JOURNAL OF
HYGIENE SCIENCES
Committed to the advancement of Clinical & Industrial Disinfection & Microbiology
VOLUME - II ISSUE - I JAN-FEB 2009

Editorial

Contents

"Success is a journey not a destination. The doing is more important than the out come. Not everyone can be Number one." (Arthur Robert Ashe).

Journal of Hygiene Sciences had started its journey a year back with an objective to create hygiene awareness globally. We were firm in our objective and had a wonderful support from our valuable readers who have made our way to success quite smooth. Hope you have enjoyed all earlier issues. Here are some more interesting topics in this issue. Keep on giving your suggestions!!

Microbiological laboratories are special, often unique work environments that may pose identifiable infectious disease risks to persons in or near them. Infections have been contracted in the laboratory throughout the history of microbiology. With the increasing concern for the well being of healthcare employees and environment the CDC developed Universal Precautions guidelines named as Biosafety in Microbiological and Biomedical Laboratories (BMBL). These biosafety guidelines were based on combination of standard and special practices, equipments and facilities recommended for use when working with various infectious agents in laboratory settings.

In Mini Review we have discussed the CDC guidelines of Biosafety in detail. As like our earlier issues our Current Trends section is enriched with an interesting topic "Gellan Gum". Just go through it you will find it interesting!! This time our In Profile section is dedicated to Edward Jenner, a person known for the vaccination of smallpox. It is because of his efforts that we are able to eradicate smallpox.

In our Bug of the Month section we have briefed about a medically and commercially important organism, *Aspergillus* species. Did You Know section is focused on Ecometric method, an easy and economic way of monitoring solid media quality on a routine basis. Microorganisms usually exist in mixed populations in soil, water and some parts of the human body. It is not feasible to identify or study the characteristics of a particular species and therefore a pure culture must be obtained in preparation of further work. Different types of Pure Culture Techniques are outlined in our Best Practices section. A full page is devoted to relax your mood as usual.

As the new year blossoms, may the journey of your life be fragrant with new opportunities, your days be bright with new hopes and your heart be happy with love! We Would Like To Wish Our Readers A Happy New Year.

Biosafety and Risk Assessment in Microbiology Laboratory

Principles of Biosafety

The term "containment" refers to safe methods for managing infectious material in the laboratory environment. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons and the outside environment from potentially hazardous environment. Primary containment is the protection of personnel and the immediate laboratory environment from exposure to infectious agents and Secondary containment is the protection of the environment external to the laboratory from exposure to infectious materials.

A microbiology laboratory is a unique environment that requires special containment facilities and practices in order to properly protect persons working with infectious agents. Safety is the primary concern of a microbiology laboratory. The agents that may be used in bioterrorism attack are by nature organisms having a greater potential to be easily disseminated by aerosolization, are more stable, and can cause higher morbidity and mortality than typical organisms identified in a microbiology laboratory. Brucellosis, typhoid, tuberculosis, hepatitis are the most commonly reported laboratory acquired infections.

Centers for Disease Control and Prevention Guidelines for Biosafety

Microbiological laboratories are special, often unique work environments that may pose identifiable infectious disease risks to persons in or near them. Infections have been contracted in the laboratory throughout the history of microbiology. With the increasing concern for the well being of healthcare employees and environment the CDC developed Universal Precautions guidelines named as Biosafety in Microbiological and Biomedical Laboratories (BMBL). These biosafety guidelines were based on combination of standard and special practices, equipments and facilities recommended for use when working with various infectious agents in laboratory settings.

To achieve biosafety three main elements of containment are followed:

a) Laboratory practice and technique
b) Safety equipment
c) Facility design.

a) Laboratory practice and technique - The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or potentially infected materials must be aware of potential hazards, and must be trained and proficient in the practices and techniques required to handle such material safely. The director or person in charge of the laboratory is responsible for providing or arranging the appropriate training of personnel.

Each laboratory should develop or adopt a biosafety or operations manual that identifies the hazards that will or may be encountered, and that specifies practices and procedures designed to minimize or eliminate exposures to these hazards. Personnel should be advised of special hazards and should be required to read and follow the required practices and procedures. A scientist trained and knowledgeable in appropriate laboratory techniques, safety procedures, and hazards associated with handling infectious agents must be responsible for the conduct of work with any infectious agents or material. This individual should consult with biosafety or other health and safety professionals with regard to risk assessment. When standard laboratory practices are not sufficient to control the hazards associated with a particular agent or laboratory procedure, additional measures may be needed. The laboratory director is responsible for selecting additional safety practices, which must be in keeping with the hazards associated with the agent or procedure. Appropriate facility design and engineering features, safety equipment, and management practices must supplement laboratory personnel, safety practices, and techniques.

b) Safety Equipment (Primary Barriers) - Safety equipment includes biological safety cabinets (BSCs), enclosed containers, and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The biological safety cabinet (BSC) is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures. Three types of biological safety cabinets (Class I, II, III) are mainly used in microbiological laboratories. Class I and Class II biological safety cabinets provide significant level of protection to environment and laboratory personnel. The Class II biological safety cabinet also provides protection from external contamination of the materials (e.g., cell cultures, microbiological stocks) being manipulated inside the cabinet. The gas-tight Class III biological safety cabinet provides the highest attainable level of protection to personnel and the environment.

Safety equipment may also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Personal protective equipment is often used in combination with biological safety cabinets and other devices that contain the agents, animals, or materials being hand led. In some situations in which it is impractical to work in biological safety cabinets, personal protective equipment may form the

primary barrier between personnel, and the infectious materials.

c) Facility Design and Construction (Secondary Barriers) - The design and construction of the facility contributes to the laboratory workers' protection, provides a barrier to protect persons outside the laboratory, and protects persons or animals in the community from infectious agents which may be accidentally released from the laboratory. Laboratory management is responsible for providing facilities commensurate with the laboratory's function and the recommended biosafety level for the agents being manipulated. The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. Secondary barriers in these laboratories may include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave), and hand washing facilities. When the risk of infection by exposure to an infectious aerosol is present, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features include specialized ventilation systems to ensure directional airflow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks as laboratory entrances, or separate buildings or modules to isolate the laboratory.

Biological Safety Levels

Work with biohazardous agents and materials can be managed safely through the use of good microbiological practices and the facilities and equipment designated for the biosafety containment levels. Biosafety practices are an important part of a program to manage the risk of exposure to potentially infectious agents. BMBL has classified four biosafety levels according to work with biohazardous agents. Each level consists of a combination of laboratory practices and techniques, safety equipment and laboratory facilities. Each combination is especially appropriate for the operations performed, the documented or suspected routes of transmission of the infectious agents and for the laboratory function and activity. The standard practices of the CDC/NIH guidelines are required at all four biosafety levels. In addition, special practices are added for the higher levels of containment.

BSL 1 - Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. Work is generally conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is neither required nor generally used. The following standard and special practices, safety equipment and facilities are maintained in a standard microbiology laboratory as per Biosafety Level 1:

- Persons should wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in the work areas. Persons who wear contact lenses in laboratories should also wear goggles or a face shield.
- Mouth pipetting is prohibited; mechanical pipetting devices should be used.
- All procedures are performed carefully to minimize the creation of splashes or aerosols.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving.
- Special containment devices or equipment such as a biological safety cabinet are generally not required for manipulations of agents assigned to Biosafety Level 1.
- It is recommended that laboratory coats, gowns, or uniforms be worn to prevent contamination.
- Each laboratory contains a sink for hand washing.
- The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.

