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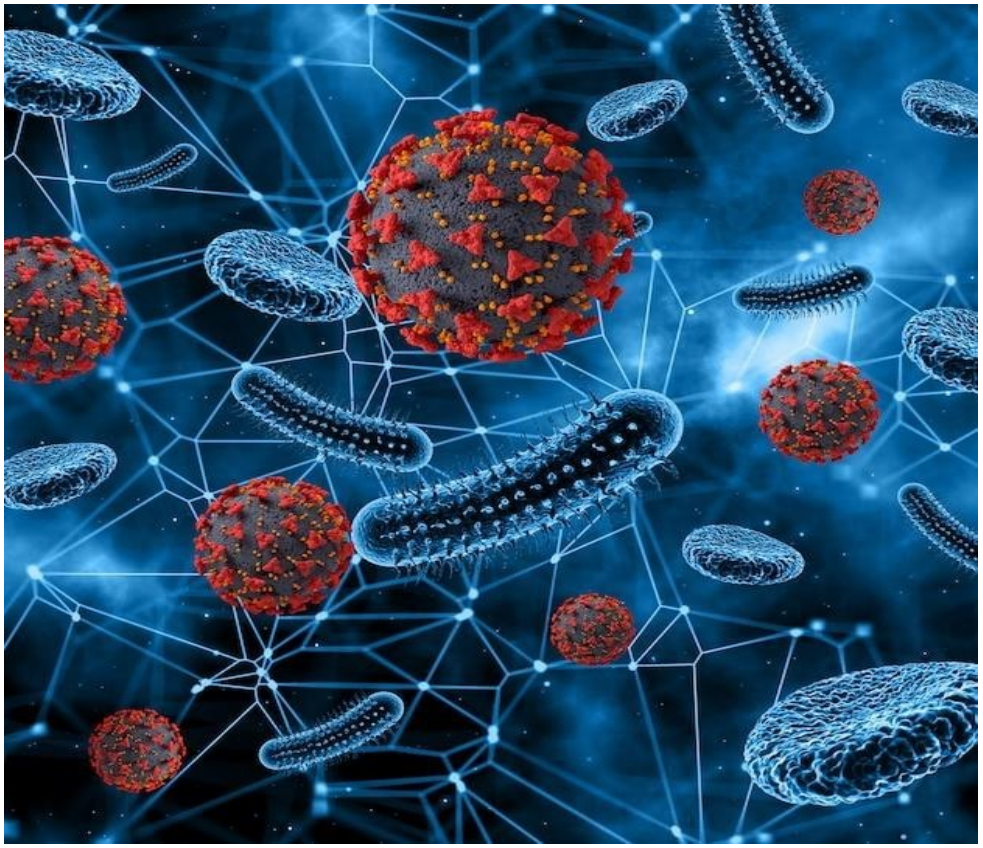
Laboratory Manual

Course Name: Fundamentals of Food Microbiology

Course code: CBM 211



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Objective 1: Introduction to Microbiology Laboratory

Setting up a basic microbiology laboratory requires careful planning and adherence to safety protocols. Below is a simplified outline of the essential components and equipment needed for a basic microbiology laboratory:

1. Laboratory Design and Safety:

a. Space Planning:

- Designate areas for different activities, including sample preparation, culturing, and analysis.

b. Safety Measures:

- Implement standard safety protocols, including the use of personal protective equipment (PPE), proper waste disposal, and emergency procedures.

2. Basic Equipment:

a. Microscopes:- Brightfield microscopes for observing bacterial and fungal cultures.
b. Incubators: - For maintaining a controlled temperature for microbial growth.
c. Autoclave: - To sterilize equipment, media, and glassware.
d. Bunsen Burners:- For aseptic techniques and flame sterilization.
e. Centrifuge:- To separate components of microbial samples.

3. Glassware and Consumables:

a. Petri Dishes:- For culturing microorganisms on solid media.
b. Test Tubes and Flasks: For liquid culture and sample storage.
c. Pipettes and Pipettors: For accurate measurement and transfer of liquids.
d. Microbiology Loop or Wire:- For streaking bacterial cultures.
e. Cotton Plugs and Corks:- For closing tubes and flasks.

