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Editorial

Contents

We have heard of the term vicious circle, which means a self-perpetuating process which returns to its starting point with no improvement. The emblem of a snake eating its own tail was commonly used in the iconography of Georgian and Victorian cemeteries which denotes the circle of life and death. A wider use of the expression was taken up by the medical profession, being used to describe conditions where one symptom affects another and the health of the patient steadily deteriorates. One of the major reasons is antibiotic resistance. The volume of antibiotic prescribed is the major factor in increasing rates of bacterial resistance rather than compliance with antibiotics. Inappropriate prescribing of antibiotics has been attributed to a number of causes, including: people who insist on antibiotics, physicians simply prescribe them as they feel they do not have time to explain why they are not necessary or else are overly cautious for medical legal reasons. For example, a third of people believe that antibiotics are effective for common cold and it is the most common reasons antibiotics are prescribed. A large number (22%) of people do not finish a course of antibiotics primarily because they feel better.

In the section mini review, applications and benefits of Mueller Hinton Agar is explained. It is used largely for testing antibiotic susceptible of micro-organisms. It has become the standard medium for the Bauer-Kirby method. Mueller Hinton Agar helps to evaluate the resistance or susceptibility of a micro-organism towards a particular drug (antibiotics). This helps in medical microbiology labs as it helps the doctors to decide on a plan of treatment or course of medication depending on the results of the susceptibility test.

In current trends section WHO guidelines for safe surgery is covered. Complications of surgical care have become a major cause of death and disability worldwide. The aim is to develop potential standards for improvements in four areas: safe surgical teams, safe anaesthesia, prevention of surgical site infection and measurement of surgical services by creating public health metrics to measure provision and basic outcomes of surgical care.

In profile segment covers biography of Dr. Shanti Swaroop Bhatnagar. He played a significant part along with Homi Jehangir Bhabha, Prasanta Chandra Mahalanobis, Vikram Ambalal Sarabhai and others in building of post-independent S&T infrastructure and in the formulation of India's science and technology policies. Bhatnagar was the Founder Director of the Council of Scientific and Industrial Research (CSIR), which was to later become a major agency for research in independent India. He was the first Chairman of the University Grants Commission (UGC).

Clostridium difficile (C. difficile) be discussed in the Bug of the month segment. It a gram-positive, spore-forming ovel, subterminial anaerobic motile bacillus. It is a bacterium that is related to the cause tetanus and botulism. Antibiotic-associated (C. difficile) colitis is an infection of the colon caused by C. Difficile occurs primarily among individuals who have been using antibiotics.

Did You Know emphasize on Sterile Disinfectants. A sound cleaning and sanitization program is needed for controlled environments used in the manufacture of Pharmacopeia articles and to prevent the microbial contamination of these articles. Sterile drug products may be contaminated via their pharmaceutical ingredients, water, packaging components, manufacturing environment, processing equipment and manufacturing operators. So maintaining the sterile environment is must and these are done by High Technology Disinfectants.

Best Practices segment will encompass Sterility testing which is defined as a test that critically assesses whether a sterilized pharmaceutical product is free from contaminating microorganisms.

JHS team thank all our readers for the support and contribution. Feedback and suggestions are always invited.

Mueller Hinton Agar

The Mueller Hinton Agar is used largely for testing antibiotic susceptible of micro organisms. It has become the standard medium for the Bauer-Kirby method and its performance is specified by the National Committee for Clinical Laboratory Standards (NCCLS). Mueller-Hinton Agar meets the requirements of WHO.

Mueller Hinton Agar helps to evaluate the resistance or susceptibility of a micro organism towards a particular drug (antibiotics). This helps in medical microbiology labs as it helps the doctors to decide on a plan of treatment or course of medication depending on the results of the susceptibility test.

This media was proposed by Mueller and Hinton in 1941 for testing the sensitivity of clinically important pathogens towards antibiotics or sulfonamides.

The use of a suitable medium for testing the susceptibility of microorganisms to sulfonamides and trimethoprim is essential. Antagonism to sulfonamide activity is demonstrated by para-aminobenzoic acid (PABA) and its analogs. Reduced activity of trimethoprim, resulting in smaller inhibition zones and inner zonal colonies, is demonstrated on unsuitable Mueller Hinton medium possessing high levels of thymidine. Both the PABA and thymine/thymidine content in Mueller Hinton Agar are reduced to a minimum, thus markedly reducing the inactivation of sulfonamides and trimethoprim when the media is used for testing the susceptibility of bacterial isolates to these antimicrobials.

An important task of the clinical microbiology laboratory is the performance of antimicrobial susceptibility testing of significant bacterial isolates. The goals of testing are to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections. In clinical microbiology laboratories in the early 1960s were using a wide variety of procedures for determining the susceptibility of bacteria to antibiotic and chemotherapeutic agents, Bauer, Kirby and others developed a standardized procedure in which Mueller Hinton Agar was selected as the test medium, finally in 1966, Bauer, Kirby, Shervis and Turck recommended Mueller Hinton medium for the study of the antibiotic susceptibility of bacteria using the disk method. The International Collaborative Study Group (1971) also recommended this medium with 5% defibrinated blood, for the international standard disc susceptibility test method. Finally, a standardized control method was published by the National Committee for Clinical Laboratory Standards for the Kirby-Bauer method.

Better diffusion of antibiotics is just one reason why Mueller Hinton Agar is used for antibiotic sensitivity test (ABST). There are several factors that affect the ABST, they are:

- disc concentration,
- diffusability of the drug,
- nature and composition of the medium,
- thickness of the medium,
- pH of the medium,
- potency of the disc.

The pH should be between 7.2 and 7.4. If the pH is too low, certain drugs loose potency (eg. aminoglycosides, quinolones and macrolides) while certain others show excess activity (eg. tetracycline). If the pH is too high then reverse reactions would occur. Excess moisture too affects the reliability of the result. Increased thymidine reverses the inhibitory effect of sulphonamides, Mueller Hinton Agar has decreased thymidine content. Increased concentration of Mg^{2+} and Ca^{2+} affects the results of aminoglycosides and tetracycline, also reduces zone size in case of P. aeruginosa. Decreased cation increases the zone size. Increased Zn would decrease the zone size of carbapenen group of antibiotics. Some fastedious organisms like Haemophilus sp, N.gonorrheae, S. pneumoniae, S. viridans and beta hemolytic Streptococcus require Mueller Hinton Agar supplemented with 15 micro gram per ml NAD, bovine hematin and yeast extract.

The starch performs as a growth factor, probably functions like a colloid protector and neutralizes toxic products that are able to form during the development of the organisms. But Mueller Hinton Agar is specifically used for testing antibiotic sensitivity as it does not contain any inhibitory substances for the growth of the organism. In biology, starch stores energy. This energy is released during respiration. Starch is insoluble in water, so it can be stored in organisms as an energy source.

The Kirby-Bauer test, known as the disk-diffusion method, is the most widely used antibiotic susceptibility test in determining what treatment of antibiotics should be used when treating an infection. This method relies on the inhibition of bacterial growth measured under standard conditions. For this test, a culture medium, specifically the Mueller-Hinton agar, is uniformly and aseptically inoculated with the test organism and then filter paper discs, which are impregnated with a specific concentration of a particular antibiotic is placed on the medium. The organism will grow on the agar plate while the antibiotic "works" to inhibit the growth. If the organism is susceptible to a specific antibiotic, there will be no growth around the disc containing the antibiotic. Thus, a "zone of inhibition" can be observed and measured to determine the susceptibility to an antibiotic for that particular organism. The measurement is compared to the criteria set by the National Committee for Clinical Laboratory Studies (NCCLS). Based on the criteria, the organism can be classified as being Resistant (R), Intermediate (I) or Susceptible (S).

Preparation Precautions and Quality Control of Mueller-Hinton agar.

The disc diffusion test not only categorises resistant, intermediate and susceptible organisms through quantitative results, but also provides a visual indication of:

- Inoculum level
- Presence of contamination
- Antagonism and synergy between adjacent antibiotics
- ß-lactamase activity

Although disc diffusion is a simple test to perform, to obtain accurate and reproducible results attention to detail and care must be taken, since relatively small changes in operating procedure can affect the results obtained.

The initial consideration must be investment in quality products with which to perform AST.

There are three main international standards for potency of antibiotics in the discs, and manufacturers do not all produce to the same standards:-

- FDA specification 67 – 150% of the stated concentration
- WHO specification 75 – 135% of the stated concentration
- DIN specification 90 – 125% of the stated concentration

If Mueller-Hinton agar is the medium of choice then this should be

produced to conform with the NCCLS M6-A which tightly controls many of the variable factors in the medium e.g. cation concentration, pH, antibiotic inhibitor levels. If these are uncontrolled, the results obtained could be greatly affected. Although no international standards currently exist for Iso-sensitest agar or Diagnostic sensitivity test agar, users should ensure that their supplier adheres to strict quality systems to ensure a reliable product e.g. ISO 9001 and ISO 9002.