BSL 2 - Biosafety Level 2 is similar to Biosafety Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs from BSL-1 in that (a) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (b) access to the laboratory is limited when work is being conducted; (c) extreme precautions are taken with contaminated sharp items; and (d) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment. Apart from the standard criteria of BSL-1 following standards and special practices, safety equipments and facilities should be maintained in BSL 2:

- Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at increased risk of acquiring infections.
- Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).
- Spills and accidents that result in overt exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
- Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:

Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, and vigorous shaking or mixing, sonic disruption, opening containers of infectious materials.

- Lockable doors should be provided for the laboratory.

BSL 3 - Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents, which may cause serious or potentially lethal disease as a result of exposure, by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents, and are supervised by competent scientists who are experienced in working with these agents.

Personnel wearing appropriate personal protective clothing and equipment conduct within biological safety cabinets or other physical containment devices, or all procedures involving the manipulation of infectious materials. The laboratory has special engineering and design features. Laboratory personnel should have specific training in handling pathogenic and potentially lethal agents and are supervised by competent, experienced scientists.

- The laboratory is separated from areas that are open to unrestricted traffic flow within the building, and access to the laboratory is restricted. Passage through a series of two self-closing doors is the basic requirement for entry into the laboratory from access corridor. Doors are lockable. A clothes change room may be included in the passageway. The interior surfaces of walls, floors, and ceilings of areas where BSL-3 agents are handled and constructed for easy cleaning and decontamination.
- Biological safety cabinets are required and are located away from doors, from room supply louvers, and from heavily traveled laboratory areas.
- A ducted exhaust air ventilation system is provided. This system creates directional airflow, which draws air into the laboratory from "clean" areas and toward "contaminated" areas. The exhaust air is not recirculated to any other area of the building.
- HEPA-filtered exhaust air from a Class II biological safety cabinet can be recirculated into the laboratory if the cabinet is tested and certified at least annually. When exhaust air from Class II safety cabinets is to be discharged to the outside thro ugh the building exhaust air system, the cabinets must be connected in a manner that avoids any interference with the air balance of the cabinets or the building exhaust system. When Class III biological safety cabinets are used they should be directly connected to the exhaust system. If the Class III cabinets are connected to the supply system, it is done in a manner that prevents positive pressurization of the cabinets (see Appendix A).
- Additional environmental protection (e.g., personnel showers, HEPA filtration of exhaust air, containment of other piped services and the provision of effluent decontamination) should be considered if recommended by the agent summary statement, as determined by risk assessment, the site conditions, or other applicable federal, state, or local regulations.

BSL 4 - Biosafety Level 4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol transmitted laboratory infections and life-threatening disease. Members of the laboratory staff have specific and thorough training in handling extremely hazardous infectious agents and they understand the primary and secondary containment functions of the standard and special practices, the containment equipment, and the laboratory design characteristics. Competent scientists who are trained and experienced in working with these agents supervise them. The laboratory director strictly controls access to the laboratory. The facility is either in a separate building or in a controlled area within a building, which is completely isolated from all other areas of the building. A specific facility operations manual is prepared or adopted. The Biosafety Level 4 laboratory has special engineering and design features to prevent microorganisms from being disseminated into the environment. Apart from the regular practices the following standard and special safety practices equipment, and facilities apply to agents assigned to Biosafety Level 4:

- Laboratory personnel receive available immunizations for the agents handled or potentially present in the laboratory.
- Baseline serum samples for all laboratories and other at risk personnel are collected and stored. Additional serum specimens may be periodically collected, depending on the agents handled or the function of the laboratory. The decision to establish a serologic surveillance program takes into account the availability of methods for the assessment of antibody to the agent(s) of concern. The program provides for the testing of serum samples at each collection interval and the communication of results to the participants.
- Personnel enter and leave the laboratory only through the clothing change and shower rooms. They should take a decontaminating shower each time they leave the laboratory. Personnel should use the airlocks to enter or leave the laboratory only in an emergency.
- All procedures within the facility are conducted in the Class III biological safety cabinet or in Class II biological safety cabinets used in conjunction with one-piece positive pressure personnel suits ventilated by a life support system.
- Liquid effluents from the dirty-side inner change room (including toilets) and cabinet room sinks, floor drains (if used), autoclave chambers, and other sources within the cabinet room are decontaminated by a proven method, preferably heat treatment, before being discharged to the sanitary sewer.
- A daily inspection of all containment parameters (e.g., directional airflow, chemical showers) and life support systems is completed before laboratory work is initiated to ensure that the laboratory is operating according to its operating parameters.

Risk Assessment

The backbone of the practice of biosafety is risk assessment. "Risk" implies the probability that harm, injury, or disease will occur. In the context of the microbiological and biomedical laboratories, the assessment of risk focuses primarily on the prevention of laboratory-associated infections. When addressing laboratory activities involving infectious or potentially infectious material, risk assessment is a critical and productive exercise. It helps to assign the biosafety levels (facilities, equipment, and practices) that reduce the worker's and the environment's risk of exposure to an agent to an absolute minimum. Risk assessment can be qualitative or quantitative. In the presence of known hazards quantitative assessments can be done.

The laboratory director or principal investigator is responsible for assessing risks in order to set the biosafety level for the work. This should be done in close collaboration with the Institutional Biosafety Committee to ensure compliance with established guidelines and regulations. In deciding on the appropriate containment for an experiment, the initial risk assessment should be followed by a thorough consideration of the agent itself and the process of manipulation. Factors to be considered in determining the level of containment include agent factors such as virulence, pathogenicity, infectious dose, environmental stability, route of spread, communicability, operations, and quantity, availability of vaccine or treatment, and gene product effects such as toxicity, physiological activity and allergenicity. The factors of interest in a risk assessment include:

- The pathogenicity of the infectious or suspected infectious agent depends on disease incidence and severity (i.e., mild morbidity versus high mortality, acute versus chronic disease). The more severe is the potentially acquired disease, the higher the risk. For example, *Staphylococcus aureus* only rarely causes a severe or life threatening disease in a laboratory situation and is relegated to BSL-2. Viruses such as Ebola, Marburg, and Lassa fever, which cause diseases with high mortality rates and for which there are no vaccines or treatment, are worked with at BSL-4. However, disease severity needs to be tempered by other factors. Work with human immunodeficiency virus (HIV) and hepatitis B virus is also done at BSL-2, although they can cause potentially lethal disease. Since aerosol is not the route of transmission, the incidence of laboratory-acquired infection is extremely low for HIV, and an effective vaccine is available for hepatitis B.
- The route of transmission (e.g., parenteral, airborne, or by ingestion) of newly isolated agents may not be definitively established. Agents that can be transmitted by the aerosol route have caused most laboratory infections. The greater the aerosol potential, the higher the risk.
- The infectious dose of the agent is another factor to consider. Infectious dose can vary from one to hundreds of thousands of units. The complex nature of the interaction of microorganisms and the host presents a significant challenge even to the healthiest immunized laboratory worker. The laboratory worker's immune status is directly related to his/her susceptibility to disease when working with an infectious agent.
- Agent stability is a consideration that involves not only aerosol infectivity (e.g., from spore-forming bacteria), but also the agent's ability to survive over time in the environment.
- The concentration (number of infectious organisms per unit volume) will be important in determining the risk. The volume of concentrated material being handled is also important. In most instances, the risk factors increase as the working volume of high-tittered microorganisms increases, since additional handling of the materials is often required.

Risk assessment is ultimately a subjective process. The investigator must make an initial risk assessment based on the risk group of an agent. Agents are classified into four risk groups according to their relative pathogenicity for healthy adult humans by the following criteria:

a) Risk Group 1 - Risk Group 1 agents are not associated with disease in healthy adult humans.
b) Risk Group 2 - Risk Group 2 agents are associated with human diseases, which are rarely serious, and for which preventive or therapeutic interventions are often available and the risk of spread is limited.
c) Risk Group 3 - These agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available.
d) Risk Group 4 - Risk Group 4 agents are likely to cause serious or lethal human disease for which prevention or therapeutic interventions are not usually available.

