JOURNAL OF
HYGIENE SCIENCES
Committed to the advancement of Clinical & Industrial Disinfection & Microbiology
VOLUME - I ISSUE - VI NOV-DEC 2008

Editorial

Contents

An aseptic processing activity is ordinarily supported by monitoring of the environmental air and surfaces in proximity to the process. The purpose of this monitoring is to confirm the acceptability of the environment during the process execution. There are number of environmental methodologies and also a number of regulatory and pharmacopeial references that delineate microbial levels considered acceptable for aseptic processing environments. One of the major routes of contamination in any pharmaceutical production facility is the air. Biological Air Sampling is routinely used to monitor the populations of air borne particles of the surrounding area. In Mini Review we have concentrated on microbiological air sampling and its significance in various industry as well as in clinical segment. Like every issue we have discussed about a new trend in our Current Trends section. Aldehydes, especially glutaraldehyde and formaldehyde, have been used as antimicrobial agents for the past several decades. Though glutaraldehyde is widely used as antimicrobial agents it has some major disadvantages. Here we have solution for that with a new molecule, Polyhexamethylene biguanide (PHMB). Though PHMB is a widely used antimicrobial agent there is little or no evidence of emerging resistance in nosocomial pathogens.

This time our In Profile section is dedicated to Sir Alexander Fleming, discoverer of lifesaving antibiotic Penicillin. The genus *Escherichia* is a typical member of the *Enterobacteriaceae*, the enteric bacteria, which are facultatively anaerobic Gram-negative rods that live in the intestinal tracts of animals in health and disease. Bug of the Month is focused on morphological and cultural characteristics, antigenic structure, and virulence characteristics of *Escherichia coli*. Clinical manifestations of *Escherichia coli* infection as well as the diagnosis and treatment are also detailed in this section. Surgical site infections are the third most commonly reported nosocomial infection and they account for approximately a quarter of all nosocomial infections. They have been responsible for the increasing cost; morbidity and mortality related to surgical operations and continue to be a major problem even in hospitals with most modern facilities and standard protocols of preoperative preparation and antibiotic prophylaxis. Centers for Disease Control and Prevention (CDC) have recommended some preventive measures, which are enlightened in Did You Know section.

This time Best Practices section covers a vital topic of microbiology, culture media preparation and its quality control. Media preparation, proper storage and quality control testing can assure a consistent supply of high quality media. Check our In Focus page. Tulip Group, which is well known for its innovation, has come up with some new products. Merry Christmas to all our readers.

Microbiological Air Sampling: A Requisite Factor For Environmental Monitoring

Environmental Monitoring & Its Importance

Environmental conditions may directly or indirectly affect the quality of the finished product. An environmental monitoring program has to be properly constituted to be of any practical use. Environmental control describes the systems functionally ensuring that clean rooms operate within predetermined limits whereas environmental monitoring describes the techniques used to measure the effectiveness of the environmental control systems and defines the procedure necessary in the event of limits being exceeded. Environmental control, particularly in sterile manufacture, is achieved by means of many factors: well designed and efficiently operated facilities and air handling systems, by the use of integral HEPA filters, well designed and well made garments for staffs, by reliable disinfection regimes, and by rigid adherence to aseptic disciplines. The purpose of routine monitoring of aseptic manufacturing facilities is to obtain some measure of the level of control being achieved. The ideal is that monitoring should be done in a way that will promptly reveal any failure of the control systems to meet their intended purpose.

All pharmaceutical manufacturing environments merit a level of environmental monitoring. The greatest emphasis and the tightest limits are applied to sterile manufacturing facilities. When different areas within sterile manufacturing facilities serve different purposes, so the environmental monitoring programs differ. In Europe, microbiological limits applying to various grades of manufacturing clean room are specified in the Guide to Good Manufacturing Practice for Medicinal Products. Monitoring should be done when the facilities are manned and operational. In chapter <1116> of United States Pharmacopeia is followed for the microbiological environmental monitoring. The manufacturer should decide the type of suitable environmental monitoring program for his/her own facility, since each plant is unique. Most manufacturers therefore develop their own in house standards. Such standards must be based on knowledge of the normal background levels of contamination.

One of the major routes of contamination in any pharmaceutical production facility is the air. Biological Air Sampling is routinely used to monitor the populations of air borne particles of the surrounding area. In the context of microbiological assessment air sampling is the collection of air borne microbial contaminants that may impact on product spoilage, product safety and human health. Air Sampling has the following significance:

- Measures the number of viable air borne particles (i.e. the concentration of microorganisms in the air).
- Evaluation of the effectiveness of control methods.
- Compliance status with respect to various occupational health standards.
- Routine surveillance.

Clean Room Classification

The design and construction of clean rooms and controlled environments are covered in Federal Standard 209E. This standard of air cleanliness is defined by the absolute concentration of airborne particles. The application of Federal Standard 209E to clean rooms and other controlled environments in the pharmaceutical industry has been used by manufacturers of clean rooms to provide a specification for building, commissioning and maintaining these facilities. Data available in the pharmaceutical industry provide no scientific agreement on a relationship between the number of nonviable particulates and the concentration of viable microorganisms. The criticality of the number of nonviable particulates in the electronic industry makes the application of Federal Standard 209E a necessity, while the pharmaceutical industry has a greater concern for viable particulates than total particulates as specified in Federal Standard 209E. The rationale that the fewer particulates present in a clean room, the less likely it is that airborne microorganisms will be presently accepted and can provide pharmaceutical manufacturers and builders of clean rooms and other controlled environments with engineering standards in establishing a properly functioning facility.

Federal Standard 209E, as applied in the pharmaceutical industry is based on limits of all particles with sizes equal to or larger than 0.5µm. Following table describes airborne particulate cleanliness classes in Federal Standard 209E as adapted to the pharmaceutical industry. The pharmaceutical industry deals with Class M 3.5 and above. According to Federal Standard 209E (United States) **USP** classification of clean room is given as follows:

Class Name		Particles equal to and larger than 0.5µm	
SI	U.S. Customary	(m^3)	(ft^3)
M1	-	10.0	0.283
M1.5	1	35.3	1.00
M2	-	100	2.8
M2.5	10	353	10.0
M3	-	1,000	28.3
M3.5	100	3,530	100
M4	-	10,000	283
M4.5	1,000	35,300	1,000
M5	-	100,000	2,830
M5.5	10,000	353,000	10,000
M6	-	1,000,000	28,300
M6.5	100,000	3,530,000	100,000
M7	-	10,000,000	283,000

Table: Airborne Particulate Cleanliness Classes* (* Adapted from U.S. Federal Standard 209E)

The number in the classification description represents the limit of particles >= 0.5 µm that may be present in a one cubic meter sample of air. Therefore, lower the number in the class description, cleaner the air. Class 100 rooms are considered to be critical areas or zones where sterile product or containers come in direct contact with the environment. Class 10,000 describes the air quality in rooms immediately outside of Class 100. These rooms are usually storage rooms, corridors and other rooms with service and support functions within the aseptic core. Class 100,000 describes the air quality for preparation areas, compounding areas and any other area immediately adjacent to the aseptic suite.

Clean areas for the manufacture of sterile products are classified according to the required characteristics of the environment. Each manufacturing operation requires an appropriate environmental cleanliness level in the operational state in order to minimize the risks of particulate or microbiological contamination of the product or materials being handled. In order to meet "in operation" conditions these areas should be designed to reach certain specified air cleanliness levels in the "at rest" occupancy state. This latter state is the condition where the installation is complete and the production equipment has been installed and is operating, but no operating personnel are present. The "in operation" state is the condition where the installation is functioning in the defined operating mode and the specified numbers of personnel are present.

According to **EU GMP**- guidelines classification of clean room is given as follows:

GMP Class	Max. Number of particles if not in operation ($1/m^3$)		Max. Number of particles in operation ($1/m^3$)	
Particle Size	0.5 µm	5 µm	0.5 µm	5 µm
A	3,500	1	3,500	1
B	3,500	1	350,000	2,000
C	350,000	2,000	3,500,000	20,000
D	3,500,000	20,000	n.d.*	n.d.*

* n.d. = not defined

Four grades are described as follows:

- Grade A: The local zone for high-risk operations, e.g., filling and making aseptic connections. Normally such conditions are provided by laminar airflow workstations.
- Grade B: In aseptic preparation and filling, the background environment for the Grade A zone.
- Grade C & D: Clean areas for carrying out less critical stages in the manufacture of sterile products.

In order to reach the B, C & D air grades, the number of air changes should be appropriate for the size of the room and the equipment & personnel present in it. At least 20 air changes per hour are usually required for a room with a good airflow pattern and appropriate HEPA filters.