[Note: Several commercial formulations of Mueller-Hinton agar are available. This medium should not be prepared from individual ingredients because this can diminish the quality. Commercial dehydrated Mueller-Hinton is carefully quality controlled before being released for sale.]

The quality of water used for reconstitution of dehydrated culture media can affect the performance of the medium being prepared. The quality of the water must be regularly monitored and maintained by the Microbiology Department.

Fresh, high quality water prepared by distillation, de-ionisation, or reverse osmosis is recommended for the satisfactory reconstitution of culture media. Tap water should not be used as it contains impurities such as calcium and magnesium and their metal ion traces. In addition chlorine and fluorine will alter the characteristics of selective media. In areas of hard water, it is advisable to soften the water prior to distilling. Condensed boiler distillate should not be used owing to the carry-over of chemicals used to treat the water. Boilers have also been known to contaminate media during autoclaving. It may be necessary to add trace elements to media in order to allow fungi to exhibit characteristic morphologies (King et al). However, tap water is too variable in its ion content to be considered.

After autoclaving, cool medium to 50°C. Measure 30 to 32 ml of medium per plate into 15 x 150-mm plates, or measure 29 to 32 ml per plate into 15 x 100-mm plates. Agar should be poured into flat-bottom glass or plastic petri dishes on a level pouring surface to a uniform depth of 4 mm. Using more or less agar will affect the susceptibility results. Agar deeper than 4 mm may cause false-resistance results, whereas agar less than 4 mm deep may be associated with a false-susceptibility report.

Freshly prepared plates may be used the same day or stored in a refrigerator (2° to 8°C) for up to 2-3 weeks. If plates are not used within 7 days of preparation, they should be wrapped in plastic to minimize evaporation. Just before use, if excess moisture is on the surface, plates should be placed in an incubator (35° to 37°C) until the moisture evaporates (usually 10 to 30 min). The moisture in the medium plays an important role in disc diffusion. When the antibiotic discs are applied to the agar, moisture from the plate is absorbed, dissolving the antibiotic, and allowing it to diffuse into the agar. Thus, a reduced moisture content will impede the flow of antibiotic and result in smaller zones. Do not leave lids ajar because the medium is easily contaminated.

Each new lot should be quality controlled before use by testing the E. coli 25922 and/or Staphylococcus aureus 25923 standard strains. These standard strains are used with every test run for Enterobacteriaceae gram-positive aerobes, respectively. The pH of each new lot of Mueller-Hinton should be between 7.2 to 7.4. If outside this range, the pH medium should not be adjusted by the addition of acid or base; the batch of plates should be discarded and a new batch of plates prepared. If the pH for every batch is too high or low, the entire lot of dehydrated medium may have to be returned to the manufacturer as unsatisfactory.

To verify that susceptibility test results are accurate, it is important to include at least one control organism (ATCC 25922 is the E. coli strain used when testing Enterobacteriaceae V. cholerae) with each test. Zone diameters obtained for ATCC 25922 should be compared with NCCLS published limits. If zones produced by the control strain are out of the expected ranges, the laboratorian should consider possible sources of error. Susceptibility tests are affected variations in media, inoculum size, incubation time, temperature, and other factors. The medium used may be a source of error if it fails to conform to NCCLS recommended guidelines. For example, agar containing excessive amounts of thymidine or thymine can reverse the inhibitory effects of sulfonamides and trimethoprim, causing the zones of growth inhibition to be smaller or less distinct. Organisms may appear to be resistant to these drugs when in fact they are not. If the depth of the agar in the plate is not 3 to 4 mm or the pH is not between 7.2 and 7.4, the rate of diffusion of the antimicrobial agents or the activity of the drugs may be affected. An increase in pH will cause a decrease in penicillin and quinolone zones; conversely, the same increase will increase zone diameters for macrolide and aminoglycosides.

If the inoculum is not a pure culture or does not contain a concentration of bacteria that approximates the McFarland standard, the susceptibility test results will be affected. For instance, a resistant organism could appear to be susceptible if the inoculum is too light. Once the inoculum has been applied to the agar plate, the discs should be applied as soon as possible. Delays here will allow the organism a chance to begin replication before the antibiotic is applied, potentially resulting in smaller zone sizes. Also, if colonies from blood agar medium are used to prepare a suspension by the direct inoculum method, trimethoprim or sulfonamide antagonists may be carried over and produce a haze of growth inside the zones of inhibition surrounding trimethoprim-sulfamethoxazole disks even when testing susceptible isolates.

If antimicrobial disks are not stored properly or are used beyond the stated expiration date, their potency may decrease; this will usually be indicated by a decrease in the size of the inhibition zone around the control strain. One more thing which is extremely important since it is well known that moisture is a major cause of antibiotic degradation desiccant in the base of the dispensers must be regularly recharged to help maintain the moisture free environment. After regeneration, the desiccant should be checked to ensure that the indicator dye has returned to its original colour. If a strong colour is not achieved, the desiccant should be replaced. After refrigeration, the dispensers need to be allowed to reach room temperature before the lid is removed to prevent condensation and moisture ingress.

As mentioned above, testing some bacteria against certain antimicrobial agents may yield misleading results because these in vitro results do not necessarily correlate with in vivo activity. Examples include narrow- and expanded spectrum cephalosporins and aminoglycosides (e.g., gentamicin) tested against Shigella, and erythromycin tested against V. cholerae.

Dispenser adjustments

Before the antibiotics are inserted into the dispenser, the height of the "skirt" at the base needs to be adjusted to match the depth of the agar. If the dispenser is incorrectly adjusted the discs can be imbedded into the agar. As a consequence, the prongs that press the discs into place on the agar surface can become contaminated which can then contaminate successive plates. If the dispenser becomes contaminated, then the prongs can be cleaned using a swab soaked in alcohol. This procedure should be performed on a weekly basis, as a preventative measure unless gross contamination takes place when immediate cleaning is required. If the dispenser is set too high, the discs may not be pressed onto the agar surface. This can lead to uneven spacing of the discs, and consequently overlapping or unreadable zones. If insufficient contact between the discs and the agar takes place moisture may not be drawn into the disc and the antibiotic would not diffuse into the plate. This would be especially problematic with large

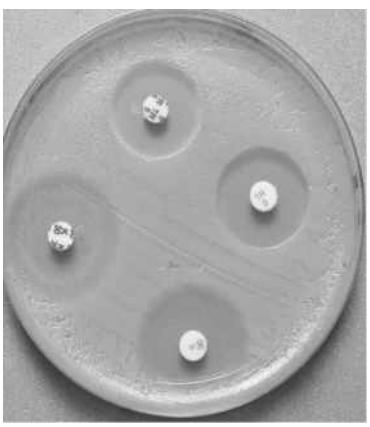
molecular weight antibiotics such as vancomycin and teicoplanin, which routinely have difficulty diffusing into agar.

MH agar is considered the best medium to use for routine susceptibility testing of non-fastidious bacteria. The CLSI disk diffusion document M2- Performance standards for Antimicrobial Disk Diffucion Susceptibility Tests recommends the use of Mueller Hinton Agar for routine susceptibility for the following reasons:

- It shows acceptable batch-to-batch reproducibility for susceptibility testing
- It is low in sulfonamide, trimethoprim, and tetracycline inhibitors
- It supports satisfactory growth of most nonfastidious pathogens
- Provides satisfactory growth of most non-fastidious pathogens and demonstrates batch-to-batch reproducibility.
- A large body of data and experience has been collected concerning susceptibility tests performed with this medium.

Especially for the susceptibility testing of non-fastidious use of media other than Mueller-Hinton agar may result in erroneous results. Also note that only the aerobic or facultative bacteria that grow well on unsupplemented MH agar should be tested using this protocol. Fastidious organisms require MH agar supplemented with additional nutrients and require that modification to this protocol be made Casein, Acid Hydrolysate is an excellent source of free amino acids with the exception of cystine, because casein contains little cystine, and tryptophan, which is destroyed by the acid treatment, as are vitamins and short peptide fragments, which are required by microorganisms for growth. Casein is a rich source of amino nitrogen, Starch is added to absorb any toxic metabolites produced, Hemoglobin provides X factor (hemin) for Haemophilus species. IsoVitaleXô Enrichment is a defined supplement that provides V factor (nicotinamide adenine dinucleotide, NAD) for Haemophilus species and vitamins, amino acids, co-enzymes, dextrose, ferric ion and other factors for improved growth of fastidious organisms; i.e., pathogenic Neisseria. Agar is the solidifying agent. Treatment with hydrochloric acid hydrolyzes the protein into primarily free amino acids with some short peptide fragments. Tryptophan and vitamins present in the casein are destroyed by the acid hydrolysis. The salt content of this peptone is very high at approximately 36%. While this peptone is used in a number of microbiological media formulations, its main usage is in Mueller Hinton Agar. Commercial medias range of Acid Hydrolysate of Casein is a hydrochloric acid hydrolysate of casein for use in preparing microbiological culture media.