4. Media and Solutions:

a. Agar Plates: - Nutrient agar for general microbial cultivation.
b. Broths and Agar Slants:- For liquid and semi-solid microbial cultures.
c. Buffer Solutions:- pH buffers for adjusting and maintaining culture conditions.
d. Sterile Water:- For preparing solutions and dilutions.

5. Staining and Microscopic Analysis:

a. Stains (Gram Stain, etc.):- For microscopic examination of microbial morphology.
b. Slides and Cover Slips:- For preparing microscope slides.

6. Safety Equipment:

a. Gloves, Lab Coats, and Safety Goggles:- Personal protective equipment for laboratory personnel.

b. Biological Safety Cabinet (BSC):- Provides a sterile working environment for handling potentially hazardous microorganisms.

7. Microbial Strains and Cultures:

a. Bacterial and Fungal Cultures:- Obtain reference strains or cultures for experiments.
b. Stock Cultures:- Maintain a collection of well-characterized microbial strains.

8. Documentation and Record-Keeping:

a. Laboratory Notebooks: For recording experimental procedures, results, and observations.

9. Basic Lab Furniture:

a. Lab Benches and Tables: - For setting up equipment and conducting experiments.
b. Storage Cabinets: - For storing chemicals, glassware, and equipment.

10. Basic Laboratory Techniques:

a. Aseptic Techniques: - Proper methods to prevent contamination during experiments.
b. Culturing Techniques: - Sterile streaking, plating, and inoculation.

11. Waste Management:

a. Biohazard Bins:- For the safe disposal of biohazardous waste.
b. Sharps Containers: - For safe disposal of used needles and other sharp objects.

Objective 2: Aseptic and Sterilization Techniques

Sterilization is the process of eliminating or destroying all forms of microbial life, including bacteria, viruses, fungi, and spores, as well as their dormant forms. The primary purpose of sterilization is to create a sterile environment, free from any living microorganisms that could cause contamination, infection, or spoilage. Sterilization is critical in various fields, including healthcare, laboratory research, food preparation, and manufacturing processes.

There are several methods for achieving sterilization, each with its own set of advantages and disadvantages. Some common sterilization methods include:

Autoclaving: Autoclaving is a widely used method of sterilization that employs high pressure and saturated steam to effectively kill or inactivate microorganisms, including bacteria, viruses, fungi, and spores. The process of autoclaving is essential in various fields, such as healthcare, microbiology, research laboratories, and the pharmaceutical industry, to ensure that equipment and materials are free from harmful contaminants. The autoclave is heated to the desired sterilization temperature, usually around 121°C (250°F) or higher. The temperature may vary depending on the specific requirements of the items being sterilized. As the temperature increases, pressure builds up within the autoclave, creating a controlled environment of high temperature and high pressure. The elevated pressure raises the boiling point of water, allowing it to reach temperatures sufficient for sterilization. The items in the autoclave chamber are exposed to these elevated temperatures and pressures for a specified period, usually ranging from 15 to 20 minutes. This exposure kills or inactivates any microorganisms present. After the sterilization cycle is completed, the autoclave is gradually depressurized and cooled to a safe temperature.

Autoclaving is highly effective for sterilization and is widely used because it is capable of penetrating porous materials and is relatively simple to operate. However, it may not be suitable for heat-sensitive items or materials that can be damaged by moisture or high temperatures. In such cases, alternative sterilization methods like chemical sterilization or radiation sterilization may be employed.

Chemical Sterilization:

The various chemicals are used for sterilization to ensure that the growth medium, equipment, and media are free from contaminants. The choice of sterilization chemicals depends on the specific materials and the level of sterilization required. Common sterilization chemicals used in microbiology include:

1. **Ethanol (Alcohol):** Ethanol is used for the surface sterilization of plant materials and equipment. It is especially effective for disinfecting tools and instruments used in **Microbial Culture**.

