IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 3, Issue 2, January 2023
Impact Factor: 7.301

Review on Microbiology and Molecular Biology

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Abstract: *Microbiology is the study of microorgan- isms biological entities too small to be seen with the unaided eye. Most major advances in microbiology have occurred within the past 150 years, and several important subdisciplines of microbiology have developed during this time, including microbial ecology, molecular biology, immunology, industrial microbiology and biotech- nology. Microorganisms of various types exist in all three domains of life (the Bacteria, Archaea and Eukarya), and they are by far the most abundant life forms on Earth. Microscopic biological agents include bacteria, archaea, protists (protozoa and algae), fungi, parasitic worms (helminths) and viruses. Although a small percentage of microor- ganisms are harmful to certain plants and animals and may cause serious disease in humans, the vast majority of microorganisms provide beneficial services, such as assisting in water purification and the production of certain foods, and many are essential for the proper functioning of Earth's ecosystems. Molecular biology has revolutionized our understanding of the diversity, function, and community structure of marine microorganisms. Increasingly, tools and techniques derived from the biomedical diagnostics and research industries are used in parallel with sensors that characterize the physical, chemical, and optical properties of ocean waters.*

Keywords: Microbiology

I. INTRODUCTION

Microbiology is defined as the study of organisms and agents that are too small to be seen clearly by the unaided eye. To be more simple, microbiology is the study of microorganisms which are the living organisms of microscopic size. Microorganisms are the living organisms that are less than 1 millimeter in diameter which cannot be seen by our naked eye. Microorganisms can be viewed through microscopes and they can exist as single cells or clusters. Microorganisms include the cellular organisms like bacteria, fungi, algae and protozoa. Viruses are also included as one of the microorganism but they are a cellular.

Best laboratory practices in microbiology

a) Personal hygiene

- Keep clothing clean and change the clothing after the lab, especially if it has been soiled by chemicals.
- Keep your hands clean during the lab.
- Always wash your hands before and after you eat.
- Wear gloves for the appropriate experiments and make sure these are kept clean for others.

b) Activity of Personal

Plan and conduct complex research projects, such as improving sterilization procedures or developing new drugs to combat infectious diseases. Perform laboratory experiments that are used in the diagnosis and treatment of illnesses.

c) Garments of Testing Areas

Assess the quality of your apparel's materials and workmanship, using a number of quality control checks and tests, including:

1. Dimensional stability/ Shrinkage to Laundering/washing
2. Skew Test
3. Distortion Test
4. Dimensional stability to Dry Cleaning
5. Appearance Rating

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6. Color fastness to Laundering
7. Color fastness to Crocking
8. Color fastness to Perspiration
9. Color fastness to Water
10. Color fastness to Light
11. Tensile strength

d) Procedure for entry and exit into microbiology laboratory and testing areas:

Procedure for Entry

1) Remove Microbiology laboratory footwear outside the change room - 1 2)Enter the Change room - after wearing the footwear provided for use in Microbiology testing area.3)Take one packet consisting of Pre- sterilized over gown and cap and two pairs of pre-sterilized hand gloves individually packed inbutter paper.4)Enter the Change Room II by opening thedoor.5)Disinfect hands by spraying 0.45 m filtered70% IPA.

Procedure for Exit

1) After completion of work, put off the Spirit lamp, remove all discarded material, material for washing and the media used for the test through UV hatch ensuring that UV light is off.2) Enter the Change Room -II and close the door of the microbiological testingarea 3)Proceed from change room - Il to change4)Remove both pair of hand gloves, over gown and cap. Fold neatly and put them into a poly bag & transfer the poly bag outside the change room.5) Remove foot wear of microbiologicaltesting area and come out from change room-l

e) General care to be taken during work:

1)Treat all microorganisms as potential pathogens.2)Maintain a curated culture collection. 3)Obtain fresh stock cultures of microorganisms annually.4)Obtain fresh stock cultures of microorganisms annually.5)Disinfect work areas before and after use.6)Wear protection. 7)Wash your hands. 9)Do not eat or drink in the lab, nor store food in areas where microorganisms are stored. 10)Clean up spills with care.

Sampling process:

Sampling is a process in statistical analysis where researchers take a predetermined number of observations from a larger population. The method of sampling depends on the type of analysis being performed, but it may include simple random sampling or systematic sampling.

a) Sampling for finished product:

1) Sampling of finished products shall be carried out online during packaging operations.2) IPQA shall sample the quantity required for analysis as per annexure-6..3) Select and pick the samples randomly from the packaging line over the period of packaging operations at different intervals.4) IPQA person shall submit the sample along with Analytical Request/Report (Annexure-I) to QC for analysis.

Bacterial endotoxin test

A bacterial endotoxin test (BET), such as LAL (limulus amebocyte lysate), is an in vitro assay used to detect bacterial endotoxins. The bacterial endotoxin test uses the lysate from blood cells from horseshoe crabs to detect bacterial endotoxins.

