.

Calibrate Carefully

Calibrate dispensing equipment carefully and often – at least every time a new container of disinfectant is opened. When calibrating, check the equipment for leaks and malfunctions. Equipment can be calibrated with water instead of the chemical to prevent waste.

Measure Accurately and Use Proper Dilution

Measure concentrates before adding them to the dilution tank. All disinfectants have a concentration that maximizes their ability to disinfect. Adding extra does not help. Using higher dilutions does not necessarily react more quickly or effectively. In fact it can increase the likelihood of injury, damage to equipment, contaminating drinking water sources, and they increase material cost. Follow manufacturer directions for the lowest concentration of disinfectant to achieve the highest level of antimicrobial activity.

Reduce Volume

Use the smallest possible amount of disinfectant to obtain the desired level of microbial control. This practice reduces waste, minimizes spills, and exposure. Mix only the amount needed, do not mix a gallon if you only need a quart.

Label Containers

Once the concentrate is diluted to the proper level label the container with the name, date, and initials of who diluted the solution to track its expiration / out date. Check the manufacturer's instructions for an out date.

Allow Time for Disinfectants to React

Follow label directions carefully. They provide information on proper dilution ratios, time required on the surface to be cleaned and application methods.

Staff Training

Train staff and clearly post the procedure for disinfectant use at the dispensing station. Ensure staff have access to, and use adequate personal protective equipment. Check the material safety data sheet (MSDS) for the suggested personal protective equipment.

Storage

Keep containers closed when not in use. Store disinfectants in original containers, on low shelves. Check containers regularly for breaks, leaks, rust or other corrosion. If a break or leak occurs, transfer the product into another container with the same labeling.

Waste

Avoid Spills, Clean up Spills

Store disinfectants in compatible containers. Use drip pans under spouts to catch and contain drips. Minimize transfer of disinfectants from container to container. Use pumps and spigots instead of pouring to decrease the likelihood of spills or skin contact. When spills occur, clean them up immediately. Ensure that spilled residues are managed properly, they may be considered hazardous waste. Refer to the product's MSDS for spill cleanup information.

Inventory Tips

Maintain appropriate inventory. Order and stock only what is needed. This will help avoid unnecessary disposal of excess or outdated disinfectant.

Dispose Waste Properly

Unused disinfectant concentrates may be considered hazardous waste and therefore should be disposed off properly.

Disinfection is most importantly done in order to reduce or completely eliminate microbes, so as to ensure health safety. However using the appropriate disinfectant, in proper dilution is also important and thus knowing how to manage daily needs for the purpose of disinfection is appropriate.

Reference

Minnesota Technical Assistance Program; Fact Sheet.

Alexis Carrel

Birth: June 28, 1873
Death: November 5, 1944
Nationality: French
Known for: Work on vascular suture and the transplantation of vessels and organs

Alexis Carrel was born at Lyons, France, on June 28, 1873. He was the son of a business man, also named Alexis Carrel, who died when his son was very young.
Alexis was educated at home by his mother Anne Ricard, and also at St. Joseph School, Lyons.

Early life

Carrel made his first acquaintance with the life saving dextrousness when he was four years old, when his father died. To supplement her income, his mother undertook embroidering to support her three children. Alexis was very impressed by her skill with the tiny needles employed. Alexis was sent to a Jesuit day school near his home in Lyon. As a schoolboy he showed an interest in biology by dissecting birds. Encouraged by an uncle, he conducted experiments in chemistry.
After taking his baccalaureat he entered at the University of Lyon in 1890. When the French President Mare – Francois – Sadi Carnot was assassinated by an Italian anarchist in Lyons on June 24, 1894, he was already specializing in surgery, and he was advised that the surgeons could not repair the president's vein which had been severed by the assassins knife. Such wounds could not at that time be successfully repaired.
From that time on Carrel became interested in techniques of suturing blood vessels and went to one of the finest embroiderists in Lyons, Madame Leroidier, to learn the use of tiny needles and thread which they employed. He developed an extraordinary skill in using the finest needles and practiced sewing with a needle and thread on paper until he was able to make stitches that would not show on either side. He used these skills in experiments on animals with vessel anastomosis, and devised a method of turning back the ends of cut vessels like cuffs, so that he could unite them end – to – end without exposing the circulating blood to any other tissue than the smooth lining of the vessel. By this device and coating his instruments, needles and thread with paraffin jelly, he avoided blood clotting that might obstruct flow through the sutured artery or vein. He avoided bacterial infection by a most exacting aseptic technique.