Mueller Hinton Agar is often abbreviated as M-H Agar, and complies with requirements of the World Health Organization. Mueller Hinton Agar is specified in FDA Bacteriological Analytical Manual for food testing, and procedures commonly performed on aerobic and facultatively anaerobic bacteria. A variety of supplements can be added to Mueller Hinton Agar, including 5% defibrinated sheep blood, 1% growth supplement and 2% sodium chloride. Mueller-Hinton Agar supplemented with yeast, NAD and haematin is used specifically for the susceptibility testing of Haemophilus influenzae.



Precautions:

- Incubation in a CO_2 enriched atmosphere is not recommended because of its pH effect on the medium. If it is imperative to use CO_2 then known control organisms should be included with the test plates to measure its effect.
- Carbohydrates should not be added to Mueller-Hinton Agar because they may influence the rate of growth of the organism and the resulting pH of the medium.
- The addition of lysed horse blood to the medium may further reduce the levels of thymidine and prevent the growth of thymidine-dependent organisms.

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WHO Guidelines for Safe Surgery

The problem: complications of surgical care have become a major cause of death and disability worldwide. Data from 56 countries showed that in 2004 the annual volume of major surgery was an estimated 187–281 million operations, or approximately one operation annually for every 25 human beings alive. This is a large and previously unappreciated volume with significant implications for public health. It is almost double the annual volume of childbirths - in 2006, there were approximately 136 million births - and is at least an order of magnitude more dangerous. While the rates of death and complications after surgery are difficult to compare since the case mix is so diverse, in industrialized countries the rate of major complications has been documented to occur in 3 - 22% of inpatient surgical procedures, and the death rate 0.4 - 0.8%. Nearly half the adverse events in these studies were determined to be preventable. Studies in developing countries suggest a death rate of 5 - 10% associated with major surgery, and the rate of mortality during general anaesthesia is reported to be as high as 1 in 150 in parts of sub-Saharan Africa. Infections and other postoperative complications are also a serious concern around the world.

An estimated 63 million people a year undergo surgical treatment for traumatic injuries, 31 million for malignancies and 10 million for obstetric complications. Problems associated with surgical safety are well recognized in developed and developing countries alike. The goal of the WHO Patient Safety Safe Surgery Saves Lives Challenge is to improve the safety of surgical care around the world by defining a core set of safety standards that can be applied in all countries and settings.

Surgical Resources and Environment: Trained personnel, clean water, consistent light source, consistent suction, supplemental oxygen, functioning surgical equipment and sterile instruments
Prevention of Surgical Site Infection
Hand washing Appropriate and judicious use of antibiotics Antiseptic skin preparation Atraumatic wound care Instrument decontamination and sterility
Safe Anaesthesia
Presence of a trained anaesthetist Anaesthesia machine and medication safety check Pulse oximetry Heart rate monitoring Blood pressure monitoring Temperature monitoring
Safe Surgical Teams
Improved communication Correct patient, site, and procedure Informed consent Availability of all team members Adequate team preparation and planning for the procedure Confirmation of patient allergies
Measurement of Surgical Services: quality assurance, peer review and monitoring of outcomes

The aim of the working groups was to identify potential standards for improvements in four areas: safe surgical teams, by promoting communication among team members to ensure that each preparatory step is accomplished in a timely and adequate fashion with an emphasis on teamwork; safe anaesthesia, by appropriate patient monitoring and advance preparation to identify potentially lethal anaesthetic or resuscitation problems before they cause irreversible harm; prevention of surgical site infection, through antisepsis and control of contamination at all levels of patient care; and measurement of surgical services, by creating public health metrics to measure provision and basic outcomes of surgical care.

The Safe Surgery Saves Lives Challenge was further guided by three principles. The first is simplicity. An exhaustive list of standards and guidelines might create a package that would improve patient safety, but such comprehensiveness would be difficult to implement and convey, and would probably face significant resistance. The appeal of simplicity in this setting cannot be overstated. Uncomplicated measures will be the easiest to institute and can have profound effects in a variety of settings. The second principle is wide applicability. Focusing on a specific resource milieu would reduce the number of issues (e.g. minimum equipment standards for resource-poor settings), but the goal of the challenge is to reach all environments and settings, from resource rich to resource poor, so that all WHO Member States can be involved. Furthermore, regular failures occur in every setting and environment and are amenable to common solutions. The third is measurability. Measurement of impact is a key component of the Second Challenge. Meaningful metrics must be identified, even if they relate only to surrogate processes, and they must be reasonable and quantifiable by practitioners in all contexts.

If the three principles of simplicity, wide applicability and measurability are followed, the goal of successful implementation will be feasible.

Surgical care is complex and involves dozens of steps which must be optimized for individual patients. In order to minimize unnecessary loss of life and serious complications, operating teams have 10 basic, essential objectives in any surgical case, which the WHO safe surgery guidelines support. (1) The team will operate on the correct patient at the correct site. (2) The team will use methods known to prevent harm from administration of anaesthetics, while protecting the patient from pain. (3) The team will recognize and effectively prepare for life-threatening loss of airway or respiratory function. (4) The team will recognize and effectively prepare for risk of high blood loss. (5) The team will avoid inducing an allergic or adverse drug reaction for which the patient is known to be at significant risk. (6) The team will consistently use methods known to minimize the risk for surgical site infection. (7) The team will prevent inadvertent retention of instruments and sponges in surgical wounds. (8) The team will secure and accurately identify all surgical specimens. (9) The team will effectively communicate and exchange critical information for the safe conduct of the operation. (10) Hospitals and public health systems will establish routine surveillance of surgical capacity, volume and results.

I. The team will operate on the correct patient at the correct site.

The universal protocol: The Universal Protocol is a three-step process in which each step is complementary and adds redundancy to the practice of confirming the correct patient, site and procedure.

Step1. Verification: This consists of verifying the correct patient, site and procedure at every stage from the time a decision is made to operate to the time the patient undergoes the operation. This should be done:

- when the procedure is scheduled;

- at the time of admission or entry to the operating theatre;
- any time the responsibility for care of the patient is transferred to another person;
- before the patient leaves the preoperative area or enters the procedure or surgical room.

Step2. Marking: The Universal Protocol states that the site or sites to be operated on must be marked. This is particularly important in case of laterality, multiple structures (e.g. fingers, toes, ribs) and multiple levels (e.g. vertebral column).
The protocol stipulates that marking must be:

- At or next to the operative site; non-operative sites should not be marked;
- unambiguous, clearly visible and made with a permanent marker so that the mark is not removed during site preparation;
- made by the surgeon performing the procedure or must be present during surgery, particularly at the time of incision;
- completed, to the extent possible, while the patient is alert and awake, as the patient's involvement is important.

Step3. 'Time out': The 'time out' or 'surgical pause' is a brief pause before the incision to confirm the patient, the procedure and the site of operation. It is also an opportunity to ensure that the patient is correctly positioned and that any necessary implants or special equipment are available. The Joint Commission stipulates that all team members be actively involved in this process. Any concerns or inconsistencies must be clarified at this stage. The checks during the 'time out' must be documented, potentially in the form of a checklist, but the Universal Protocol leaves the design and delivery to individual organizations. The 'time out' also serves to foster communication among team members.

II. The team will use methods known to prevent harm from administration of anaesthetics, while protecting the patient from pain.
In order that no patient be harmed by anaesthesia, several goals must be met:

- Anaesthesia services should be made safer.
- Training and facilities for anaesthesia should be improved in many parts of the world.
- Safety in obstetric anaesthesia should be a priority, as obstetric patients are at particularly high risk from anaesthesia.
- Standardized global definitions of anaesthesia mortality should be developed.
- Every avoidable death is a tragedy, and lessons should be learnt from each instance of death during anaesthesia in order to reduce the risk of recurrence.

The provision of safe anaesthesia depends on careful preparation, which is facilitated by a systematic approach to reviewing the patient, machine, equipment and medications. This is ideally based on a formal check of the anaesthesia system. In addition to the personnel involved in delivering anaesthetic, the anaesthesia system includes:

- any machine or apparatus that supplies gases, vapours, local anaesthesia or intravenous anaesthetic agents to induce and maintain anaesthesia;
- any equipment necessary for securing the airway;
- any monitoring devices necessary for maintaining continuous evaluation of the patient;
- the patient himself or herself, correctly identified, consensual and evaluated preoperatively.