Microbiological Monitoring

In contrast, microbiological monitoring is a continuous assessment of the water quality, provided that the applied analytical method is meaningful, simple, rapid and cost-effective. Adenosine triphosphate (ATP) is a marker for cell viability used in a number of hygienic applications in many industries.

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a) Microbiology laboratory

The isolation and characterization of microorganisms inflicting infections performed by the microbiology laboratory play two important functions.1)Clinical – Managing the infections on a regular basis2)Epidemiological – Deep knowledge of an infective microbe present in a patient is helpful in investigating its source and mode of transmission

b) production area & sterile area validation

Process Validation is defined as the. collection and evaluation of data, from the. process design stage throughout. production, which establishes scientific evidence that a process is capable of consistently delivering quality products & STERILITY. Confirm the sterility of each sterilized batch of medium by incubating a portion of the media at the specified incubation temperature for 14 days. No growth of microorganisms occurs.

II. GROWTH PROMOTION TEST OF AEROBES, ANAEROBES, AND FUNGI.

Water Analysis

Microbiological water analysis is a method of analyzing water to estimate the numbers of bacteria present and to allow for the recovery of microorganisms in order to identify them. The method of examination is the plate count.

Cleaning, Sterilization and Disinfection

Cleaning is the removal of visible soil (e.g., organic and inorganic material) from objects and surfaces and normally is accomplished manually or mechanically using water with detergents or enzymatic products. Thorough cleaning is essential before high-level disinfection and sterilization because inorganic and organic materials that remain on the surfaces of instruments interfere with the effectiveness of these processes. Decontamination removes pathogenic microorganisms from objects so they are safe to handle, use, or discard.

Sterilization describes a process that destroys or eliminates all forms of microbial life and is carried out in health-care facilities by physical or chemical methods. Steam under pressure, dry heat, EtO gas, hydrogen peroxide gas plasma, and liquid chemicals are the principal sterilizing agents used in health-care facilities. Sterilization is intended to convey an absolute meaning; unfortunately, however, some health professionals and the technical and commercial literature refer to “disinfection” as “sterilization” and items as “partially sterile.” When chemicals are used to destroy all forms of microbiologic life, they can be called chemical sterilants.

Disinfection describes a process that eliminates many or all pathogenic microorganisms, except bacterial spores, on inanimate objects (Tables 1 and 2). In health-care settings, objects usually are disinfected by liquid chemicals or wet pasteurization. Each of the various factors that affect the efficacy of disinfection can nullify or limit the efficacy of the process.

a) Microbiology Laboratory and Other Areas

Failure to properly disinfect or sterilize equipment carries not only risk associated with breach of host barriers but also risk for person-to-person transmission (e.g., hepatitis B virus) and transmission of environmental pathogens (e.g., Pseudomonas aeruginosa).

b) Glass Wares

Use warm tap water and a brush with soapy water to scrub the inside of curved glassware. This waste water can go down the sink. Remove soapsuds with deionized water to avoid harsh water stains. The DI water rinse should form a smooth sheet when poured through clean glassware.

c) Apparatus

Autoclaves, clean-in-place (CIP) and sterilization-in-place (SIP) systems, dry heat sterilizers and ovens, steam sterilizers, media sterilizers, and UV chambers all work to sterilize equipment and supplies.

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Preparation, Filling and Disposal of Microbial Culture Media

Media preparation from dehydrated commercial formulations should be performed by following manufacturer's instructions. The formulation of basic ingredients, like peptones, yeast extracts, agar, buffering substances, and antibiotics, is modified to achieve consistency of the medium. The required amount of dehydrated medium or individual ingredients are dissolved in distilled water by continuous stirring followed by heating (if necessary). Media containing agar should be adequately soaked with proper agitation before heating. The pH must be adjusted, and the medium is dispensed into appropriate containers for sterilization by moist heat in an autoclave. Heat-sensitive substances (e.g., proteins, enzymes) are sterilized by using membrane filters.

Culture media must be stored at specified temperatures to prevent modification of the composition, and no longer than the product shelf-life. Aseptic preparation and storage are essential to protect culture media from microbial infection. Water loss on storage can be minimized by impermeable wrapping and/or storage at 5 °C ± 3 °C. Chemical degradation, e.g. oxidation or antimicrobial loss, can be retarded by protection from light, heat, and dehydration.

Disposal of-

a) Microbial culture

We also recommend to soak/rinse the used culture tubes and inoculation sticks in a dilute solution of Lysol or bleach before disposing them in the trash. The bench should be wiped down with a cleaning agent

b) Used media plates

Run an empty Autoclave sterilization cycle at 15 lbs for 15 minutes after the sterilization of used media. Record the disposal of microbial culture media as a "Disposal Load " in the separate record for disposal of microbial culture media. Assign separate load number for the disposal load.