2. **Hydrogen Peroxide (H2O2):** Hydrogen peroxide is sometimes used for surface sterilization and can also be added to the culture medium to prevent contamination. It helps eliminate fungal from the bacterial culture.

Radiation Sterilization: Radiation sterilization is a method used to sterilize equipment, other items by exposing them to ionizing radiation, typically gamma rays or high-energy electron beams. This process is highly effective at destroying the microorganisms, making it a reliable method for achieving sterility. Here are the key points about radiation sterilization:

Types of Ionizing Radiation:

1. **Gamma Radiation**: Gamma radiation is produced from the decay of radioactive isotopes, most commonly cobalt-60 (^60Co) or cesium-137 (^137Cs). It is widely used for radiation sterilization because of its high penetrating power and consistent dose delivery.
2. **Electron Beam (E-beam) Radiation**: Electron beam radiation uses a stream of high-energy electrons generated by an electron accelerator. E-beams are often used in situations where the radioactive materials associated with gamma radiation are undesirable.

Filtration: Filtration and sterilization are critical processes in microbiology to ensure that the growth medium and equipment are free from contaminants, which can negatively affect the health and development of other microbiology. In Microbiology, the growth medium used for cultures needs to be free from particulate matter and contaminants. Filtration can also be used to sterilize heat-

sensitive materials, such as some vitamins and organic additives commonly used in culture. This is achieved through the use of sterile filtration. Nylon membranes with appropriate pore sizes can be used for the isolation and culturing of microorganisms from culture. This is important for research and diagnostics. The liquid medium is passed through a sterile membrane filter with specific pore sizes (0.25 to 0.45 micrometer). The choice of the specific nylon membrane pore size and its application in microbial culture will depend on the requirements of the experiment or procedure. Nylon membranes are known for their strength, durability, and chemical resistance, making them suitable for a range of applications in culture and other laboratory settings.

Flame Sterilization: In microbial culture, instruments and equipment such as scalpels and forceps are often flame-sterilized before use. This process involves passing the metal parts of the tools through a flame to kill any potential contaminants. Proper aseptic techniques are crucial in maintaining sterility throughout the culture process.

Laminar Flow Hood: A laminar flow hood, also known as a laminar flow cabinet or clean bench, is a piece of laboratory equipment designed to provide a controlled and sterile environment for various

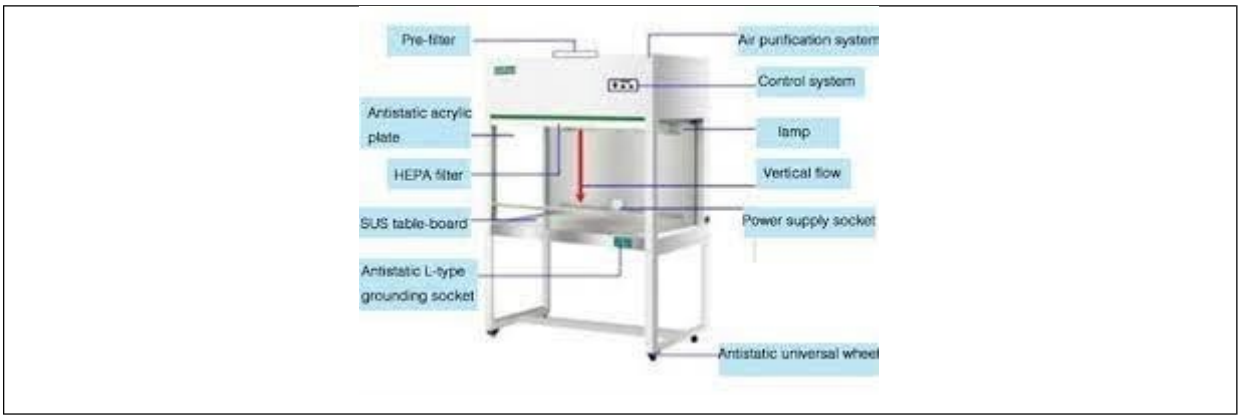
applications, including microbial culture. It is an essential tool for maintaining aseptic conditions during experiments and procedures.