Professional Interest

In 1889 he took the degree of Bachelor of Letters at the University of Lyons; in 1890 the degree of Bachelor of Science and in 1900 his Doctor's degree at the same University. He then continued his medical work at the Lyons Hospital and also taught Anatomy and Operative Surgery at the University, holding the post of Prosector in the Department of Professor L. Testut. Specializing in Surgery, Carrel began experimental work in this subject in Lyons in 1902, but in 1904 he went to Chicago and in 1905 worked in the Department of Physiology in the University of Chicago under Professor G. N. Stewart.
Though Carrel was a deft surgeon, he was denied tenure at the University of Lyon, where his colleagues thought he was peculiar after he wrote an account of a miracle he said he had witnessed at Lourdes. He performed some of the earliest blood transfusions between humans, and transplanted kidneys and legs between dogs. In his most famous experiment, he removed a small amount of tissue from an embryonic chicken's heart, immersed it in nutrients, and was able to keep this tissue alive in his lab. Decades later, after Carrel's own death in 1944, the chicken tissue was intentionally allowed to die.
In 1906 he was attached to the Rockefeller Institute for Medical Research, New York, as an Associate Member, becoming a Full Member in 1912. In this Institute he carried out most of the experiments which earned him, in 1912, the Nobel Prize in Physiology or Medicine.
During the 1914-1919 War, Carrel served as a Major in the French Army Medical Corps and at this time he helped to devise the well-known Carrel-Dakin method of treating war wounds, which was widely used.
Carrel's researches were mainly concerned with experimental surgery and the transplantation of tissues and whole organs. As early as 1902 he published, in the *Lyons Medical*, a technique for the end-to-end anastomosis of blood vessels and in 1910 he demonstrated that blood-vessels could be kept for long periods in cold storage before they were used as transplants in surgery. Earlier, in 1908, he had devised methods for the transplantation of whole organs and later, in 1935, in collaboration with Charles Lindbergh, the airman who was the first to flow across the Atlantic, he devised a machine for supplying a sterile respiratory system to organs removed from the body, Lindbergh having solved the mechanical problems involved. He discussed this aspect of his work and its implications in his book *The Culture of Organs*. Carrel also published the well-known book entitled *Man, the Unknown* and, in collaboration with Georges Debelly, a book on *Treatment of Infected Wounds*.
In collaboration with the French surgeon Theodore Tuffier, who was a pioneer of thoracic surgery, Carrel performed on the heart a successful series of valvotomies, and in collaboration with Burrows he grew sarcoma cells in tissue cultures by the technique of Harrison.
Carrel was honored by memberships of learned societies in the U.S.A., Spain, Russia, Sweden, The Netherlands, Belgium, France, Vatican City, Germany, Italy and Greece, and by honorary doctorates of the Universities of Belfast, Princeton, California and New York, and Brown and Columbia Universities. He was a Commander in the Legion d'Honneur of France and in the Leopold Order of Belgium, a Grand-Commander in the Swedish Order of the Polar Star, and the recipient of other decorations in orders from Spain, Serbia, Great Britain and the Holy See.
He was married to Anne-Marie-Laure Gourlez de La Motte, the widow of M. de La Meyrie. They had no children.
Carrel had a myocardial infarction in 1943 and another one immediately before the liberation. He was removed from all his offices, and he and his wife were placed under guard in Paris as collaborators. He died on November 5th, 1944, from a myocardial infarction, shortly after the French radio accused him of fleeing his guards to avoid trial and of being a collaborator. "Cold, privations and isolation," he had written to a friend, had brought suffering to him and his wife. Another friend of the Nobel laureate eulogized, "He died really of a broken heart; he could not stand the accusations made against him and his sensitive soul broke under them."
A Carrel Foundation was later established at Georgetown University with the help of Charles Lindbergh. Its objective was "to promote the study and dissemination of the ideas expounded during his lifetime by the late Alexis Carel; to preserve manuscripts, records, apparatus, and other memorabilia left by or which relate to the late Alexis Carrel; to sponsor research projects which shall deal with subjects in which the late Alexis Carrel was interested; and the advancement and diffusion of knowledge concerning science, religion, and humanity."