Bioburden Determination

Bioburden is defined as the number of microorganisms living on a surface that has not been sterilized. The aim of bioburden testing (microbial limit testing) is to measure the total number of viable microorganisms (total microbial count) prior to sterilization. Products used in the pharmaceutical or medical field require control of microbial levels during processing and handling. We test for total aerobic microbial count (TAMC) and total combined yeast and mold count (TYMC).

a) Primary packing material

Primary packaging materials include glass, shrink film, flexible pouches, aluminum cans, and various other packaging materials.

Microbial limit test:

The microbial limit test (MLT) is performed to assess how many and which of certain viable microorganisms are present in non-sterile pharmaceutical, healthcare or cosmetics manufacturing samples that range from raw materials to finished products. The test provides information about the safety of the tested product by determining if quantitative limits for certain microorganisms are exceeded. It covers testing for bile tolerant gram-negative bacteria as well as for Escherichia coli, Salmonella, Pseudomonas aeruginosa, Staphylococcus aureus, clostridia and Candida albicans. The samples are prepared by mixing multiple portions taken randomly from the ingredient or product to be tested. If the samples contain antimicrobial substances, these have to be eliminated beforehand by dilution, neutralization, filtration, inactivation or other appropriate means. Great care must be taken to prevent microbial contamination from the outside.

Antimicrobial sensitivity test:

An important task of the clinical microbiology laboratory is the performance of antimicrobial susceptibility testing of significant bacterial isolates. The goals of testing are to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections.

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HEPA filter integrity testing and line clearness

The HEPA (High Efficiency Particulate Air) filter integrity test is typically performed on supply air or exhaust air HEPA filters using a photometer to scan the filter surface for pinhole leaks that could allow the transmission of contaminant particles that would be unacceptable in a critical application. HEPA (High Efficiency Particulate Air) is a filtration system that is used to extract contaminants or particulates from the air in a cleanroom. When installed, it will contribute greatly to maintaining or increasing air cleanliness levels.

III. INTRODUCTION TO MOLECULAR BIOLOGY

Molecular biology is the branch of biology that seeks to understand the molecular basis of biological activity in and between cells, including biomolecular synthesis, modification, mechanisms, and interactions. The study of chemical and physical structure of biological macromolecules is known as molecular biology.

Molecular biology was first described as an approach focused on the underpinnings of biological phenomena - uncovering the structures of biological molecules as well as their interactions, and how these interactions explain observations of classical biology.

In 1945 the term molecular biology was used by physicist William Astbury. In 1953 Francis Crick, James Watson, Rosalind Franklin, and colleagues, working at Medical Research Council unit, Cavendish laboratory, Cambridge (now the MRC Laboratory of Molecular Biology), made a double helix model of DNA which changed the entire research scenario. They proposed the DNA structure based on previous research done by Rosalind Franklin and Maurice Wilkins. This research then lead to finding DNA material in other microorganisms, plants and animals.

Molecular biology is not simply the study of biological molecules and their interactions; rather, it is also a collection of techniques developed since the field's genesis which have enabled scientists to learn about molecular processes.[6] In this way it has both complemented and improved biochemistry and genetics as methods (of understanding nature) that began before its advent. One notable technique which has revolutionized the field is the polymerase chain reaction (PCR), which was developed in 1983.[6] PCR is a reaction which amplifies small quantities of DNA, and it is used in many applications across scientific disciplines.

The central dogma of molecular biology describes the process in which DNA is transcribed into RNA, which is then translated into protein.

Molecular biology also plays a critical role in the understanding of structures, functions, and internal controls within individual cells, all of which can be used to efficiently target new drugs, diagnose disease, and better understand cell physiology.[10] Some clinical research and medical therapies arising from molecular biology are covered under gene therapy whereas the use of molecular biology or molecular cell biology in medicine is now referred to as molecular medicine.

a) RTPCR

Reverse transcription polymerase chain reaction (RT-PCR) is a laboratory technique combining reverse transcription of RNA into DNA (in this context called complementary DNA or cDNA) and amplification of specific DNA targets using polymerase chain reaction (PCR).[1] It is primarily used to measure the amount of a specific RNA. This is achieved by monitoring the amplification reaction using fluorescence, a technique called real-time PCR or quantitative PCR (qPCR). Combined RT-PCR and qPCR are routinely used for analysis of gene expression and quantification of viral RNA in research and clinical settings.