Airflow: Laminar flow hoods create a unidirectional, sterile airflow that moves from the back to the front of the hood, minimizing the risk of airborne contaminants entering the workspace.

HEPA or ULPA Filters: These hoods are equipped with high-efficiency particulate air (HEPA) or ultra-low penetration air (ULPA) filters that remove particulates and microorganisms from the incoming air. HEPA filters remove particles down to 0.3 micrometers in size, while ULPA filters are more efficient and capture particles down to 0.12 micrometers.

Work Surface: The work surface within the hood is typically made of stainless steel or other easy-to-clean materials. It is designed to be free from obstructions and contaminants, providing a clean area for various tasks.

UV Lamp: Some laminar flow hoods have built-in ultraviolet (UV) lamps for periodic sterilization of the work surface. UV light helps inactivating microorganisms on exposed surfaces.



Uses of a Laminar Flow Hood in Microbial Culture:

Aseptic Techniques: Laminar flow hoods are crucial for maintaining aseptic conditions during **Microbial Culture** procedures. They create a sterile environment that minimizes the risk of contamination, ensuring that **Microbial Culture** remain free from unwanted microorganisms.

Media Preparation: Microbial Culture media often require precise preparation. A laminar flow hood can be used to weigh and mix media components, ensuring sterility and accuracy in media formulation.

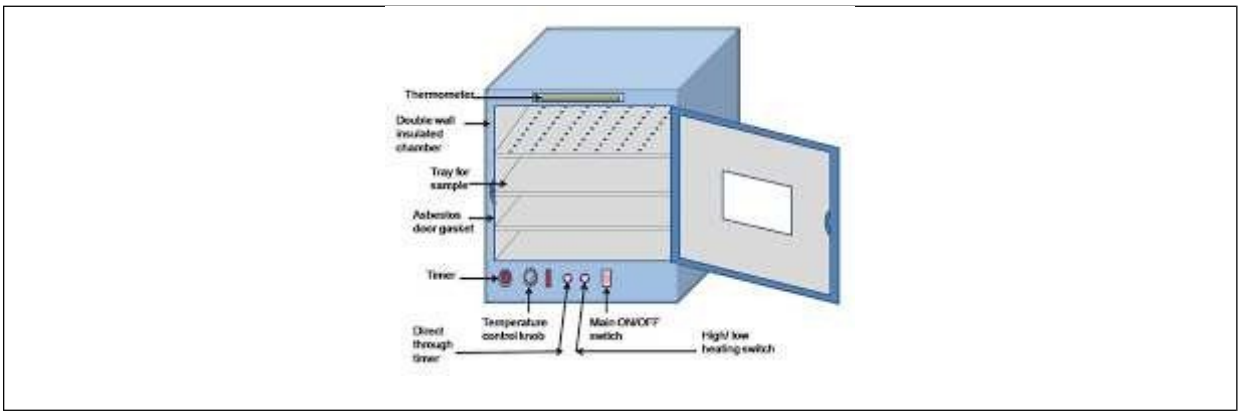
Subculture and Transfer: The hood allows for the subculture and transfer of microbes from one vessel to another, such as from one culture dish to another, without introducing contaminants.

Sterile Instrument Handling: Laminar flow hoods are used to store and handle sterile instruments, such as scalpels, forceps, and other tools used in **Microbial Culture**.

Laminar flow hoods are essential in **Microbial Culture** and other life science applications where maintaining a sterile environment is critical. They help ensure the success of experiments, minimize contamination risks, and promote the growth and propagation of healthy culture. Proper care and regular maintenance are necessary to keep the hood's airflow and sterility within the required specifications.

Glove Box: In some specialized **Microbial Culture** applications, a glove box with a controlled environment (usually nitrogen gas) is used to maintain sterility and prevent contamination during the handling of sensitive **Microbial Culture**.