The basic limitation of particulate counters is that they measure particles of 0.5µm or larger. While airborne microorganisms are not free floating or single cells, they frequently associate with particles of 10-20µm. Microbial counts within controlled environments vary with the sampling location and the activities being conducted during sampling.

Test frequencies (air sampling) for environmental monitoring:

The frequency of air monitoring depends on the criticality of the specified sites. Following table (acc to USP) shows the frequencies of sampling in decreasing order of frequency of sampling and in relation to the criticality of the area of the controlled environment being sampled.

Aseptic production (clean room area)	**Evaluation frequency**
Class 100	Every shift
Class 10.000	Daily
Class 100.000	2 times per week
Class 100.000 (Non-product/ container contact)	1 time per week

Microbiological Air Sampling: One of the Methods of Environmental Monitoring

Environmental monitoring can be done by various methods: microbiological air sampling, microbiological surface sampling, physical particle monitoring, monitoring of pressure differentials, etc. Following construction of a clean room, it must be tested to ensure that it is providing the required quality of environment. These verification tests are rigorously performed and are similar to the tests used to monitor clean room subsequently. The monitoring tests ensure that the clean room continues to provide satisfactory operation. To ensure that the pharmaceutical clean room is providing the required environmental standards, the following are determined.

Air Quality

The air supplied to the clean room must not contribute to particulate or microbial contamination within the room. The HEPA filters for the inlet air must be tested to ensure that neither the filter fabric nor the filter seals are leaking. Air handling equipment for any controlled or critical area should be sized to provide adequate volumes of incoming and exhaust air in order to achieve and maintain appropriate air quality. Air quality should be commensurate with the type of process (aseptic or non sterile), risk of product exposure to the environment, and criticality of microbial limit expectations.

Air Movement

Adequate ventilation through out the clean room can be

determined by air movement tests. These are carried out at the time of clean room validation. The outflow of air from a clean room with a higher standard of cleanliness to an area with a lower standard is indicated by the pressure differential between the rooms.

Air Velocity

The velocity of the air at several points in a clean room area of critical importance should be determined. This is done both at validation of the clean room and at timed intervals. The procedure involves the use of an anemometer.

Airborne Particulate And Microbial Monitoring

The particle count and the microbial bioburden of the clean room provide the basis for the air classification system for grading a clean room as discussed above. The points for sampling and the number of samples taken at each position are determined by the size and the grade of the clean room. There should be very few viable organisms present in the clean room air.

Operators within the clean room disperse large numbers of skin particles. Many of these particles are contaminated with bacteria. Sampling for microbial contamination is necessary when people are present in the clean room during production. Monitoring of the microbial contamination during production will ensure that that both the use of clean room clothing by the operators and the air ventilation system are producing the required environmental standards.

Air sampling can be done in two ways: active air sampling and passive air sampling. Active air sampling is intended to provide an index of the number of microorganisms per unit volume of air space in clean rooms. If a good air handling system, with integral HEPA filters in place, serves the clean room air borne, microbial contamination arises from personnel operating within the clean room.

Most active air sampling should be done when the clean room is operational. If a clean room has been non operational for a few days (e.g. a long weekend) or a few weeks (e.g., a scheduled shut down for vacation or maintenance), it is beneficial to start sampling a few days prior o production start up. All active air samplers will disrupt airflow to some extent. They should be located carefully, and when they are operated, they must not counteract protective airflow patterns in significant parts of the clean room.

It is generally accepted by scientists that air borne microorganisms in controlled environments can influence the microbiological quality of the intermediate or final products manufactured in these areas. Different types of air samplers are used for sampling procedure.

Slit-to-Agar Air Sampler (STA) - The principle of the slit sampler is that of inertial impaction; particles moving in an air stream have an individual inertia and may be deflected onto a surface where they may be trapped by impaction. Slit samplers are provided with pumps that draw air from the area being sampled through a narrow slit.

The effect of the slit is to increase the velocity of the airflow and hence the inertial velocity of the any particle being carried in the air stream. The accelerated particles are directed onto the surface of a plate of basal medium (e.g. nutrient agar) that is rotated continuously and progressively. Pumps provided with slit samplers usually operated at fix rates, and an on/off button controls the volume of air sampled.

Sieve Impactor - The apparatus consists of a container designed to accommodate a Petri dish containing a nutrient agar. The cover of the unit is perforated, with the perforations of a predetermined size. A vacuum pump draws a known volume of air through the cover and the particles in the air containing microorganisms impact on the agar medium in the Petri dish. Some samplers are available with a cascaded series of containers containing perforations of decreasing size.

Sieve impactors are available in single or multistage designs, which facilitate both enumeration and sizing of aero biologic contaminants. All single stage systems impact microorganisms aspirated by the sampler through a matrix of multiple inlet orifice, directly on to an agar medium for development from a single plate with no further subculture steps required for enumeration. In the case of multistage impactors, each vertically stacked stage contains an individual agar plate. As the sample air transits the device at a constant volume, sample velocities increased at each stage, resulting in gradient deposition and accurate sizing of microorganisms of smaller diameters and lower mass.

Advantages of sieve samplers are generally high particle deposition rates, and ability to size particles and vary sampling time and volume. Sieve impactors demonstrate superior collection efficiencies when compared to other methods of aero biologic testing.

Centrifugal Sampler - This type of sampler contains a propeller or turbine that pulls a known volume of air into the instrument and then pushes it outward onto a nutrient agar strip. After sampling the strip is placed back into its original sterile plastic cover and incubated for a specified time at a specified temperature range. Centrifugal samplers operate by accelerating air with entrained particles until a critical velocity is reached for a given particle size. The blades of an impeller force the air onto agar surface. The volume of air sampled may be difficult to determine because the air mixes as it enters and exits the sampler. Centrifugal sampler may disrupt the linearity of airflow in the controlled zone where it is placed for sampling.

Sterilizable Microbiological Atrium - The unit is a variant of the single stage sieve impactor. The unit's cover contains uniformly

spaced orifices approximately 0.25 inch in size. The base of the unit accommodated one Petri dish containing a nutrient agar. Vacuum pump controls the movement of air through the unit, and a multiple unit control center as well as a remote sampling probes are available.

Surface Air System Sampler - This integrated unit consists of an entry section that accommodates an agar contact plate. Immediately behind the contact plate is a motor and turbine that pulls air through the unit's perforated cover over the agar contact plate and beyond the motor, it is exhausted. Multiple mounted assemblies are also available.

Gelatin Filter Sampler - The unit consists of a vacuum pump with an extension hose terminating in a filter holder that can be located remotely in the critical space. The filter consists of random fibers of gelatin capable of retaining airborne microorganisms. After a specified exposure time, the filter is aseptically removed and dissolved in an appropriate diluent and then plated on an appropriate agar medium to estimate its microbial content.

Passive air sampling is done by means of settle plates - Petri dishes containing agar medium are exposed to the environment for a prolonged period of time and microorganisms are deposited on the agar surface by gravity. Large and heavy particles tend to settle out due to gravitational forces; with increasing air movement only the very heaviest particle settle out. This limits the value of the method in laminar flow protected areas or other clean rooms where still air is not intended. This method is used only for qualitative purpose. This method is inefficient since organisms are deposited on plates by chance. This method is also affected by airflow and particle sizes in the environment that may not be controllable.

Andersen's Air Sampler: A Popular Sampler for the Collection and Enumeration of Viable Air Borne Particles

Andersen's Sampler was designed to count viable airborne particles are counted. Andersen's Sampler consists of a series of six stages through which the sample of air or aerosol is consecutively drawn. The device is pressure sealed with gaskets and three adjustable spring fasteners. Each stage contains a plate perforated with 400 holes and immediately below a Petri dish of agar culture medium. Air is drawn through the device at the rate of 1 cubic foot per minute (cfm) and a jet of air from each of these holes plays on the surface of the medium. The size of the holes is constant for each stage; consequently, the jet velocity is uniform in each stage but increases in each succeeding stage.

When the velocity imparted to a particle is sufficiently great, its inertia will overcome the aerodynamic drag and the particle will leave the turning stream of air and be impinged on the surface of the medium; otherwise the particle remains in the stream of air and proceeds to the next stage. The sampler is so designed that when operated at 1 cfm any airborne particle, a fraction of 1 micron or larger, will be collected on one stage or another, depending on its aerodynamic dimensions. If the particle size spectrum of the aerosol sample is sufficiently broad, particles will be collected on all stages of the sampler. Each succeeding stage will remove a top fraction (largest particles) of the remaining particles; the last stage completes the collection of bacterial particles.