Isolation of-

a) DNA

Deoxyribonucleic acid (DNA) isolation is an extraction process of DNA from various sources. Methods used to isolate DNA are dependent on the source, age, and size of the sample. Despite the wide variety of methods used, there are some similarities among them.

b) RNA

RNA isolation generally consists of several steps: (1) cell lysis and homogenization, (2) quenching of biochemical processes, (3) nucleic acid partitioning, (4) RNA retrieval and crude purification, and (5) assessing the quality of the extracted RNA

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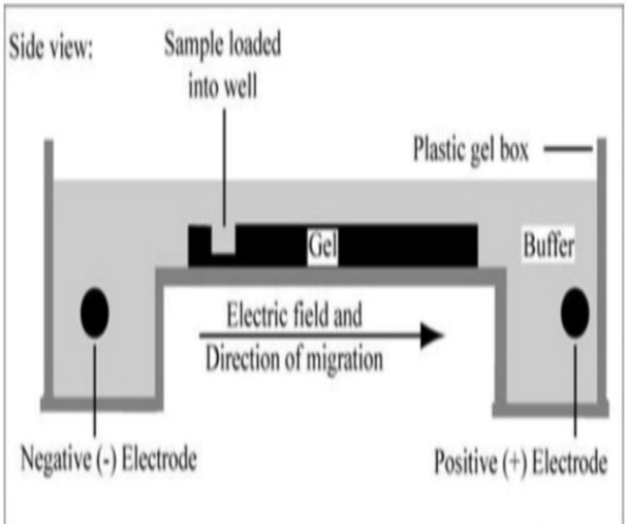
C) Protein

In order to extract the protein from the cells where it is present, it is necessary to isolate the cells by centrifugation. In particular, centrifugation using media with different densities may be useful to isolate proteins expressed in specific cells.

Agarose gel electrophoresis

Agarose (complex carbohydrate) provides a matrix through which DNA molecules migrate.

- Larger molecules move through the matrix slower than small molecules
- The higher the concentration of agarose, the better the separation of smaller molecules



- Gel electrophoresis is a technique for the analysis of nucleic acids and proteins and preparation and analysis of DNA
- Gel electrophoresis separates molecules on the basis of their rate of movement through a gel under the influence of an electrical field
- We will be using agarose gel electrophoresis to determine the presence and size of PCR products.
- Agarose gel electrophoresis is a method to separate DNA, or RNA molecules by size.
- This is achieved by moving negatively charged nucleic acid molecules through an agarose matrix with an electric field (electrophoresis).
- Shorter molecules move faster and migrate farther than longer ones.

Introduction to rDNA and production of therapeutic protein like insulin, interferon etc.

- Proteins are the most abundant organic molecules of the living system. They have significant role in structural and functional organisation of the cell.
- Proteins that result from the expression of recombinant DNA within living cells are termed recombinant proteins.
- Recombinant DNA technology involves taking genetic material from one source and recombining it in vitro with another source followed by introducing of recombined material into host cell.
- Once a Recombinant DNA is inserted into bacteria, these bacteria will make protein based on this rDNA. This protein is know as Recombinant Protein.

Recombinant DNA (rDNA) molecules are DNA molecules formed by laboratory methods of genetic recombination (such as molecular cloning) to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in biological organisms. Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure; they differ only in the sequence of nucleotides within that identical overall structure. Consequently, when DNA from a foreign source is linked to host sequences that can drive DNA replication and then introduced into a host organism, the foreign DNA is replicated along with the host DNA.

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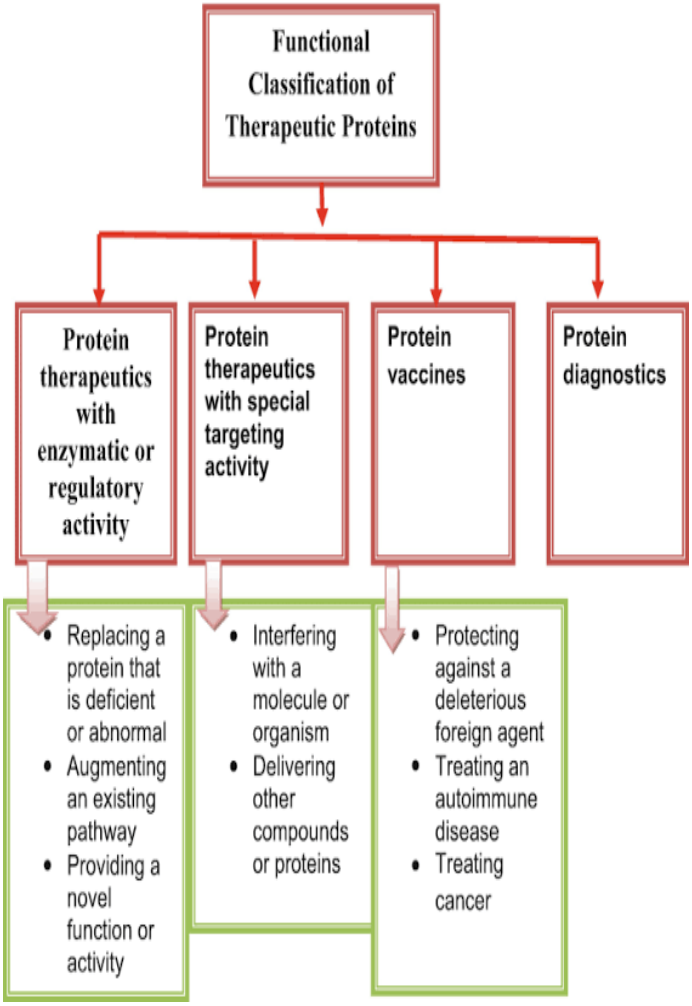
The steps involved in rDNA technology are:

1. Isolation of DNA
2. Fragmentation of the DNA using the enzyme Restriction endonucleases
3. Isolation of the desired DNA fragment
4. Amplification of the gene of interest
5. Ligation of the DNA fragment into a suitable vector by the enzyme DNA ligases
6. Transfer of DNA into the host cell
7. Screening
8. Culturing the host cells on a suitable medium on a large scale
9. Extraction of the desired product
10. Downstream processing of the products



Preparation of culture media and technique of inoculation:

Microbial culture media preparation is the process of mixing nutrients, agents for buffering and maintaining the osmotic balance, as well as selective inhibitors or indicators to create an agar or broth that supports the growth and the differentiation of microorganisms. Different methods of inoculation include stab culture, slant culture, agar culture and broth culture.

Steps in preparation of culture media:

1. Select culture media recipe from database
2. Recalculate ingredient quantities according to the required culture media volume
3. Weigh main ingredients into the container

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4. Weigh trace ingredients on a high accuracy balance and add to the container
5. Pour in deionized water up to around 80% of the required volume
6. Mix to dissolve the ingredients, gentle heating may be required
7. Check the pH using a pH meter and adjust if necessary
8. Top up the culture media to the required volume
9. Label the container
10. Sterilize in autoclave
11. Document results

Immobilization of Enzyme

An immobilized enzyme is an enzyme attached to an inert, insoluble material—such as calcium alginate (produced by reacting a mixture of sodium alginate solution and enzyme solution with calcium chloride). This can provide increased resistance to changes in conditions such as pH or temperature. It also lets enzymes be held in place throughout the reaction, following which they are easily separated from the products and may be used again - a far more efficient process and so is widely used in industry for enzyme catalysed reactions. An alternative to enzyme immobilization is whole cell immobilization.

Basics of tissue culture and application of cell cultures in pharmaceutical industry and research:

Tissue culture, a method of biological research in which fragments of tissue from an animal or plant are transferred to an artificial environment in which they can continue to survive and function. The cultured tissue may consist of a single cell, a population of cells, or a whole or part of an organ.

Application of Cell Cultures :Cell culture is an important tool in cellular and molecular biology, offering good model systems for investigating normal cell physiology and biochemistry (e.g., metabolic research, ageing), the effects of medicines and toxic chemicals on cells, and mutagenesis and carcinogenesis. It is also utilised in medication screening and research, as well as large-scale biological compound synthesis (e.g., vaccines, therapeutic proteins). Cell culture is especially useful for all of these studies because of the uniformity and reproducibility of results produced from clonal cells.

Use of biological indicator:

A biological indicator provides information on whether necessary conditions were met to kill a specified number of microorganisms for a given sterilization process, providing a level of confidence in the process. Endospores, or bacterial spores, are the microorganisms primarily used in BIs

Efficacy of antimicrobial preservation:

Antimicrobial preservatives are added to products to prevent or limit microbial contamination, which can occur during normal conditions of storage and use. The efficacy of an antimicrobial preservative may be enhanced or diminished by the active constituent of the preparation, or by the formulation in which it is incorporated, or by the container and/or closure being used as the final packaging material.

Maintenance and identification of standard culture /isolation and preservation methods for pure cultures:

Microorganisms in a pure culture are those belonging to a single species. Purification of microorganisms from a mixture using pure culture techniques is called purification. There are a number of important pure culture techniques, which include,

1) Streak plate method
2)Micromanipulator method
3) Spread plate method
4) Pour plate method

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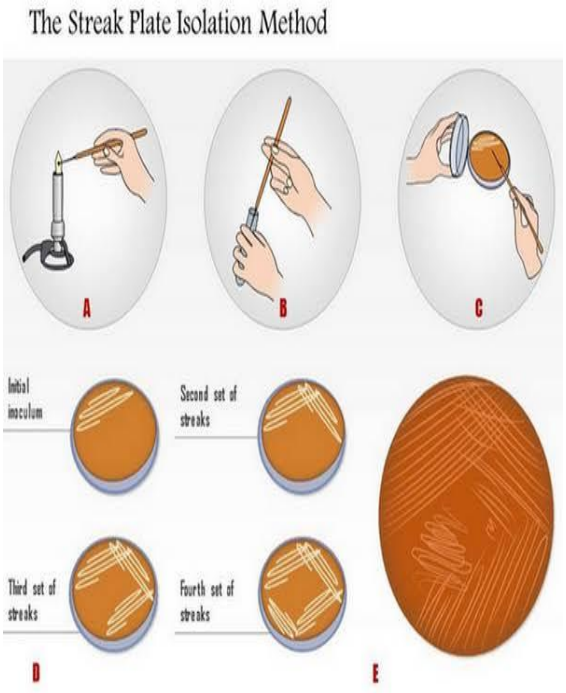
1) Streak plate method:

- The most common method is the isolation of pure bacteria.
- The mixed culture is streaked across the agar medium with the aid of an inoculation loop or needle tip.
- A thin layer of inoculum is applied, and subsequent streaks separate the microorganisms.
- A colony is allowed to grow on these plates.
- Striking establishes a dilution gradient across the face of a Petri plate when bacteria are deposited on agar surfaces.
- Confluent growth takes place in the part of the medium that contains the fewest bacterial cells because of this dilution gradient.



2) Micromanipulator method

- Single cells are isolated usi this technique.
- There are micromanipulators that enable one to remove a single cell from a mixture of cells.
- With the help of the instrument, a single bacterial cell (usually from a hanging drop preparation) can be taken out of the fluid.
- Micrometer adjustments enable the micropipette of the micromanipulator to be positioned right, left, forward, and backward.
- In order to place drops of diluted culture on sterile coverslips, a series of hanging drops is placed by a micropipette.
- Gently suction is used to draw the cells into the micropipette, and then the drop of sterile medium is placed on a sterile coverslip.

3) Spread plate method

- The medium used in this method is sterile, typically water or physiological saline, not melted agar. This method differs from the pour plate method in that there is no dilution in melted agar.
- Using a sterilized bend-glass-rod, one drop of such diluted liquid from each tube is mixed with a sterilized bend-glass-rod, and spread evenly over the agar plate.
- On the agar medium plates, colonies can grow well-isolated on some plates, while others can grow on less-isolated plates.

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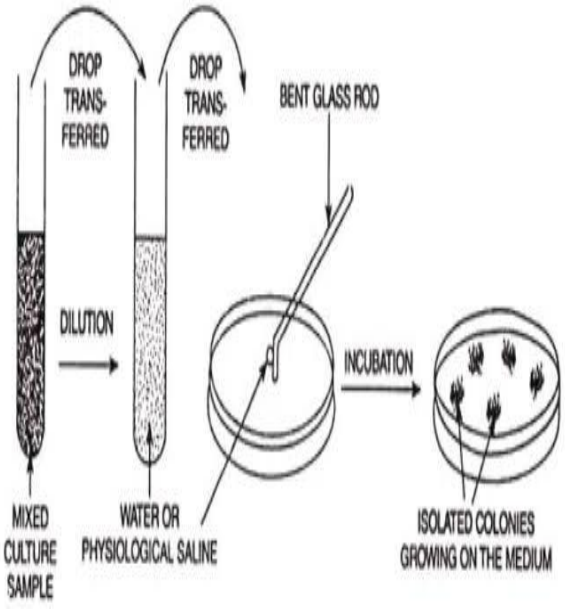
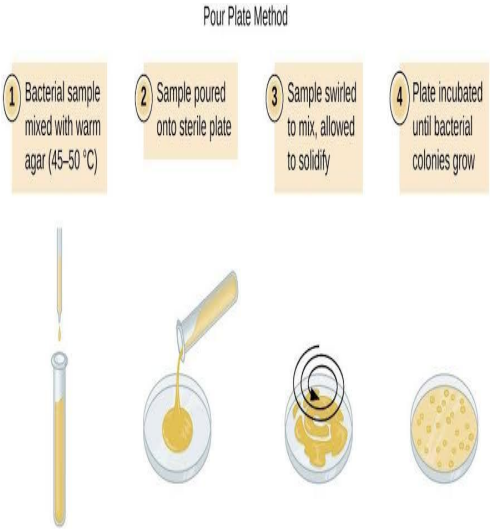
- A diluted drop of liquid is spread over the medium to separate individual microorganisms.
- Each colony is then removed from the plate and transferred to new medium to ensure purity.



4) Pour plate method

- Agar medium is melted and mixed with diluted samples before plating.
- In agar plates, the main purpose is to provide a thorough distribution of bacteria throughout the medium by diluting the inoculum in successive tubes of liquefied agar.
- Mixed cultures of bacteria are directly diluted in liquid agar tubes at 42 - 45 deg * C in the presence of melted agar medium.
- The melted medium is thoroughly mixed with the bacteria.
- Incubate the tubes' contents separately in different Petri plates after they have solidified.
- The development of bacterial colonies is characterized by both subsurface and surface colonies forming within the agar medium.
- A loop is then used to pick up the isolated colonies and streak them onto another Petri plate so they will remain pure.