Dry Heat Sterilization: Dry heat sterilization is a method of sterilizing equipment and materials by exposing them to high temperatures in the absence of moisture. It is often used in **Microbial Culture** to sterilize heat-resistant items, such as glassware, metal instruments, and some types of growth media or substrates. A hot air oven, also known as a dry heat sterilizer, is a piece of laboratory equipment used for sterilizing various items, including glassware and metal tools, by subjecting them to high temperatures in a dry environment. It is widely used in laboratories, healthcare settings, and **Microbial Culture** culture facilities to eliminate microorganisms and achieve sterility.



The choice of sterilization method depends on the materials or equipment being sterilized, their heat sensitivity, and the specific requirements of the application. Proper sterilization procedures are crucial in various industries to ensure the safety and quality of **Microbial Culture** procedure.

Objective 3: Luria Bertani Media preparation protocol

Introduction

In order for bacteria to be successfully cultured, they must be grown in the appropriate media. LB, also known as Lysogeny broth, is a nutrient rich broth that is a standard for culturing Escherichia coli, as it allows for quick growth and high yields. Therefore, the proper preparation of LB will be crucial to maintaining our bacterial stock throughout the summer. Furthermore, addition of agar to LB broth creates a gel for bacteria to grow upon, and is therefore used for plating bacterial cultures on petri dishes.

Materials

Reagents Note: This protocol makes 500mL of broth or ~25 plates.

- 5 Bacto-tryptone
- 2.5g yeast extract
- 5g NaCl
- 7.5g agar (Only necessary if making LB agar plates)
- 500mL of dH2O (distilled water)

Equipment

- 1L Pyrex bottle
- 1L graduated cylinder
- Filter paper and scoopula
- Stack of sterile plates (this protocol makes approximately 25)
- Bunsen burner/ethanol burner
- 70% EtOH wash bottle
- Paper towels/wipes

Procedure

Part 1: Making the LB broth

This part can be carried out at a regular lab bench.

1. Obtain a clean 1L pyrex bottle
2. Obtain a graduated cylinder with 500mL of dH2O and add to the bottle. Record the amount added.
3. Using filter paper, separately measure out 5g of NaCl, 5g of Tryptone, and 2.5g of yeast extract
   on a scale and add them to the bottle. Swirl the bottle in a circular motion to mix. Remember to
   re-calibrate your scales in between measurements.
4. If you are making LB agar plates, weigh and add 7.5g of agar and swirl to mix.
5. Record theamount added.

Note the contents do not necessarily need to be completely in solution before autoclaving.

Part 2: Autoclaving

1. Lightly seal the top of the beaker with aluminium foil, and label the beaker with autoclave tape
   stating LB (agar)–[your name]–[date]–[media number].
2. Use appropriate transportation protocols to bring the LB bottle into the autoclave room. (Remember to store the beaker in an autoclavable basin, in case of spills).

3. Check the water level on the autoclave, if necessary. Autoclave on the liquid setting for approximately 20 min.
4. The contents of the beaker will be hot after autoclaving, therefore take the necessary measures to prevent burns.
5. After autoclaving, allow the LB media to cool to 55°C before handling.
6. Use laser thermometer to check the temperature of the glass.
7. The LB broth can be stored in sterile conditions at room temperature, and should be good for 3-4 months.
8. Flame the lip of the bottle each time the LB is used. If the LB contains antibiotics, store in a -4°C freezer.
9. However, it is not recommended to store LB with antibiotics as the antibiotics will degrade over time

Part 3: Pouring the plates (for LB agar)

While pouring the plates, it is crucial to maintain a sterile environment. This should be done with a sterile environment provided by a lit Bunsen burner.

Note: steps 1-3, in addition to the clean up from Part 1, can be done while waiting for autoclave.

1. Sterilize the workspace with 70% EtOH before depositing your materials. Light the Bunsen burner.

2. Obtain a stack/roll of empty plates. The plates should still be in their plastic sleeve/wrapping, as they should be sterile. Don't throw out the wrapping as it can be used to store the plates. It is essential that you minimize any chance of contaminating the plates. Make sure that you open the package at the top and expose the plates as minimally as possible.

3. Once you take the plates out, store them upside down on your lab bench. Label the plates with [your name]—[date]–[media number]–[antibiotic]. Once labelled, you may stack the plates to free up workspace.

