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Editorial

Contents

Yet another issue and a lot more of information to keep you updated on many new facets that have been and are keeping pace with the need for a hygienic and clean practice of many new urgencies.
With the globe facing newer and adverse challenges it is a necessity that we keep ourselves updated on all facts and are prepared to rid of all myths. Though we look forward to better health care and hygiene, lets not forget what we already have.

Mini Review gives a description of tetanus, which has been a scourge on humanity for ages and is referred to as 'disease of antiquity', never the less this disease and its manifestations still continue to haunt us at a time when there are developments that have taken place around the globe to combat such emergencies. There are situations in which slack attitudes and negligence can lead to fatalities or irreversible damage to a tetanus victim and hence there is a need that we are aware of the signs, symptoms and immediate measures that we can take to be effectively helpful in order to prevent further mishaps.

Years after doctors and surgeons have come to a conclusion for antiseptic surgery, there may be conditions that are not dealt with seriously that can cause a patient her life, therefore at all times there should be maximum attention paid in this direction; that a life saving or a life sustaining activity such as a surgery should be considered an optimum priority for hygiene. Our section on 'Current Trends' deals with Antisepsis in Surgery which gives some quick tips on maintaining such a condition that is best for both; the patient as well as the health care provider.

A vaccine for the viral disease, Yellow fever was given by none other than Max Theiler, a Nobel laureate of Medicine and Physiology in 1951, who was a member of the Royal College of Surgeons (FRCS), he is the scientist who is in 'In Profile' for this issue.

Diphtheria has claimed many lives prior to the era, when, vaccination is a prophylactic measure and antibiotics a cure. *Corynebacterium diphtheriae* is the etiological of diphtheria, also called Klebs – Loeffler bacillus, is a Gram positive, aerobic organism that is immobile and in ramified aggregates appears like Chinese alphabets, is the 'Bug of the Month'. It is interesting how this organism causes toxin associated complications which can be the sole cause of death of an individual.

'Did you know' that Lowenstein Jensen (LJ) medium which is used so commonly and effectively for the growth of mycobacterial species is a complex media which contains egg and is by itself one of the most significant growth media for the genus?. Well, though sometimes the media is readily available for isolation of mycobacteria, its good to know the underlying principle of preparation and the precautions that are taken to ensure proper sterilization and best performance.

Best ideas are Great when you can share and speak about them, in the most appropriate manner. So when we open our mouths to talk, we also open a route of communication, which can make the other feel interested or disgusted. So lets have Great ideas and have good means to share the word. Our 'Best Practices' gives an insight on Oral Hygiene, to keep our mouths smelling good and clean, and the confidence to give those Wow!!!! Smiles and happy laughs.....So go ahead, and believe that when you smile...the world smiles with you.

As you enjoy yet another issue of our effort to communicate, lets remember that communication and contact is best Two Sided. Let's hear from you.....your ideas and suggestions are always appreciated.

Tetanus: A Scourge

Tetanus is referred to as, 'disease of antiquity'. However though the scourge of tetanus may have been virtually eradicated from the West, it still continues to stalk the developing countries, including India.

Tetanus can have localized as well as generalized symptoms, one of the signature symptoms may involve a locking of the jaw, therefore tetanus is also called 'lockjaw', this may be the result of striated skeletal muscle spasms .

Microbiology

The disease is caused by Gram positive obligate anaerobic bacillus, *Clostridium tetani*, which has a characteristic tennis racket appearance due to the presence of a terminal endospore. The bacterium is unable to survive in the presence of oxygen, therefore free oxygen can have lethal effects on the bacterium. As the bacterium matures it develops the spore. *Clostridium tetani* is found abundantly in soil, manure, and even in contaminated heroin.

Epidemiology

The disease takes a toll in places and areas where there is poverty, lack of education, overcrowding with poor or no sanitation, and inadequate health services, other factors which may aggravate the condition include: a susceptibility to physical injury, lack of facilities and education to care for and manage such injuries, and the inadequacy of effective immunization programmes. Even physical injury that is caused in cutting umbilical cord or even an operative procedure may be responsible for a tetanus infection. So also agricultural workers who work barefoot on soil rich in *C. tetani* spores are also at a high risk of acquiring a wound that can act as a 'portal of entry' for the bacterium.

However it may be noted that the high risk groups vary from one country to another. In developing countries, neonates form the single most important high risk group, and hence the epidemiology of neonatal tetanus is considered separately. Other important risk groups include the infants and children that undergo circumcision.

High risk groups in Western or developed countries chiefly include patients in the older age groups. This is because there is a fall in the protective antitoxin levels in the blood of older patients, unless they have received booster doses of tetanus toxoid.

Immunocompromised individuals, particularly those with HIV infection, constitute a high risk group everywhere in the world.

Signs and Symptoms

Generalized tetanus: is the most common type of tetanus. The generalized form usually presents with a descending pattern. The first sign is trismus, or lockjaw, and the facial spasms called risus sardonicus, followed by stiffness of the neck, difficulty in swallowing, and rigidity of pectoral and calf muscles. Other symptoms include elevated temperature, sweating, elevated blood pressure, and episodic rapid heart rate. Spasms may occur frequently and last for several minutes with the body shaped into a characteristic form called opisthotonos. Spasms continue for 3 – 4 weeks, and complete recovery may take months.

Though the clinical picture gives the impression of a downward movement of the toxin in the spinal cord, this is indeed not so. The sequence of involvement of the skeletal muscles in generalized tetanus is determined by the transport of toxin through the general neural pathway to the CNS. Toxin reaches the CNS through neural pathways of varying lengths. The neural pathways connecting the muscles of the jaw, face and neck, to their motor centers in the brain stem and spinal cord are the shortest, so that the toxin reaches these centers earliest. The effect of tetanus toxin is therefore first manifest in these muscles, and then in muscles innervated by longer neural pathways, e.g. muscles of the trunk and extremities. The use of the term, 'descending tetanus' for the usual form of generalized tetanus is thus incorrect, there being no true descending spread of the toxin down the spinal cord. In fact, generalized tetanus can be looked upon as the occurrence of multiple local tetanus due to uptake of toxin from the blood by nerve terminals throughout the body.

Ascending tetanus: is a form of the disease which first involves the lower limb and spreads upwards to involve the whole body. In this form of tetanus, probably very little toxin enters the bloodstream, the regional neural pathway being far more important than the general neural pathway for toxin transport to the CNS. After first producing local tetanus, the toxin ascends along the spinal cord and the medulla to produce an ascending form of the disease. In most patients, however, the toxin after first producing local tetanus does find its way into the bloodstream and is thus distributed to all the muscles of the body. The general neural pathways involving retrograde axonal transport through nerves in the whole musculature, are thus soon involved in the spread of the toxin to the CNS.

Neonatal tetanus: is a form of generalized tetanus that occurs in newborns. Infants who have not acquired passive immunity because the mother has never been immunized are at a high risk. It usually occurs through infection of the unhealed umbilical stump, particularly when the stump is cut with a non – sterile instrument.

Local tetanus: is an uncommon form of the disease, in which patients have persistent contractions of muscles in the same anatomic area as the injury. The contractions may persist for many weeks before gradually subsiding. Local tetanus is generally milder, only about 1% cases are fatal, but it may precede the onset of generalized tetanus.

Cephalic tetanus: is a rare form of the disease, generally occurring either due to injury to the head or with otitis media (ear infections) in which *C. tetani* is present in the flora of the middle ear. There is an involvement of cranial nerves, especially in the facial area.

Physiopathology

C. tetani produces two toxins – tetanolysin and tetanospasmin. The term ' tetanus toxin', in clinical parlance, is reserved for tetanospasmin, a very potent neurotoxin which reaches the nervous system and is responsible for the disease.

C. tetani is strictly an anaerobe, and in order to germinate,

multiply, and produce exotoxin, it requires a low oxygen tension. Healthy tissues are well oxygenated with a high oxygen – reduction potential (Eh), so that even if spores gain entry to such tissues, germination cannot occur. Wounds which have areas of necrosis, ischaemic wounds, or wounds contaminated by soil, manure or foreign bodies, have a low oxygen – reduction potential, and form fertile breeding ground for germinating spores of *C. tetani*. Wounds secondarily infected by other bacteria, also allow germination of tetanus spores. Tetanus toxin is only released after autolysis occurs.

The incubation period of tetanus averages 6 – 14 days, but varies from 2 days to several weeks. The incubation period and severity of the disease are chiefly influenced by conditions present within the wound. The severity is related to the amount and rate of toxin production in the wound, the total amount of toxin that reaches the central nervous system, and the rate at which it does so. These factors are dependent on the local conditions in a wound that favors germination of tetanus spores and the elaboration of tetanus toxin. It is also probable that different strains of *C. tetani* may produce varying quantums of toxin. A valuable clinical guide to the severity of the disease is the period of onset – the time interval between the first reflex spasm – as this interval is related to both the amount of toxin and the rate at which it reaches the central nervous system.

Spread of Toxin:

Toxin elaborated by germinating *C. tetani* spores in a wound, or injected under the skin of an experimental animal, spreads in the following manner:

Entry into muscle: toxin enters the underlying muscle, which is demonstrated by the specific muscle – blocking effect of the anti – toxin – tetanus fails to develop when the toxin is injected into the skin over a muscle which has been previously injected with antitoxin. From the affected muscle, the toxin spreads to adjacent muscles, so that the area over which it ascends the neural pathways increases, and the increasing number of nerves are involved in the transport of the toxin to the central nervous system.

Spread into the lymphatic system: toxin from a tissue depot quickly spreads to the regional lymph nodes. Injecting and 'blocking' the regional lymph nodes with antitoxin, prevents the development of fatal tetanus in mammals. From the lymph nodes, the toxin ultimately drains via the lymphatic system into the bloodstream.