Highly recommended steps are:

- The first and most important component of peri-anaesthetic care is the continuous presence of a vigilant, professionally trained anaesthesia provider.
- Supplemental oxygen should be supplied for all patients undergoing general anaesthesia. Tissue oxygenation and perfusion should be monitored continuously using a pulse oximeter with a variable-pitch pulse tone loud enough to be heard throughout the operating room.
- The adequacy of the airways and of ventilation should be monitored continuously by observation and auscultation. Whenever mechanical ventilation is employed, a disconnect alarm should be used.
- Circulation should be monitored continuously by auscultation or palpation of the heart beat or by a display of the heart rate on a cardiac monitor or pulse oximeter.
- Arterial blood pressure should be determined at least every 5 minutes and more frequently if indicated by clinical circumstances.
- A means of measuring body temperature should be available and used at frequent intervals where clinically indicated (e.g. prolonged or complex anaesthesia, children).
- The depth of anaesthesia (degree of unconsciousness) should be assessed regularly by clinical observation.

III. The team will recognize and effectively prepare for life-threatening loss of airway or respiratory function.
A failed airway has been defined as three unsuccessful attempts at oro-tracheal intubation by a skilled practitioner or failure to maintain acceptable oxygen saturation (usually 90%) in an otherwise normal patient. Mortality from anaesthesia-related procedures frequently can be due to failure to recognize and address airway and ventilation problems that compromise the patient's oxygenation. While many strategies can be used to manage a difficult airway - such as mask ventilation, insertion of a laryngeal mask airway, endotracheal intubation, fibre-optic intubation and, in the most extreme cases, creation of a surgical airway - simultaneous failure of these approaches is fatal.

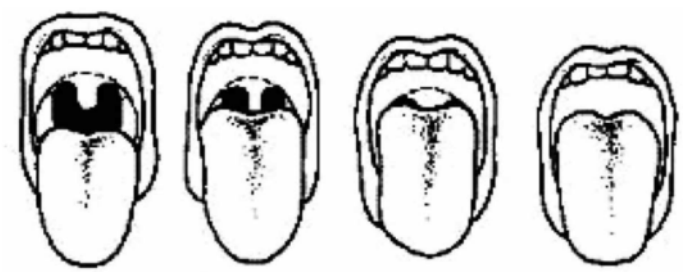
Technique	Failure rate (%)
Bag mask ventilation	0.16
Supraglottic airway insertion	2-6
Intubation	0.05–0.35
Intubation requiring multiple attempts or blades with optimal external laryngeal manipulation occurs in 1-18% of intubations	
Intubation requiring multiple attempts or blades with optimal external laryngeal manipulation and also requiring multiple laryngoscopists occurs in 1-4% of intubations	
Intubation and ventilation	0.0001–0.02

Airways assessment
Failure to evaluate the airway and anticipate problems is widely accepted as the most important factor in ventilation and oxygenation failure.
The most useful bedside test for predicting a difficult intubation in an apparently normal patient is a combination of the Mallampati classification and thyromental distance.

Thyromental distance: Patil and Zauder first described

measurement of the thyromental distance in 1983. This objective test is based on a measurement taken with a ruler or thyromental gauge from the thyroid notch to the undersurface of the mandible with the head fully extended. In an adult, laryngoscopy and intubation should be straightforward if the thyromental distance is > 6.5 cm, challenging if it is 6.0–6.5 cm (especially if associated with prominent teeth, receding jaw, temporomandibular joint problems or cervical spine abnormalities), and often impossible if the thyromental distance is < 6.0. In fact, difficult intubation can occur with both extremes of the distance.

Mallampati classification: The Mallampati test is a subjective evaluation of the ratio of oral cavity volume to tongue volume. Mallampati et al. originally proposed three oropharyngeal classes, but modified this to comprise four classes on the basis of experience with the technique. The test is performed on a sitting patient with the head in a neutral position, mouth fully opened and tongue fully extended and involves evaluating the visibility of anatomical structures as shown in figure. The difficulty of intubation is then classified, a Class 1 airway being the easiest to manage and control by intubation, and a Class 4 airway being potentially the most difficult.



Management of the airway

Guidelines for managing a difficult airway are numerous and many strategies exist to manage the airway during induction. The general themes of all the guidelines and recommendations are similar: avoid hypoxia; prevent trauma; use pre-planned strategies; attempt to identify a difficult airway preoperatively; be prepared with equipment, assistance and skill; be practised in a range of techniques; have backup plans; confirm endotracheal intubation; prepare a clear extubation strategy; and, if the airway is difficult, consider managing patients while they are awake. The essential requirement for managing a difficult airway is a skilled practitioner with adequate assistance, a clear plan of action and suitable equipment.

Face-Mask ventilation: Ventilation with a face mask is a fundamental skill in anaesthesia. Success depends on the ability to maintain a patient airway while holding an airtight seal with a bag-mask. It requires proficiency acquired with practice. The advent of the laryngeal mask airway reduced the need to use face-mask ventilation in the maintenance of anaesthesia. In countries with a ready supply of laryngeal mask airways, this skill may be less widespread than formerly.

Supraglottic airway ventilation: The laryngeal mask airway has become the device of choice for supraglottic airway ventilation. Its growing popularity, where it is available, is testament to its superiority to manual face-mask ventilation. Again, skill and practice are required to appropriately insert it and safely maintain it in position, and inadequate supraglottic airway ventilation occurs after 2 - 6% of insertions. Appropriate patient selection is also essential to avoid problems and complications. Factors associated with difficult supraglottic airway use include restricted mouth opening, upper airway obstruction at or below the level of the larynx, a disrupted or distorted airway, stiff lungs and a stiff cervical spine.

Endotracheal intubation: Endotracheal tubes have become fundamental to the practice of anaesthesia, particularly since the advent of neuromuscular blockade. Its usefulness for maintaining the patency of the airway in anaesthetized patients is undisputed. The skill required to accurately insert and properly maintain an endotracheal tube comes from substantial practice, as well as thorough knowledge of the anatomy of the upper airways and comfort with its many physiologic variations. Difficult endotracheal intubation occurs when multiple attempts are required, either in the presence or absence of disease.

Fibre-optic intubation: The ability to cannulate the airways by flexible bronchoscopy is a skill required of all anaesthetists. It is considered the gold standard for managing an airway expected to be difficult. The indications for its use are numerous: endotracheal intubation of normal and difficult airways, placing selective segmental blockers and tubes such as for thoracic cases, assessing airway function and diagnosing pathology, monitoring during tracheostomy, changing the endotracheal tube, confirming tube placement, broncho-alveolar lavage, placing nasogastric tubes, facilitating other airway management techniques such as retrograde intubation and laryngeal mask airway placement in difficult patients, avoiding extension of the neck or dental damage, performing intubation with topical anaesthesia and improving experience and teaching. Relative contraindications are important to recognize however, and include an acute life-threatening airway obstruction, an uncooperative conscious patient, copious secretions or blood in the airway, an airway-obstructing abscess or friable tumour and distortion of anatomy that limits the airway space.

After intubation, the anaesthetist should always confirm endotracheal placement by listening for breath sounds as well as gastric ventilation and monitoring the patient's oxygenation with a pulse oximeter. Patients undergoing elective surgery should be fasting prior to anaesthesia. Those at risk of aspiration should be pre-treated to reduce gastric secretion and increase pH. Airway disasters, while uncommon, are lethal and entirely preventable with appropriate planning, adequate pre-induction airway evaluation and careful preparation of the patient and equipment. The skill, experience and judgement of a practised anaesthetist and the timely and appropriate support of assistants can avert airway catastrophes and prevent death from anaesthetic administration. All anaesthetists should have a strategy for intubation of the difficult airway.

IV. The team will recognize and effectively prepare for risk of high blood loss.

Loss of a large volume of blood, especially when associated with haemodynamic instability, has been clearly associated with poor surgical outcome. Controlling haemorrhage and mitigating its clinical effects by appropriate fluid resuscitation are important components of intraoperative care.

Resuscitation of hypovolaemic patients

Patients who present for surgery in a volume-depleted state should be resuscitated before surgery whenever possible. Intravenous

	Class I	Class II	Class III	Class IV
Blood loss	≤ 750 ml	750–1500 ml	1500–2000 ml	> 2000 ml
% of blood volume lost	15%	15–30%	30–40%	> 40%
Pulse rate	< 100	> 100	> 120	> 140
Blood pressure	Normal	Normal to decreased	Decreased	Markedly decreased
Mental status	Normal to slightly anxious	Mildly anxious	Anxious and confused	Confused or lethargic
Urine output	Normal	Reduced	Minimal	Nil
Fluid replacement	Crystalloid	Crystalloid	Crystalloid and blood	Crystalloid and blood

access should be obtained promptly and resuscitation begun in an efficient fashion to minimize delays in performing the operation. Fluid deficits should be remedied by infusion of crystalloid solutions. In certain circumstances, some of the fluid deficit can be replaced by oral intake; however, this is often undesirable in gastrointestinal conditions, impending general anaesthetic or other clinical concerns. Monitoring of fluid status should be instituted wherever feasible, tailored to the specific clinical situation and include regular evaluation of haemodynamic parameters, such as pulse rate and blood. It may also include urinary catheterization, central venous cannulation and other invasive monitoring. Communication among the clinicians caring for the patient in the pre-, intra- and post- operative periods will improve resuscitation and allow for appropriate timing of the operation.