4. Allow the LB media to cool before pouring. The LB will start to settle at ~30°C.

5. If you are preparing selective media, add antibiotic to the mixture. Swirl the flask in a circular motion to mix. If you don't know whether or not you are preparing selective media.

- Use concentrated liquid stocks for the antibiotics.

6. Recommended antibiotic concentrations:
   - Chloramphenicol (CAM): 25µg/mL
   - Ampicillin (AMP): 100µg/mL
7. Take an empty plate and open it slightly. You do not need to open it all the way to pour the agar.

8. Pour agar until 2/3 of the plate has been covered, or approximately half of the plate has been filled when viewed from the side. Pour the agar slowly to prevent the formation of bubbles. Swirl the plate in a circular motion to distribute the media evenly on the plate.

9. If you pour too much LB, you will not be able to produce 25 plates. If you don't pour enough

media, it may minimize bacterial growth.

10. After pouring, set the plates to cool in stacks of 4-5 to save space and flip the plates to prevent condensation forming on the agar. Don't stack plates too high - we want to minimize the risk of spills. Allow the plates to cool for at least 20 minutes until the agar has solidified.

11. Rinse the Pyrex bottle with water before the remnants solidify and become hard to remove.

12. The plates can then be stacked and stored in plastic bags (ideally, reuse the plastic bags that the plates came in.
13. Store LB agar plates in a 4°C freezer. They should be good for 1-2 months.

Observation: Observation of Culture

Date of Media Preparation	**Date of Culture**	**Observation recorded**

Acknowledgements
● Addgene: Protocol – Making LB Agar Plates for Bacteria. From: https://www.addgene.org/plasmid-protocols/bacterial-plates
● Department of Ecology and Evolutionary Biology, UCLA. Making LB Agar Plates. From: https://www.eeb.ucla.edu/Faculty/Barber/Web Protocols/LB Agar Plates.pdf
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● Sezonov G, Joseleau-Petit D, D'Ari R. Escherichia coli Physiology in Luria-Bertani Broth. Journal of Bacteriology. 2007;189(23):8746-8749. doi:10.1128/JB.01368-07

Objective 4: Gram Staining of Microorganisms

Bacterial species are often distinguished from one another on the basis of Gram' reaction.
In this process:

- A bacterial smear is heat fixed on glass slide, stained with crystal violet (30 sec), rinsed gently and treated with iodine solution and finally rinsed with ethanol.
- When the bacteria retain the crystal violet stain after rinsing, the bacteria are called gram positive; and those which do not retain the stain are called gram negative.
- The later are then counter stained with pink colour safranin.
- The ability of bacteria to retain crystal violet stain or not, depends upon fundamental structure of cell wall.

In gram positive bacteria, the basal wall is made up of thick layer of peptidoglycan consisting of chain of alternating N-acetyl muramic acid (NAM) and N-acetyl glucosamine (NAG) units cross linked by tetrapeptide and pentaglycine units.

In gram negative bacteria, the thin basal peptidoglycan layer is covered by a layer mostly composed of lipo-polysaccharides and the cell wall also contains lipoproteins.

Gram staining is a fundamental technique in microbiology used to differentiate bacteria into two groups based on the characteristics of their cell walls: Gram-positive and Gram-negative.

Materials Needed:

- Bacterial culture
- Microscope slides
- Bunsen burner
- Inoculating loop
- Crystal violet (primary stain)
- Gram's iodine (mordant)
- Ethanol or acetone (decolorizing agent)
- Safranin (counterstain)
- Water
- Microscope

Procedure:

- Preparation of Smear:
- a. Place a small drop of water on a clean microscope slide.
- b. Aseptically transfer a small amount of bacterial culture to the water drop using a sterile inoculating loop.
- c. Spread the bacterial culture over the slide to create a thin, even smear.
- d. Allow the smear to air-dry completely.

Heat Fixation:

a. Pass the smear through the flame of a Bunsen burner several times to heat-fix the bacteria. This helps the cells adhere to the slide.