Enjoy the humour

A farmer's wife had become deranged as they carried her out of the house in a strait jacket he said: "I sure don't know what got into her – she ain't been out of the kitchen in twenty five years.

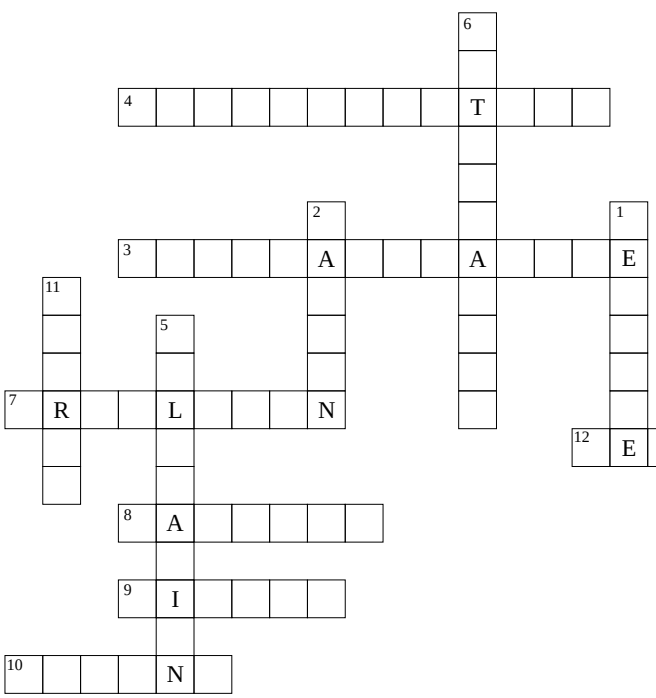
Two soldiers were carrying a stretcher with a wounded man on it.
Bill: "I say, Joe, how did you manage to keep him alive?"
Joe: "Brandy!"
Bill: "What! Don't you know that the doctor said you were not to give brandy to any of the wounded?"
Joe: "I didn't! I promised him some!"

"I think," the new secretary announced triumphantly, "that the boss has decided to keep me!"
"Has he said anything?" her friend asked.
"No," she replied happily, "but this morning he brought me a dictionary."

Thoughts to live by

- No student ever attains very eminent success by simply doing what is required of him: it is the amount and excellence of what is over and above the required, that determines the greatness of ultimate distinction. (Charles Kendall Adams).
- Without forgiveness life is governed by... an endless cycle of resentment and retaliation. (Roberto Assagioli)
- Health is the thing that makes you feel that now is the best time of the year. (Franklin P. Adams)
- Far and away the best prize that life has to offer is the chance to work hard at work worth doing. (Theodore Roosevelt)
- Let us not look back in anger, nor forward in fear, but around in awareness. (James Thurber)

Track your brain



Across

3. Agar is a natural _____ (14) produced by marine algae.
4. _____ (13) implies the complete destruction of all forms of microorganisms including spores.
7. Antibacterial chemicals such as _____ (9) are added to soaps, cleaners and other products.
8. Carrel in collaboration with Burrows grew _____ (7) cells in tissue culture by the technique of Harrison.
9. Acute glomerulonephritis is an immune complex disease of the _____ (6).
10. _____ (6) is the scientist who modified EMB agar to its present composition.
12. _____ (9) waste is highly hazardous, mutagenic, teratogenic and / or carcinogenic.