Spread in the bloodstream: toxin absorption into the bloodstream takes place mainly via the lymphatics, but probably also occurs directly through the capillaries near the depot of toxin. In man, the greater part of the toxin is also probably absorbed into the blood. The spread of toxin in the bloodstream is an important route, yet it is not an indispensable condition for the development of lethal tetanus. Thus tetanus toxin may be neutralized or blocked by an appropriate dose of tetanus antitoxin given intravenously; however, if there is a sufficient depot in a muscle, lethal ascending tetanus develops because of transport of the toxin to the nervous system along neural pathways. The final and chief pathway that the toxin follows to reach the CNS, is a retrograde transport within axons of neural pathways – both locally where the toxin is produced, and from muscles all over the body, the toxin being distributed to these muscles via the bloodstream. It is important to realize that tetanus toxin is transported in sensory and autonomic neurones as well as motor neurones. Toxin transported in a retrograde direction is peripheral sensory nerves, stops and accumulates in the posterior root ganglion; that transported within adrenergic nerves reaches the lateral grey horns of the thoracic spinal cord, where the cell bodies of preganglionic sympathetic neurons are located. Toxin may ascend in the epineurium and the perineurium of nerves within the muscles, but it is the toxin which is transported upwards within the intra – axonal compartment that reaches and accumulates within the brain stem and spinal cord.

Within the spinal cord and brain stem, the toxin leaves the anterior horn cells and the motor nuclei in the brain stem to cross the synaptic cleft and reach the terminals of the inhibitory neurones, where it is bound to receptors on the pre-synaptic membranes.

Diagnosis

The diagnosis of tetanus is solely based on the clinical findings; there is no laboratory test which is diagnostic for the disease. The blood count may show a mild polymorphonuclear leucocytosis and the sedimentation rate may be moderately raised. The urine often shows a trace of albumin. In several cases, it has been often observed a mild rise in serum transaminase and a significant increase in the creatinine phosphokinase. Absence of a wound does not in any way exclude tetanus.

Even if a wound is present in a patient with tetanus, cultures for tetanus bacilli on anaerobic media are rarely positive, the bacilli being probably phagocytosed or perhaps lodged deeper in interstitial tissues. A reasonably reliable method to demonstrate *C. tetani* in tissues is to inoculate the debrided tissue into the hind leg of mice (in concordance with Koch's postulates, of identifying an etiological agent of a disease).

However to medical practitioners who have a good experience of treating and knowing tetanus symptoms would consider a true risus sardonicus in particular for all of pathognomonic purposes associated with the disease. Risus sardonicus is also referred to as trismus sardonicus or alternatively simply trismus. It is also important to distinguish trismus produced by tetanus from spasms caused due to local pathologies in the mouth, throat and inflammation of the temporomandibular joint. Alveolar or peritonsillar abscesses in particular can produce severe trismus and should be excluded by a careful local examination. Severe trismus is invariably associated with the typical facial expression of tetanus – a risus sardonicus.

However there is only one substance that is known so far that can mimic the true symptoms of tetanus, Strychnine. The substance is a white crystalline alkaloid that is used to poison small vertebrates like rodents. The major differentiating feature is that in between the spasms or seizures produced by strychnine poisoning , the muscles lack the extreme rigidity which is always observed in tetanus, however the difference may be apparent to a physician who has experience in dealing with either cases. Meningitis and meningo – encephalitis can also produce trismus, rigidity, seizures, neck retraction and opisthotonos. A true risus sardonicus is, however, never present, and in case of doubt these diseases can be differentiated by a cerebrospinal fluid examination which is always normal in tetanus.

Prognosis and Mortality

The prognosis and mortality of tetanus depend on the severity of the disease. It is doubtful whether conservative management of severe tetanus, ie, sole reliance on sedation and muscle relaxants, significantly alters the mortality of the severe forms of the disease. The ultimate result in such patients seems to be predetermined,

even before treatment on conservative lines can be instituted.

Prognostic factors

Numerous prognostic criteria are evident from the history as well as from clinical observations in the early phase of the disease.
The major factors that influence or predict the course of the illness are as follows:

- Incubation period and period of onset: The value of the incubation period in prognosis was recognized as early as 1898. As a general rule, a short incubation period points to the ultimate evolution of severe tetanus and is associated with a greater risk of death. Even so, it is important to stress the fact that at times even patients with long incubation periods have high mortality rates.

- Clinical Severity: The prognosis of Grade I tetanus is excellent, of Grade II tetanus good, and of Grades III and IV tetanus, grim. The grading of the severity of tetanus is generally apparent within 4 days of administration to the hospital. However, in some instances, what initially appears to be Grade I or II tetanus may graduate to Grade III or even IV after a week or ten days.

  The two main clinical features that point to an adverse prognosis are the presence of spasms, and the occurrence of autonomic disturbances, characterized by sharp fluctuations in the heart rate and systemic arterial blood pressure. The occurrence of spasms is the single most adverse clinical factor in determining the prognosis. The stronger the spasms and the greater their frequency, the worse the prognosis. Patients who have a well-nigh continuous spasms with barely any intermission. Almost always die when on conservation management. Occasional or short – lasting spasms, as in Grade II tetanus do not indicate a high mortality. The marked difference in the prognosis of Grades I and II tetanus as compared to grades III and IV tetanus is chiefly related to the occurrence and severity of the spasms.

- Autonomic storms: constitute an independent and adverse prognostic feature. They always occur against a background of frequent spasms so that a combination of frequent spasms and autonomic cardiovascular disturbances (Grade IV tetanus) are of very grave importance. Bradyrhythms carry a worse prognosis than tachyrhythms, and this fact holds true even in patients under intensive care after induced paralysis and on ventilator support.

- Period of apnoea: are of grave prognostic significance when patients with Grade III tetanus are being treated conservatively. This clinical feature, however, loses its prognostic importance in patients on the ventilator after induced paralysis.

- Laryngeal spasms: are also of serious prognostic significance, and are an important cause of death in patients with Grade II tetanus, except when a prior elective tracheostomy has been performed on these patients.

- Complications: which adversely affect prognosis even when patients are on ventilator support are pneumonia, aspiration pneumonia, major lobar atelectasis where expansion of the lobe is difficult or delayed, the adult respiratory distress syndrome (ARDS), and sepsis (Sepsis may be a result of nosocomial infections that may be acquired in the medical facility).

- Pyrexia: particularly over 39 deg. C, and unrelated to obvious infection, is a bad prognostic sign. Sudden episodes of hyperpyrexia which may remain undetected unless rectal or core temperatures are monitored, are indeed of ominous importance, and constitute an important cause of sudden, otherwise unexplained death.

- Renal shutdown: particularly when associated with severe hypotension, also carries a high mortality, though some patients have been salvaged by expert management which includes dialysis.

- Age: prognosis is worse and the mortality higher in newborns. In contrast, death rate is lowest in older children, and young adults, it again progressively rises after 50 years. A background of ischaemic heart disease, or of cerebrovascular disease worsens the prognosis in older patients.

- Poor Nutrition: When severe tetanus (Grades III and IV) occurs against a background of a poor nutritional state, the prognosis is doubly grim. Recovery can never ensue under such circumstances, unless good nutrition with a very high caloric intake is maintained.

- Management Strategies: Intensive care management with the use of induced paralysis and ventilator support has markedly altered the prognosis and reduced the mortality in severe tetanus. In spite of the adverse prognostic features described, recovery can occur in the majority of seriously ill patients.

Treatment and Management

General Facets

Mild (Grade I) tetanus poses no serious problems in management except when complicated by a serious wound which in itself can cause death. It, however, needs to be re – stressed that mild tetanus can graduate over a period of 8 to 10 days to moderate (Grade II) or even severe (Grades III and IV) tetanus. All patients with tetanus, therefore, need to be hospitalized and, whenever facilities exist, they should be admitted to an Intensive Care Unit (ICU) or other special units set aside for this purpose.

Transport of a patient with tetanus can be hazardous. In patients with mild or moderately severe tetanus, sedation and decrease in muscle rigidity can be achieved by injecting diazepam or chlorpromazine intramuscularly, prior to transporting the patient. In cases where there is improper transfer of patients with severe tetanus the rate of fatalities are higher, therefore tracheostomy may be advisable prior to the transfer.

The first principle on admission to hospital is to gauge the severity of tetanus since the grade of severity determines the mode of management. Attempts at eliciting a detailed or exhaustive history are best avoided. It is sufficient to determine the incubation period if possible, and the period of onset if spasms are present. In fact, quietly observing the patient yields as much, if not more information than the history. Careful observation over 10 minutes for example, may enable the clinician to detect mild spasms which otherwise would have been missed. The presence or absence of spasms, and their frequency and severity if present, are of crucial importance.

Patients with tetanus need not be confined to darkened rooms or wards. Though bright sunlight is best avoided, the use of heavy dark curtains as well as other measures to cut off all light, are unnecessary. Tetanus wards are best kept cool and well ventilated. Air conditioning the wards in tropical climes is more a necessity rather than a luxury, particularly in summer.

Also good nursing is of utmost significance in treating patients suffering from tetanus. The nurses attending to such patients should be well trained in recognizing any signs and symptoms that may prompt the next stage of the disease. Routine medical examinations and frequent sessions of sponging the patient should be avoided. However, immediately after the admission of the patient, as soon as basic care has been instituted, it is vitally important to thoroughly clean the skin of all dirt and dust, particularly in poor laborers who may not have bathed for many days. The nurses should also ensure that the bed, the patient's linen, as well as the floor of the tetanus ward are kept scrupulously clean.

Nurses should be trained in a manner by which they can monitor vital parameters, for instance should be able to read electrocardiograms and recognize arrhythmias. They are also trained to detect abnormal physical signs in the chest, and to anticipate and detect complications which they often bring to the doctor's notice. Also they should be trained well in the 'no touch' technique.