Prevention of blood loss

Some procedures, such as caesarean section or major vascular surgery, inevitably involve heavy blood loss. Other circumstances can also predispose a patient to unusually heavy bleeding during an operation, such as reoperation or dissections known to be difficult. The first step in mitigating blood loss during an operation is prevention. Known coagulation deficits should be corrected before surgery whenever clinically possible. The surgical, anaesthetic and nursing personnel involved in an operation should all be aware of the potential for major blood loss before the procedure and be prepared for it. Ensuring appropriate intravenous access is a critical step and allows the anaesthetist to respond to fluctuations in blood pressure. Access may take the form of large-bore peripheral lines, central venous catheters or some combination of the two. If the expected blood loss is greater than 500 ml for an adult or 7 ml/kg in children, the observed standard of practice dictates the insertion of two wide-bore intravenous lines or a central venous catheter (also preferably large bore) to allow for adequate resuscitation. When the need for a blood transfusion is anticipated, operating teams should communicate early with the blood bank to ensure prompt availability of cross-matched blood products. When the patient is bleeding before surgery, it is imperative that all members of the operating team be aware of the source and estimated volume of blood loss.

Management of blood loss

If surgery is undertaken in an emergency or urgently for haemorrhage, complete preoperative resuscitation is often neither practical nor desirable, and resuscitation must be coupled with surgery to stem the haemorrhage. Again, large-bore intravenous access must be obtained and resuscitative measures instituted as soon as possible before operation. Volume resuscitation includes infusion of crystalloid solutions and transfusion of blood products or other volume expanders. Evidence is accumulating for the effectiveness of transfusing fresh-frozen plasma, when available, for each one or two units of packed red blood cells to combat coagulopathy. While increasing the amount of fresh-frozen plasma used, this may decrease the overall use of blood products by decreasing the amount of packed red blood cells required. Where appropriate and available, mechanisms to collect and re-transfuse shed blood may be used. In some situations, temporizing measures should be taken to control bleeding in order to allow fluid resuscitation to catch up with accumulated blood loss before definitive surgical management. In other situations, intra-abdominal packing to temporize bleeding is prudent and may allow for correction of coagulopathy, hypothermia and acidosis. In such 'damage control' surgery, abdominal re-exploration follows 24-72 hours after the initial surgical exploration. The team of anaesthetists, surgeons and nurses must all be aware of the plan for resuscitation so that they can take appropriate measures to reduce the morbidity of haemorrhage. Hypovolaemia represents a situation in which clear, unhindered communication is essential to optimize patient care. Coordination of care during resuscitation and the operation combined with an anaesthetic plan based on the patient's physiological state can make a profound difference in intra-operative management.

Recommendations

Before inducing anaesthesia, the anaesthetist should consider the possibility of large-volume blood loss, and, if it is a significant risk, should prepare appropriately. If the risk is unknown, the anaesthetist should communicate with the surgeon regarding its potential occurrence.

Before skin incision, the team should discuss the risk for large volume blood loss and, if it is significant, ensure that appropriate intravenous access is established.

A member of the team should confirm the availability of blood products if needed for the operation.

Rest of the objectives for Safe Surgery will be covered in next issue…

Reference:
WHO guidelines for safe surgery : 2009 : safe surgery saves lives.
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Dr. Shanti Swaroop Bhatnagar

(February 21, 1894 – January 1, 1955)

Early life

Bhatnagar was born in Shahpur, now in Pakistan. His father Parmeshwari Sahai Bhatnagar died when he was only eight months old and he spent his childhood in the house of his maternal grandfather, an engineer, where he developed a liking for science and engineering. He used to enjoy building mechanical toys, electronic batteries, string telephones. From his maternal family he also inherited a gift of poetry, and his Urdu one-act play Karamati won the first prize in a competition.

Education and early research

He went to England on a research fellowship after completing his Master's Degree in India. He received his DSc. from the University College London in 1921. After returning to India, he was awarded a professorship at Benaras Hindu University. He was knighted in 1941 by the British Government as a reward for his research in science. On March 18, 1943 he was elected as a Fellow of the Royal Society. His research interests included emulsions, colloids, and industrial chemistry, but his fundamental contributions were in the field of magneto-chemistry. He used magnetism as a tool to know more about chemical reactions. He also composed a beautiful kulgeet (University song) which sung with great reverence prior to functions held in the university.

Work in India

Prime Minister Nehru was a proponent of scientific development, and after India's independence in 1947, the Council of Scientific and Industrial Research (CSIR) was set up under the chairmanship of Dr. Bhatnagar. He became the first Director-General of the CSIR. He became known as "The Father of Research Laboratories" and is largely remembered for having established various chemical laboratories in India. He established a total twelve national laboratories such as Central Food Processing Technological Institute, Mysore, National Chemical Laboratory, Pune, The National Physical Laboratory, New Delhi, The National Metallurgical Laboratory, Jamshedpur, The Central Fuel Institute, Dhanbad, just to name a few.

Shanti Swaroop Bhatnagar played a significant part along with Homi Jehangir Bhabha, Prasanta Chandra Mahalanobis, Vikram Ambalal Sarabhai and others in building of post-independent S&T infrastructure and in the formulation of India's science and technology policies. Bhatnagar was the Founder Director of the Council of Scientific and Industrial Research (CSIR), which was to later became a major agency for research in independent India. He was the first Chairman of the University Grants Commission (UGC).

He was Secretary, Ministry of Education and Educational Adviser to Government. Bhatnagar played an important role both in the constitution and deliberations of the Scientific Manpower Committee Report of 1948. Bhatnagar was a University Professor for 19 years (1921-40) first at the Banaras Hindu University and then at the Punjab University and he had a reputation as a very inspiring teacher and it was as a teacher that he himself was most happy. His research contribution in the areas of magneto chemistry and physical chemistry of emulsion were widely recognized. He also did considerable work in applied chemistry. He played an instrumental role in the establishment of the National Research Development Corporation (NRDC) of India, which bridges the gap between research and development. Bhatnagar was responsible for the initiation of the Industrial Research Association movement in the country. He constituted the one-man Commission in 1951 to negotiate with oil companies for starting refineries and this ultimately led to the establishment of many oil refineries in different parts of the country. He induced many individuals and organizations to donate liberally for the cause of science and education.

From Banaras Bhatnagar moved to Lahore where he was appointed as University Professor of Physical Chemistry and Director of University Chemical Laboratories. He spent 16 years in the Panjab University, Lahore and this period was the most active period of his life for original scientific work. While his major fields of study were colloidal chemistry and magneto-chemistry he did considerable work in applied and industrial chemistry. In 1928 Bhatnagar, jointly with K.N. Mathur, invented an instrument called the Bhatnagar-Mathur Magnetic Interference Balance. The balance was one of the most sensitive instruments for measuring magnetic properties. It was exhibited at the Royal Society Soiree in 1931 and it was marketed by Messers Adam Hilger and Co, London.

Bhatnagar did considerable work in applied and industrial chemistry. The first industrial problem undertaken by Bhatnagar was the development of a process to convert bagasse (peelings of sugarcane) into food cake for cattle. One of the important achievements of Bhatnagar in applied and industrial chemistry was the work he did for an oil company. The company in their drilling operations confronted a peculiar problem, wherein the mud used for drilling operation when came in contact with the saline water got converted into a solid mass which hardened further. This solidification of the mud rendered all drilling operations impossible. Bhatnagar realized that this was a problem in colloidal chemistry and developed a suitable method to solve it. The problem was elegantly solved by the addition of an Indian gum which had the remarkable property of lowering the viscosity of the mud suspension and of increasing at the same time its stability against the flocculating action of electrolytes.

Meghnad Saha wrote to Bhatnagar in 1934 saying, 'you have hereby raised the status of the university teachers in the estimation of public, not to speak of the benefit conferred on your Alma Mater'.

After his death, CSIR established the Shanti Swaroop Bhatnagar Award for eminent scientists in his honour.

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Enjoy the humour

1) A man wasn't feeling well so he went to the doctor. After examining him the doctor took his wife aside, and said, "Your husband has a very sensitive heart. I am afraid he's not going to make it, unless you treat him like a king, which means you are at his every beck and call, 24 hours a day and that he doesn't have to do anything himself. On the way home the husband asked with a note of concern "what did he say?" "Well", the lady responded, "he said it looks like you probably won't make it."