The flow rate of 1 cfm is achieved with a small vacuum pump or with a vacuum system. The collection and assessment of aerosol samples is very simple. Six Petri dishes, each containing enough agar medium appropriate for the microorganisms which may be encountered, are placed in the instrument and the sample of air is drawn; the plates are then removed, inverted in their covers, incubated and counted. When samples of aerosols are being drawn from a chamber, airflow through the instrument should not be interrupted until air washing with clean air from outside the chamber has replaced all of the sample air within the sampler. Air should not be drawn through the sampler unless Petri plates are in place because this may lodge dirt in the small holes of the lower stages.

Colonies on plates are scattered over the plates and should be counted in the usual manner, except when the plates are heavily loaded, in which case counting may be done through a dissecting type microscope before the colonies merge. A number of fields or segments of the plate are counted, and the total number of colonies for the plate is calculated. In stages 3-6, the colonies conform to the pattern of jets and are counted by either the 'positive hole' method or by the microscope method.

The positive hole method is essentially a count of the jets which delivered viable particles to the Petri plates and conversion of this count by use of the positive hole conversion table (Feller's correction table). This table is based upon the principle that as the number of viable particles being impinged on a given plate increases, the probability of the next particle going into an empty hole decreases. The values in the table were calculated from the basic formula (Feller, 1950): $P_r = N\,[1/N+1/N-1+1/N-2\ldots\ldots 1/N-r+1]$

Where Pr is the expected number of viable particles to produce, r is positive holes and N is the total number of holes per stage (400). The above formula assumes that the flow of particles stops at the instant a particle enters the r^{th} hole. Since, in the actual case of sampling, the flow of particles stops at random, the expected number of particles present if r positive holes are observed, would be equal to or greater than Pr but less than P_{r+1} and the average would be $(P_r + P_{r+1}^{-1})/2$. This correction has been applied in the construction of the table.

Media Recommended for Environmental Monitoring

As per USP chapter <1116> Soyabean Casein Digest Agar (SCDA) is the standard medium for sampling or quantitation of microorganisms in controlled environments. Incubation

temperature is 30-35°C. Yeasts and moulds may also be specifically sought out. Sabouraud Dextrose Agar is used especially for yeasts and moulds. Incubation temperature is maintained at 20-25°C.

Sometimes media are supplemented with additives to overcome or to minimize the effects of sanitizing agents or of antibiotics if used or processed in these environments. The duration of incubation generally recommended is 48-72 hours. Environmental control media should be validated for their ability to support growth throughout their shelf lives. Media are often prepared, sterilized and stored for days and weeks before melting and pouring as environmental monitoring plates.

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Encyclopedia

Bioremediation can be defined as any process that uses microorganisms or their enzymes to return the environment altered by contaminants to its original condition. Bioremediation may be employed in order to attack specific contaminants, such as chlorinated pesticides that are degraded by bacteria, or a more general approach may be taken, such as oil spills that are broken down using multiple techniques including the addition of fertilizer to facilitate the decomposition of crude oil by bacteria.

By definition, bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment. The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site. Contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes. Biodegradation of a compound is often a result of the actions of multiple organisms. When microorganisms are imported to a contaminated site to enhance degradation we have a process known as bioaugmentation. For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products.

Naturally occurring bioremediation and phytoremediation have been used for centuries. George M Robinson reportedly invented bioremediation technology using microorganisms. Bioremediation technologies can be generally classified as *in situ* and *ex situ*. *In situ* bioremediation involves treating the contaminated material at the site while *ex situ* involves the removal of the contaminated material to be treated elsewhere.

Not all contaminants are readily treated through the use of bioremediation; for example, heavy metals such as cadmium and lead are not readily absorbed or captured by organisms. The integration of metals such as mercury into the food chain may make things worse as organisms bioaccumulate these metals.

Advantages of Bioremediation

- Bioremediation is a natural process and is therefore perceived by the public as an acceptable waste treatment process for contaminated material such as soil. Microbes able to degrade the contaminant increase in numbers when the contaminant is present; when the contaminant is degraded, the biodegradative population declines. The residues for the treatment are usually harmless products and include carbon dioxide, water, and cell biomass.
- Theoretically, bioremediation is useful for the complete destruction of a wide variety of contaminants. Many compounds that are legally considered to be hazardous can be transformed to harmless products. This eliminates the chance of future liability associated with treatment and disposal of contaminated material.
- Instead of transferring contaminants from one environmental medium to another, for example, from land to water or air, the complete destruction of target pollutants is possible.
- Bioremediation can often be carried out on site, often without causing a major disruption of normal activities. This also eliminates the need to transport quantities of waste off site and the potential threats to human health and the environment that can arise during transportation.
- Bioremediation can prove less expensive than other technologies that are used for clean-up of hazardous waste.

Disadvantages of Bioremediation

- Bioremediation is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation.
- Biological processes are often highly specific. Important site factors required for success include the presence of metabolically capable microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants.

PHMB: *A Proven Molecule In The Modern Era of Disinfection*

Disinfection describes a process that eliminates many or all pathogenic microorganisms with the exception of bacterial spores. It is generally a lethal process than sterilization. It eliminates virtually all recognized pathogenic microorganisms, but not necessarily all microbial forms (e.g. bacterial endospores) on inanimate objects. Disinfectants are the chemical agents that either inhibit microbial activities and growth or are lethal and kill the microorganisms.

Brief History of Disinfection

The scientific application of disinfectants is limited to, at most, the past 150 years. However, the empiric practices that were more or less effective go back to ancient times. Sulfur dioxide was the first reported disinfectant for fumigation. 865 AD, ethanol was discovered and used in 1763 in a Paris Hospital for dressing wound. In 1811, Iodine was discovered and used in wounds in 1839 by Davies. Later on phenol and quaternary ammonium compounds were discovered and came into commercial use. Aldehydes are also widely used as disinfectants.

Aldehydes As A Widely Used Disinfectant

Aldehydes, especially glutaraldehyde and formaldehyde, have been used as antimicrobial agents for the past several decades. In a microbiological context, glutaraldehyde has been recommended for the disinfection of certain types of medical equipment and anesthetic equipment. Glutaraldehyde is capable of acting as a sterilizing agent but only after prolonged periods of contact. Glutaraldehyde has long been used for the high level disinfection of endoscopes. Though glutaraldehyde is widely used as antimicrobial agents it has some major disadvantages.

Disadvantages of Glutaraldehyde:

- Short activated life - The solution itself is unstable once alkalinized and has a short activated life of 14 days. This instability leads to eventual polymerization of free aldehyde, resulting in little microbial activity of the disinfectant and the possible support of *Pseudomonas* growth. Glutaraldehyde's life can be further reduced by dilution during rinse in machine washers or if more than one hundred scopes have been manually processed through a soak trough.
- Loss of activity in heat - The loss of reactive aldehydes in alkaline solution on storage is increased as the solution is heated.
- Fixative properties - Glutaraldehyde's fixative properties has advantage in some applications of the solution. But, as a sterilizer of operation theatre equipment, this can be a problem. Any organic matter or other media with protein content present on the surface of the instrument as it is immersed in glutaraldehyde will be bonded to the surface and be very difficult to remove.
- Absorption of glutaraldehyde into plastics and rubbers, which can cause localized toxicity (e.g. colitis with the use of flexible endoscopes).
- Will only sterilize the surface presented Glutaraldehyde can only treat surface that are wetted by the solution. A surface could be protected from glutaraldehyde by a layer of gel or other solution used in the last procedure. With endoscopes, it is sometimes difficult to position them in the glutaraldehyde container so that they remain immersed in the solution and be certain that there are no bubbles in any of the internal channels.
- Equipments need to be thoroughly cleaned before immersion- All materials presented to the glutaraldehyde must be clean, on account of its fixative properties and since only surfaces wetted by glutaraldehyde will be treated. If equipment were covered in gel or organic matter, either the microbial load would be too great for the solution, the microbes would be protected inside organic matter which would now be firmly bonded to the instrument. Cleaning is time consuming, and in the case of flexible scopes, requires some skills.
- Risk to patients - Glutaraldehyde is too risky for soaking respiratory equipment. Care must be taken with asthmatic patients in an OT room containing glutaraldehyde troughs, as it may cause an attack. Bronchoscopes must be particularly well rinsed to avoid respiratory sensitivity.
- Adversely toxic to staffs - Glutaraldehyde fumes are notably irritating and toxic to skin, mucous membranes and particularly respiratory tracts. Respiratory tract irritation is observed at concentrations as low a 0.3 ppm. Therefore good ventilation is strongly recommended when glutaraldehyde is used. Prolonged exposure in glutaraldehyde cause skin irritation and sensitization causing dermatitis. Direct eye contact causes blepharitis, conjunctivitis and corneal injury. Other less common reactions in presence of glutaraldehyde include headaches, dizziness and slowed reactions.