Classification of agents on the basis of hazard, is based on the potential effect of a biological agent on a healthy human adult and does not account for instances in which an individual may have increased susceptibility to such agents, e.g., preexisting diseases, medications, compromised immunity or pregnancy.

A final assessment of risk based on these considerations is then used to set the appropriate containment conditions for the experiment. The containment level required might be equivalent to the Risk Group classification of the agent or it may be raised or lowered as a result of the above considerations.

Management of Biological Safety and Biosecurity

The responsibility for the safety of staff lies with the supervisors and directors of the microbiology laboratory, it can be advantageous to identify an individual(s) to specifically manage biological safety issues. In many laboratories, this role is either informally assigned to a qualified individual who performs these duties on a part-time basis (e.g., senior microbiologist) or the role is shared by a number of individuals. This role can also be formally assigned to a dedicated Biological Safety Officer who has a working knowledge of the laboratory practices and procedures within the facility. The Biological Safety Officer should liaise with the Committee through regularly scheduled meetings and can present specific safety problems, concerns or policy/protocol improvements to be considered and addressed. The Committee is also available to the Biological Safety Officer for risk assessments, disputes about biological safety matters or

other matters that may be of a biological safety nature. The structure for the management of biological safety issues within each facility should be determined locally and will vary according to the level of coordination and the associated resources necessary for implementation. Determining factors include the following: the size of the facility, the concentration of multiple laboratories in the facility, the containment levels within the facility and experimental or diagnostic animal activities within the facility.

Facilities handling infectious agents need not only a biosafety program but also a biosecurity plan in place. While biosafety deals with all aspects of containment to prevent any exposure to and accidental release of pathogens, biosecurity is implemented to prevent the theft, misuse or intentional release of pathogens. Whether it is for the advancement of science or the diagnosis of agents causing disease or the misuse of these technologies, there is unfortunately a dual use potential in the nature of the work (i.e., procedures, equipment, etc.) that takes place with these agents.

A protocol for reporting and investigating security incidents e.g., missing infectious substances, unauthorized entry, should be addressed. A mechanism needs to be in place for the reporting and removal of unauthorized persons. Biosecurity incident and emergency plans should include response to intentional (bomb threats etc.), unintentional (accidental release) and natural events (power outages, severe weather). Training needs to be provided to all relevant personnel. Expert advice from security and/or law enforcement experts should be sought in the development of threat assessments and security protocols specific to each facility. The threat assessment and security practices should be regularly reviewed and updated to reflect new threats that may be identified.

Encyclopedia

Lyophilization is a process in which water is removed from a product after it is frozen and placed under a vaccum, allowing the ice to change directly from solid to vapour without passing through the liquid phase. Lyophilization has been successfully used in pharmaceutical protein formulations, as well as in biological cells. Lyophilization is defined as a stabilizing process in which the substance is first frozen and then the quantity of the solvent is reduced first by sublimation (primary drying) and then by secondary drying to values that will no longer support biological growth or chemical reactions. Lyophilization is a multistep operation in which each step is critical.

- Sample Preparation - During preparation, sample (cells) is collected and washed with an isotonic physiological solution and then resuspended in a solution with lyoprotective agents, which are chemicals used for preventing cells from injury during the lyophilization process. The biological cells or tissues may exhibit significant degradation and loss of activity during the freeze-drying process. The lyoprotective agents are selected for different cells and tissue types. The following are commonly used lyoprotective agents: sugars such as sucrose, glucose; polyhydric alcohols such as polyethylene glycol (PEG), glycerol; polar solvents such as dimethyl sulphoxide (DMSO) etc.

- Freezing Process - The next step is the freezing process, which is critical for the survival of the cells and tissues. The goal of the freezing process is to separate the solvent from solutes. For an aqueous system, the water will form ice crystals and solutes will be confined to the interstitial region among the ice crystals.

- Primary Drying - Primary drying is a process in which the water is removed by sublimation. The pressure in the freeze dryer is reduced by the vaccum system and heat is applied to the sample formulation to initiate sublimation. Completion of the primary drying process occurs when all of the ice crystals have been removed from the formulation and the volume occupied by the resulting cake is equivalent to that of the frozen matrix.

- Secondary Drying - In the secondary or final drying phase, the point is to reduce the residual moisture content in the product as much as necessary to ensure a permanently storable state of the product. At the completion of primary drying, some water will still be adsorbed onto the surface of the material.

After lyophilization, a formulation must be protected from the environment. Mostly the material in the container is sealed by a stoppering mechanism contained in the freeze dryer, which depresses the closure into the container. Upon completion of the stoppering of the containers, the products can be safely removed from the freeze dryer and the stopper can be crimped sealed with a metal or coloured plastic cap to provide a permanent seal for the product.

Reconstitution - Usually the lyophilized samples can be rehydrated at normal temperature. In most cases, rehydration is performed by addition of an exact amount of aqueous solution that was previously extracted from the material during lyophilization.

Gellan Gum: *A New Trend Among Gelling Agents*

Natural gums have been used in commerce since the beginning of recorded history. Biopolymers are used as gelling agents because of their ability to cause the aqueous phase to gel. A numbers of water-soluble polymers have the ability to form gels. The gels may be weak and unable to support themselves or strong such that they retain their shape on removal from their container. The gels are referred to as physical gels since they are formed by intermolecular association through, for example, hydrogen bonding, hydrophobic association or ion mediated cross linking and should be differentiated from chemical gels which can be formed by covalent cross linking and should be differentiated from chemical gels which can be formed by covalent cross linking of polymer chains using chemical reagents such as glutaraldehyde.

Intermolecular association of the polymer chains gives rise to the formation of junction zones and their lifetime will depend on the number of polymer segments that are involved. For some polymers, chain association is a cooperative process with several consecutive segments involved and this leads to the formation of strong gel. Certain helix forming biopolymers for example, agarose, carrageenan, gellan gum and gelatin form strong gels on cooling. These polymers adopt a disordered conformation at high temperatures but on cooling undergo a conformational change and stiff ordered helices are formed which self associated to form a gel. The process is thermally reversible and the gels melt on heating. The melting temperature is often higher than the gelation temperature since melting only occurs after disaggregation of the helices. Other biopolymers such as alginate and pectin form strong gels through cation mediated cross-linking. The cations, e.g., calcium ions, interact with the carboxylate groups on a polymer chain and induce intermolecular cross-linking. The junction zones formed are strong and the gels tend to be thermally irreversible.

Gel formation only occurs above the critical minimum concentration required to give connectivity, which is specific for each polymer type. The properties of individual polymer gels vary considerably in strength and elasticity due to differences in the number and nature of the junction zones and the degree of chain aggregation.

Most food systems consist of aqueous mixtures of biopolymers that interact in several ways to produce various attributes that impact on quality, texture and stability. Polysaccharides constitute an important group of biopolymers that interact under specific conditions to form three-dimensional networks, or structures, that bind the aqueous phase. These structures play important roles in texture, flavour release and stability of a wide range of food products including, desserts, jellies and confectionary goods. Biopolymers are able to accomplish this through their ability to swell and absorb water and to reorder their molecular configuration through inter and intrapolymer interactions and entanglements.