2) A doctor, an engineer, a rabbi and a lawyer were debating who was the world's first professional.
The Doctor said, "It must have been a doctor. Who else could have helped with the world's first surgery of taking a rib from Adam to create Eve, the first woman?"
"No," said the rabbi. "It must have been a rabbi, since the Lord needed someone to help preach his message to Adam and the world."
"Wait," said the engineer. "The world was created in 6 days from nothing. Do you know what a master engineering feat that must have been to create the whole world into an organised civilized place from utter chaos?"
"Yes, but who created the chaos?" asked the lawyer….

3) A Sunday school teacher asked the children just before she dismissed them to go to church, "And why is it necessary to be quiet in church?"
Little Johnny replied, "Because people are sleeping."

4) A man is flying in a hot air balloon and realises he is lost. He reduces height and spots a man down below. He lowers the balloon further and shouts:
"Excuse me, can you tell me where I am?"
The man below says:
"Yes you're in a hot air balloon, hovering 30 feet above this field."
"You must work in Information Technology," says the balloonist.
"I do," replies the man. "How did you know?"
"Well," says the balloonist, "everything you have told me is technically correct and yet it's of no use to anyone."
The man below says, "You must work in management."
"I do," replies the balloonist, "but how did you know?"
"Well", says the man, "You don't know where you are, or where you're going, but you expect me to be able to help; and you're in the same position as you were before we met, but now it's my fault.

TechStuff

3D Technology
A 3-D (three-dimensional) film is a motion picture that enhances the illusion of depth perception.
3-D films have existed in some form since the 1950s and later experienced a worldwide resurgence in the 1980s and '90s driven by IMAX high-end theatres and Disney themed-venues.
Stereoscopic cameras and softwares are required to create 3D films.
3D spectacles are used for viewing 3D images and also 3D films.
The two main types of 3D spectacles are "Passive and Active".

Passive 3D glasses
Passive 3D glasses do not require a power source to view the 3D content and the two major types of these are anaglyph and polarized.

Anaglyph glasses are most commonly seen with one red and one green/blue lens. They are the least advanced of all methods of delivering 3D and because they use colour to separate the images, some or all colour information is lost to the viewer, so are rarely used now.

Polarized 3D glasses
To present a stereoscopic motion picture, two images are projected superimposed onto the same screen through different polarizing filters. The viewer wears low-cost eyeglasses which also contain a pair of polarizing filters oriented differently (clockwise/counter clockwise with circular polarization or at 90 degree angles, usually 45 and 135 degrees, with linear polarization). As each filter passes only that light which is similarly polarized and blocks the light polarized differently, each eye sees a different image. This is used to produce a three-dimensional effect by projecting the same scene into both eyes, but depicted from slightly different perspectives.
Polarized 3D glasses come in two forms, linear polarized and circular polarized.
Linear polarized glasses require the user to maintain a vertical head position. Tilting the head, left or right can break the 3D effect.
Circular polarization does away with this problem but requires a special projector and filters and will not be used on 3D televisions.

Active 3D glasses
Active 3D glasses require a power source for them to function. Shutter glasses are the active 3D glasses most viewers will use for 3D content. They use LCD lenses that alternately open and shut each lens to show each eye a different image. Shutter glasses used to be connected to the TV by a wire to provide both synchronization and power but now most are powered by small batteries and receive synchronization signals via an infrared beam similar to a TV remote control. The technology involved, makes shutter glasses considerably more expensive than passive glasses.

Clostridium difficile

Clostridium difficile (C. difficile) is a gram-positive, spore-forming ovel, subterminial anaerobic motile bacillus. It is a bacterium that is related to the cause tetanus and botulism. The C. difficile has two forms, an active, infectious form that cannot survive in the environment for prolonged periods, and a nonactive, "noninfectious" form, called a spore, that can survive in the environment for prolonged periods. Although spores cannot cause infection directly, when they are ingested they transform into the active, infectious form.

Scientific Classification
Kingdom – Bacteria
Phylum – Firmicutes
Class – Clostridia
Order – Clostridiales
Family – Closstridiaceae
Genus – Clostridium
Species – difficile
Binomial name – Clostridium difficile

History
1893 – First case of pseudomembraneous colitis reported as diphtheritic colitis – "Bacillus difficile" isolated.
1970 – Antibiotic-associated colitis identified.
1977 – C. difficile identified as cause from Birmingham General Hospital
1978 – C. difficile toxins identified in humans.
1979 – Therapy with vancomycin or metronidazole
2000 – Increased incidence and virulence

Antibiotic-associated (C. difficile) colitis is an infection of the colon caused by C. Difficile occurs primarily among individuals who have been using antibiotics. It is the most common infection acquired by patients while they are in the hospital. More than three million C. difficile occur in hospitals in the US each year. After a stay of only two days in a hospital, 10% of patients will develop infection with C. difficile. C. difficile may be acquired outside of hospitals in the community. It is estimated that 20,000 infections with C. difficile in the community each year in the U.S.

Pathogenicity
C. difficile spores are found frequently in: hospitals, nursing homes, extended care facilities, and nurseries for newborn infants. They can be found on bedpans, furniture, toilet seats, linens, telephones, stethoscopes, fingernails, rings (jewelry), floors, infants' rooms, and diaper pails.
They even can be carried by pets. Thus, these environments are a ready source for infection with C. difficile.

Epidemiology
C. difficile spores lie dormant inside the colon until a person takes an antibiotic. The antibiotic disrupts the other bacteria that normally are living in the colon and preventing C. difficile transforming into its active, disease causing bacterial form. As a result, C. difficile into its infectious form and then produces toxins (chemicals) that inflame and damage the colon. The inflammation results in an influx of white blood cells to the colon. The severity of the colitis can vary. In the more severe cases, the toxins kill the tissue of the inner lining of the colon, and the tissue falls off. The tissue that falls off is mixed with white blood cells (pus) and gives the appearance of a white, membranous patch covering the inner lining of the colon. This severe form of C. difficile is called pseudomembranous colitis because the patches appear like membranes, but they are not true membranes. Not everybody infected with C. difficile colitis. Many infants and young children, and even some adults, are carriers (they are infected but have no symptoms) of C. difficile. C. difficile not cause colitis in these people probably because; (1) the bacteria stay in the colon as non-active spores, and (2) the individuals have developed antibodies that protect them against the C. difficile .

Symptoms
Patients with mild C. difficile may have a grade fever, mild diarrhea (5-10 watery stools a day), mild abdominal cramps and tenderness.
Patients with severe C. difficile colitis may have a high fever (temperature of 102°F to 104°F), severe diarrhea (more than 10 watery stools a day) with blood and severe abdominal pain and tenderness.
Severe diarrhea also can lead to dehydration and disturbances in the electrolytes (minerals) in the body. Rarely, severe colitis can lead to life-threatening complications such as megacolon (markedly dilated colon), peritonitis (inflammation of the lining of the abdominal), and perforation of the colon.

Which antibiotics cause C. difficile ?
Although the antibiotic clindamycin (Cleocin) has been widely recognized as causing C. difficile colitis, many commonly prescribed antibiotics also cause colitis. Examples of antibiotics that frequently cause C. difficile colitis include:
Ampicillin, Amoxicillin, and Cephalosporins [such as cephalexin (Keflex)].
Antibiotics that occasionally cause C. difficile colitis include:
Penicillin, Erythromycin, Trimethoprim, and Quinolones such as Ciprofloxacin (Cipro).
Antibiotics that rarely if ever cause C. difficile colitis include:
Tetracycline, Metronidazole (Flagyl), Vancomycin (Vancocin), and Aminoglycosides [such as Gentamicin (Garamycin)].
In fact, metronidazole and vancomycin are two antibiotics that are used for treating C. difficile colitis; however, there are rare reports of C. difficile colitis occurring several days after stopping metronidazole.
While most C. difficile colitis in the US is caused by antibiotics, C. difficile also can occur in patients without exposure to antibiotics. For example, patients with ulcerative colitis and Crohn's disease have been known to develop C. difficile colitis without exposure to antibiotics.
Since many antibiotics can cause C. difficile infection, all antibiotics should be used prudently. Self-administration or using antibiotics without an accurate diagnosis or a proper reason should be discouraged. On the other hand, benefits of properly prescribed antibiotics for the right reasons usually far outweigh the risk of developing C. difficile colitis.
Antibiotics can sometimes cause diarrhea that is not due to C. difficile. The reason for the diarrhea is not clear. The practical implication is that not all diarrhea associated with antibiotics should be considered to be due to C. difficile and treated as such.