Down

1. _____ (7) is a commonly used nitrogen source in microbiological media.
2. Acid hydrolysate of _____ (6) are commonly used as sources of amino acids.
5. _____ (10) sterilization operates through the exclusion rather than destruction of microorganisms.
6. _____ (11) uses exposure to steam, generally under pressure to kill microorganisms.
11. _____ (6) refers to syringes, disposable scalpels, blades etc in health care.

Check your Answers on Page 16

Streptococcus species

Streptococcus is a genus of facultatively anaerobic, spherical Gram positive bacteria belonging to the phylum Fimicutes and the lactic acid bacteria group. Streptococci are catalase negative. Cellular division occurs along a single axis in these bacteria, and thus they grow in chains or pairs, hence the name from Greek, 'streptos', meaning easily bent or twisted, like a chain (twisted chain).

Streptococci are subdivided into groups by antibodies that recognize surface antigens. These groups may include one or more species. The most groupable streptococci are A, B and D. Among the groupable streptococci, infectious disease (particularly pharyngitis) is caused by group A. *Streptococcus pneumonia* (a major cause of human pneumonia) and *Streptococcus mutans* and other so – called viridans streptococci (among the causes of dental caries) do not possess group antigens.

Three types of hemolysis reaction are seen after growth of streptococci on sheep blood agar (alpha, beta, gamma). Alpha refers to partial hemolysis with a green coloration (from production of an unidentified product of hemoglobin) seen around the colonies; beta refers to complete clearing and gamma means there is no lysis. Group A and B streptococci are beta hemolytic, whilst D are usually alpha or gamma. *Streptococcus pneumonia* and viridans ("green") streptococci are α hemolytic. Thus the hemolysis reaction along with one physiological characteristic is different for a presumptive clinical identification.

History

In the early part of the 1900s streptococcal cross – infection became a focus of attention. The dissemination of *Streptococcus pyogenes* by scarlet fever patients was clearly documented in 1927, but it was not until the advent of serological typing of this bacterium that the high incidence of cross – infection in scarlet fever wards was confirmed by Gunn and Griffith. The epidemiology of puerperal fever due to *S. pyogenes* was also further elucidated by means of serological typing. This work fully established the importance of nasal and throat carries in the transmission of streptococcal disease. It also showed that streptococcal infection occurred in burns and maternity wards and implicated airborne dust in the spread of infection. The period from 1935 to 1950, which was marked by intensive inquiry into streptococcal cross – infection, also saw a great decline in the importance of this type of infection. While much of this decline was almost certainly due to the introduction of the sulphonamides and penicillin, and by improved methods in hospital hygiene, the streptococcus was probably also undergoing a spontaneous decrease in virulence.

Among those bacteria that are not inhibited by quinolones are viridans streptococci. In the past these have seldom caused serious infections in neutropenic patients and have responded to a variety of antibacterial regimens, but in the recent years they have emerged as a cause of significant infections. The routine adminstration of antacids or H_2 antagonists may be another important risk factor. Several studies have demonstrated that the concentration of organisms recovered from the stomach increases with increasing pH, and Gram positive bacteria are predominant among them. These serious viridans streptococcal infections are of special concern because they may cause renal failure or acute respiratory distress syndrome resulting in the death of the patient, after the acute infection appears to have been controlled.

Group A streptococcus (*S. pyogenes*)

This organism traditionally causes suppurative, but non invasive pharyngitis, and less frequently the skin infection, impetigo. In the middle part of the 1900's, the serious complications of group A streptococcal infections began to decline dramatically and had greatly decreased by the 70's. Thus, interest in this organism waned. In the 80's and 90's, there has been an upsurge in classical "rheumatic fever" (a non suppurative disease of the heart) but also new forms of streptococcal disease which included both "invasive" bacteremia, a toxic shock – like syndrome (as seen with *S. aureus*) and so called "flesh eating" bacteria.