The significant commercial success of xanthan gum in food, industrial and oil field applications has provided the springboard for the development of a new generation of microbial polysaccharides. The search for these polysaccharides has resulted in the development of several proprietary new gum possessing novel and useful properties that equate with commercial potential. The first of these is S-60 polysaccharide or gellan gum, the generic name for this commercial product. Gellan gum was first used in food in Japan, 1988 where microbial polysaccharides are considered natural materials. The FDA has approved it for use in the United States and Europe.

Structure

Gellan gum is an extracellular polysaccharide produced commercially as a fermentation product of the bacterium *Sphingomonas elodea* (ATCC 31461) previously referred as *Pseudomonas elodea*. It is a linear anionic heteropolysaccharide with a molecular weight of 500kDa. Gellan gum is a linear polysaccharide with a tetrasaccharide repeat unit of glucose, glucuronic acid and rhamnose in the molar ratio of 2:1:1. The polymer is produced with two acyl substituents present on the 3-linked glucose, namely L-glyceryl, positioned at O (2) and acetyl at O (6). On average there is one glycerate per repeat unit and acetate per every two repeats.

Chemical analysis proved that glycerate substitution predominates over that with acetate. Moreover, glycerate substitution dramatically influences gellan properties since its bulk hinders chain associations and accounts for the change in gel texture brought about by de esterification.

Manufacture

Gellan gum is produced by a pure culture of the bacteria *Sphingomonas elodea*. It is manufactured in an aerobic, submerged, fermentation process. The fermentation medium for gum formation includes a carbon source such as glucose, nitrogen source and the desired inorganic salts. For fermentation under sterile conditions to be successful, aeration, agitation and controlled temperature and pH need to be maintained. The fermentation broth increases in viscosity as the glucose is metabolized by the bacteria and the gum is secreted. Once fermentation is complete (when the carbon source is exhausted), the viable bacteria are killed by heat treatment before processing

the broth to obtain the polysaccharide. Treatment of the pasteurized broth with alkali removes the acyl substituents on the gellan gum backbone. Then cellular debris is removed and the gum is recovered by precipitation with alcohol. Thus, a non-substituted form with a high degree of gellan gum purity is achieved. In fact two forms of gellan gum are produced: the fully acylated native form (High Acyl Gellan Gum) and deacylated form (Low Acyl Gellan Gum).

Functional Properties of Gellan Gum

There are following three steps to be considered for the successful formulation of gellan gum gel:

a) Dispersion
b) Hydration
c) Gelation

a) Dispersion - The first step in preparing gellan gum solution is to ensure that the gum particles are properly dispersed in the solvent and do not clump together. Poor dispersion will result in incomplete hydration and loss of gum functionality. Both forms of gellan gum are insoluble in cold water although it will tend to swell in water of low calcium content. As the ion concentration in the water increases, dispersion becomes even easier.

b) Hydration - The presence of divalent ions in the polysaccharide has an inhibiting effect on its hydration. In cold deionized water, only partial hydration is obtained. It is necessary to heat the dispersion to at least 70ºC to achieve complete hydration. The inhibiting effect of the divalent ions is confirmed by the fact that complete hydration can be achieved in cold deionised water by using a pure monovalent salt form of the gum. The presence of divalent cations in most water supplies further restricts hydration at ambient temperature. Therefore in most practical situations, gellan gum can be dispersed easily without hydrating.

The extent to which hydration takes place in cold water depends upon the cation concentration. The presence of monovalent ions can also inhibit the hydration of gellan gum, but the levels necessary are considerably higher than those of the divalent ions. Thus, the latter may be sequestered with compounds such as sodium or potassium citrate or various sodium or potassium phosphates; enabling gellan gum to be dissolved at lower temperatures, without introducing sufficient monovalent ions to interfere with hydration. It is even possible to hydrate gellan gum fully at ambient temperature with the use of sufficient sequestrant. The temperature at which low acyl (LA) gellan gum hydrates is dependent on the type and concentration of ions in solution. The hydration of High Acyl (HA) gellan gum is much less dependent on the concentration of ions.

c) Gelation - The initial step of gellan gelation occurs through the formation of double helices, followed by their ion-induced association. Heating and cooling solutions of gellan gum allow the fibrils to form as neighboring molecules form a double helix. In the presence of ions, these fibrils aggregate into a three dimensional structure, forming a gel. As a hot solution cools gellan gum undergoes a disorder-order transition. This transition attributes to a coil-helix transition. In low acyl gellan gum, gel promoting cations such as sodium, potassium, calcium and magnesium promote aggregation of the gellan double helices to form a three dimensional network and the subsequent gels are hard & brittle.

The acyl substituents have profound effect on the structure and rheological characteristics of high acyl gellan gum gels. The high acyl gellan gum undergoes similar disorder to order transition as the solution is cooled, but further aggregation of the helices is limited by the presence of the acetyl group. The subsequent gels are therefore soft and elastic.

Application

Gellan gum has wide applications in various fields e.g., in microbiological media, tissue-culture media, foods and pet foods, deodorant gels, films and coatings and capsules, bakery products, photographic emulsions and microcapsules.

Gellan gum is used in low calorie jams and jellies because in addition to provide good acid stability, clarity and flavour release. In some starch-based products, it is possible to replace a portion of the starch with gellan gum and improve flavour release. Gellan gum can be used as a fining agent for alcoholic beverages including beers, wines and fortified wines. The delicate texture, acid stability and intense flavour impact imparted by gellan gum is utilized in citrus flavoured desert gels, while gel clarity is a key feature in car deodorant gels.

In soft gelatin capsules and photographic emulsions, blends of gelatin and low acyl gellan gum have been found to be a reasonable replacement for gelatin alone. The inclusion of gellan gum changes its solubility in the gut. Low acyl gellan can also serve as a component of microcapsules. The use of gellan gum as a substitute of agar has been widely accepted. The advantages of gellan gum over agar relate to their thermostability, enabling long incubations at higher temperatures. Gellan gum gives equivalent strength at lower concentration than agar.

Gellan culture medium is advantageous in that it reduces the time required for plate preparation, it produces a drier medium and in case of some mesophilic species, it reduces required incubation time. The presence of sulphur or other impurities in the agar affect plant tissue culture's growth. Gellan gum is pure enough to use it in tissue culture. Transparency of gel is also another advantage in tissue culture. Gellan gum exhibits good resistance to contamination by molds, easy washing from the plant tissue for transplantation and the ability to observe stages in culture development. Gellan gum is likely to become more popular in the near future.

Edward Jenner

Birth: May 18, 1749

Death: January 26, 1823

Nationality: English

Known For: Vaccination for small pox

Small pox killed up to fifty percent of the patients in earlier days, while victims who survived might be left sterile, blind or disfigured. In organized medicine, practical helplessness against the scourge ended with the introduction of the first vaccine in 1796 by Edward Jenner.

Edward Jenner was born on May 17, 1749 in the small village of Berkeley in Gloucestershire. He is the third son of six children of Sarah and Stephen Jenner, a clergyman and landowner. After the death of his parents when he was just five years old, his uncle raised him. After a classical elementary education he was apprenticed, at about age thirteen, to Daniel Ludlow, a local surgeon, with whom he studied for about seven years. In 1770 Edward moved to London to continue his education. He studied surgery and anatomy under the surgeon John Hunter. Later on he worked at St. George's Hospital. Returning to his native countryside, by 1773 he became a successful general practitioner and surgeon. He spent the rest of his career as a doctor in his native town.