PHMB: A Better Alternative than Traditional Aldehydes

PHMB is an acronym for Polyhexamethylene biguanide. Biguanides are an important class of cationic surface-active antimicrobial agents. PHMB is a polymeric biguanide and a polycationic linear polymer comprising a hydrophobic backbone with hexamethylene chains.

Mode Of Action

As a biguanide PHMB can be classified as membrane active agent. The antimicrobial effect of PHMB can be described by the following sequence:

- Rapid attraction towards the bacterial surface.
- Binding to a receptive site on the surface.

- Overcoming bacterial defense mechanism.
- Attraction towards the cytoplasmic membrane.
- Leakage of low molecular weight cytoplasmic components and inhibition of membrane bound enzymes.
- Extensive disruption of cytoplasmic membrane and leakage of macromolecular components.
- Precipitation of cell contents.

Though PHMB is a widely used antimicrobial agent there is little or no evidence of emerging resistance in nosocomial pathogens. It is important to note that the nature of the interaction with the cell following binding to receptive sites determines activity and the potential for resistance development and this differs between the major classes of cationic agents. Monocationic agents may be affected by efflux pumps but polycationic PHMB may not.

The outermost surface of bacterial cells universally carries a net negative charge, often stabilized by the presence of divalent cations such as Mg^{2+} and Ca^{2+}. This is associated with the teichoic acid and polysaccharide elements of Gram-positive bacteria, the lipopolysaccharide of Gram-negative bacteria, and the cytoplasmic membrane itself. It is not therefore surprising that many antimicrobial agents are cationic and have a high binding affinity for bacterial cells. Often, cationic antimicrobials require only a strong positive charge together with a hydrophobic region in order to interact with the cell surface and integrate into the cytoplasmic membrane. Such integration into the membrane is sufficient to perturb growth and at the treatment levels associated with antiseptic formulations is sufficient to cause the membrane to lose fluidity and for the cell to die.

In PHMB molecule biguanide groups are separated by six carbons (a hexamethylene chain), a length that allows them to join together two head groups but does not allow them to bend and integrate into the membrane. When PHMB lands on a bacterial membrane, it settles on the surface, preferring to bind with acidic phospholipids as one particular category of lipid. As the PHMB binds to the membrane, it drags acidic phospholipids to that location to reorganize the membrane, creating an acidic lipid domain. This way PHMB exerts surface membrane activity and efflux is completely ineffective at removing PHMB because it has not entered the lipid domain that efflux pumps require. This nonspecific mechanism of action makes antimicrobial resistance development highly unlikely.

Spectrum of Action

PHMB is a broad-spectrum antimicrobial agent. It is highly effective against Gram positive and Gram negative bacteria as well as fungi. Organic matter does not affect activity of PHMB against microorganisms. The antimicrobial activity of PHMB does not change against ESBL (extended spectrum beta lactamase) producing organisms. PHMB retains activity in hard water and it is stable over a wide pH (1-11) range.

Toxicity

Toxicity of PHMB has been studied extensively in mammalian systems to support its use in medical products. PHMB has low acute systemic toxicity via the oral and dermal routes. PHMB is poorly absorbed through human skin. It exhibits low skin and eye irritancy potential at low use concentrations. PHMB has been tested in a series of in vitro and in vivo mutagenicity assays and found not to be mutagenic. Upon absorption, PHMB is excreted rapidly from the body with no potential for bioaccumulation.

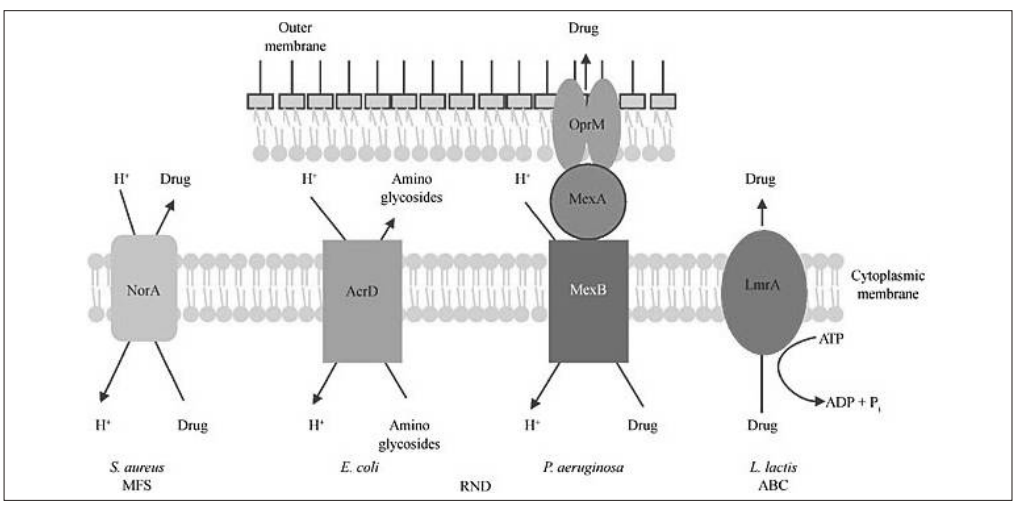


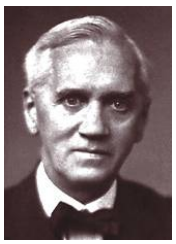
Fig.: Schematic illustration of the main types of bacterial drug efflux pumps.

Advantages of PHMB

PHMB has the following advantages over glutaraldehyde as a disinfectant:

- Shelf life of PHMB is longer than glutaraldehyde and organic matter does not affect the activity of PHMB.
- PHMB is not affected by sunlight, water, temperature and pH fluctuations. This stability allows PHMB as a better antimicrobial agent.
- PHMB is less toxic than glutaraldehyde. So the risk of the patient as well as healthcare worker is reduced.
- It is a broad-spectrum antimicrobial agent and due to its non-specific mechanism of action microorganisms are unable to grow resistance against it.

So PHMB has been accepted widely as an effective antimicrobial agent in the modern era of disinfection.



Alexander Fleming

Birth: August 6, 1881

Death: March 11, 1955

Nationality: Scottish

Known For: Discovery of Penicillin

Penicillin is one of the earliest discovered and widely used antibiotic agents, derived from the *Penicillium* mold. Famous Scottish scientist Alexander Fleming discovered it.

Sir Alexander Fleming was born at Lochfield near Darvel in Ayeshire, Scotland on August 6, 1881, the son of a farmer. He attended Louden Moor School, Darvel School and Kilmarnock Academy before moving to London where he attended the Polytechnic. After completing his Polytechnic, London in 1897, he took an office job few years. In 1901 he quit his job and went to St. Mary Hospital with scholarship to study medicine. He qualified with distinction in 1906. Then he started working in Almroth Wright's research team as a research assistant with a strong interest in bacteriology. He gained M.B., B.S., (London) with gold medal in 1908 and became a lecturer at St. Mary's until 1914.

During the war between Britain and Germany in 1914, Fleming joined the British Royal Army Medical Corps to develop a cure to reduce the number of soldiers dying from infected wounds. He served through out World War I as a captain in the Army Medical Corps was mentioned in dispatches and returned back to St. Mary's in 1918. He was then elected professor of bacteriology in 1928.

After the war, Fleming actively searched for antibacterial agents having witnessed the death of many soldiers from septicemia resulting from infected wounds. In an article for the medical journal *The Lancet* during World War I Fleming strongly opposed for the use of antiseptics in wound. He explained antiseptics worked well on the surface, but deep wounds tended to shelter anaerobic bacteria from the antiseptic agent. Sir Almroth Wright strongly supported Fleming's findings but most army physicians over the course of World War I continued to use antiseptics even in cases where this worsened the condition of the patient. Early in his medical life, Fleming became interested in the natural bacterial action of the blood and in antiseptics. He was able to continue his studies throughout his military career and on demobilization he settled to work on antibacterial substances, which would not be toxic to animal tissues. In 1921, he discovered in an important bacteriolytic substance, which he named lysozyme. About this time, he devised sensitivity titration methods and assays in human blood and other body fluids, which he subsequently used for the titration of penicillin.