Diagnosis

History: A history of antibiotic use is important in the diagnosis of C. difficile. Patients taking antibiotics (or recently having taken antibiotics) who abdominal pain, cramps and diarrhea are usually tested for C. difficile. However, doctors do not always wait for the appearance of diarrhea to start testing for C. difficile in rare instances C. difficile cause abdominal pain and tenderness without diarrhea.

Laboratory tests: Patients with C. difficile colitis often have elevated white blood cell counts in the blood, and, in severe colitis, the white blood cell counts can be very high (20,000 to 40,000). Patients with C. difficile colitis also often have white blood cells in their stool when a sample of stool is examined under a microscope.

The most widely used test for diagnosing C. difficile colitis is a test that detects toxins produced by C. difficile in a sample of stool. There are two different toxins, toxin A and toxin B, both capable of causing colitis. Accurate tests for both toxins are available commercially for use in all laboratories. Unfortunately, like most tests in medicine, these tests for toxins are not perfect; both false positive tests (finding toxins when there is no C. difficile) and false negative tests (not finding s when C. difficile is present) can occur. Therefore, other tests such as flexible sigmoidoscopy and colonoscopy often are necessary to look for pseudomembranes that are characteristic of C. difficile colitis.

Flexible sigmoidoscopy and colonoscopy: Flexible sigmoidoscopy is an examination in which a doctor inserts a flexible fiberoptic tube with a light and a camera on its end into the rectum and sigmoid colon. (The sigmoid colon is the segment of the colon that is closest to the rectum.) In most patients with C. difficile colitis, the doctor will find pseudomembranes in the rectum and the sigmoid colon. However, some patients with C. difficile colitis will have pseudomembranes only in the right colon (the segment of the colon farthest from the rectum). Patients with pseudomembranes confined to the right colon require colonoscopy in order to see the pseudomembranes. (A colonoscope is a longer version of the flexible sigmoidoscope that is long enough to reach the right colon.)

X-Rays: X-ray examinations and computed tomography (CT) examinations of the abdomen will occasionally demonstrate thickening of the wall of the colon due to inflammation, but these x-ray findings also are non-specific and only demonstrate that colitis is present. They do not demonstrate the cause of the colitis, for example, C. difficile.

Treatment

Treatment of C. difficile colitis includes: (1) correction of dehydration and electrolyte (mineral) deficiencies, (2) discontinuing the antibiotic that caused the colitis, and (3) using antibiotics to eradicate the C. difficile bacterium.

In patients with mild colitis, stopping the antibiotic that caused the infection may be enough to cause the colitis and diarrhea to subside. In most cases, however, antibiotics are needed to eradicate the C. difficile.

Antibiotics that are effective against C. difficile include metronidazole (Flagyl), and vancomycin (Vancocin). These two antibiotics usually are taken orally for 10 days. Both antibiotics are equally effective. With either antibiotic, fever usually will resolve in one or two days, and diarrhea in three or four days.

The choice of which antibiotic to use depends on the individual patient's situation and the preferences of the treating doctor. Some doctors will prescribe metronidazole first because it is much cheaper than vancomycin. Vancomycin is reserved for patients who do not respond to metronidazole, are allergic to metronidazole, or develop side effects from metronidazole. Other doctors will prescribe vancomycin first for severe colitis because vancomycin can achieve much higher antibiotic levels in the colon than metronidazole (and higher antibiotic levels theoretically would be more effective in killing bacteria).

What is new in C. difficile?

The prevalence of C. difficile infection has been increasing steadily particularly in the elderly. There have been reports from several hospitals of a newer, more virulent strain of C. difficile bacteria that produces large amounts of both toxins A and B and as well as a third toxin. This strain produces more severe colitis than the usual strains. Patients infected by this strain are more seriously ill, require surgery more frequently, and die from the infection more frequently than patients infected with the usual strains. Currently, the commercially available diagnostic tests cannot distinguish this strain from the usual strains.

Traditionally, antibiotic use is often considered the most important factor for the development of C. difficile colitis. Increasingly though doctors are diagnosing C. difficile colitis in patients without antecedent antibiotic exposure. This is especially true in patients with Crohn's disease or ulcerative colitis. In one study of 92 patients with ulcerative colitis and Crohn's disease relapse, 10 patients tested positive for C. difficile.

Another change that is occurring with C. difficile infection is that it is no longer restricted to patients in hospitals or nursing homes. Up to one-quarter of infections begin out of the hospital.

Doctors are witnessing increasing difficulty in treating C. difficile colitis. Firstly, resistance to metronidazole is on the rise. Secondly, colitis (along with symptoms of diarrhea and cramps) is taking longer to resolve and may require higher doses of vancomycin. Thirdly C. difficile colitis relapse (with recurrent diarrhea) is common. More troublesome still, many patients experience multiple relapses, often requiring prolonged (months) antibiotic (such as vancomycin) treatment.

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Sterile Disinfectants

A sound cleaning and sanitization program is needed for controlled environments used in the manufacture of Pharmacopeia articles and to prevent the microbial contamination of these articles. Sterile drug products may be contaminated via their pharmaceutical ingredients, water, packaging components, manufacturing environment, processing equipment and manufacturing operators. So maintaining the sterile environment is must and these are done by High Technology Disinfectants.

Disinfectants are substances that are applied to non-living objects to destroy microorganisms that are living on the objects. Disinfection does not necessarily kill all microorganisms, especially non-resistant bacterial spores; it is less effective than sterilization, which is an extreme physical and/or chemical process that kills all types of life.

"Biocide" is a general term describing a chemical agent, usually broad spectrum that inactivates microorganisms. Because biocides range in antimicrobial activity, other terms may be more specific, including "-static," referring to agents which inhibit growth e.g., bacteriostatic, fungistatic, and sporistatic and "-cidal," referring to agents which kill the target organism e.g., sporicidal, virucidal, and bactericidal. Disinfectants can be sporostatic but are not necessarily sporicidal.

In Pharmaceuticals the areas has been classified to many classes according to the bioburden and it as follows

Class 100,000:- particle count not to exceed a total of 100,000 particles per cubic foot of a size 0.5µ and larger.

Class 10,000:- particle count not to exceed a total or 10,000 particles per cubic foot of a size 0.5µ and larger.

Class 1,000:- particles count not to exceed a total of 1000 particles per cubic foot of a size 0.5µ and larger.

Class 100:- particles count not to exceed a total of 100 particles per cubic foot of a size 0.5µ and larger.

Paul Villard, a French chemist and physicist, discovered gamma radiation in 1900, while studying radiation emitted from radium. Alpha and beta "rays" had already been separated and named by the work of Ernest Rutherford in 1899, and in 1903 Rutherford named Villard's distinct new radiation "gamma rays." GAMMA RAYS (denoted as γ), is electromagnetic radiation of high frequency (very short wavelength). They are produced by sub-atomic particle interactions such as electron-positron annihilation, neutral ion decay, radioactive decay (including isomeric transition which involves an inhibited gamma decay), fusion, fission or inverse Compton scattering in astrophysical processes. Gamma rays have frequencies above 10 exahertz (10^{19} Hz), and therefore have energies above 100 keV and wavelength less than 10 picometers, often smaller than an atom.

Gamma irradiation is a safe technology that can penetrate dense material to eliminate the presence of microorganisms, without damaging or changing the temperature of the material. Gamma sterilization has significant advantages compared to other methods of sterilization. Gamma sterilization: has better assurance of sterility than sterile filtration and aseptic processing; is a low temperature process; is more penetrating than E-beam irradiation; and unlike poison gas technologies gamma irradiation does not leave residue behind.

Gamma rays have wavelength shorter than the wavelength of ultraviolet light. X rays, which have wavelength of 0.1 to 40 nm, and gamma rays, which have even shorter wavelength, are forms of ionizing radiation, so named because it can dislodge electrons from atoms, creating ions. (Longer wavelengths comprise non-ionizing radiation.) These forms of radiation also kill microorganisms and viruses and ionizing radiation damages DNA and produces peroxides, which act as powerful oxidizing agents in cells.

As there will be bio burden on the surface of the disinfectant also e.g.; on surface of bottle, on the cover of pack etc., it is very important to sterilize the disinfectant also before taking to the highly sterile areas like class 100, e.g.: eye ointment filling areas, injection filling areas, and also it is necessary to remove the outer cover of the disinfectant bottle before taking inside these areas.