Jenner worked in a rural community and most of his patients were farmers or worked on farms with cattle. In the 18th century smallpox was a very common disease and was a major cause of death. The main treatment was by a method, which had brought success to a Dutch physiologist Jan Ingenhaus and was brought to England in 1721 from Turkey by Lady Mary Wortly Montague. This method involved inoculating healthy people with substances from the pustules of those who had a mild case of the disease, but this often had fatal results. In 1788 an epidemic of smallpox hit Gloucestershire and during this outbreak Jenner observed that those of his patients who worked with cattle and had come in contact with the much milder disease called cowpox never came down with smallpox. Jenner needed a way of showing that his theory actually worked. Jenner was given the opportunity on the 14 May 1796, when a young milkmaid called Sarah Nelmes came to see him with sores on her hands like blisters. Jenner identified that she had caught cowpox from the cows she handled each day. Jenner took some pus from a sore on the hand of that milkmaid. With this he vaccinated, via superficial incisions, an eight-year-old boy, James Phipps. Thereafter, James came down with a fever and a pustule sore that scabbed and scarred. Six weeks later when James recovered, Jenner challenged the reaction by injecting James with matter from smallpox sores, taken from a recent case. This was an extremely dangerous experiment. If James lived Jenner would have found a way of preventing smallpox. If James developed smallpox and died he would be a murderer. To Jenner's relief James survived. His experiment had worked. Encouraged, Jenner tried several more cases over the next year, with the same success.

Reaction to Jenner's work on smallpox was initially skeptical. Royal Society rejected his paper about his procedure for inoculation. In consequence Jenner prepared *An Inquiry into the Causes and Effects of the Variolae Vaccinae*, which he published privately in 1798. Within several years Jenner's book had been translated into the major European languages. Promoting the smallpox vaccine became Jenner's principal activity by the turn of the nineteenth century. However the obvious advantages of vaccination and the protection it provided won out, and vaccination soon became widespread. Eventually vaccination was accepted, and in 1840 the British government banned variolation and provided vaccination free of charge. As his work became appreciated, Jenner was showered with honours. Jenner's continuing work on vaccination prevented him from continuing his ordinary medical practice. He was supported by his colleagues and the King in petitioning Parliament and was granted £10,000 for his work on vaccination. In 1806 he was granted another £20,000 for his continuing work.

In 1803 in London he became involved with the Jennerian Institution, a society concerned with promoting vaccination to eradicate smallpox. In 1808, with government aid, this society became the National Vaccine Establishment. Jenner became a member of the Medical and Chirurgical Society on its foundation in 1805, and subsequently presented to them a number of papers. Returning to London in 1811 he observed a significant number of cases of smallpox after vaccination occurring. He found that in these cases the severity of the illness was notably diminished by the previous vaccination. In 1821 he was appointed Physician Extraordinary to King George IV, a considerable national honour, and was made Mayor of Berkeley and Justice of Peace. Jenner did not patent his discovery as it would have made the vaccination more expensive and out of the reach of many. It was his gift to the world. Jenner carried out research in a number of other areas of medicine and was also keen on fossil collection and horticulture.

Jenner lived through a mild stroke in 1820 but did not survive another one three years later, and died on 26th January 1823. Smallpox was a major killer before Edward Jenner's vaccination that was to change medical history. Whilst Jenner's vaccination did not eradicate smallpox, it had a marked impact on fatality rates. Today, smallpox has become the only contagious disease ever to be effectively eliminated worldwide from the human population.

References

Doctors and Discoveries by John G. Simmons.

Enjoy the humour

- A man got 2 wishes from GOD. He asked for the Best wine and Best Woman.
  Next moment, he had the Best Wine and Mother Teresa next to him.
  Moral: BE SPECIFIC
- A sher is getting married in the jungle. There is a big bash and all animals are dancing to the tune of loud music being played.
  In a corner a chooha too is dancing.
  He is asked, "Are bhai choohe, aap kyu nach rahe ho?"
  "Mere chote bhai ki shadi hai....Nachunga Nahin?"
  "Sher kabse aapka bhai hone laga?"
  "Shadi se pehle main bhi sher tha!", replied chooha.
- "Do you drink?" the girl's father inquired of his prospective son-in-law."
  "First tell me whether it is a question or an invitation" asked son-in-law.

Thoughts to live by

- Honesty and transparency make you vulnerable. Be honest and transparent anyway. (Mother Teresa)
- Imagination is more important than knowledge. For while knowledge defines all we currently know and understand, imagination points to all we might yet discover and create. (Albert Einstein)
- Strong people make as many mistakes as weak people. Difference is that strong people admit their mistakes, laugh at them, learn from them. That is how they become strong. (Richard Needham)
- Patience and perseverance have a magical effect before which difficulties disappear and obstacles vanish. (John Quincy Adams)
- Goals are not only absolutely necessary to motivate us. They are essential to really keep us alive. (Robert H. Schuller)

Track your brain

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

SERIES I

(a) I L O S T C A I D R

(b) X R T D O E E S

(c) S E L R I A I T

(d) A T M L

Write down the name of the selective medium for *Pseudomonas* species. (Agar)

SERIES II

(a) R S L I T E E

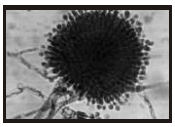
(b) D S O O E E C N P

(c) M F I O E T

(d) N C D A I A D

What do you call the chemical agents that are applied to non-living objects to destroy (kill) microorganisms?

Check your Answers on Page 16



Aspergillus species

Aspergillus is a filamentous and ubiquitous fungus found in nature. It is commonly isolated from soil, plant debris, and indoor air environment. While a teleomorphic state has been described only for some of the *Aspergillus* spp., others are accepted to be mitosporic, without any known sexual spore production. The genus *Aspergillus* includes over 185 species. Around 20 species have so far been reported as causative agents of opportunistic infections in man.

Economic Importance

Species of *Aspergillus* are important medically and commercially. Some species can cause infection in humans and other animals. Other *Aspergillus* species are important in commercial microbial fermentations. Perhaps the most well known application of *A. niger* is as the major source of citric acid. This organism accounts for over 99% of global citric acid production. *A. niger* is also commonly used for the production of native and foreign enzymes. Another use for *A. niger* within the biotechnology industry is in the production of magnetic isotope-containing variants of biological macromolecules for NMR analysis.

Morphology & Cultural Characteristics

Aspergillus species are saprophytic, thermotolerant fungi that grow on organic debris. The basic microscopic morphology is same for all species. However, some other microscopic structures are unique to certain species and constitute the key features for species identification together with the surface color of the colony. Hyphae are septate and hyaline. The conidiophores originate from the basal foot cell located on the supporting hyphae and terminate in a vesicle at the apex. Vesicle is the typical formation for the genus *Aspergillus*. The morphology and color of the conidiophore vary from one species to another. Covering the surface of the vesicle entirely ("radiate" head) or partially only at the upper surface ("columnar" head) are the flask-shaped phialides, which are either uniseriate or attached to the vesicle directly or are biseriate and attached to the vesicle via a supporting cell, metula. Over the phialides are the round conidia (2-5 µm in diameter) forming radial chains.

Other microscopic structures include sclerotia, cleistothecia, aleuriconidia, and Hulle cells. These structures are of key importance in identification of some *Aspergillus* species. Sclerotium is a compact mass of hardened mycelium which stores reserve food material. Cleistothecium is produced during the sexual reproduction stage of some *Aspergillus* species. It is a round, closed structure enclosing the asci which carry the ascospores. The asci are spread to the surrounding when the cleistothecium bursts. Aleuriconidium is a type of conidium produced by lysis of the cell that supports it. The base is usually truncate and carries remnants of the lysed supporting cell. These remnants form annular frills at its base. Hulle cell is a large sterile cell bearing a small lumen. Similar to cleistothecium, it is associated with the sexual stage of some *Aspergillus* species.