In 1928, while working on influenza virus, he observed that mould had developed accidentally on a *Staphylococcus* culture plate and that the mould had created a bacteria-free circle around itself. He was inspired to further experiment and he found that a mould culture prevented growth of staphylococci, even when diluted 800 times. He named the active substance penicillin. He investigated its positive anti-bacterial effect on many organisms, and noticed that it affected bacteria such as staphylococci, and indeed all Gram-positive pathogens. Fleming published his discovery in 1929 in the British Journal of Experimental Pathology, but little attention was paid to his article. Fleming continued his investigations, but found that cultivating penicillium was quite difficult, and that after having grown the mould, it was even more difficult to isolate the antibiotic agent. Fleming's impression was that because of the problem of producing it in quantity It was two other scientists however, Australian Howard Florey and Ernst Chain, a refugee from Nazi Germany, who developed penicillin further so that it could be produced as a drug. At first supplies of penicillin were very limited, but by the 1940s it was being mass-produced by the American drugs industry. Fleming cautioned about the use of penicillin in his many speeches around the world. He cautioned not to use penicillin unless there was a properly diagnosed reasons for it to be used, and that if it were used, never to use too little, or for too short a period, since these are the circumstances under which bacterial resistance to antibiotics develops.

Sir Alexander wrote numerous papers on bacteriology, immunology and chemotherapy, including original descriptions of lysozyme and penicillin. They have been published in medical and scientific journals. Fleming, a Fellow of the Royal College of Surgeons (England), 1909, and a Fellow of the Royal College of Physicians (London), 1944, has gained many awards. Fleming was awarded the Hunterian Professorship by the Royal College of Surgeons of England. Fleming was knighted in 1944. In 1945 Fleming was presented the Nobel Prize for Medicine. He served as President of the Society for General Microbiology; he was a Member of the Pontifical Academy of Science and Honorary Member of almost all the medical and scientific societies of the world. He was Rector of Edinburgh University during 1951-1954, Freeman of many boroughs and cities and Honorary Chief Doy-gei-tau of the Kiowa tribe. He was also awarded doctorate, *honoris causa,* degrees of almost thirty European and American Universities.

On 11th March 1955, Fleming died suddenly at his home in London of a heart attack. He was cremated and his ashes interred in St. Paul's Cathedral. His discovery of penicillin had changed the world of modern medicine by introducing the age of useful antibiotics; penicillin has saved, and is still saving, millions of people.

Enjoy the humour

- ***Teacher:*** *Tell me a sentence that starts with an "I".*
  Student: *I is the....*
  Teacher: *Stop! Never put 'is' after an "I". Always put 'am' after an "I".*
  Student: *OK. I am the ninth letter of the alphabet.*

- *A big cat surprised a family of mice. Father Mouse jumped and said, "Bow-wow!" The cat ran away. "What was that, Father?" asked Baby Mouse. "Well, son, that's why it's important to learn a second language."*

- ***Teacher:*** *Why are you late?*
  Student: *There was a man who lost a hundred dollar bill.*
  Teacher: *That's nice. Were you helping him look for it?*
  Student: *No. I was standing on it.*

Thoughts to live by

- Nobody can go back and start a new beginning, but anyone can start today and make a new ending.

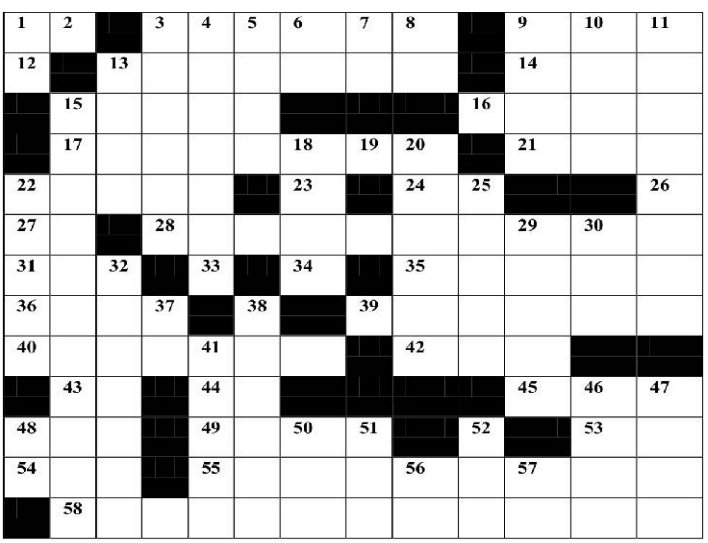
- Destiny is not a matter of chance,
  It is a matter of choice;
  It is not a thing to be waited for
  It is a thing to be achieved.

- Greed is a fat demon with a small mouth and whatever you feed it is never enough.

- The only safe ship in a storm is leadership.

- Love at first sight is easy to understand; it's when two people have been looking at each other for a lifetime that it becomes a miracle.

Track your brain



Across

1. Disease caused by *Mycobacterium tuberculosis* (abbr). 3. Leprosy's other name, _____'s disease. 9. On the list of CDC notifiable diseases: toxic syndrome. 13. Warts appearing in this area are not notifiable, but recommended for surveillance. 14. Prefix with the procedures involving ultrasound. 15. Pieces of a Whole. 16. Famous tennis player, who died of AIDS, Arthur ____. 17. Caused by *Treponema pallidum.* 21. Make a knot. 22. One who hoard money greedily. 24. Surprise! (interj). 27. Utah. 28. Gram negative bacteria responsible for typhoid and paratyphoid ever, food poisoning, gastroenteritis and septicemia. 31. Meeting (abbr). 35. Medical device used to draw fluid from an internal body cavity to the surface. 36. It may range from mild discomfort to agony. 39. Hydrophobia, transmitted to humans by a bite from an infected animal. 40. _____ fever's medical name is Scarlatina. 42. Intermittent self-catheterization prevent urinary infection and incontinence. 43. It joins alternatives. 44. Prefix denoting absence. 45. Virus responsible for papilomatosis. 48. Lysergic acid diethylamide. 49. Serum glutamic pyruvic transaminase is used in the diagnostic of acute liver disease. 53. Emoticon for mouth full. 54. Twelve, in ancient Rome. 55. An organism with a membrane bounded nucleus containing the nucleic acid. 58. Plaster used by patients whose vertebrae were damaged by tuberculosis.

Down

1. T cell. 3. It causes the common cold sore. 4. Caused by *Bacillus anthracis.* 5.Law term, for unless, if not. 6. Standard. 7. Early antigen of Epstein Barr virus. 8. Non licet (abbr). 9. To subject to an examination. 10. Prefix denoting split. 11. _____ syndrome follows a major hemorrhage in pregnancy. 13. Male homosexuals. 15. Notifiable disease caused by *Chlamaydia psittaci.* 18. Caused by the spirochete *Borrelia burghdorferi*, named after a town Connecticut. 20. Paramyxovirus named after a city in central Japan. 22. Also called infectious parotitis. 25. Plants with non woody stems. 29. Laigh (abbr). 30. The opposite of truth. 32. Causes giardiasis, disease on the surveillance list. 37. Near (abbr). 38. Flavivirus, on the CDC surveillance list. 41. Light amplification by stimulated emission of radiation. 46. Pal, in Paris. 47. Vietnamese. 48. Lux. 50. Personal computers. 51. Cap worn by Muslims. 52. Burkitt lymphoma results from chromosomal translocations involving this gene. 56. The ancient Egyptian god of sun. 57. Jocular alteration of all correct.

Check your Answers on Page 16

Escherichia coli

The genus *Escherichia* is named after Theodor Escherich, who first described the colon bacillus under the name *Bacterium coli commune* in 1885. It was later renamed *Escherichia coli*, and for many years the bacterium was simply considered to be a commensal organism of the large intestine. It was not until 1935 that a strain of *E. coli* was responsible for an outbreak of diarrhea among infants. The genus *Escherichia* is a typical member of the *Enterobacteriaceae*, the enteric bacteria, which are facultatively anaerobic Gram-negative rods that live in the intestinal tracts of animals in health and disease.

Morphology & Cultural Characteristics

Escherichia coli is a short, straight Gram negative non-spore forming rod arranged singly or in pairs. Usually the strains are motile by peritrichate flagella, some of the strains are non motile. Capsules and fimbriae are found in some strains. *Escherichia coli* is a facultative aneaerobe capable of fermentative and respiratory metabolism. It grows on a wide temperature range 10-40ºC but the optimum temperature for growth is 37ºC. It grows readily on a wide range of simple culture media and on simple synthetic media.