After the initial measures have been instituted, it is considered essential that a dose of antitoxin be administered to the patient, however a skin sensitivity test should be done prior to the injection to test for any visible reaction, except in newborns where such a test may not show. Human tetanus immunoglobulin (HTIG) is generally considered to be superior to the equine antiserum, and produces no hypersensitivity reaction, and if available should be given in preference to the latter.

Penicillin and Tetracycline are the mainstay of the treatment for tetanus. Also in cases of moderate tetanus metronidazole is recommended. Patients treated with the latter had a significantly, lower mortality rate, and a shorter stay in hospital.

Conservative Treatment – use of sedatives and Muscle Relaxants
This therapy constitutes the mainstay of the traditional conservative management of tetanus, and its aim is to reduce rigidity and control spasms without significantly depressing respiration. It is however also the treatment of choice in mild and moderate tetanus. In countries like ours this mode of treatment is used even in cases of severe tetanus. Sedatives and muscle relaxants are used in a manner such that there is no depression in respiratory functions which is the main cause why there are tetanus associated deaths. Drugs used in this respect mainly include diazepams, chlorpromazine, phenobarbitone, paraldehyde and mephenesin.

Tracheostomy: is mandatory in severe (Grades III and IV) tetanus. It should also be preferably done in Grade II (moderate) tetanus because even in an intensive care setting, an important preventable cause of death is a sudden prolonged laryngeal spasm leading to fatal asphyxia.

Corticosteroids in tetanus: Betamethasone have been beneficial in tetanus with significant reduction in mortality.

Active immunization with Tetanus toxoid as a treatment measure
It is important to know that a tetanus attack does not confer immunity against the disease. All patients after recovery from tetanus should, therefore, be actively immunized with tetanus toxoid. The first dose should be given during convalescence and subsequent booster doses at recommended intervals. Alternatively active immunization with tetanus toxoid can be started as soon as the disease is diagnosed and the second dose of the toxoid is administered approximately 4 to 6 weeks after the first, at the time of discharge; the third dose is scheduled 4 weeks after the second.

Prevention
The scourge of tetanus can be almost totally eliminated particularly in developing countries, by active immunization with alum-adsorbed tetanus toxoid (ATT) which has an estimated failure rate below 4 per 100 million immunocompetent individuals, and by proper care and management of wounds. The emphasis in prevention should unquestionably be on active immunization. It is the most effective prophylactic measure and is the only method of preventing tetanus following trivial and unnoticed injuries.
Doctors and paramedical personnel recruited from cities to serve in rural areas are never half as effective as when those involved in health care are locally recruited from the rural areas concerned. Preventive medicine in the villages of poor countries does not necessarily require formal medical education, and the sooner this is realized, the better it will be for improving health in these countries.

Tetanus neonatorum would be well-nigh extinct if every village had well-informed (if not given formal trained and educated) women who spread the information of hygienic midwifery and improve the standards of hygienic care of both the mother and the newborn infant.

Active immunization: In an individual younger than 7 years of age, primary immunization is optimally started during and is achieved by the intramuscular injection of the triple vaccine – Diphtheria, Pertussis and Tetanus (DPT). Three doses of the vaccine are given at 4 to 8 weekly intervals starting when the infant is 8 weeks old. In certain cases a fourth dose is also recommended. In countries like India a three dose program of the vaccine are carried out. Protection is absolute for one year, but partial protection remains for many years. Even so a booster dose at 4 to 6 years of age is always given. A compulsory and well implemented vaccination programme against tetanus in infants, with a booster dose at 4 to 6 years of age, would greatly increase the number to immunized individuals, especially those from the high risk population.

Primary immunization: In pregnant women is achieved by two injections of Td toxoid at monthly intervals preferably during the second or third trimester. There is admittedly no convincing evidence that the Td toxoid administered in the first trimester may be harmful to the fetus.

It is noted that an attack of tetanus does not confer immunity, so that the patient needs to be actively immunized after recovery. The first dose of tetanus toxoid should be given during convalescence and the next two doses at the recommended intervals.

Immunization after injury: When a patient presents with a wound or injury, tetanus prevention commences with cleaning the wound with copious irrigation, removal of any foreign body and debridement of the devitalized tissues. The use of antibiotics depends on the nature of the wound and is a matter of clinical judgement. The nature of immunization depends on the type of wound and the extent of past immunization as judged from a carefully taken history. However medical practitioners classify a wound as tetanus-prone or non tetanus-prone. A tetanus-prone wound is a wound older than six hours and is contaminated, ischaemic or infected. A non tetanus-prone wound is less than six hours old, is clean, superficial and linear. This principle may not hold good in countries like India, since some trivial injuries that may not be visible or sometimes even forgotten by the patient may lead to a portal for tetanus infection. Therefore it might be better to consider wounds as minor, uninfected (nontetanus-prone) and major, infected (tetanus-prone).

Overall Critical care

The current mode of management of severe tetanus (induced paralysis with ventilator support) has prolonged the natural history of the disease to well over 3 to 6 weeks. Overall critical care thus assumes increasing importance. This involves both clinical awareness and a follow-up of relevant investigations.

Reference:

Tetanus by Farokh Erach Udwadia; 1994.

Encyclopedia

Millipore Filters

This type of filters are made from pure and biologically inert cellulose esters. They are prepared as thin porous, circular membranes of about 150 µm thickness. The filters have different porosity. The assemblage consists of funnel shaped inlet and a tube like outlet. In between these two the filter is fitted.

The outlet can be connected to a vacuum pump to suck known amount of air. After collecting required volume of air through the filter, it can directly be placed onto the surface of a solid medium. After incubation colonies formed can be counted.

Millipore filters are in use in various industrial sectors including chemical, analytical and agriculture. These also find application in food processing, biotechnology and environment related aspects.

The filter can be selected on the following basis:

- Particle retention efficiency
- Fluid flow rate
- Particulate loading.

General guidelines for selecting a filtration system:

1. Select the filter material which is chemically compatible with the fluid to be filtered and that best fits your application.
2. Determine if you need a single-stage or multistage filtration train. For multistage filter systems, select the final filter first. Multistage filtration is necessary with plugging streams.
3. When choosing the final filter select the smallest microorganism to retain then choose the appropriate pore size.
4. The pre-filter train (clarification and pre-filtration) before the final filter is then chosen to give the best combination of retention and capacity. The final pre-filter is chosen to have a retention close to that of the final filter.
5. Check the differential pressure to see if it is compatible with the filter and the process. The effective final differential pressure across the cartridge (change-out pressure drop) is a function of the particle/filter interaction. In cases of fluids containing deformable particles, the final differential pressure should be lower than the maximum differential pressure that is known for the particular material/fluid.

Normal Flow Filtration (NFF): Filtration systems for normal flow filtration processing generally consists of a series of filter cartridges, with each cartridge in the series protecting and extending the life of the next filter cartridge. Choosing the correct type of each application will lead to an optimized filtration train and reduced overall filtration costs. Due to many different types of feed solutions and filters, choosing the best filter train offers a unique challenge.

Before designing a liquid filtration system, select the design basis. The two most common parameters involved in filter sizing are throughput (also referred to as capacity, expressed in volume of fluid filtered before filter change-out is required) and flow rate.

The sizing method depends largely on the nature of the fluid to be filtered, and how plugging it will be for the final filter selected based on the microorganism removal requirements.

Sizing is typically based on flow rate in applications where the fluid has low amounts of particles and colloids, and therefore has low or non-plugging for the selected filter. It is the case in most bottled water processes, where 0.22 µm or 0.45 µm membrane final filtration is selected for microorganism removal. In such cases, sizing will be calculated by selecting a flow rate value per unit of filter area. This value is selected so that the initial differential pressure created across a new filter at this flow rate is low. Filter manufacturers provide a chart showing the pressure differentials as a function of flow rate for a specific filter type and a surface area. Based on these charts, the flow rate per unit of filter area can be selected, and the total surface area required for the process is obtained by dividing the flow rate of the process line by this flow rate per unit of filter area.

For applications where the fluid will have a medium of large amount of plugging particles (some wines and most beers fall into this category), it will be necessary to study the plugging profile of the filter by using small scale testing in order to perform the necessary filter sizing calculations. The 0.45 µm pore size is used to recover bacteria and other microorganisms for many samples and environments – almost to the exclusion of other pore sizes. Only rarely are other sizes used for growth and recovery of microbes.

Overall: Recovery is much more complex than the retention of microorganisms on the surface of the membrane filter and the influence of pore size. It is a combination of factors that may include:

- The microorganism species and its condition – each microorganism has the potential to react differently.
- The sieving effects of the pore size as it relates to the retention of microorganisms.
- Types of medium and the selectivity.
- Structure and chemistry of the membrane filter.
- Environmental conditions (example; moisture, incubation and temperature).

If pore sizes other than those indicated by industry standards are used, they should be validated on relevant samples and media, and compared to 0.45 µm. The 0.45 filters gave the most consistent recoveries across a variety of test systems and did not allow passage of the standard 0.2 sterilizing filter challenge microorganism, *B. diminuta*, under typical filtration conditions.

Antisepsis in Surgery

Antisepsis, the word has originated from Greek. Anti = against, Sepsis = putrefaction (decomposition). Infection in surgical practice remains one of the most common causes of increased morbidity and mortality. In the era of multi-resistant organisms, a clear understanding of the techniques used to prevent surgical infections is very important. Asepsis in surgery mainly involves 3 things – asepsis (freedom from infection or prevention of contact with microorganisms), aseptic technique (instruments, air, drapes, gloves and gowns are free from microorganisms) and antisepsis prevention (of infection by inhibiting or arresting the growth and multiplication of germs / infectious agents).