Group A streptococcal infections affect all ages with peak incidence at 5 – 15 years of age. The serious complications (including rheumatic fever and invasive bacteremia) were felt to affect primarily those with some underlying defect in their immune system (including infants, elderly people and those immunocompromised). However, it is clear now that previously healthy children and adults are defintely at risk of serious complications.

Rheumatic fever

Rheumatic fever, is an inflammatory disease affecting primarily the heart and joints. Although severe it can take an extended period of time to develop. The mechanism of chronic immunopathology of rheumatic fever is not resolved. M protein cross – reacts with heart myosin leading to autoimmunity. Also the group A streptococcal cell wall is highly resistant to degradation in the host. These antigens persist for months in vivo and experimentally elicit diseases that resemble rheumatic arthritis and carditis. Rheumatic arthritis should not be confused with the most common rheumatic disease – rheumatoid arthritis. Early termination of throat infections with penicillin therapy decreases the incidence of the subsequent development of rheumatic carditis.

Acute glomerulonephritis

This is an immune complex disease of the kidney.

Scarlet fever

Scarlet fever is an infection that was once a very serious childhood disease, but now is treatable. Streptococci produce a toxin that leads to a red colored rash which is characteristic of the disease and can be considered as a hallmark. The main risk factor is infection with the bacteria that causes strep throat.

Bacteremia and toxic shock

The invasive and sometimes fatal forms of the disease with a toxic shock like disease (including rash, fever, and shifting of fluid from the blood stream to peripheral tissues with resulting edema) and / or necrotizing myositis and fascitis. Production of pyrogenic toxins (A, B and C) are a hallmark of these strains. Pyrogenic toxin is a superantigen (a mitogen) for T cells causing

non – specific activation of the immune system. This may be involved in the pathogenesis. This disease is still uncommon but can progress very quickly (a few days) and is life threatening.

General Features in Pathogenesis

The identity of the adhesin allowing adhesion to the respiratory epithelium (via fibronectin) is somewhat controversial. Lipoteichoic acid is localized in the cell membrane of many bacteria. For group A streptococci, much is also present in the fimbriae on the cell exterior. Classical work suggests lipoteichoic acid is the group A streptococcal adhesin although more recently a role for an "F (fibronectin – binding) protein" has been suggested.

Group A in the absence of fibrinogen fix complement to the peptidoglycan layer and, in the absence of antibodies, are not phagocytosed. The M protein (also found in fimbriae) binds fibrinogen from serum and blocks the binding of complement to the underlying peptidoglycan. This allows survival of the organism by inhibiting phagocytosis. However in immune individuals, neutralizing antibodies reactive with M protein elicit phagocytosis which results in killing of the organism. This is a major mechanism by which immunity is able to terminate group A streptococcal infections. M protein vaccines are thus a major candidate for use against rheumatic fever. The capsule of group A streptococci classically was stated to have limited anti – phagocytic activity. Many of the virulent strains are highly mucoid and the capsules are important in pathogenesis.

Unfortunately, certain M protein types cross – react antigenically with the heart and may be responsible for rheumatic carditis. The fear of autoimmunity has rightly inhibited the use of group A streptococcal vaccines. However, distinct protective versus cross – reactive epitopes have been defined and the availability of a vaccine appears likely. M proteins vary antigenically between strains thus immunity to one M protein does not imply general immunity to all *S. pyogenes* strains. M typing along with other antigens (T and R) are used for serotyping.

Laboratory Diagnosis

Direct detection – the antigen is extracted from a throat swab. The antigen extract will then bind with antibody specific to the group A streptococcal carbohydrate. This has classically involved agglutination of antibody coated beads. However, simpler tests have been recently introduced.

Lancefield grouping of isolated beta hemolyitc colonies.

Colonies are beta hemolytic and their growth is inhibited by bacitracin.

Patient serum shows antibodies to streptolysin O or other streptococcal antigens. This is important if delayed clinical sequelae occur.

Beta hemolysis is caused by two hemolysins O and S; the former is inactive in the presence of oxygen. Thus, stabbing of the plate increases the intensity of the hemolysis reaction.