On solid media colonies are non-pigmented and may be smooth or rough. Colonies are large, thick, grayish white, moist, smooth opaque and partially translucent discs. On MacConkey's Agar, colonies are bright pink due to lactose fermentation. Many strains are hemolytic on blood agar. *E. coli* can ferment glucose, lactose, mannitol, maltose and many other sugars and produce acid and gas as end product. They respond for IMViC test in a following way: it gives positive result for Indole and Methyl Red test but negative result for Voges Proskauer and Citrate test.

Antigenic Structure

The serological classification of *E. coli* depends on a number of antigens, somatic lipopolysaccharide O antigens, capsular K-antigens, flagellar-H antigens and fimbrial-F antigens. The K antigen is the acidic polysaccharide antigen located in the 'envelope' or microcapsule. Several different serotypes of *E. coli* are found in the normal intestine. Most of them do not have K antigens.

Virulence Characteristics

Several virulence factors have been identified in *E. coli*. The pathogenic processes that operate in a given infection always involve more than one virulence factor. Following virulence factors are involved in pathogenesis of *E. coli*:

Fimbrial and Non fimbrial Adhesins

Adhesins are major contributor to *Escherichia coli* virulence. These adhesions help *E. coli* for colonization. At least 21 different adhesin types have been described for pathogenic *E. coli*. Most of the clinical strains of *E. coli* consist of pili. These pili allow the *E. coli* to attach to mannose receptors associated with the host cell surface. Many other pilus types with unique receptor specificities are expressed by different *E. coli* isolates and are associated with specific disease states.

Iron Acquisition

The ability of a microorganism to attach to and multiply at the site of infection is critical to the process of colonization. The multiplication phase of this process requires that sufficient nutrients are available at the site of attachment. As a form of non-specific immunity, the human host sequesters iron by binding this element with high affinity to tranferrin or to lactoferrin. For a microorganism like *E. coli* to survive in human tissues, it must be able to compete for host iron stores. To achieve this, *E. coli* cells produce siderophores (low molecular weight, non proteinaceous compounds that have extremely high affinity for irons). These compounds are able to tranfer iron from host to the bacteria and help to multiply it at the site of attachment.

Toxin

E. coli produces two kinds of exotoxin hemolysins and enterotoxins. Hemolysins do not appear to be relevant in pathogenesis though they are produced more commonly by virulent strains than by avirulent strains. Enterotoxin has role in pathogenesis of diarrhea. Three distinct types of *E. coli* enterotoxins have been identified heat labile toxin (LT), heat stable toxin and verotoxin also known as Shiga like toxin. The LT is a large oligomeric protein complex composed of one enzymatically active subunit surrounded by five identical binding subunits. The toxin binds to ganglioside GM_1 enterocyte receptors and causes an increase in cAMP (cyclic adenosine monophosphate) that results in the exclusion of fluid to the lumen of the gut, causing diarrhea.

Clinical Manifestations

E. coli is one of the most important members of the *Enterobacteriaceae*. Strains are predominant among the commensal bacteria in the healthy human intestine. From their normal site in the human body they are able to cause frequent opportunistic pathogen infections: they are often present in appendix abscesses, peritonitis, cholecystitis and septic wounds. They cause bacteraemia and endotoxic shock and occasionally meningitis in neonates.

E. coli causes four main types of clinical syndromes:

- Urinary tract infection

- Diarrhea
- Pyogenic infections
- Septicemia

Urinary tract infection: *E. coli* causes 90% of the urinary tract infections. The bacteria colonize from the feces or perineal region and ascend urinary tract to the bladder. Bladder infections are more common in females than males due to shortened urethra. The microorganisms are propelled into the bladder from the periurethral region during sexual intercourse. With the aid of specific adhesins they are able to colonize the bladder.

Diarrhea: As a pathogen *E. coli* is best known for its ability to cause intestinal diseases. At least five different types of diarrheagenic *E. coli* are now recognized: Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enterohemorrhagic *E. coli* (EHEC) and Enteroaggregative *E. coli* (EAEC).

Enteropathogenic *E. coli* (EPEC) - EPEC induce a profuse, watery diarrhea. They are a leading cause of infantile diarrhea in developing countries. Outbreaks have been linked to the consumption of contaminated drinking water as well as some meat products. Adherence of EPEC strains to the intestinal mucosa is a very complicated process. Pathogenesis of EPEC involves a plasmid-encoded protein refereed to as EPEC adherence factor that enables localized adherence of bacteria to intestinal cells and a non-fimbrial adhesin, intimin mediates the final stage of adherence.

Enterotoxigenic *E. coli* (ETEC) - ETEC diarrhea is mainly associated with tropical and developing countries and affects susceptible travelers to these places. ETEC strains produce heat labile enterotoxin (LT) or heat stable enterotoxin (ST) or both. In addition, they form specific adhesive fimbrial proteins that promote attachment to specific receptors on the intestinal epithelium. It causes traveller's diarrhea.

Enteroinvasive *E. coli* (EIEC) - EIEC strains are similar to *Shigella* species. They can penetrate the epithelial cells of the large intestine and multiply intracellularly, giving rise to blood and mucus in the stool. EIEC cause about 5% of all cases of diarrhea in areas of poor hygiene.

Enterohemorrhagic *E. coli* (EHEC) or Verotoxigenic *E. coli* (VTEC) - EHEC are recognized as the primary cause of hemorrhagic colitis or bloody diarrhea. EHEC are characterized by the production of vero toxin or Shiga toxin. Although many different serotypes of *E. coli* are known to produce Vero cytotoxin, those of O157: H7 and O157: H^- are so far the most common types causing human infections. Unpasteurized milk and food, especially improperly cooked hamburgers, have been identified as important vehicles in North America.

Enteroaggregative *E. coli* (EAEC) - The distinguishing feature of EAEC strains in their ability to attach to tissue culture cells in an aggregative manner. They have been associated with persistent diarrhea, especially in developing countries.

Pyrogenic Infections: *E. coli* form the most common cause of intra abdominal infections, such as peritonitis and abscesses resulting from spillage of bowel contents. They also cause pyogenic infections in the perianal area. They are an important cause of neonatal meningitis.

Septicemia: Blood stream invasion by *E. coli* may lead to fatal conditions like septic shock and systemic inflammatory response syndrome (SIDS).

Diagnosis & Treatment

All patients thought to have *E. coli* infection should have a routine CBC (Complete Blood Count) count with differential to evaluate for leukocytosis. Gram stain results determine if the organism is Gram-negative, but findings do not distinguish among the other aerobic Gram-negative bacilli that cause similar infectious diseases. It grows on MacConkey Agar and gives pink colour colony as a lactose fermenter. EMB Agar and Endo Agar are the selective medium for *Escherichia coli*. IMViC test is also carried out to detect *E. coli*. Serodiagnosis is done to identify the strain of *Escherichia coli*. Bacterial infections are usually treated with antibiotics. Antibiotics, which may be used to treat *E. coli* infection, include amoxicillin as well as other semi-synthetic penicillins, many cephalosporins, carbapenems, trimethoprim-sulfamethoxazole, ciprofloxacin and aminoglycosides. Researchers have actively been working to develop safe, effective vaccine to lower the worldwide incidence of *E. coli* infection.

Prevention & Control

The best ways to prevent an *E. coli* infection include practicing good hygiene and food safety habits. Washing hands with high-level disinfectant prior to handling food or after exposure to feces is very important. This practice can remove bacteria and keep away from being spread or ingested accidentally, thereby preventing infection. Utensils should be cleaned properly after coming into contact with raw meat. Fruits and vegetables should be washed properly before consuming.

References

Textbook of Microbiology. R.Ananthanarayan & C.K.J. Paniker. Sixth edition, 2003. Edited by C.K.J. Paniker.
Practical Medical Microbiology, Mackie & McCartney, 13th Edition 1989, Edited by J.G. Collee, J.P. Daguid.

SSI: Preventive Guidelines recommended by CDC

By definition, a surgical site infection (SSI) is an infection that develops within thirty days after an operation or within one year if an implant was placed and the infection appears to be related to the surgery. Surgical site infections are the third most commonly reported nosocomial infection and they account for approximately a quarter of all nosocomial infections. They have been responsible for the increasing cost; morbidity and mortality related to surgical operations and continue to be a major problem even in hospitals with most modern facilities and standard protocols of preoperative preparation and antibiotic prophylaxis. Surgical site infections include superficial site infections (skin and subcutaneous tissue), deep site infections (involving fascia and muscle layers) and organ or space infections (such as abscesses, bone infections, etc.).