Infection in surgical practice remains one of the most common causes of increased morbidity and mortality. In the era of multi-resistant organisms, it is important for surgeons to have a clear understanding of the techniques used to prevent surgical infections.

History

In the middle of the nineteenth century, post-operative sepsis infection accounted for the death of most of the patients undergoing major surgery. Joseph Lister, a British surgeon, was busy researching the inflammation of wounds, at the Glasgow infirmary. In 1865 when, Louis Pasteur suggested that decay was caused by living organisms in the air, which on entering matter caused it to ferment, Lister made the connection with wound sepsis. A meticulous researcher and surgeon, Lister recognized the relationship between Pasteur's research and his own that microbes present in the air were the likely cause of the putrefaction and needed to be destroyed before they enter the wound. Lister had heard that in Carlise, sewage was being treated with 'carbolic acid' and that fields treated with the affluent were free of a parasite causing disease in cattle. Lister started to clean wounds and dress them using a solution of carbolic acid. And in 1867, he announced at a British Medical Association meeting, that his wards at the Glasgow Royal Infirmary had remained clear of sepsis for nine months. Initially his methods met with indifference and hostility but gradually his methods were accepted. In 1870 Lister's antiseptic methods were used by German doctors during the Franco-Prussian war. In Germany, by 1878, with a German physician Robert Koch demonstrating the usefulness of steam for sterilizing surgical instruments and dressings, German surgeons began to practice antiseptic surgery, which involved keeping wounds free from micro-organisms by the use of sterilized instruments and materials. With the introduction of antiseptic methods, surgery entered its modern phase.

Routes of sepsis in surgery:

There are mainly three possible routes for entry of organisms into operating wound - pre operative, during the operation and post operative. Pre operatively patients can get infected either from the health-care workers, other patients or cross infection from other surgical wounds, infected linen, blankets, utensils etc. During operation chances of infection are mainly from - improperly prepared skin of the patient (which may harbor organisms in the ducts of sweat glands, sebaceous glands and in the sides of hair follicles), improperly cleaned and disinfected hands and surgical gloves of the surgeons, drainage from the wound, improperly sterilized and prepared operation theater, surface material, operating theater personnel etc. Post operative infections are generally caused due to several of the mentioned reasons, and also due to the improper hygiene of the wound, post surgery. Also post operative sepsis may occur depending on factors that may predispose the wound to infections such as in a diabetic individual who may be more prone to a chronic wound.

The introduction of antiseptic surgery is considered one of surgery's major advances. In sanitized and antiseptic hospital environments living, potentially deadly, microbes are inactivated or often destroyed. Patients are safer from primary and secondary infections.

Areas of the body treated by general surgery include the stomach, liver, intestines, appendix, breasts, thyroid gland, salivary glands, some arteries and veins, and the skin. The brain, heart, eyes and feet, to name only a few are areas that require specialized surgical repair.

Microbiology that underlies sepsis in surgery

The patient's own endogeous flora, which are present on the skin , mucus membranes or hollow viscera. The usual pathogens on the Gram positive cocci (like Staphylococci); however, Gram negative aerobes and anaerobic bacteria contaminate skin in the groin and perineal areas.

The interventions emphasize five distinct practices, including: education and training of health care providers who place and care for catheters, utilizing maximum sterile barrier precautions during catheter placement, skin preparation using 2% chlorhexidine, avoiding routine replacement of central lines as a strategy to reduce infection, and using antiseptic or antibiotic coated lines in the event that infection rates remain high despite adherence to the above measures.

Practices to avoid sepsis during surgery

Skin preparation: hair removal should be done just prior to surgery (not the night before), and ideally should be done with clipper rather than a razor. Many surgeons even recommend a shower with antiseptic soap the night before surgery. The patient's skin should be treated at least for five minutes with the antiseptic solution prior to making the incision

Antibiotics: may not be needed at all in surgical procedures, however they can be routinely used in certain antiseptic surgery. Some operators may inject the patients with 1g chloramphenicol intravenously immediately before the surgery to prevent infection during the course of the surgical procedure.

Operating room: the operating room should be adequately sterilized with effective air sanitizers containing antimicrobial chemicals such as benzalkonium chloride preferably in an alcohol base such as isopropyl alcohol. In addition to these the entry and exit of personnel in the room should be limited, since the constant movement can aid in dust particles being displaced, which in turn can be a cause of nosocomial (hospital induced) infections.

Wound / bandage care: wound and bandage care would involve post operative precautions because such wounds can give rise to

sepsis and surgical site infections (SSI).
Though all surgical wounds are contaminated with microbes, but in most cases, infection does not develop because innate host defenses are quite efficient in the elimination of contaminants. A complex interplay between host, microbial and surgical factors ultimately determines the prevention or establishment of a wound infection.

For diabetics: maintaining a normal blood glucose levels is of utmost importance during the surgery and the post operative period. Elevated levels of blood sugar are linked to a higher risk of post surgical infections.
Watch for signs of infection: one should always be watchful for the clinical signs and symptoms of post operative infections, such as the formation of pus.
Antiseptic surgery and antiseptic solutions: use chlorhexidine 5% concentrate to make two solutions (1) weak solution of 1/2000 of the active agent in water. Use this for soaking towels, etc. (2) A strong solution for instruments. Make up small quantities of solutions frequently, make them up hot, and clean out the containers well between the batches.
Sterilizing equipment and drapes: soak everything which will come in contact with the wound in the antiseptic solutions for at least 30 minutes. Soak sutures and gloves in the solution overnight.
Swab the trolley with the antiseptic solution, or put the instruments on a solution soaked towel. Keep two bowls near the operating table, one containing water and the other containing antiseptic solution. When instruments have been used, wash them in water and keep them in the solution until you use them again. Shake off the excess solution before you use them.

Patient tissue handling: Handle the patient's tissue as little as you can, and try to keep the solution out of the wound as much as possible. Don't let it get into the body cavities.
If the wound is well sutured and is not expected to discharge, leave it open to the air. This is better than covering it with a questionable sterile dressing.

Following are the categories of antiseptics and disinfectants that are commonly used in surgical emergencies:

Group	Agent	Uses
Alcohols	Ethyl alcohol 70% Isopropyl alcohol 70%	Skin disinfectant
Quaternary ammonium compounds	Benzalkonium chloride Cetrimide Methylbenzethonium chloride Benzethonium chloride Cetalkonium chloride Cetylpyridinium chloride Dofanium chloride Domiphen bromide	Skin disinfectant Irrigations Eye drop preservative
Chlorhexidine and other diguanides	Chlorhexidine gluconate Chlorhexidine acetate	Pre-op skin disinfectant Treat wounds Bladder irrigations
Polymeric biguanidines	Polyhexamethelene biguanidine (PHMB)	Wound management Inter – operative irrigations
		Post operative dressings Surgical bath / Sitz bath Routine antisepsis during minor incisions, catheterization, scopy, erc.
Antibacterial dyes	Proflavine hemisulphate Triphenylmethane Brilliant green Crystal violet	Skin disinfectant Treat wounds and burns
Peroxides and permanganates	Hydrogen peroxide solution Potassium permanganate solution Benzoyl peroxide	Wound cleanser Gargles and mouthwashes Irrigations Skin disinfectant
Halogenated phenol derivatives	Chlorocresol Chloroxylenol Chlorophene Hexachlorophane/ hexachlorphene Triclosan	Skin disinfectant Medicated soaps and solutions
Quinolone derivatives	Hydroxyquinoline sulphate Potassium hydroxyquinoline sulphate Chlorquinaldol Dequalinium chloride Di-iodohydroxyquinoline	Treat wounds Throat lozenges Skin disinfectant

Uses of antiseptics:
Antiseptics are mainly used to reduce levels of microorganisms on the skin and mucus membranes. The skin and mucus membranes of the mouth, nose and vagina are home to a large number of what are usually harmless microorganisms. However, when the skin or mucus membranes are damaged or breached in surgery, antiseptics can be used to disinfect the area and reduce the chances of infection.
Common and general uses of Antiseptics in surgery:

- Hand washing – chlorhexidine gluconate and povidone iodine solution are often used in hand scrubs.
- Pre – operative skin disinfection – antiseptics applied to the operation site to reduce the resistant skin flora.
- Inter – operative irrigation – antiseptics are applied to disinfectant drainage from an open exposed wound.
- Mucus membrane disinfection – antiseptic irrigation may be instilled into the bladder, urethra or vagina to treat infections or cleanse the cavity prior to catheterization.
- Preventing and treating infected wounds and burns – antiseptic preparation are available over the counter to treat minor cuts, abrasions and burns.
- Treating mouth and throat infections – dequalinium chloride has both antibacterial and antifungal properties and is the active ingredient in throat lozenges.

Careful antiseptic surgery can help prevent many complications and avoid many mishaps that may arise during or after the surgery.

Max Theiler

Birth: January 30, 1899
Death: August 11, 1972
Nationality: South African / Swiss
Known For: Vaccine against Yellow Fever

Max Theiler was born on January 30, 1899, in Pretoria, South Africa, one of the four children of Sir Arnold and Emma Theiler. His father was a well known veterinary scientist. He attended local schools except for one year in Basle, Switzerland (His father was of Swiss origin), then went on to Rhodes University College, Grahamstown and the University of Capetown Medical School (1916 – 1918). Max then went to England to study at St. Thomas' Hospital and at the London School of Tropical Medicine, receiving his medical degree in 1922. In the same year he became a Licentiate of the Royal College of Physicians and a member of the Royal College of Surgeons.