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Microbial Assay

Microbial assays or microbiological assays could be a sort of bioassays designed to analyse the compounds or substances that have impact on micro-organisms. They help to estimate concentration and efficiency of antibiotics. Also facilitate in determination of the simplest anti-biotic appropriate for patient recovery

a) Antibiotic

The microbiological assay of an antibiotic is based upon a comparison of the inhibition of growth of micro-organisms by measured concentrations of the antibiotics under examination with that produced by known concentrations of a standard preparation of the antibiotic having a known activity.An antibiotic can be determined by chromatographic methods or microbiologically. The activity of an antibiotic is estimated by comparing the inhibition of the growth of sensitive microorganisms caused by known concentrations of a reference substance and the antibiotic to be tested, respectively.

b) Vitamin B12

THE first indication that vitamin B1 was a growth factor for micro- organisms was provided by the work of Shorb, who showed that it was essential for the growth of Lactobacillus lactis Dorner and that the amount of growth was proportional to the amount of vitamin B, present. Shorb developed a turbidimetric method of assay, but this has since been found to give somewhat unsatisfactory results; in the first place the organism is rather unstable, secondly, it will apparently grow without vitamin B under anaerobic or reducing conditions,3,4 and, thirdly, thymidine and other deoxyribosides also stimulate the growth of the organism, making it essential to separate these from vitamin B12 prior to carrying out the assay.

Cuthbertson, Pegler and Lloyds obtained more satisfactory results with L. lactis Dorner by using the cup-plate method of Bacharach and Cuth- bertson. In this method petri dishes containing a solid medium seeded with the test organism are used, holes are cut into the agar and filled with dilutions of the solution to be tested and the plates are then incubated.

Test on disinfectant:

The process of establishing documented evidence that a disinfectant will consistently remove or inactivate known or possible pathogens from samples. This complex test is done to determine the effectiveness of disinfectants.

Different methods for Testing of Disinfectants

There are present different tests which can be used to test the efficiency of disinfectants such as;

1)Phenol coefficient test

2)Carrier tests

3)Capacity test

4)In-use test

Objectionable microorganisms:

Microorganisms can be recovered from the environment, utility systems, raw materials, components, manufacturing equipment, in-process testing, or in the final product. An objectionable microorganism can be defined as a microorganism that can cause illness in the patient or that can adversely affect a nonsterile product.2 This includes the potential to degrade a product's stability

Sterility testing:

Sterility testing is required to ensure viable contaminating microorganisms are not evident in a product. This testing is conducted by direct inoculation or membrane filtration methods and can be performed in an isolator or cleanroom environment.

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Instruments:

a)Autoclave:

An autoclave is a machine that provides a physical method of sterilization by killing bacteria, viruses, and even spores present in the material put inside of the vessel using steam under pressure. Autoclave sterilizes the materials by heating them up to a particular temperature for a specific period of time. The autoclave is also called a steam sterilizer that is commonly used in healthcare facilities and industries for various purposes. The autoclave is considered a more effective method of sterilization as it is based on moist heat sterilization

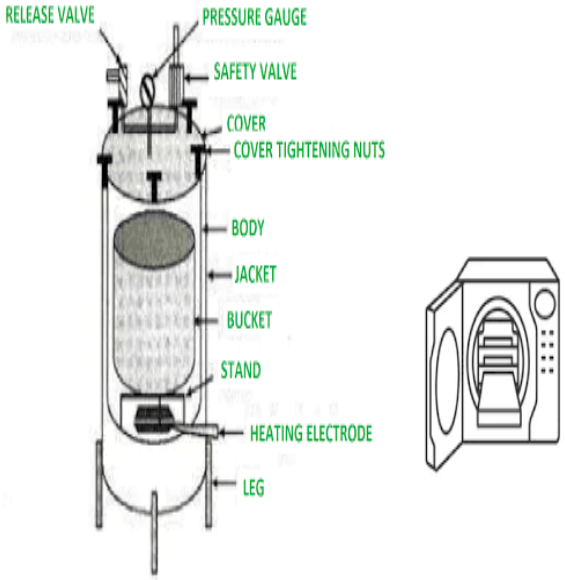
Use of autoclave:

1)They are used to decontaminate specific biological waste and sterilize media, instruments, and labware.

2)Regulated medical waste that might contain bacteria, viruses, and other biological materials is recommended to be inactivated by autoclaving before disposal.

3)In medical labs, autoclaves are used to sterilize medical equipment, glassware, surgical equipment, and medical wastes.