Group B streptococcus (*S. agalactiae*)

These organisms cause neonatal meningitis and septicemia after transmission from the vaginal flora of the mother.

The organism can be identified on the basis of beta hemolysis, hydrolysis of hippurate and the CAMP reaction (CAMP is an abbreviation for the names of the four individuals who originally described the test).

Group D streptococcus

Growth on bile – esculin produces a black precipitate derived from esculin; many other bacteria will not grow in the presence of bile. Group D streptococci are divided into those that will grow in 6.5% saline (enterococci) and those that will not (non enterococci). Enterococci much more commonly cause human disease than non enterococci. Enterococci are often resistant to penicillin. Enterococci are distantly related to other streptococci and have been moved into the genus *Enterococcus*, the most commonly isolated is *E. (S) faecalis*. As the name implies enterococci are found in the gut flora and infection often follows from fecal contamination. A significant cause of urinary tract infections (much less common than *E. coli*) and also opportunistic infections (including intra – abdominal, septicemia and endocarditis). Colonies are usually alpha or gamma hemolytic.

Other beta hemolytic groups

Groups C and G (and rarely group F) occasionally cause human disease (particularly pharyngitis).

Minute Colony Streptococci

The normal human flora contains organisms that may be group A, C, F or G or are non groupable (*S. anginosus* / *S. milleri*). Their role in human disease is unclear.

Viridans Streptococci

These are a diverse group of species commonly found orally (including *S. mutans*) and cause endocarditis after release into the bloodstream from tooth extraction. They are also involved in dental caries. They are alpha hemolytic and negative for other tests. They are non groupable.

Laboratory Diagnosis in General

Streptococci are readily cultured from blood agar plates where α or β haemolysis may be seen. The colonies may be mucoid, matt, or glossy. Pour plates should be used in order to be certain of detecting β-haemolysis. Lancefield typing may be carried out on extracted carbohydrate C antigens classically by the precipitin test with specific rabbit antisera. However, a simple method may be used to identify group A since group A strains are exquisitely sensitive to bacitracin.

The patient's sera may be tested for the presence of anti-M antibodies by a variety of serological tests. However, test results may be often misleading because of cross-reacting antigens. Serological tests for antibodies to extracellular products are much easier to perform and are widely used to obtain evidence of recent streptococcal infection. The antistreptolysin test measures antibodies against streptolysin O. Antibodies against streptokinase, hyaluronidase, or DNAse may be also be used.

Treatment in General

β-haemolytic streptococci are among the most susceptible pathogens to antimicrobial agents. Penicillin is the drug of choice in the treatment of *S. pyogenes* infection. Where a history of hypersensitivity to penicillin exits, erythrocmycin may be used instead. Prophylactic penicillin is often given to patients with rheumatic fever in order to prevent a recurrence.

References: Microbiology and Immunology; Bacteriology; Chapter 12: Streptococci.

These Abbreviations!

Microbiology encompasses a vast diversity of fields that utilize methods which are specific to the type study. To make notes and observation easier or even coding simpler, the use and implementation of abbreviations becomes indispensable, both for making observations and respective interpretations.

Therefore in this article, the aim is to mention most of the important abbreviations that could aid in better understanding of the subject in a booklet (for instance price lists). Although there will be a glossary which mentions the abbreviations used in the text, always having an acquired knowledge is helpful.

ISO – International Organization for Standardization; is an international standard setting body composed of representatives from various national standards organizations. The organization itself, is a non government organization, however in practice, ISO acts as a consortium with strong links to governments.

BIS – Bureau of Indian Standards; is the national standards organization of India under the aegis of Ministry of Consumer Affairs, Food & Public Distribution, Government of India. As a corporate body it has members drawn from national and state politics, industry, scientific and research institutions, and consumer organizations.

IP – Indian Pharmacopoeia; the commission is committed to the promotion of the highest standards for drugs for use in the prevention of diseases in human beings and animals keeping in view the special features of the pharmaceutical industry in India.