In 1922 he joined the Department of Tropical Medicine at the Harvard Medical School, Boston, Massachusetts, first as an assistant then being appointed as an instructor. In 1930 he joined the staff of the international Health Division of the Rockefeller Foundation, becoming in 1951, Director of Laboratories of the Rockefeller Foundation's Division of Medicine and Public Health, New York.
His early work at Harvard, dealt with amoebic dysentery and rat bite fever. He also worked on the problem of yellow fever, a subject in which he had become interested whilst still in London. This was to become his major interest. By 1927 he and his colleagues had proved that the cause for yellow fever was not a bacterium but a filterable virus. He also demonstrated that the disease could be readily transmitted to mice. Previously, laboratory work on this topic had been done using monkeys as experimental animals; the use of mice enabled the cost of such research to be greatly reduced. In 1930, when he joined the Rockefeller Foundation, that body was engaged in a broad attack on the problem of yellow fever.
The Head of the department, Andrew Watson Sellards, had a particular interest in yellow fever. Following the success of the researchers at Rockefeller foundation, he and his collaborators – then working in Dakar, French West Africa – had isolated the virus in monkeys. Sellards brought this isolate called the 'French strain', to his laboratory in the United States. In his early work at Harvard, Theiler showed that the spirochete *Leptospira icteroides* has no involvement in yellow fever. Although the Reed commission had already documented that the etiological agent for the disease was a virus, a theory that this spirochete was involved had been persuasively argued by Hideyo Noguchi. Theiler's finding conclusively disproved this. Theiler also did some preliminary comparative immunological studies of yellow fever viruses from West Africa and South America. Theiler then propagated the French strain of virus in the brains of mice. This was an important finding since it lowered the cost of the research and also decreased the cumbersome use of monkeys to study the virus. Because of this contribution, the Rockefeller Foundation welcomed Theiler when he applied for a position for its International Health Division (formerly the International Health Board), in 1930. Theiler enjoyed the environment of the Foundation and remained associated with it until he retired in 1964. Here, Theiler and his colleagues worked on vaccines against the disease and eventually developed a safe, standardized vaccine, 17D, one advantage of which was its ready adaptability to mass production.

Theiler's path of Discovery

After Theiler's 1930 discovery that yellow fever virus can be propagated by passage in the mouse brain, he found that repeated passages in mice led to a progressive shortening of the incubation time and, importantly a successive reduction of the pathogenicity of the virus in monkeys. Theiler then developed a convenient test for measuring protective antibodies in mice. The technique also allowed a quantitative demonstration of the presence of antibodies in humans. This proved to be an important tool for mapping the epidemiology of infections and evaluating candidate vaccines. After Theiler's work on yellow fever, it became evident that mice could be used for similar research purposes and thus the initial work done by Theiler proved to be a stepping stone for many other researches and the like experiments.

Theiler and collaborators first demonstrated that the attenuation of virus obtained by passages in mice was not sufficient. This diminished the viscerotropic properties of the virus, which are the main source of the symptoms associated with yellow fever, but the capacity of the virus to attack the brain increased. To get around this problem attempts were made to use minimal doses of virus, but this approach also failed. Theiler and Whitman demonstrated that, paradoxically lower doses of virus gave a higher frequency of encephalitis in monkeys.
The critical experiments that solved this problem were performed by Theiler and his collaborators during 1935 – 1937. Different virus strains with different properties were carried through several hundred passages in different types of tissue cultures and repeatedly tested for their neurotrophic activity. The breakthrough came when the 'Asibi strain' of virus – the first ever isolated – was passed repeatedly in minced chicken embryos from which the central nervous system had been removed. Between the 89th and the 114th passage, a virus variant suddenly emerged that lacked both the viscerotrophic and the neurotrophic effects. Fortunately, the properties of this virus were stable, and its neurovirulence was not regained upon repeated passages in chicken embryo cultures containing brain material.

The first field trial was with the vaccine, started under the aegis of the Rockefeller Foundation in Brazil in 1938, was highly successful. And the continued use of more than 400 million doses for over 60 years of the 17D virus vaccine has proven it to be a remarkably safe and an effective product. The World Health Organization (WHO) guidelines regarding the vaccine have remained unchanged. Today the vaccine is still produced using the original methods: it is passaged in embryonated chicken eggs and stored as a frozen homogenate.
His other work for the Institute have been connected with the causes and immunology of certain disorders which include Weil's disease. He had also been engaged in research on dengue fever and Japanese encephalitis. The problem of poliomyelitis had been of great interest to him and he had discovered an apparently identical disorder in laboratory mice which is now sometimes called Theiler's disease (encephalomyelitis).

Dr. Theiler has been a contributor to two books, Viral and Rickettsial Infections of Man (1948) and Yellow fever (1951). In the same year Theiler's discovery earned him the Nobel prize in the category of Physiology and Medicine. He has also written numerous papers in the *American Journal of Tropical Medicine* and *Annals of Tropical Medicine and Parasitology*.
Honors awarded to him include the Chalmer's Medal of the Royal Society of Tropical Medicine and Hygiene (London, 1939), the Flattery Medal (Harvard, 1945), and the Lasker award of the Lasker Foundation (1949).

He married Lillian Graham in 1928. They have one daughter.
Max Theiler died on August 11, 1972.

References:

http://nobelprize.org/nobel_prizes/medicine/laureates/1951/theiler-bio.html

Enjoy the humour

The student reporter had just submitted his editorial for the day. Leaving the room, he began to reflect upon what he had written and decided to go back and change something in it.

"I have a few corrections to make on the editorial that I submitted," he told the editor.

The editor reached into the waste basket and pulled out the articles.

"All right, but make it snappy, the waste basket will be emptied in five minutes.

"Did you hear," asked the senior, "about our cross eyed professor getting fired?"
"No," replied the innocent freshman. "Why was he let out?"
"Because he couldn't control his pupils."

Thoughts to live by

- Attitude is more important than the past, than education, than money, than circumstances, than what people do or say. It is more important than appearance, giftedness, or skill. (W.C. Fields)
- A mind at peace, a mind centered and not focused on harming others, is stronger than any physical force in the universe. (Wayne Dyer)
- There are three constants in life.....change, choice and principles. (Stephen Covey)
- Give whatever you are doing and whoever you are with the gift of your attention. (Jim Rohn)
- How people treat you is their karma. How you react is yours. (Wayne Dyer)

Track your brain

Place one letter in each box or circle using the clue given. Using the alphabets in the circle complete the hint given below.

SERIES I

Causes mechanical complications in diphtheria.

			U				E				N	

Substance known to truly mimic symptoms of tetanus.

S	T	R				N			

Inflammation of the gums, that may result in pain, swelling and bleeding.

		N						S

The present formulation of the egg media, for the cultivation of TB bacilli was put forth by the researcher.

	E		S		

Temporary deodorizers to mask bad breath.

SERIES II

Is sometimes called Theiler's disease.

E							O		Y	E					

Is commonly incorporated into LJ antibiotic media.

				M			

Is a porous material that is commonly used in biological filter materials.

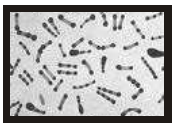
C								E

Is used as a skin disinfectant.

			R					

Tetanus related complications are attributed to this toxin.

Check your Answers on Page 16



Corynebacterium diphtheriae

Corynebacterium diphtheriae is a pathogenic bacterium that causes diphtheria. It is also known as Klebs – Loffler bacillus, because it was discovered by German bacteriologists Edwin Klebs (1834 – 1912) and Friedrich Loffler (1852 – 1915) in the year 1884.

Morphology

C. diphtheria is an aerobic, Gram positive organism, characterized by non – encapsulated, non – sporulated, immobile, straight or curved rods with a length of 1 – 8 µm and width of 0.3 to 0.8 µm, which form ramified aggregations in culture (looking like 'Chinese characters' or cuneiform arrangement) and sometimes which have clubbed ends. This may occur due to the incomplete separation of daughter cells after binary fission. The bacterium may contain polymetaphosphate aggregates called Volutin granules. These granules are more strongly Gram positive than the rest of the bacterial cell. Stained with Loffler's methylene blue, the granules take up a bluish purple color and hence they are called metachromatic granules, alternatively they are also referred to as Babes Ernst granules. They are often situated at the poles of the bacilli and are called polar bodies. Other stains such as Albert's, Neisser's and Ponder's stains have been devised for demonstrating the granules clearly.

Classification

Four subspecies are recognized: *C. diphtheriae mitischodis*, *C. diphtheriae intermedius*, *C. diphtheriae gravis* and *C. diphtheriae belfanti.* The four subspecies differ slightly in their colonial morphology and biochemical properties such as the ability to metabolize certain nutrients, but all may be toxigenic (and therefore cause diphtheria) or non toxigenic.

According to the clinical severity, diphtheria may be classified as: Malignant or hypertoxic, in which there is severe toxemia, with marked cervical adenitis (bull neck). Death is due to circulatory failure. There is high incidence or paralytic sequelae in those who recover. Sepsis, which leads to ulceration, cellulitis and even gangrene around the pseudomembrane; and hemorrhagic lesions, which is characterized by bleeding from the edge of the membrane, epistaxis, conjunctival hemorrhage, purpura and generalized bleeding tendency which increases fatality.

Culture characteristics

Growth is scanty on ordinary media. Enrichment with blood, serum or egg is necessary for good growth. The optimum temperature for growth is 37^{o}C (range 15 – 40^{o}C) and optimum pH 7.2. The usual media employed for cultivation of the diphtheria bacillus are Loffler's serum slope, and colonies can be seen rapidly within 6 – 8 hours, long before other bacteria grow. Colonies are at first small, circular, white, opaque discs, but enlarge on continued incubation and may acquire a distinct yellow tint. Several modifications of tellurite blood agar have been utilized, such as McLeod's and Hoyle's media.