The major macroscopic features remarkable in species identification are the growth rate, color of the colony and thermotolerance. Except for *Aspergillus nidulans* and *Aspergillus glaucus,* the growth rate is rapid to moderately rapid. While *Aspergillus nidulans* and *Aspergillus glaucus* grow slowly and reach a colony size of 0.5-1cm following incubation at 25°C for seven days on Czapekdox agar. Those of the remaining species are 1-9cm in diameter in the specified setting. These variations in growth rate help in species identification. *Aspergillus* colonies are downy to powdery in texture. The surface color may vary depending on the species. The reverse is uncolored to pale yellow in most of the isolates. However, reverse color may be purple to olive in some strains of *Aspergillus nidulans* and orange to purple in *Aspergillus versicolor*. *Aspergillus fumigatus* is a thermotolerant fungus and grows well at temperatures over 40°C. This property is unique to *Aspergillus fumigatus* among the *Aspergillus* species. *Aspergillus fumigatus* can grow at a temperature range of 20 to 50°C.

Species	Surface	Reverse
Aspergillus clavatus	Blue-green	White, brownish with age
Aspergillus flavus	Yellow-green	Golden to red brown
Aspergillus fumigatus	Blue-green to gray	White to tan
Aspergillus glaucus	Green with yellow areas	Yellowish to brown
Aspergillus nidulans	Green, buff to yellow	Purplish red to olive'
Aspergillus niger	Black	White to yellow
Aspergillus terreus	Cinnamon to brown	White to brown
Aspergillus versicolor	White at the beginning, turns to yellow, tan, pale green or pink	White to yellow or purplish red

Pathogenicity & Clinical Manifestations

Aspergillus species are well known to play a role in three different clinical settings in man: (i) opportunistic infections (ii) allergic states and (iii) toxicoses. Immunosuppression is the major factor predisposing to development of opportunistic infections. These infections may present in a wide spectrum, varying from local involvement to dissemination and as a whole called aspergillosis. These include (1) mycotoxicosis due to ingestion of contaminated foods; (2) allergy and squeal to the presence of conidia or transient growth of the organism in body orifices; (3)

colonization without extension in preformed cavities and debilitated tissues; (4) invasive, inflammatory, granulomatous, narcotising disease of lungs, and other organs; and rarely (5) systemic and fatal disseminated disease. The type of disease and severity depends upon the physiologic state of the host and the species of Aspergillus involved. The etiological agents are cosmopolitan and include *Aspergillus fumigatus, A. flavus, A. niger, A. nidulans* and *A. terreus.*

Pulmonary Aspergillosis

The clinical manifestations of pulmonary aspergillosis are many, ranging from harmless saprophytic colonization to acute invasive disease. Allergic aspergillosis is a continuum of clinical entities ranging from extrinsic asthma to extrinsic allergic alveolitis to allergic bronchopulmonary aspergillosis (hypersensitivity pneumonitis) caused by the inhalation of *Aspergillus* conidia. Features include asthma, intermittent or persistent pulmonary infiltrates, peripheral eosinophilia, positive skin test to *Aspergillus* antigenic extracts, positive immunodiffusion precipitin tests for antibody to *Aspergillus*, elevated total IgE, and elevated specific IgE against *Aspergillus*. Plug expectoration and a history of chronic bronchitis are also common. Non-invasive aspergillosis or aspergilloma (fungus ball) is caused by the saprophytic colonization of pre-formed cavities, usually secondary to tuberculosis or sarcoidosis. Features often include hemoptysis with blood stained sputum, positive immunodiffusion precipitin tests for antibody to *Aspergillus*, and elevated specific IgE against *Aspergillus.*

Chronic narcotising aspergillosis is an indolent, slowly progressive, "semi-invasive" form of infection seen in mildly immunosuppressed patients, especially those with a previous history of lung disease. Diabetes mellitus, sarcoidosis and treatment with low-dose glucocorticoids may be other predisposing factors. Common symptoms include fever, cough and sputum production; positive serum antibody precipitins may also be detected.

Disseminated Aspergillosis

Hematogenous dissemination to other visceral organs may occur, especially in patients with severe immunosuppression or intravenous drug addiction. Abscesses may occur in the brain (cerebral aspergillosis), kidney (renal aspergillosis), heart, (endocarditis, myocarditis), bone (osteomyelitis), and gastrointestinal tract. Ocular lesions (mycotic keratitis, endophthalmitis and orbital aspergilloma) may also occur, either as a result of dissemination or following local trauma or surgery.

Aspergillosis of the paranasal sinuses

Two types of paranasal sinus aspergillosis are generally recognized. (1) A non-invasive "aspergilloma" form, primarily seen in non-immunosuppressed individuals. Predisposing factors include a history of chronic sinusitis and poorly draining sinuses with excessive mucus. (2) An invasive form, usually seen in the immunosuppressed patient. This form has a similar clinical setting to that seen in rhinocerebral zygomycosis; and symptoms include fever, rhinitis and signs of invasion into the orbit.

Cutaneous Aspergillosis

Cutaneous aspergillosis is a rare manifestation that is usually a result of dissemination from primary pulmonary infection in the immunosuppressed patient. However, cases of primary cutaneous aspergillosis also occur, usually as a result of trauma or colonization. Lesions manifest as erythematous papules or macules with progressive central necrosis.

Laboratory Diagnosis

Sputum, bronchial washings and tracheal aspirates from patients with pulmonary disease and tissue biopsies from patients with disseminated disease are taken as specimens to diagnose the disease. Direct microscopy is done for sputum specimens. Sputum can be observed under microscope with wet mount in 10% KOH. Tissue sections should be stained with H&E, GMS and PAS digest. The presence of hyaline, branching septate hyphae, consistent with *Aspergillus* in any specimen, from a patient with supporting clinical symptoms should be considered significant. Biopsy and evidence of tissue invasion is of particular importance. Clinical specimens should be inoculated onto primary isolation media, like Sabouraud's dextrose agar. Colonies are fast growing and may be white, yellow, yellow-brown, brown to black or green in colour.

Aspergillus species are well recognized as common environmental airborne contaminants, therefore a positive culture from a non-sterile specimen, such as sputum, is not proof of infection. However, the detection of *Aspergillus* (especially *A. fumigatus* and *A. flavus)* in sputum cultures, from patients with appropriate predisposing conditions, is likely to be of diagnostic importance and empiric antifungal therapy should be considered. Unfortunately, patients with invasive pulmonary aspergillosis, often have negative sputum cultures making a lung biopsy a prerequisite for a definitive diagnosis. Immunodiffusion tests for the detection of antibodies to *Aspergillus* species have proven to be of value in the diagnosis of allergic, aspergilloma, and invasive aspergillosis. However, they should never be used alone, and must be correlated with other clinical and diagnostic data. Mixed and individual antigenic extracts and antisera to the common *Aspergillus* species are commercially available from a number of sources. Reliable antigen detection tests for invasive aspergillosis are currently not available.

Treatment

Voriconazole is currently first-line treatment for invasive aspergillosis. There are other drugs that can be used to treat invasive aspergillosis in patients who cannot take voriconazole or who have not responded to voriconazole. These include itraconazole, lipid amphotericin formulations, caspofungin, micafungin, and posaconazole. Whenever possible, immunosuppressive medications should be discontinued or decreased.

Ecometric Method

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. Responsibility for quality rests primarily with manufacturer of culture media. Acknowledging this, reputable manufacturers have adapted the principles of Total Quality Management (TQM), which provides the framework for quality procedure that controls raw materials sourced world-wide, all manufacturing operations, as well as laboratory analysis and control, the follow up of complaints and defects. Quality assurance manager should pay attention to the internal quality control tests to be carried out on each batch of culture media, involving a range of physical, chemical and microbiological tests.