USP – United States Pharmacopoeia is a non profit organization that develops information relating to various aspects of drug use and disseminates this information to practitioners, pharmacists, and others who make decisions about health care. USP also plays a role in the naming of both pharmaceutical ingredients and products.

EP – European Pharmacopoeia of the Council of Europe is a pharmacopoeia developed by the European Directorate for the Quality of Medicines.

BP – British Pharmacopoeia is the official collection of standards for UK medicinal products and pharmaceutical substances. Produced by the British Pharmacopoeia Commission Secretariat, part of the Medicines and health care products Regulatory Agency, the BP makes an important contribution to public health by setting publicly available standards for the quality of medicines.

Levine – is the scientist who modified EMB agar to its present composition, and therefore is added as the prefix to the media.

FDA – Food and Drug Administration (or USFDA) is an agency of the United States Department of Health and Human Services, one of the United Stated federal executive departments, responsible or protecting and promoting public health through the regulation and supervision of food safety, tobacco products, dietary supplements, prescription and over the counter pharmaceutical drugs (medications), vaccines, bio – pharmaceuticals, blood transfusions, medical devices, electromagnetic radiation emitting devices (ERED), veterinary products, and cosmetics.

BSI – British Standards Institution is a multinational business service provider whose principal activity is the production of standards and the supply of standards related services.

BCP – Bromocresol Purple is a pH indicator, the most common solution is 0.04 % aqueous. The other uses of BCP include, its use in medical laboratories to measure albumin. It is also used as an addition to acid stop baths used in photographic processing as an indicator that the bath has reached neutral pH and needs to be replaced.

m- Endo agar LES – McCarthy, Delaney and Grasso formulated Endo Agar LES (Lawrence Experimental Station) for testing water for coliform bacteria by a two step membrane filter procedure using Lauryl Tryptose Broth as a preliminary enrichment. They recovered higher numbers of coliforms by this method compared with the one step technique using m endo broth.

The American Public Health Association specifies using m Endo agar LES in the standard total coliform membrane filtration procedure for testing drinking water and bottled water. It is also specified for use in the completed phase of the standard total coliform fermentation technique.

The US Environmental Protection Agency species using m Endo Agar LES in the total coliform methods for testing water using single step, two step and delayed incubation membrane filtration methods.

PPLO – Pleuropneumonia like organism. Any of the numerous parasitic, pathogenic microorganisms of the genus Mycoplasma that lack a true cell wall, are Gram negative, and need sterols such as cholesterol for growth. In humans, one species is a primary cause of nonbacterial pneumonia. Also called pleuropneumonia like organism.

PNY – Is a medium comprising of Peptone, NaCl (Sodium chloride) and Yeast extract used for the cultivation and isolation of Lactobacillus species. This medium is often referred to as PNY medium on commercial price lists etc.

SIM – Refers to a medium for Hydrogen Sulphide detection, Indole production and Motility. These are distinguishing characteristics which aid in the identification of the Enterobacteriaceae, especially Salmonella and Shigella. SIM medium is therefore useful in the process of identification of enteric pathogens. The indole is detected by the addition of chemical reagents following the incubation period. Motility detection is possible due to the semisolid nature of the medium. Growth radiating out from the central stab line indicates that the test organism is motile.

SSA – Salmonella Shigella Agar is moderately selective and differential media for the isolation of pathogenic enteric bacilli, especially those belonging to the genus Salmonella. Differentiation of enteric organisms is achieved by the incorporation of lactose in the medium. Organisms that ferment lactose produce acid which, in the presence of the neutral red indicator, results in the formation of red colonies. Lactose non fermentors form colorless colonies. The latter group contains the majority of the intestinal pathogens, including Salmonella and Shigella.

Handling, Storage, and Transportation of Health Care Waste

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

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

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

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

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

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

Health Impact

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

Waste segregation and packaging

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

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

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

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

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

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

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

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

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

container at the point of production.