The pathogens isolated from infections differ, primarily depending on the type of surgical procedure. In clean surgical procedures, in which the gastrointestinal, gynecologic, and respiratory tracts have not been entered, *Staphylococcus aureus* from the exogenous environment or the patient's skin flora is the usual cause of infection. In other categories of surgical procedures, including clean contaminated, contaminated, and dirty, the polymicrobial aerobic and anaerobic flora closely resembling the normal endogenous microflora of the surgically resected organ are the most frequently isolated pathogens.

Host-derived factors are important contributors to the risk of SSI, including increased age, obesity, malnutrition, diabetes mellitus, and poor control of the hyperglycemic stress response in non-diabetic patients, hypocholesterolemia, hypoalbuminemia, and several other factors that is not accounted for specifically by NNIS. Among the factors that are controllable in many cases are poor preoperative nutrition, remote infection at the time of elective surgery, inappropriate prescribing of prophylaxis of antibiotics and injudicious use of drains and catheters. In 1999, CDC's (Centers for Disease Control and Prevention) Health Care Infection Control Practices Advisory Committee published revised guidelines for the prevention of infections.

Preoperative Issues

Following preoperative issues should be followed to lower the surgical site infection:

a. Preoperative Antiseptic Showering - A preoperative antiseptic shower or bath decreases skin microbial colony counts. Chlorhexidine gluconate containing products require several applications to attain maximum antimicrobial benefit; so repeated antiseptic showers are usually indicated.
b. Preoperative Hair Removal - Preoperative shaving of the surgical site the night before an operation is associated with a significantly higher SSI risk than either the use of depilatory agents or no hair removal. The increased SSI risk associated with shaving has been attributed to microscopic cuts in the skin that later serve as foci for bacterial multiplication.
c. Patient Skin Preparation in Operating Room - Patient's skin should be prepared with antiseptics before an incision. Chlorhexidine gluconate and alcohol containing products are commonly used for skin preparations. Before the skin preparation of a patient is initiated, the skin should be free of gross contamination. The patient's skin is prepared by applying an antiseptic in concentric circles, beginning in the area of the proposed incision. The prepared area should be large enough to extend the incision or create new incisions or drain sites.
d. Preoperative Hand/ Forearm Antisepsis - Members of the surgical team who have direct contact with the sterile operating field or sterile instruments or supplies used in the field wash their hands and forearms by performing a traditional procedure known as scrubbing (or the surgical scrub) immediately before donning sterile gowns and gloves. Ideally, the optimum antiseptic used for the scrub should have a broad spectrum of activity, be fast acting, and have a persistent effect.

Intraoperative Issues

Some intraoperative issues are recommended to reduce the risk of surgical site infections:

a. Operating Room Environment - Operating room air may contain microbial-laden dust, lint, skin squames, or respiratory droplets. The microbial level in operating room air is directly proportional to the number of people moving about in the room. Therefore, efforts should be made to minimize personnel traffic during operations. Operating rooms should be maintained at positive pressure with respect to corridors and adjacent areas. Conventional operating room ventilation systems produce a minimum of about 15 air changes of filtered air per hour. When visible soiling of surfaces or equipment occurs during an operation, an Environmental Protection Agency (EPA) approved hospital disinfectant should be used to decontaminate the affected areas before the next operation. Surgical instruments can be sterilized by steam under pressure, dry heat, ethylene oxide, or other approved methods.
b. Surgical Attire and Drapes - Scrub suits, masks, surgical caps, sterile gloves, gowns and drapes should be used by health care workers to reduce the spreading of infection.

Post Operative Issues

a. Incision Care - The type of postoperative incision care is determined by whether the incision is closed primarily (i.e., the skin edges are re-approximated at the end of the operation), left open to be closed later, or left open to heal by second intention. When a surgical incision is closed primarily, as most are, the incision is usually covered with a sterile dressing for 24 to 48 hours. Beyond 48 hours, it is unclear whether a dressing must cover an incision or whether showering or bathing is detrimental to healing. When a surgical incision is left open at the skin level for a few days before it is closed (delayed primary closure), a surgeon has determined that it is likely to be contaminated or that the patient's condition prevents primary closure (e.g., edema at the site). When such is the case, the incision is packed with a sterile dressing. When a surgical incision is left open to heal by second intention, it is also packed with sterile moist gauze and covered with a sterile dressing.
b. Discharge Planning - Many patients are discharged very soon after their operation, before surgical incisions have fully healed. The intent of discharge planning is to maintain integrity of the healing incision, educate the patient about the signs and symptoms of infection, and advise the patient about whom to contact to report any problems.

References

Guideline for prevention of Surgical Site Infection, 1999. *Infection Control and Hospital Epidemiology*. **20(4):** 247-278.

Culture Media Preparation and Its Quality Control

A growth medium or culture medium is a liquid or gel designed to support the growth of microorganisms and cells. Microorganisms need nutrients, a source of energy and certain environmental conditions in order to grow and reproduce. In the environment, microbes have adapted to the habitats most suitable for their needs, in the laboratory; however, these requirements must be met by a culture medium.

Culture media are the basis for most microbiological tests. So the quality of the culture media is a critical factor for the success of microbiology laboratory. Media preparation, proper storage and quality control testing can assure a consistent supply of high quality media. As per USP chapter <1117> good laboratory practices in a microbiology laboratory also contains the activities of media preparation and quality control.

Media Preparation

Culture media may originate from following three sources: laboratory prepared from raw materials, commercially manufactured presented either in a dehydrated or ready to use form. The first one is now rarely been used in pharmaceutical industry and microbiological laboratory. Dehydrated culture media and ready to use media is widely used for quality assurance in industrial as well as clinical segment. It is important to choose the correct media or components in making media based on the use of accepted sources or references for formulas. Preparation and evaluation of culture media is a continuous process extending from the raw material, through manufacture to the final product use on the bench.

Storage of incoming raw materials

All incoming raw materials should be purchased from an approved supplier and dated on receipt. Particular care should be given to the sourcing of media used in the production of pharmaceutical materials with respect to the bovine spongiform encephalopathy (BSE) status of herds providing beef extracts. Raw materials should be stored as advised by the manufacturer; humid environment should be avoided.

Water

Water quality can markedly influence the performance of culture media. Purified water is most often used for media preparation, but in certain cases the use of deionized or distilled water is appropriate. Tap water is unsuitable for the preparation of culture media. Water quality should be regularly monitored.

Preparation of Culture Media

Good laboratory practice should be maintained for the preparation of culture media. Media should be prepared according to master formulae and written procedures. Documentation should be completed for each batch of prepared media; a batch number and expiry date is always allocated. Manufacturer's instructions should be closely followed.

Consistent preparation of media requires accurate weighing of dehydrated media or media constituents. A calibrated balance with the appropriate weight range for the ingredients should be used. When dissolving powders in water, the aqueous phase (1/3 volume) should be added first, followed slowly by the powder, dispersed by gentle swirling and finally the remaining liquid can be used to wash down any adhering powder. Balances should be cleaned thoroughly after use; they also require weekly calibration and regular maintenance. Equipment used for dispensing culture should be cleaned properly to prevent the contamination of foreign substances.

Measurement of pH

The properties of a given culture medium are pH dependent. Incorrect pH may result not only in physical changes such as precipitation of components or soft gelling of agar, but also significant chemical changes. Incorrect pH may also affect the recovery of stressed cells and influence cell growth. Since the pH is temperature dependent, measurements are best taken at a standardized temperature i.e. room temperature (25ºC). The pH of media should be in a range of ± 0.2 of the value indicated by the manufacturer. A flat pH probe is recommended for agar surfaces and an immersion probe is recommended for liquids. pH meters should be calibrated weekly by using standardized buffer solutions. During autoclaving the solution that has been adjusted to be a little on the alkaline side of neutrality tend to fall by about 0.1 unit.

Cleaning of glass apparatus

Preparation of media in poorly cleaned glassware can allow inhibitory substances to enter the media. Inhibitory substances can come from detergent residue after cleaning glassware. Cleaning process should remove debris and foreign matter.

Sterilization

The sterilization of culture media is a critical control point in assuring their quality. The media must be sufficiently processed to ensure sterility, but any over processing may affect their nutritive properties and result in the accumulation of toxic substances. Thus, in pharmaceutical production, individual sterilization cycles should be properly validated with thermocouples to ensure that all containers in the load achieve the required temperature.

Commercially prepared media should provide documentation of the sterilization method used. The manufacturer should provide the sterility assurance level (SAL) of the media against a recognized biological indicator. For each batch processed the accompanying temperature time chart recording should indicate

the correct conditions in the load have been achieved. Manufacturers recommend an autoclave cycle of 121ºC for 15 minutes using a validated autoclave. The sterilization time will be dependent on the media volume and autoclave load. Storage of the media in the autoclave after the liquid cycle is completed is not recommended after cooling, as it may damage the media. Improper heating or sterilizing conditions for commercially prepared or internally prepared media may result in a difference in colour change, loss of clarity, altered gel strength or pH drift from the manufacturer's recommended range. Membrane filtration is used to sterilize heat sensitive media and components.