Staining:

a. Flood the smear with crystal violet (primary stain) and let it stand for 1 minute.
b. Rinse the slide with water to remove excess crystal violet.
c. Flood the smear with Gram's iodine (mordant) and let it stand for 1 minute.
d. Rinse the slide with water to remove excess iodine.
e. Decolorize the smear by quickly flooding it with ethanol or acetone. Decolorize until no more color runs off (usually 5-10 seconds). Be careful not to over-decolorize, as this can result in false interpretations.
f. Rinse the slide immediately with water.
g. Counterstain the smear with safranin for 1-2 minutes.
h. Rinse the slide with water.

Microscopic Examination:

a. Blot the slide gently with bibulous paper to remove excess water.
b. Allow the smear to air-dry.
c. Examine the slide under the microscope using the oil immersion lens.

Interpretation:

Gram-Positive Bacteria: Retain the crystal violet-iodine complex and appear purple/blue.
Gram-Negative Bacteria: Lose the crystal violet-iodine complex during decolorization and take up the safranin counterstain, appearing red/pink.

Notes:

- The success of Gram staining depends on the age and thickness of the bacterial smear, proper heat-fixation, and precise timing during staining steps.
- Gram staining results are crucial for preliminary bacterial classification and can aid in selecting appropriate antibiotics.
- Ensure that all materials used are sterile to prevent contamination.
- It's essential to follow laboratory safety protocols and use appropriate personal protective equipment.
- This procedure provides a basic overview of the Gram staining technique. However, specific variations may exist depending on the bacterial species and the laboratory's standard operating procedures. Always refer to the specific guidelines provided by your laboratory or instructor.
- **Observation: Observation of Culture**

Date of Media Preparation	**Date of Culture**	**Observation recorded**

Objective 5: Fungus Culture on PDA

Culturing fungi on Potato Dextrose Agar (PDA) is a common and widely used method in mycology for growing and maintaining fungal cultures. Here's a step-by-step procedure for culturing a fungus on PDA:

Materials Needed:

- Potato Dextrose Agar (PDA) plates
- Fungal spore or mycelial sample
- Sterile loop or inoculating needle
- Sterile petri dishes
- Parafilm or laboratory film
- Bunsen burner or alcohol lamp
- Incubator set to appropriate temperature for the fungus

Procedure:

- Preparation of PDA Plates:

  a. Prepare PDA according to the manufacturer's instructions.
  b. Pour the molten PDA into sterile petri dishes to solidify, creating PDA plates.

- **Fungal Inoculation:**

  a. Use a sterile loop or inoculating needle to transfer a small amount of fungal spores or mycelium from the original culture to the center of the PDA plate.
  b. Spread the spores or mycelium evenly on the surface of the agar, using a back-and-forth streaking motion.

- **Incubation:**

  a. Seal the PDA plate with Parafilm or laboratory film to prevent contamination.
  b. Incubate the plate at the appropriate temperature for the growth of the fungus. Incubation temperatures vary depending on the fungal species.

Observation and Subculturing:

a. Check the PDA plate regularly for fungal growth.
b. Once the fungus has grown sufficiently, observe its characteristics, such as colony morphology, color, and texture.

Subculturing (Optional):

a. If you want to maintain the culture or conduct further experiments, transfer a small piece of the growing mycelium to a new PDA plate using a sterile loop or needle.
b. Repeat the process for continuous subculturing.

Notes: **Sterilization: Ensure all equipment and materials are sterilized to prevent contamination. Flame the loop or needle using a Bunsen burner or alcohol lamp before and after each use.**
Aseptic Technique: Practice aseptic technique to avoid introducing contaminants during inoculation and subculturing.

Incubation Conditions: Incubate the cultures in an incubator set to the optimal temperature for the fungal species. Incubation times can vary, ranging from a few days to several weeks.
This procedure provides a general guideline for culturing fungi on PDA. It's important to adjust the steps based on the specific requirements of the fungus you are working with. Always refer to literature specific to the fungal species and follow the guidelines provided by your laboratory or institution.