Toxin Production

C. diphtheria is pathogenic only in humans and produces diphtheria toxin, a proteic exotoxin, with a molecular weight of 62 kilodaltons which ADP ribosylates host EF – 2, resulting in the inhibition of protein synthesis and thus is responsible for the signs of diphtheria. The inactivation of this toxin with an antitoxic serum (antitoxin) is the basis of the antidiphtheric therapeutic vaccination. However, not all strains are toxigenic; the ability to produce the exotoxin is conferred on the bacterium when it is infected by a bacteriophage (a mechanism termed as 'lysogenic activation'). Therefore with such a mechanism a non toxigenic strain can become toxigenic and cause a potential infection.

Virulent strains of diphtheria bacilli produce a very powerful exotoxin. The pathogenic effects of the bacillus are due to the toxin. Almost all strains of gravis and intermedius (about 95 – 99 percent) are toxigenic, while only about 80 – 85 percent of mitis strains are so. The proportions vary with the origin of the cultures tested. Strains of all three types are invariably virulent when isolated from acute cases. Avirulent strains are common among convalescents, contacts and carriers, there is considerable variation in the amount of toxin produced by the different strains, some strains producing the toxin abundantly and while others, only poorly. But the toxin produced by all strains of diphtheria bacilli is considered to be qualitatively similar. The strain almost universally used for toxin production is the 'Park Williams 8' strain, which has been variously described as a mitis and an intermedius strain.

The diphtheria toxin when released is inactive because the active site on fragment A is masked . Activation is probably accomplished by proteases present in the culture medium and infected tissues. All the enzymatic activity of the toxin is present in fragment A. Fragment B is responsible for binding the toxin to the cells. The antibody to fragment B is protective by preventing the binding of the toxin to the cells.

The toxin is labile. Prolonged storage, incubation at 37^{o}C for 4 – 6 weeks, treatment with 0.2 – 0.4 percent formalin or acid causes the loss of toxicity but not antigenicity. It is capable of producing antitoxin and reacting specifically with it. The toxigenicity of the diphtheria bacillus depends on the presence in it of a symbiotic bacteriophage, the beta phage, which acts as the genetic determinant controlling toxin production.

Resistance

Cultures may remain viable for two or more weeks at 25^{o}C – 30^{o}C. The culture is readily destroyed by heat, in ten minutes at 58^{o}C and in a minute at 100^{o}C. It is more resistant to the action of light desiccation and freezing than most non sporing bacilli. The bacilli has been cultured from dried bits of pseudomembrane after 14 weeks. It remains fully virulent in blankets and floor dust for five weeks. It is easily destroyed by antisepsis. It is moderately susceptible to sulfonamides and quite susceptible to penicillin, erythromycin and the broad spectrum antibiotics.

Transmission

Diphtheria is commonly transmitted most often from person to person spread from the respiratory tract. Rarely, transmission may occur from skin lesions or articles soiled with discharges from lesions of infected persons (fomites). The reservoir is mainly human carriers, which are usually asymptomatic.

Transmission may occur as long as virulent bacilli are present in discharges and lesions. The time is variable , but organisms usually persist 2 weeks or less, and seldom more than 4 weeks, without antibiotics. Chronic carriers may shed organisms for six months or more.

Symptoms

Usually, diphtheria occurs in the throat. Early symptoms include a sore throat and a mild fever. A membrane that forms over the throat and tonsils can make it hard to swallow. The infection also causes the lymph glands and tissue on both sides of the neck to swell to an unusually large size. Some people can be infected but not appear ill. They can also spread the infection.

Pathogenicity

The incubation period in diphtheria is commonly 3 – 4 days, but may on occasion be as short as one day. In carriers, the incubation period may be prolonged. The site of infection may be: 1) faucial, 2) laryngeal, 3) nasal, 4) otitic, 5) conjunctival, 6) genital – vulval, vaginal or preputial, and 7) cutaneous – mainly around the mouth or nose; sometimes diphtheritic whtlow or ulcer may occur. Faucial diphtheria is the commonest type and may vary from mild catarrhal inflammation to very widespread involvement.

Susceptible persons may acquire toxigenic diphtheria bacilli in the nasopharynx. The organism produces a toxin. The organism produces a toxin that inhibits cellular protein synthesis and is responsible for local tissue destruction and membrane formation. The toxin produced at the site of the membrane is absorbed into the bloodstream and then distributed to the tissues of the body. The toxin is responsible for low platelet counts (thrombocytopenia) and protein in the urine (proteinuria). Clinical disease associated with non – toxin producing strains is generally milder.

Diphtheria is a toxemia. The bacilli remain confined to the site of entry, where they multiply and form the toxin. The toxin causes local necrotic changes and the resulting fibrinous exudate, together with the disintegrating epithelial cells, leucocytes, erythrocytes and bacteria, constitute the pseudomembrane, which is characteristic of diphtheritic infection. The mechanical complications of diphtheria are due to the toxin induced pseudomembrane.

Complications

Most complications of diphtheria, including death, are attributable to effects of the toxin. The severity of the disease and complications are generally related to the extent of local disease. The toxin, when absorbed, affects organs and tissues distant from the site of invasion.

The common complications are:

- Asphyxia due to mechanical obstruction of the respiratory passage by the pseudomembrane for which an emergency tracheostomy often becomes necessary;
- Acute circulatory failure, which may be peripheral or cardiac;
- Postdiphtheritic paralysis, which typically occurs in the third or fourth week of the disease;
- Palatine and ciliary, but not pupillary, paralysis is characteristic, and spontaneous recovery is the rule; and septic, such as pneumonia and otitis media.
- Relapse may occur in about one percent of cases.

  The most frequent complications of diphtheria are myocarditis and neuritis; Myocarditis: may present as abnormal cardiac rhythms and can occur early in the course of the illness or weeks later, and can lead to heart failure. If myocarditis occurs early, it is often fatal. Neuritis: most often affects motor nerves and usually resolves completely. Paralysis of the soft palate is most frequent during the third week of illness. Eye muscles, limbs, and diaphragm paralysis can occur after the fifth week. Secondary pneumonia and respiratory failure may result from diaphragmatic paralysis.

Laboratory diagnosis

Laboratory confirmation of diphtheria is necessary for the initiation of control measures and for epidemiological purposes, but not for the treatment of individual cases. Specific treatment should be instituted immediately on suspicion of diphtheria, without waiting for laboratory tests. Any delay may be fatal.

Laboratory diagnosis consists of isolation of diphtheria bacillus and demonstration of its toxicity. One or two swabs from the lesion, collected under vision, using a tongue depressor should be submitted to the laboratory. Diphtheria bacilli may not always be demonstrable in smears from the lesions, nor can they be confidently differentiated from some commensal corynebacterium normally found in the throat. Hence smear examination alone is not sufficient for diagnosing diphtheria but is important in identifying Vincent's angina. It has been reported that toxigenic diphtheria bacilli may be identified in smears by immunofluorescence.

For culture: the swabs are inoculated on Loffler's serum slope, tellurite blood agar and a plate of ordinary blood agar, the last for differentiating streptococcal or staphylococcal pharyngitis, which may simulate diphtheria. If the swab cannot be inoculated promptly it should be kept moistened with sterile horse serum so that the bacilli will remain viable. The serum slope may show growth in 6 – 8 hours, but, if negative, will have to be incubated for 24 hours. Smears stained with methylene blue or one of the special stains (Neisser or Albert stain) will show the bacilli with metachromatic granules and typical arrangement. Tellurite plates will have to be incubated for at least two days before being considered negative, as growth may sometimes be delayed. The tellurite medium is particularly important in the isolation of diphtheria bacilli from convalescents, contacts and carriers as in these cases they may be outnumbered by other bacteria.

In the event that prior antibiotic therapy may have impeded a positive culture in a suspect diphtheria case, two sources of evidence may aid in presumptive diagnosis: (1) isolation of *C. diphtheriae* from culturing of close contacts, and/or (2) a low nonprotective diphtheria antibody titer in sera obtained prior to antitoxin administration (less than 0.1 I.U.). This is done by commercial laboratories and requires several days. To isolate *C. diphtheriae* from carriers, it is best to inoculate a Löffler's or Pai's slant with the throat swab. After an incubation period of 18–24 hours, growth from the slant is used to inoculate a medium containing tellurite.

Treatment

Treatment is with erythromycin orally or by injection (40 mg/kg/day; maximum, 2 gm/day) for 14 days, or procaine penicillin G daily, intramuscularly (300,000 U/day for those weighing 10 kg or less and 600,000 U/day for those weighing more than 10 kg) for 14 days. The disease is usually not contagious 48 hours after antibiotics are instituted. Elimination of the organism should be documented by two consecutive negative cultures after therapy is completed.

Epidemiology

Diphtheria was formerly an important pediatric disease all over the world, but following the development of effective prophylactics and mass immunization, the disease has been virtually eradicated from most advanced countries.

Infection is rare in early infancy because of the passive immunity obtained from the mother, and in adults due to the active immunity acquired by repeated subclinical infection. The disease is commoner in rural than in urban areas. It is typically a disease of schools and institutions where children of the susceptible age are herded together. Asymptomatic carriage of the bacillus in the throat or nose is common. Carriers transmit the infection to their contacts. Fomites do not seem to play an important role though in special situations toys and pencils may act as vehicles of infection. Nasal carriers harbor the bacilli for longer periods than throat carriers.

In nature, diphtheria is virtually confined to man, though cows may on occasion be found to have diphtheritic infection of the udder. The infection in such cases is invariably transmitted by the milker. The infection may be spread through the milk of infected cows.

Prophylaxis

Diphtheria can be controlled by a programme of mass immunization, as has been done in most advanced nations. The methods of immunization available are active, passive or combined. Susceptibility to diphtheria can be detected by the Schick test. When the diphtheria toxin is injected intradermally into a susceptible person, it causes a local reaction, while in an immune individual, no reaction ensues as the toxin is neutralized by the antitoxin in circulation.