Physical tests should confirm that the powder meets its specification in terms of colour, odor, particle size, homogeneity, flow characteristics and moisture content. Chemical test would include the colour, clarity, solubility and pH of the product when reconstituted with deionized water, sterilized and cooled, as required. Media containing agar must meet the required specification of gel strength. Various other tests may also be carried out which relate specifically to the medium concerned. With regard to microbiological tests, there are no specified or mandatory growth tests by which the performance characteristics of culture media are measured in pharmaceutical microbiology. Most manufacturers provide a list of suitable test organisms for the end-user to monitor the performance of the media concerned. The comparison of test versus reference medium is an essential part of any quality control testing scheme, thereby ensuring that any variation in inoculum, viability or phenotypic characteristics are controlled for each test.

Ecometric method described by Mossel *et al* is an easy and economic way of monitoring solid media quality on a routine basis. A Standard Procedure for routine quality control of microbiological performance of solid culture media. In this procedure, in a reproducible manner solid media is plated out with the test microorganism and the degree of growth is recorded. After appropriate incubation the highest rate of dilution that still leads to growth can be assessed and the results expressed as an absolute growth index (AGI). The relative growth index (RGI), the proportion of the AGI on the test medium with that of the test medium compared with that on a control medium, can be used to describe the productive and selective properties of a particular medium.

Ecometric method can be utilized to check both the growth as well as inhibition characteristics of media. This method can be used to compare results with previous batches of the same medium or between selective and non-selective media.

- A loopful (1µl) of an overnight broth culture is spread on to the surface of pre-dried plates in the manner illustrated in figure, the loop moving through sections one to five without flaming or recharging (Fig: 1).

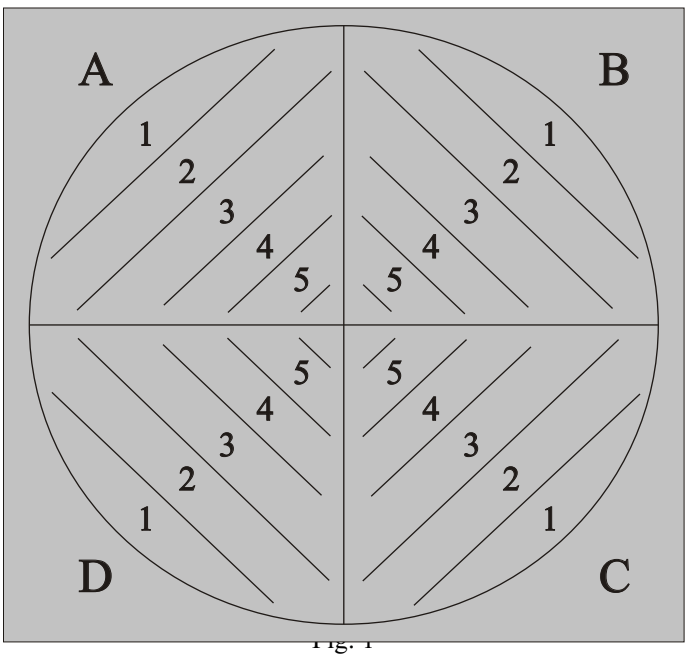


Fig: 1

- The procedure has to be repeated for the control plate.
- After incubation the last point should be noted in both test and control on which growth occurs. These are the end points of test and control media.
- These readings can be used to calculate the absolute growth index (AGI) and relative growth index (RGI) of the medium. The AGI is obtained by noting the end points (see Table 1).
- The RGI is a comparison of the AGI of the test plate and control plate. RGI = AGI test / AGI control x100.

Absolute Growth Index (AGI)

A1=5	B1=10	C1=15	D1=20
A2=25	B2=30	C2=35	D2=40
A3=45	B3=50	C3=55	D3=60
A4=65	B4=70	C4=75	D4=80
A5=85	B5=90	C5=95	D5=100

Table1

A medium's performance is regarded as satisfactory if all test strains grow appropriate for the medium being tested, and colonial morphology and reactions produced in the medium are typical for the organism on that particular type of medium. Trained or specially skilled microbiologist should carry out the streaking in order to avoid undesirable errors.

Pure Culture Technique

In microbiology, laboratory culture containing a single species of organism is known as pure culture. A pure culture is usually derived from a mixed culture (containing many species) by methods that separate the individual cells so that, when they multiply, each will form an individually distinct colony, which may then be used to establish new cultures with the assurance that only one type of organism will be present. Pure cultures may be more easily isolated if the growth medium of the original mixed culture favours the growth of one organism to the exclusion of others. Microbiologists have developed special techniques and equipment to isolate and grow pure cultures of microorganisms that are free from contaminating forms.

Brief History

A lot of early work on Pure culture technique was done by Brefeld. He introduced the practice of Single cell isolation and carried out a lot of work on Pure culture technique. The earliest method for pure culture isolation of bacteria however was that put forth by Joseph Lister which relied on the use of serial dilutions.

Significance of Pure Culture

Microorganisms usually exist in mixed populations in soil, water and some parts of the human body. It is not feasible to identify or study the characteristics of a particular species and therefore a pure culture must be obtained in preparation of further work. As the name implies pure culture contains only a single species of microorganisms. Pure culture of an organism is also helpful in designing a functional habitat for it.

Different Methods of Pure Culture Technique

Various types of techniques have been designed to achieve this goal. A variety of techniques have been developed whereby isolation into pure culture can be accomplished. Each technique has certain advantages and limitations, and there is no single method that can be used for all bacteria.

Streak Plate Technique

The quadrant streak plate technique is a relatively inexpensive and rapid method for separating bacteria in a mixed population of high cell density. It requires only a single plate of growth medium and it yields excellent distribution of bacterial colonies.

Procedure of quadrant streak plate method is described as follows:

a) A nutrient agar plate is selected for the isolation of pure strain from a mixed culture. The plate is properly labeled before streaking.

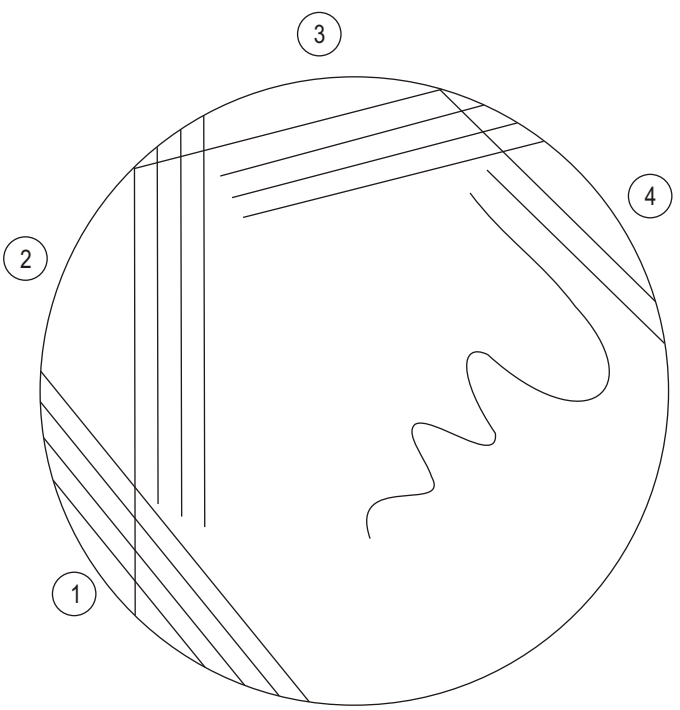
b) Aseptically obtain a loopful of the mixed population and lightly streak it several times along one area (quadrant) of the plate. Try not to cut into the agar surface and avoid airborne contamination by lifting the lid of the plate only enough to permit entry of the loop.

c) Replace the lid. Sterilize the loop to destroy any remaining bacteria. To ensure that it is cool, touch the loop to the center of the plate or between the agar and edge of the plate. Pass the loop once across the previous streaks to pick up some bacteria and continue streaking into a second area of the plate. Replace the lid.

d) Sterilize the loop as before, and then cool it. To pick some bacteria pass the loop one time through the second area and continue streaking into third area. And the same procedure should be followed for the fourth area.

e) Invert the streaked (inoculated) plates and incubate them for 24-48 hours at appropriate temperature. The plates are inverted so that moisture accumulates on the lid rather than on the agar surface, where it may cause colonies to run together. Examine the plates for the well isolated and separated colonies.