4)Similarly, autoclaves are used for the sterilization of culture media, autoclavable containers, plastic tubes, and pipette tips..



b) Hot air oven:

- A hot air oven is a laboratory instrument that uses dry heat to sterilize laboratory equipment and other materials.
- That equipment cannot be wet or material that will not melt, catch fire, or change form when exposed to high temperatures are sterilized by using the dry heat sterilization method.
- Hot air oven also known as forced air circulating oven.
- Some examples of material which can not be sterilized by employing a hot air oven such as surgical dressings, rubber items, or plastic material.
- We can sterilize Glassware (like petri dishes, flasks, pipettes, and test tubes), Powder (like starch, zinc oxide, and sulfadiazine), Materials that contain oils, Metal equipment (like scalpels, scissors, and blades) by using hot air oven.
- To destroy microorganisms and bacterial spores, a hot air oven provides extremely high temperatures over several hours.
- The widely used temperature-time relationship in hot air ovens to destroy microorganisms are 170 degrees Celsius for 30 minutes, 160 degrees Celsius for 60 minutes, and 150 degrees Celsius for 150 minutes.

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
Volume 3, Issue 2, January 2023
Impact Factor: 7.301

- Most of the medical industries use hot air ovens to sterilize laboratory instruments and material due to its simple standard operating procedure and low price. It also provides quick-drying processes.
- The process of dry heat sterilization using a hot air oven Originally developed by Louis Pasteur.
- The temperature range of a hot air oven is 50 to 300 ° C. It can be controlled by using a temperature regulator.
- The forced air circulation provided by the oven ensures the temperature uniformity throughout the oven.
- In a hot air oven first, the surface of the material is sterilized then the temperature slowly enters the center of the item.

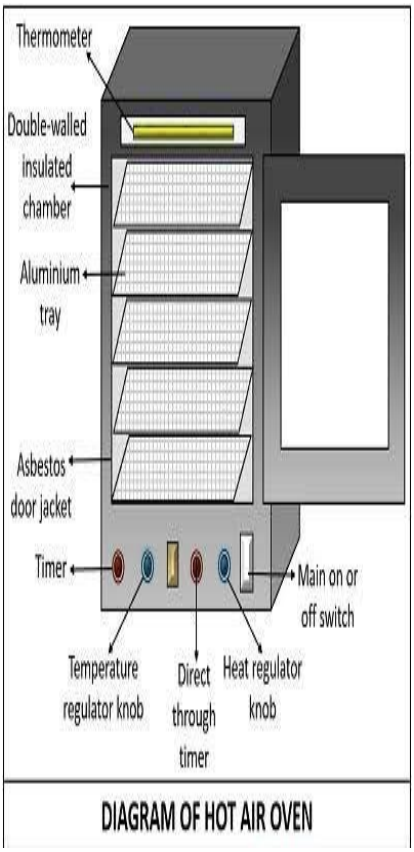


DIAGRAM OF HOT AIR OVEN

C) Laminar Air Flow Cabinet

1. A Laminar flow hood/cabinet is an enclosed workstation that is used to create a contamination-free work environment through filters to capture all the particles entering the cabinet.
2. These cabinets are designed to protect the work from the environment and are most useful for the aseptic distribution of specific media and plate pouring.
3. Laminar flow cabinets are similar to biosafety cabinets with the only difference being that in laminar flow cabinets the effluent air is drawn into the face of the user.
4. In a biosafety cabinet, both the sample and user are protected while in the laminar flow cabinet, only the sample is protected and not the user

IJARSCT
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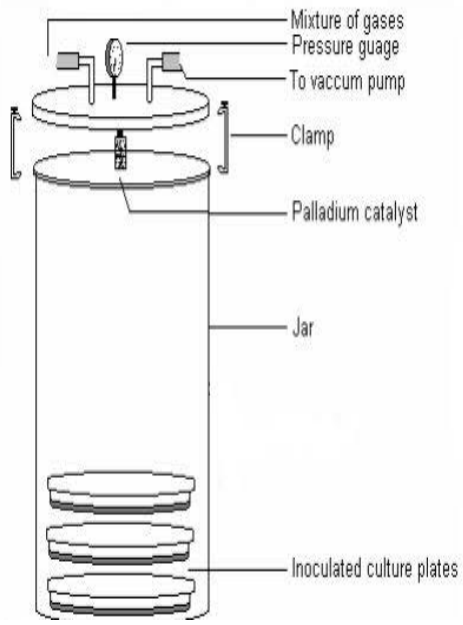
d) Anaerobic jar:

Anaerobic jar is an instrument used in microbiology laboratories to generate anaerobic conditions (anaerobiosis) to culture obligates anaerobes such as Clostridium spp.

Anaerobiosis obtained by McIntosh and Fildes' anaerobic jar is one of the excellent and most widely used methods for anaerobiosis, but it requires costly special apparatus and a vacuum pump. The availability of gas supply is another major drawback of this system. Currently, it is being replaced by a more convenient GasPak system.



IJARSCT
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