LJ Medium

Lowenstein Jensen (LJ) medium, is a highly selective, egg containing medium, used for the growth of *Mycobacterium tuberculosis*. The procedure by which this medium is prepared is a critical factor which decides the nature of growth that may be obtained.

Lowenstein originally formulated a medium for the cultivation of mycobacteria in which congo red and malachite green were incorporated for the partial inhibition of other bacteria. This formulation had been further changed by other research workers. The present formula, developed by Jensen, varies from the initial composition with reference to the following parameters:

- Citrate and phosphate content
- Does not contain congo red, instead has an increased malachite green concentration.

Media composition for 1600 mL

Potato starch: 30. 0 g	Asparagine: 3.6 g
KH_2PO_4: 2.4 g	Magnesium citrate: 0.6 g
Malachite green: 0.4 g	MgSO4.7H2O: 0.24 g
Homogenized whole egg: 1.0L	Glycerol: 12.0 mL

Preparation of homogenized whole egg

Composition per liter: Fresh whole eggs: 18 – 24

The eggs must be, less than a week old. The shells of the eggs have to be carefully scrubbed with soap and allowed to stand in a soap solution for 30 minutes. The eggs have to be thoroughly rinsed under running tap water. The eggs are then soaked in 70% ethanol for 15 minutes. Prior to breaking the eggs clean sterile containers have to be kept ready. After breaking the eggs under sterile conditions, homogenize by shaking. Filter through four layers of sterile cheesecloth into a sterile graduated cylinder. Measure out 1.0 L also under sterile conditions.

Preparation of Medium

Add glycerol to 600.0 mL of distilled / deionized water. Mix thoroughly. Add remaining components, except fresh egg mixture. Mix thoroughly. Gently heat while stirring and bring to a boil. Autoclave for 15 minutes at 15 psi pressure – 121 deg. C, cool to 50 deg. C. Aseptically add 1.0 L of homogenized whole egg. Mix thoroughly. Distribute into sterile screw – capped bottles. Place tubes in a slanted position. Inspissate at 85 deg. C (moist heat) for 45 minutes.

For the cultivation and differentiation of Mycobacterium species. *Mycobacterium tuberculosis* appears as granular, rough and dry colonies. *Mycobacterium kansasii* appears as smooth to rough photochromogenic colonies. *Mycobacterium gordonae* appears as smooth yellow – orange colonies. *Mycobacterium smegmatis* appears as wrinkled, creamy white colonies. Also used for the cultivation and maintenance of Gordona species, Nocardia species, Rhodococcus species, and *Tsukamurella paurometabolum.*

Additionally the media can also be supplemented with 5 % sodium chloride since the ability to tolerate 5 % sodium chloride is a characteristic of certain strains of mycobacteria (eg., *M. fortuitum* and *M. chelonae* subsp. *abscessus*). Most rapid growers, the slowly growing *M. triviale* and some strains of *M. flavescens* also grow on NaCl containing media. The inability of *M. chelonae* subsp. *chelonae* to grow helps differentiate it from other members of the *M. fortuitum* complex. (eg., *M. chelonae* subsp. *Abscessus*). In certain cases, LJ media may be incorporated with antibiotics like Streptomycin. For instance 0.1 mg of streptomycin is added per 10.0 mL.

Preparation of Streptomycin solution:

Add streptomycin to distilled / deionized water and bring volume to 10.0 mL. Mix thoroughly. Filter sterilize.

Preparation of the antibiotic incorporated media:

Will be the same as the preparation without the antibiotic. The antibiotic will be introduced into the composition of the media just before the media is distributed into the screw capped bottle.

The other antibiotics that are incorporated into the media are isoniazid, rifampin, pyrazinamide and ethambutol.

The incorporation of antibiotics in LJ media are done mainly for two purposes: (1) To test the sensitivity of the particular strain of Mycobacterium. (2) To make the media even more selective for the growth of the strain of interest.

With the increase in drug resistance, that has been seen in *Mycobacterium tuberculosis* strains, it is essential that only the antibiotics to which the infecting strains are sensitive should be used in the treatment regime.

Advantages of Egg media

- It is easy to prepare.
- It is the least expensive of all media available and which support good growth of tubercle bacilli.
- It may be stored in the refrigerator for several weeks provided it was made from fresh eggs and culture bottle caps are tightly closed to minimize drying by evaporation.
- Contamination during preparation is limited because it is inspissated after being placed in bottles. In addition, the malachite green added to the media suppresses the growth of non-mycobacterial organisms.

Disadvantages of Egg media

- It may take as long as eight weeks before cultures become positive, especially if specimens contain few bacilli or if decontamination procedures have been overly harsh.
- When contamination does occur, it often involves the total surface of the medium and the culture is usually lost.

A few general points that must be remembered to obtain good quality media and also avoid contamination of reagents and media are as follows:

1. Keep the environment as clean as possible. Swab the work surface with a suitable disinfectant (eg. 5% methylated spirit) before dispensing sterile reagents and media. Clean the floor with a wet mop to limit dust.
2. Use sterile glassware and equipment.
3. Use reagent grade chemicals and reagents unless otherwise specified.
4. Check the temperature of inspissators and hot air ovens.
5. Follow strict aseptic techniques when preparing media, eg. flaming flasks and tubes.
6. When preparing the media, carefully clean the egg shells before breaking.
7. Do not overheat the medium during inspissation.
8. Do not leave prepared media exposed to light (including ultra – violet light), but store in the refrigerator in the dark when not in use.
9. Do not skimp on the volume of the medium. Place 6 – 8 ml of egg medium in each bottle or 20 ml into each test tube.

Oral Hygiene : Must Know

Oral hygiene refers to the maintenance of the health of the teeth, gums and the mouth in general, it is essential to maintain a good oral hygiene, in order to prevent, rather avoid dental problems and bad breath.

Tooth and gum disease are largely caused by plaque, a sticky combination of bacteria and food. Plaque begins to accumulate on teeth within 20 minutes after eating. If this plaque is not removed thoroughly each day, tooth decay will flourish. Over time plaque will harden into tartar.

Effects of poor oral hygiene

Poor oral hygiene allows the accumulation of acid producing bacteria on the surface of the teeth. The acid demineralizes the tooth enamel causing tooth decay (cavities). Dental plaque can also invade and infect the gums causing gum disease and periodontitis.

In both these conditions, the final effect of poor oral hygiene is the loss of one or more teeth. One should not wait till he or she begins to loose teeth.

Many health problems of the mouth, such as oral thrush, trench mouth, bad breath and others are considered as effect of poor dental hygiene. Most of these dental and mouth problems may be avoided by just maintaining good oral hygiene.

Plaque and Tartar lead to a number of problems namely:

- Cavities: holes that damage the structure of the teeth.
- Gingivitis: swollen, inflamed, bleeding gums.
- Periodontitis: destruction of the ligaments and bone that support the teeth, often leading to tooth loss.
- Halitosis: bad breath
- Abscesses, pain, inability to use teeth.
- A variety of health problems outside the mouth, from preterm labor to heart disease.

The Big 'Q'

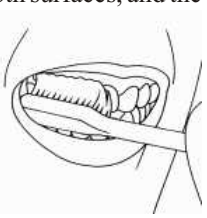
What is good oral hygiene?

Good oral hygiene results in a mouth that looks and smells healthy. This means:

- Your teeth are clean and free from debris.
- Gums are pink and do not hurt or bleed when you brush or floss
- Bad breath is not a constant problem.

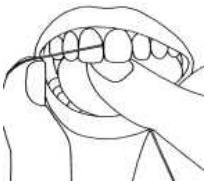
How do I brush my teeth?

1. Place your toothbrush at a 45 – degree angle against the gums.
2. Move the brush back and forth gently in short (tooth – wide) strokes.
3. Brush the outer tooth surfaces, the inner tooth surfaces, and the chewing surfaces of the teeth.
4. Use the 'toe' of the brush to clean the inside surfaces of the front teeth, using a gentle up-and-down stroke.
5. Brush your tongue to remove bacteria and freshen your breath.



How do I floss my teeth?

1. Break off about 18 inches of floss and wind most of it around one of your middle fingers. Wind the remaining floss around the same finger of the opposite hand. This finger will take up the floss as it becomes dirty. Hold the floss tightly between your thumbs and forefingers.
2. Guide the floss between your teeth using a gentle rubbing motion. Never snap the floss into the gums.
3. When the floss reaches the gum line, curve it into a C shape against one tooth. Gently slide it into the space between the gum and the tooth.
4. Hold the floss tightly against the tooth. Gently rub the side of the tooth, moving the floss away from the gum with up and down motions.
5. Repeat this method on the rest of your teeth.
6. Don't forget the back side of your last tooth.



People who have difficulty handling dental floss may prefer to use another kind of interdental cleaner. These aids include special brushes, picks or sticks. If you use interdental cleaners, ask your dentist about how to use them properly, to avoid injuring your gums.

Regular teeth cleaning by a dentist removes plaque that may develop even with regular brushing and flossing, especially in areas that are difficult for you to reach on your own. Professional cleaning includes scaling and polishing. This uses various instruments or devices to loosen and remove deposits from the teeth. Routine examination may include dental X – rays.

What about Mouth rinses?

Mouth rinses are used for a variety of reasons: to freshen breath, to help prevent or control tooth decay, to reduce plaque, to prevent and reduce gingivitis, to reduce the speed that tartar forms on the teeth.

Antibacterial mouth rinses and toothpastes reduce the bacterial count and inhibit bacterial activity in dental plaque, which can cause gingivitis, an early, reversible form of periodontal (gum) disease.

Fluoride mouth rinses help reduce and prevent tooth decay. Clinical studies have demonstrated that use of a fluoride mouth rinse and fluoride toothpaste can provide extra protection against tooth decay over that provided by fluoride toothpaste alone. Fluoride mouth rinse is not recommended for children age six or younger because they may swallow the mouth rinse.