The basis behind this technique is to achieve gradual dilution of the biomass so that at some places well-separated single colonies are obtained. Being unicellular each colony is derived by the clonal multiplication of one cell, and being haploid each one of them will be genetically pure.



Pour Plate Technique

Pour plate technique was originally developed by Koch, in which the cells were mixed with molten and cooled gelatin (now replaced with agar) then immobilizes cells so that each one of them could produce an isolated colony. The technique has been

slightly modified in which a diluted sample is used to ensure the development of isolated colonies.

a) In pour plate method the mixed culture is diluted directly in tubes of liquid agar medium. The medium is maintained in a liquid state at a temperature of 45°C to allow thorough distribution of the inoculum.

b) The inoculated medium is dispensed into Petri dishes, allowed to solidify, inverted and incubated.

c) A series of agar plates give decreasing numbers of colonies resulting from the dilution procedure in the pour plate technique.

The pour plate technique has certain disadvantages. For instance, some of the organisms are trapped beneath the surface of the medium when it gels and therefore both surface and subsurface colonies develop. The subsurface colonies can be transferred to fresh media only by first digging them out of the agar with a sterile instrument.

Another disadvantage is that the organisms being isolated must be able to withstand temporary exposure to the 45°C temperature of the liquid agar medium. For instance pour plate method would be unsuitable for isolating psychrophilic bacteria.

Spread Plate Technique

The spread plate technique is applied when the microbe to be counted grows best on the surface of the culture medium.

a) In this method the mixed culture is not diluted in the culture medium; instead it is diluted in a series of tubes containing a sterile liquid, usually water or physiological saline.

b) A small sample is then removed from each dilution tube and is placed on the surface of an appropriate solid growth medium. One Petri dish is used for each sample.

c) The inoculum on each plate is then spread as a lawn over the entire surface of the plate using a sterile spreader. The inoculum is spread uniformly by holding the stick at a set angle on the agar and rotating the agar plate or rotating the stick until the inoculum is distributed. Often a sterile bent glass rod is used for this purpose.

d) These inoculated plates are then incubated to allow colonies to grow.

This technique is also used in quantitative enumeration of bacteria. In this, the numbers of colonies are counted from a plate where the colonies are well dispersed. When the same is multiplied with the degree of dilution, total cell count of the original sample can be determined. This technique is also useful in estimating the viable cell counts as colony forming units (CFUs).

When the individual colonies are isolated on a culture medium, the individual colony of interest can further be confirmed as 'pure' by repeatedly streaking a single isolated colony on a fresh medium preferably using a selective medium and observing the growth and other characteristics of the desired organism.

Special Methods for Isolation of Pure Culture

Other than traditional methods, there are some special techniques for isolation of pure culture. Following two methods are in use for isolation of pure culture:

Capillary Pipette Method

Several small drops of a suitably diluted culture medium are put on a sterile glass-coverslip by a sterile pipette drawn to a capillary. One then examines each drop under the microscope until one finds such a drop, which contains only one microorganism. This drop is removed with a sterile capillary pipette to fresh medium. The individual microorganism present in the drop starts multiplying to yield a pure culture.

Micromanipulator Method

Micromanipulators have been built, which permit one to pick out a single cell from a mixed culture. This instrument is used in conjunction with a microscope to pick a single cell (particularly bacterial cell) from a hanging drop preparation. The micro-manipulator has micrometer adjustments by means of which its micropipette can be moved right and left, forward, and backward, and up and down. A series of hanging drops of a diluted culture are placed on a special sterile coverslip by a micropipette.

Now a hanging drop is searched, which contains only a single microorganism cell. This cell is drawn into the micropipette or microprobe by gentle suction and then transferred to a large drop of sterile medium on another sterile coverslip. When the number of cells increases in that drop as a result of multiplication, the drop is transferred to a culture tube having suitable medium. This yields a pure culture of the required microorganism.

The advantages of this method are that one can be reasonably sure that the cultures come from a single cell and one can obtain strains with in the species. The disadvantages are that the equipment is expensive, its manipulation is very tedious, and it requires a skilled operator. This is the reason why this method is reserved for use in highly specialized studies.

Like many aspects of science though Pure Culture Technique has its own limitations for instance the presence of Mycoplasmas and viruses cannot be detected. However the advantages attributed to the technique of Pure culture have outweighed the disadvantages.

Virtually all areas of research on pharmaceuticals, nutrition, ecology, agricultural production and parasitology bear witness to the usefulness of pure culture technique.

In our Best Practices section we have discussed about Pure Culture Techniques. To isolate the pure strains nutrient agar is commonly used. Here is the range of Nutrient Agar and Broth available with Accumix.

- **Nutrient Agar**
  A general-purpose culture medium for the cultivation of bacteria, which may also be enriched by incorporating 10% v/v sterile blood or other biological fluids.
- **Nutrient Agar IP**
  A general purpose culture medium for the cultivation of microorganisms in compliance with IP.
- **Nutrient Agar 1.5% ISO**
  A general purpose culture medium for the cultivation of fastidious bacteria after enrichment by incorporating 10% v/v sterile blood or other biological fluids in compliance with ISO specification ISO/DIS 13720: 1995.
- **Nutrient Agar pH 6.8**
  A medium for the cultivation of a wide variety of bacteria and for the enumeration of microorganisms in water, sewage, feces and other materials.
- **Nutrient Agar with 1% Peptone**
  A general purpose medium for the examination of water and dairy products.
- **Nutrient Broth**
  A general-purpose culture medium for the cultivation of bacteria, which may also be enriched by incorporating 10% v/v sterile blood or other biological fluids.
- **Nutrient Broth with 1% Peptone**
  A general purpose medium for the examination of water and dairy products.
- **Nutrient Broth Medium IP**
  A general-purpose medium for aerobes in compliance with IP.

SERIES I

CLOSTRIDIA

DEXTROSE

LISTERIA

MALT

CETRIMIDE

SERIES II

STERILE

ENDOSCOPE

FOMITE

CANDIDA

DISINFECTANT

ACITAR™ - Expect more.

ACITAR is high level instrument disinfectant & sterilizing solution. It is colorless to pale yellow colored liquid with characterstic odour. It is acidic glutaraldehyde solution that does not require activation. Fortified with benzalkonium chloride, it provides high level disinfection & sterilization in medical settings. High use dilution make it an economical alternative to alkaline glutaraldehyde solution.

COMPOSITION

2% w/v Glutaraldehyde
5% w/v Benzalkonium chloride
Corrosion inhibitors

ACITAR

Broad spectrum antimicrobial activity
Sporicidal in just 5 hours
High level disinfection in just 15 minutes
Excellent synergistic action
30 days reuse life
Corrosion inhibitor present
Ready to use

USAGE DIRECTION

For high level disinfection: Immerse all the cleaned instrument in 20% v/v solution of ACITAR for a contact time of 15 minutes.

For sterilization: Immerse all the cleaned instrument in undiluted solution of ACITAR for a contact time of 5 hours.

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