Checking of prepared media

Prepared media should be checked by appropriate inspection of plates and tubes for:

- Cracked containers or lids
- Unequal filling of containers
- Dehydration resulting in cracks or dimpled surfaces on solid medium
- Hemolysis
- Excessive darkening or colour change
- Crystal formation from possible freezing
- Excessive number of bubbles
- Microbial contamination
- Status of redox indicators
- Lot number and expiry date checked and recorded
- Sterility of the media

Storage of Media

Media should be stored according to manufacturer's instructions. All media should be used within their given shelf lives. The shelf life of media is determined by a number of factors: the type of media and the container as well as the storage conditions. Agar should not be stored at or below 0ºC, as freezing could damage the gel structure. Agar plates begin to deteriorate from the moment of preparation; correct packaging with low temperature storage slows the rate of deterioration. Before prolonged storage, agar plates should be placed into a sealed package or to container to retard the moisture loss. Plates showing shrinkage or wrinkling of agar should be discarded and wet plates should be dried until visible moisture has disappeared. Using boiling water bath or free steaming in an autoclave should do remelting of solid media. The molten agar medium should be kept in a monitored water bath at a temperature of 45-50ºC for not more than 8 hours. Media should not be re-melted by the direct application of heat.

Quality Control of Culture Media

Quality control in the preparation and evaluation of culture media in the laboratory is essential. The aim of quality control is to ensure that media conform to predetermined standards whereas evaluation implies the determination of their efficacy under the conditions of intended usage. The performance of media prepared in a laboratory or by a manufacturer is highly dependent on preparation. Improper media preparation can cause unsatisfactory conditions for microbial growth or recovery.

Quality control test should be carried out on each batch of culture media, involving a range of physical, chemical and microbiological tests. Physical test should confirm that the powder meets its specification in terms of colour, odour, particle size, homogeneity, flow characteristics and moisture content. Chemical test would include clarity, solubility and pH of the product. Media containing agar must meet the required specification for gel strength.

Microbiological tests are done to check the recovery of the particular organism in that reference media. Expiration dates on media should have supporting growth promotion testing to indicate that the performance of the media still meets acceptance criteria up to and including the expiration date. The length of shelf life of a batch of media will depend on the stability of the ingredients and formulation under specified conditions as well as the type of the container and closure. When a batch of media does not meet the requirements of growth promotion testing, an investigation should be initiated to identify the cause. This investigation should include a corrective action plan to prevent the recurrence of the problem. Special should be taken with media, used in environmental monitoring.

Factors Influence the Quality of Dehydrated Culture Media

Major sources of trouble include errors in weighing and measuring, use of wrong ingredients, incorrect pH adjustment, and presence of inhibiting substances in glasswares. Some of the common faults and their possible cause listed bellow:

- Loss of growth promotion capacity - Over sterilization, Incomplete mixing, Contamination with metallic salts, Incorrect molarity due to careless pH adjustment.
- Darkening of the medium or colour change - Over sterilization, Drift in pH.
- Clumping of Media - Storage of media in humid condition, Improper sealing of container.
- Decreased gel strength - Incorrect proportions of product to volume of water, Incomplete mixing, Repeated remelting.
- Change in pH - Hydrolysis of ingredients, Over sterilization, Impure water or glassware used, Incomplete mixing.
- Precipitation - Chemical incompatibility, Over sterilization, Prolonged holding of melted agar media at high temperature.

Before using media must be at correct temperature. Performance of the media is checked by standard ecometric method. Records should be maintained of all media received and used in the laboratory. Finally, disposal of used culture media should follow local biological hazard safety procedure.

References

United States Pharmacopeia, NF 26. The United States Pharmacopeial Convention. Rockville, MD. Edition 31, 2008, Volume 1.

In Mini Review we have discussed about the significance of environmental monitoring. Environmental conditions have a great impact on industrial as well as in clinical field. It may directly or indirectly affect the quality of the finished product. One of the major routes of contamination in any pharmaceutical production facility is the air. GMP, FDA, USP and ISO 14698 Guidelines recommend air monitoring for sterile areas of pharmaceutical industry.

Microxpress™ has come up with an advanced microbial air monitoring system **AccuBas™ Ax1** based on Andersen's Impaction Principle. **AccuBas™ Ax1** is a user-friendly biological air sampler for hospital, pharmaceutical, biotech, food and beverage industry.

Features	Benefits
Accommodates commercially available standard petriplates.	▪ Flexible usage
User friendly software	▪ Easy to handle.
Programmable delay time	▪ No turbulences created test-person ▪ Reproducible results in sterile area.
Programmable Air speed	▪ Application oriented and convenient.
Battery operation capacity upto 4 hours	▪ Facilitates onsite testing.
Portable and light weight	▪ Convenient to use
Data storage (sampling quantity, time and other parameters) up to 256 samples	▪ Easy to compare with previous data.

1 T	2 B		3 H	4 A	5 N	6 S	7 E	8 N		9 T	10 S	11 S
12 C		13 G	E	N	I	T	A	L		14 E	C	H
	15 P	A	R	T	S				16 A	S	H	E
	17 S	Y	P	H	I	18 L	19 I	20 S		21 T	I	E
22 M	I	S	E	R		23 Y		24 E	25 H			26 H
27 U	T		28 S	A	L	M	O	N	E	29 L	30 L	A
31 M	T	32 G		33		34 E		35 D	R	A	I	N
36 P	A	I	37 N		38 D		39 R	A	B	I	E	S
40 S	C	A	R	41 L	E	T		42 I	S	C		
	43 O	R		44 A	N					45 H	46 P	47 V
48 L	S	D		49 S	G	50 P	51 T		52 M		53 O	I
54 X	I	I		55 E	U	C	A	56 R	Y	57 O	T	E
	58 S	A	Y	R	E	S	J	A	C	K	E	T

BioShields™ introducing first time in India,

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The resistance-free antiseptic.

Nusept is new generation, powerful, non stinging, microbicidal antiseptic solution. The active ingredient is Polyhexamethylene biguanide (PHMB). Due to it polymeric nature its mode of action is non specific & hence there is no resistance development studies reported against PHMB.

Nusept

- Resistance-free
- Eliminates biofilm
- Broad spectrum antimicrobial activity
- Excellent residual action
- Non-stinging & skin safe

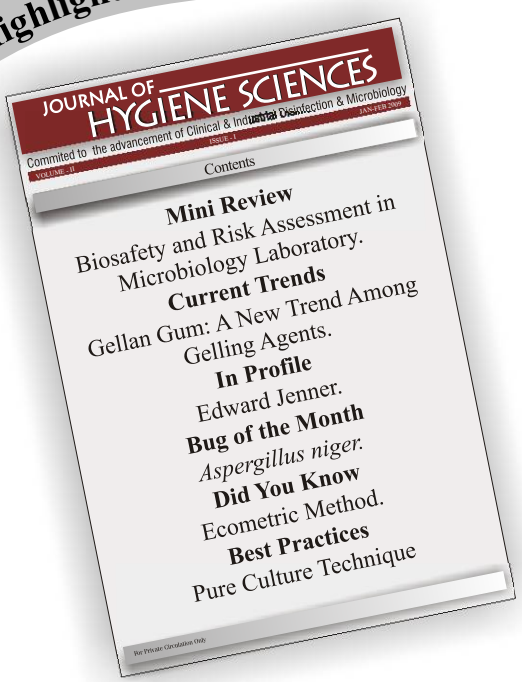
Nusept has got multiple applications in hospital, industries and general household as an antiseptic and disinfectant.

First aid	For cuts and wounds
Surgical/hygienic bath	Linen disinfection
Floor mopping	Hygienic shave
Kitchen disifection	Antidandruff

Nusept is externally evaluated from Haffkine Research Institute, Mumbai.

Nusept is also externally evaluated for dermal & oral toxicology studies from RCC Laboratories India Pvt Ltd, Hydrabad.

Highlights of the coming issue



Printed and published by D.G. Tripathi, Edited by Amrita Roy for and on behalf of Tulip Diagnostics(P) Ltd., Tulip House, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex, Post Office Goa, 403 202, India. Fax- 0832 2458544, E-mail: sales@tulipgroup.com, Website- www.tulipgroup.com.

Bimonthly Journal Published Exclusively For Tulip Group Customers