A mouth rinse basically contains the following ingredients:

Water, alcohol, cleansing agents, flavoring ingredients and coloring agents.

Active ingredients vary depending on the type of mouth rinse, but they can be placed into four general groups:

1. Antimicrobial agents act directly on oral bacteria to help reduce plaque, decrease the severity of gingivitis and control bad breath.
2. Fluoride helps reduce tiny lesions (tooth decay) on tooth enamel and make teeth more resistant to decay.
3. Astringent salts can serve as temporary deodorizers that mask bad breath.
4. Odor neutralizers act by chemically inactivating odor causing compounds.

Mouth rinses containing antibacterial agents such as chlorhexidine and cetylpyridinium chloride may play an important role in reducing the levels of halitosis – producing bacteria on the tongue, and chlorine dioxide and zinc containing mouth rinses can be effective in neutralization of odoriferous sulfur compounds.

Dentists suggest that brushing and flossing of teeth is advisable prior to using a mouth rinse. Adequate amount of rinse as specified on the container or as instructed by your dentist is used. With your lips closed and the teeth kept slightly apart, swish the liquid around with as much force as possible. Many rinses suggest swishing for 30 seconds or more. Finally, thoroughly spit the liquid from your mouth.
Teeth should be as clean as possible before applying an anti – cavity rinse to reap the full preventive benefits. Consumers should not rinse, eat or smoke for 30 minutes after using rinses, as these practices will dilute the fluoride and rinse it away.

Healthy teeth are clean and have few cavities. Healthy gums are pink and firm. To maintain healthy teeth and gums, follow these steps:

1. Brush your teeth at least twice daily, preferably after every meal and at bedtime.
2. Floss at least once per day.
3. Schedule an appointment with the dentist for a routine cleaning and examination. Many dentists recommend having the teeth professionally cleaned every 6 months.
4. Keep dentures, retainers and other appliances clean. This includes regular brushing and may include soaking them in a cleansing solution.

Ask your Dentist:

1. What toothbrush you should use, and where your problem areas are located.
2. How to properly floss your teeth. Overly vigorous and improper flossing may injure the gums.
3. Whether you should use any special appliances or tools such as water irrigation and or electric toothbrushes. These may sometimes help supplement (but not replace) brushing and flossing.
4. Whether you could benefit from particular toothpastes or mouth rinses. In some cases over-the-counter pastes and rinses may be doing you more harm than good, depending on your condition.

Constant halitosis can be used as one of the most important indicator of bad oral health and thus to summarize the need for oral hygiene there are a few quick tips on how to avoid halitosis.

- Brush and floss regularly.
- Clean and replace your toothbrush regularly. Its best to change your toothbrush every three months.
- Brush your tongue as it can becomes coated. To avoid coating, you should clean your tongue regularly.
- Limit your protein intake. Since a high protein diet is low in carbohydrates. This causes the body to use its fat reserves for energy. Acetone is formed in the blood when the body uses fat for energy instead of carbohydrates. It is released through the body in urine and causes a fruity smell on the breath.
- Have regular dental check – ups. Tooth decay and oral infections are causes of bad breath. Routine, careful cleaning of your teeth, gums and tongue will help to prevent bad breath. You should see your dentist at least twice a year.
- Have an annual physical. You should discuss any symptoms of illness with your doctor. Your doctor can conduct tests to diagnose problems which may cause bad breath. Some medical conditions such as diabetes and ulcers can cause this problem.
- If you smoke, quit. Smoking not only causes bad breath but the smell also lingers on your clothing and in your hair. Prolonged smoking causes the teeth to turn yellow.
- Check your medications. Ask your doctor if any of the medications you are taking cause bad breath or dry mouth. There may be another one for the condition that you have. If not, your doctor may have suggestions as to how you can prevent halitosis.
- Onion, garlic and curry have strong odors that remain on your breath after eating for at least 24 hours. Although the odors are strong, they do not result in continuing problem of bad breath. Foods high in protein like red meat can get stuck in your teeth. High protein foods are also difficult to digest and this is a problem if you have an ulcer or other gastric disorders. This can cause acid reflux which produces a strong odor.
- Make sure your dentures are clean and fit properly. Food particles can get trapped in your dentures and if they don't fit properly in the gaps in your open mouth. Your dentures should be cleaned regularly to get rid of bacteria that cause bad breath.
- Drink plenty of water. Dry mouth is a symptom of bad breath. Moisture cleans the tissues of the mouth. When your mouth is dry, the bacteria form and stay in place. Drink more water and less coffee or alcohol. These two beverages can cause offensive odors. Chewing sugar – free gum will also help to increase the saliva in your mouth.
- Saliva is the ingredient in the mouth that ensures that the bacteria and food particles do not get accumulated in the mouth and are flushed away, this phenomenon slows down during the night, due to which there is an initial bad taste and odor in the mouth in the morning which is easily cleared off by brushing and the intake of breakfast.
- Avoid mouthwashes with flavorings, dyes and alcohol.
- Avoid stress and seek out ways relax and resolve stressful conditions in your life.
- Get sufficient sunlight, exercise and sleep.
- Avoid spicy foods and those that leave residues or get stuck in the teeth (alcohol, cheese, meat, sweets).
- Chew Parsley after meals, it is very rich in chlorophyll, a natural mouthwash.

Importance of good oral hygiene

Prevention is always better than cure. Good oral hygiene habits will keep away most of the dental problems, saving you from toothache and costly dental treatments. The interesting part is that it can be achieved by dedicating only some minutes every day to dental hygiene care.

Unfortunately, most of us remember the importance of oral hygiene instructions, only when a problem occurs. Good oral hygiene has to be maintained not only when we are needed to do so. But it should be a life long habit, and should be inculcated at an early age.
Awareness regarding the importance of oral hygiene has significantly increased in the developed countries, but contrary to that, the modern dietary lifestyle habits are posing a greater risk for oral health. Daily preventive oral care, with proper brushing and flossing, will help stop dental problems before they develop and are much less painful, expensive, and worrisome than treating conditions that have been allowed to progress.

Maintaining good oral hygiene is one of the most important things you can do for your teeth and gums. Healthy teeth not only enable you to look and feel good, they make it possible to eat and speak properly. Good oral health is important to your overall well – being.

The sooner a good habit is started, the better. So lets start today and now to care for our overall health.

Rappaport Vassiliadis Salmonella Enrichment Broth USP

A medium recommended for selective enrichment of Salmonella species under condition of high osmotic pressure and low pH with modest nutritional requirement.

Summary

Rappaport *et al*., formulated an enrichment medium for *Salmonella* that was modified by Vassiliadis *et al*., Rappaport Vassiliadis (1 & 2) Salmonella Enrichment Broth is a selective enrichment for *Salmonella* species. This medium is selective for *Salmonella* species because they are typically resistant to malachite green, high osmotic pressure and low pH. *S. typhi* and *S. choleraesuis* are sensitive to malachite green and may be inhibited. This medium is also recommended by United States of Pharmacopeia.

Principle

Soya peptone provides the essential nutrients for the growth of the bacteria. Phosphate salts act as buffer to maintain the pH. Magnesium chloride maintains the high osmotic pressure and *Salmonella* generally survive at little high osmotic pressure. Malachite green inhibits other microorganisms other than *Salmonella*.

Ingredients in grams per liter*

Soya peptone	4.5
Magnesium chloride hexahydrate	29.0
Sodium chloride	8.0
Dipotassium phosphate	0.4
Potassium dihydrogen phosphate	0.6
Malachite green	0.036
Final pH (at 25ºC) 5.2±0.2	

*Formula adjusted to suit performance parameters.

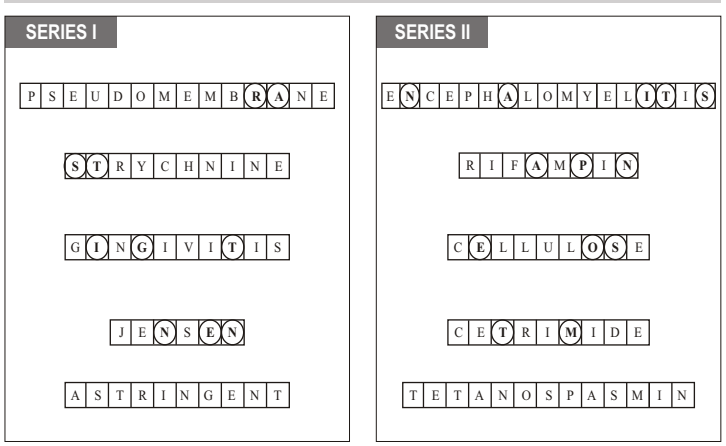
Direction

1. Bring the refrigerated medium bottle to room temperature.
2. Use as per requirements.

Benefits of Ready Prepared Media

- **Convenient and flexible usage of media.**
- **Minimizes usage of equipments.**
- **Reduced cross contamination.**
- **Less time consuming.**

Availability: 12 X 10 ml Pack Size.



TOTASEP
Disinfectant for Hospital, Hotel and Food Industry

Description:

TOTASEP is a colourless, non-perfumed liquid with a potent broad spectrum antimicrobial action combined with excellent cleaning power. Its non-toxic and environment friendly properties makes it ideal for food industry and food contact areas and equipments.

Composition:

3% w/v poly (hexamethylenebiguanide) hydrochloride and 10% w/v Didecyl dimethyl ammonium chloride .

Benefits :

- Sporicidal activity in just 30 mins
- True "Flash Sterilization"possible
- Aldehyde-free – No harmful effects
- Bioburden tolerant
- Resistance-free
- Uses 2 novel molecules; PHMB + DDAC -Powerful synergistic action
- Tough against biofilms
- Excellent material compatibility

Availability: 1000 ml and 5000 ml

Highlights of the coming issue

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