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

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

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

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

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

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

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

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

On – site collection, transport, and storage of waste

Collection

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

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

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

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

Storage

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

Temperature climate:	72 hours in winter
	48 hours in summer
Warm climate:	48 hours during the cool season
	24 hours during the hot season

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

Recommendations for storage facilities for health care waste

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

References

WHO: Wastes from Health Care Facilities

Microxpress Range of Products available for the culture of *Streptococci* species

Dehydrated Culture Medium

Azide Dextrose Broth

A selective medium for detection and cultivation of Streptococci in water, sewage, milk and other materials.

Bile Esculin Agar

A differential medium for isolation and presumptive identification of Group D Streptococci / Enterococci from foods.

Bile Esculin Azide Agar

A medium for selective isolation and presumptive identification of fecal Streptococci.

Blood Agar Base

A non selective general purpose medium to which blood may be added for use in isolation and cultivation of Streptococci and other fastidious pathogenic organisms like Neisseria, etc and also for detection of hemolytic activity.

Reddys Differential Agar, Modified (Lactic Streak Agar)

A medium for the qualitative and quantitative differentiation of lactic Streptococci.

Streptococci Selection Medium

A medium for selective isolation and cultivation of Streptococci.

Todd Hewitt Broth

Medium used for cultivation of group A hemolytic Streptococci used for serological studies.

Supplements

Staph – Strepto Supplement

An antibiotic supplement recommended for the selective isolation of Streptococcus species.

Strepto Supplement

An antibiotic supplement recommended for the selective isolation of Streptococcus species.

Ready Prepared Media

Hartley Broth

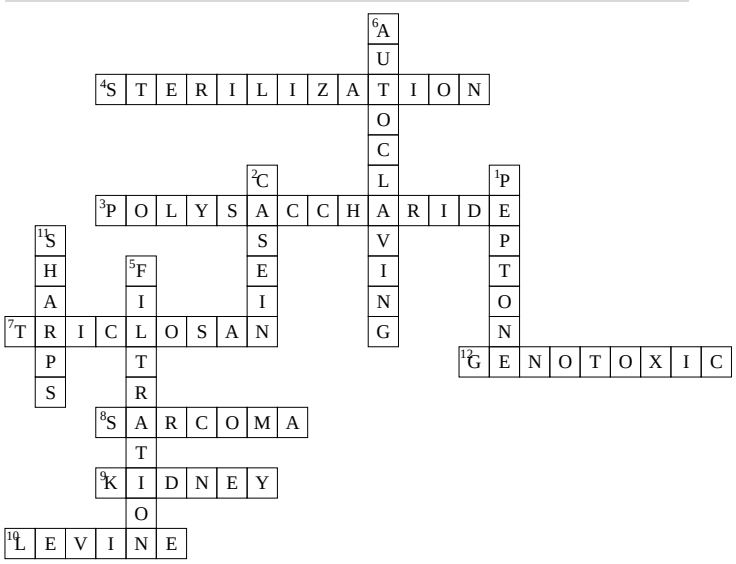
A medium for cultivation of wide variety of bacteria from blood specially fastidious Streptococci and *Corynebacterium diphtheriae*.

Biochemical Identification Test kit

Strep Identification Kit

A panel of 12 tests for identification of Streptococcus species (kit contains sterile broth for esculin hydrolysis, Voges Proskauer , arginine utilization, PYR hydrolysis, ONPG utilization and 7 different carbohydrates – glucose, arabinose, sorbitol, mannitol, sucrose, raffinose, ribose). Reagents supplied with the kit: Baritt reagent A, Baritt reagent B and creatinine for VP test, PYR reagent.

Track your brain



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 DIRECTIONS :

Surgical, postoperative, non surgical dressings	–	Use undiluted
Pre & post surgery, skin cleaning & disinfection	–	Use undiluted
Surgical/Sitz bath	–	Add 50 ml of NUSEPT™ in 1L of water & use
Antisepsis during minor incisions, catheterization,	–	Use undiluted scopy, first aid, bites, cuts stings etc
Midwifery, nursery & sickroom	–	Use undiluted
General surface disinfection	–	Add 100 ml of NUSEPT™ in 1L of water and gently mop the floor or surfaces

Highlights of the coming issue

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