Mechanism of disinfectant activity against microbial cells

Target	Disinfectant
Cell wall	Formaldehyde, hypochlorite and mercurials
Cytoplasmic membrane, action on membrane potential	Anilides and hexachlorophene
Membrane enzymes, action on electron transport chain	Hexachlorophene
Action on ATP	Chlorhexidine and ethylene oxide
Action on enzymes with SH groups	Ethylene oxide, glutaraldehyde, hydrogen peroxied, hypochlorite, iodine and mercurials.
Action on general membrane permeability	Alcohols, chlorexidine and quaternary ammonium compounds
Cell contents, general coagulation	Chlorhexidine, aldehydes, hexachlorophene and quaternary ammonium compounds,
Ribosomes	Hydrogen peroxide and mercurials
Nucleic acids	Hypochlorites
Thiol groups	ethylene oxide, glutaraldehyde, hydrogen peroxide, hypochlorite, mercurials
Amino groups	Ethylene oxide, glutaraldehyde and hypochlorite
General oxidation	Ethylene oxide, glutaraldehyde, and hypochloirite

Sterility Testing

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

According to Indian Pharmacopoeia (1996) the sterility testings are intended for detecting the presence of viable forms of microorganisms in or on the pharmacopoeial preparations. In actual practice, one invariably comes across certain absolutely important guidelines and vital precautionary measures that must be adhered to strictly so as to accomplish the utmost accuracy and precision of the entire concept of sterility testing for life-saving secondary pharmaceutical products (drugs). A few such cardinal factors, guidelines, and necessary details are as enumerated here. Sterility testing of medical devices is required during the sterilization validation process as well for routine quality control. ISO Standards for both Gamma and Electron Beam Sterilization employ sterility testing as a measure of the adequacy of sterilization parameters. An understanding of sterility testing is beneficial in terms of designing a validation process. This journal presents the general concepts and problems associated with sterility testing as well as the various testing methodologies.

INTRODUCTION

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

ENVIRONMENTAL CONCERNS RELATED TO STERILITY TESTING

The environment should be as stringently controlled as an aseptic processing environment. An aseptic processing environment (clean room) is used to dispense sterile pharmaceuticals into presterilized containers. A clean room is generally a room that delivers laminar flow air which has been filtered through microbial retentive High Efficiency Particulate Air (H.E.P.A.) filters. The room is maintained under positive pressure and has specifications for room air changes per hour. An environment used for sterility testing should be similar in design to an aseptic processing environment; there should be an anteroom for gowning and a separate area for the actual sterility testing. The testing area should meet ISO Class 5 particulate control requirements (specified in USP 1116 chapter).

METHODOLOGIES

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

The ISO radiation sterilization microbial methods describe a sterility test which is a modification for the USP method. This test is specific for the detection of aerobic organisms which have been exposed to sub-lethal sterilization cycles. This ISO sterility test method is recommended for the validation of both gamma and electron beam sterilization processes. ISO recommends that the sterility test be validated by using known sterile products.

PROCESSES

Prior to actual sterility testing, it is prudent to send an example sample to the testing laboratory so the laboratory can determine the appropriate testing procedure. Each product should have a unique procedural specification for testing. The procedure should be very specific in terms of which items to test (in the case of kits) and indicate the Sample Item Portion (SIP). The SIP is the percentage of the complete product tested. Medical devices come in all shapes and sizes. For large and cumbersome devices it is very difficult to test them in their entirety. Therefore, the test laboratory will determine a SIP which is a portion of the sample expressed in fractional terms (i.e. 0.1 for 10 percent of the sample). This number is used in gamma and electron beam dose setting methods. The SIP portion should be validated by sterility testing. Combination products have unique challenges. A combination product is defined as one that has a drug component integrated with a medical device. For example, a drug coated stent. The agency office of combination products would determine which regulatory branch is officiating the product. Official USP sterility testing of combination products is required for all sterile drug products. The drug product component applied aseptically creates the largest challenge to laboratory personnel. Biologics must be aseptically processed and cannot be terminally sterilized.

The USP Sterility Test contains two qualifying assays which must be performed prior to sterility testing. They are the "Suitability Test" (Growth Promotion Test) and the "Validation Test" (Bacteriostasis and Fungistasis Test). The Suitability Test is used to confirm that each lot of growth media used in the sterility test procedure will support the growth of less than 100 viable microorganisms. If the media cannot support the growth of the indicator organisms, then the test fails. Secondly, a portion of each media lot must be incubated and assessed for sterility according to the incubation parameters (time, temperature) established by the method. If the media is found to be non-sterile, then the test fails. The Validation Test is used to determine if the test sample will inhibit the growth of microorganisms in the test media. The USP describes three general methods for sterility testing: 1) Membrane Filtration; 2) Direct Transfer (Product Immersion); and 3) Product Flush.

MEMBRANE FILTRATION STERILITY TESTING

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

DIRECT TRANSFER STERILITY TESTING

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

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

PRODUCT FLUSH STERILITY TESTING

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

INTERPRETATION OF STERILITY TEST RESULTS

The technician must be trained as to how to detect growth during the incubation period. Growth is determined by viewing the media, which is generally clear and transparent, against a light source. Turbid (cloudy) areas in the media are indicative of microbial growth. Once growth is detected, the suspect vessel is tested to confirm that the turbidity present is due to microorganisms and not due to disintegration of the sample. Sometimes samples produce turbidity because of particulate shedding or chemical reactions with the media. Once a suspect container has been tested, it should be returned to the incubator for the remainder of the incubation period. Samples that render the media turbid are transferred on Day 14 of the test and incubated for 4 days. Growth positive samples require further processing such as identification and storage.

STERILITY TEST FAILURE INVESTIGATION

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

1. clean room environmental test (EER) data;
2. media sterilization records;
3. technician training records;
4. the relative difficulty of the test procedure;
5. control data (open and closed media controls);
6. technician sampling data (microbial counts on gloves and/or garments post testing).

CONCLUSION

Sterility testing requires high levels of control with regards to CFR Quality Systems Requirements, Good Laboratory Practices, environment and employee practices. It is essential that meticulous technique be employed. Sterility testing is an integral part of sterilization validation as well as a routine quality control. False positive results are common and should be planned for.

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Microxpress

Introducing.....

Gamma Irradiated Sterile Disinfectants

Historically pharmaceutical, biotechnology and medical device manufacturers have been using disinfectants and filter through 0.22 micron filter for further usage. Lack of availability of good quality sterile disinfectants is a major hurdle facing GMP plants today, which is being overcome on an ad hoc basis by taking recourse to 0.22 micron filtration of available disinfectants, solutions and products. Such ad hoc methods give rise to the issue of credibility of the performance of such products and require ongoing validation of process that are used to aseptically filter the disinfectants through 0.22 micron filter. Thus gamma sterilized products takes care of the aforesaid procedures and guarantees availability of sterile, effective and proven products for clean room usage.

Hand Disinfection

Cat No.	Products	Activity	Application
ANX0500	Alconox	Bactericidal, fungicidal and virucidal	Personal hand hygiene
PLG0500	Purellium Gel - C	Bactericidal, fungicidal and virucidal	Personal hand hygiene
TST0500	Triosept	Bactericidal, fungicidal and virucidal	Personal hand hygiene

Environment and Surface Disinfection

Cat No.	Products	Activity	Application
AST0500	Aerosept – C	Bactericidal, fungicidal and virucidal	For disinfecting laminar hoods, table tops, workstations, air and surface disinfection in ciritcal areas.
MLE0500	Microlyse – C	Bactericidal, fungicidal and virucidal	For floor mopping and surface disinfection
NST0500	Nusept – C	Bactericidal, fungicidal and virucidal	For surface disinfection and general purpose disinfection
NVC0500	Novacide	Bactericidal, fungicidal and virucidal	For surface disinfection
ATR0500	Acitar	Bactericidal, fungicidal and virucidal	For environment (fumigation) and surface disinfection

Pack Size Available – 750 ml

BioShields Presents Nusept

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

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

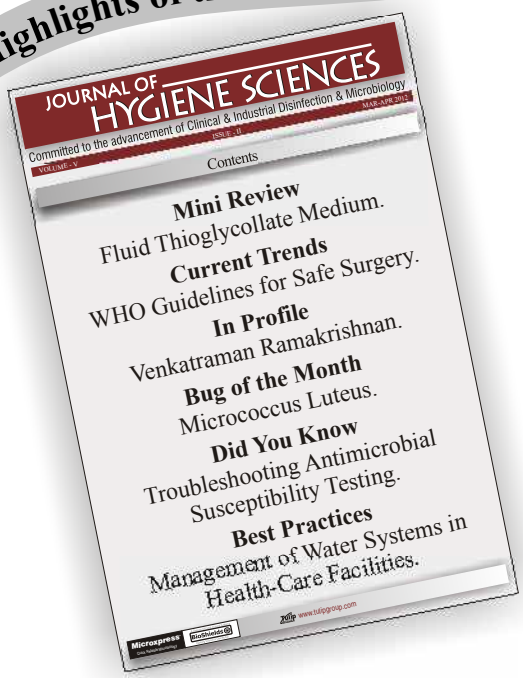
ACTIVITY : Broad spectrum: Bactericidal, Fungicidal & Virucidal.

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

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

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

Highlights of the coming issue



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