Observation: Observation of Culture

Date of Media Preparation	**Date of Culture**	**Observation recorded**

Objective 6: Purification of microorganisms by serial dilution Maintenance of microorganisms

Purification of microorganisms, often achieved through serial dilution, is a common practice in microbiology. Maintaining these microorganisms involves subculturing and storing them under appropriate conditions. Below is a step-by-step guide for the purification of microorganisms by serial dilution and their subsequent maintenance:

Purification by Serial Dilution:

Initial Culture:

a. Start with the original microbial culture that contains a mixture of microorganisms.
b. Ensure the initial culture is well-mixed to represent the diversity of microorganisms present.

Serial Dilution:

a. Prepare a series of dilutions by transferring a small volume of the original culture (e.g., 1 mL) to a larger volume of sterile diluent (e.g., saline or broth).
b. Mix thoroughly after each dilution to achieve a uniform distribution of microorganisms.
c. Plate a small aliquot from each dilution onto solid agar plates (e.g., agar plates with a suitable growth medium).

Isolation of Colonies:

a. Incubate the agar plates at an appropriate temperature for the growth of the microorganisms.
b. After incubation, observe the plates and select individual colonies that appear distinct from each other.

Single Colony Isolation:

a. Using a sterile loop or needle, streak a single colony onto a new agar plate to obtain a pure culture.
b. Repeat this process until pure colonies are obtained.

Maintenance of Microorganisms:

Subculturing:
a. Regularly subculture microorganisms to maintain their viability and purity.
b. Choose an appropriate growth medium and incubation conditions based on the requirements of the microorganisms.

Storage:

a. For short-term storage, maintain cultures on agar slants or in agar stabs at a refrigerated temperature (4°C).
b. For long-term storage, consider cryopreservation techniques such as freezing cultures in glycerol or other cryoprotective agents at ultra-low temperatures (-80°C) or in liquid nitrogen.

Documentation:

a. Keep detailed records of the microorganisms, including the strain or species, source, date of isolation, and any relevant characteristics.
b. Maintain a log of subculturing and storage activities.

Quality Control:

a. Periodically perform quality control checks on the stored cultures, such as purity and viability assessments.
b. Replace older cultures with fresh isolates as needed to prevent genetic drift or loss of viability.

Safety Measures:

a. Adhere to safety protocols when handling microorganisms, including the use of appropriate personal protective equipment (PPE).
b. Follow institutional guidelines for handling and storing microorganisms.

Remember to tailor these procedures based on the specific requirements of the microorganisms you are working with and follow the safety guidelines established by your laboratory or institution. Regularly updating and maintaining a well-organized culture collection ensures the availability of pure and viable microorganisms for research and experimentation.

Observation: Observation of Culture

Date of Media Preparation	**Date of Culture**	**Observation recorded**

Objective 7: Detecting faecal coliform

Detecting faecal coliforms is crucial for assessing water quality and identifying potential contamination with faecal matter. Faecal coliforms are a subgroup of coliform bacteria that are commonly found in the intestines of warm-blooded animals, and their presence in water is indicative of faecal contamination. The most widely used method for detecting faecal coliforms is the membrane filtration technique combined with selective media. Here's a step-by-step guide:

Membrane Filtration Technique for Faecal Coliform Detection:
Materials Needed:

- Water samples
- Sterile filtration apparatus
- Membrane filters (47 mm, 0.45 µm pore size)
- Sterile forceps
- Incubator
- Coliform-selective agar (e.g., mFC agar, mEndo agar)
- Petri dishes
- Pipettes and pipette tips
- Sterile water or phosphate-buffered saline (PBS)
- Sterile gloves
- Bunsen burner or alcohol lamp
- pH meter
- Calibrated thermometer
- Procedure:

Sample Collection:

a. Collect water samples in sterile containers.
b. Note the source, date, time, and any relevant information.

Preparation of Membrane Filtration Apparatus:

a. Sterilize the filtration apparatus and membrane filters.
b. Assemble the filtration apparatus.

Filtration:

a. Filter a known volume of water through a sterile membrane filter (typically 100 mL).
b. Use a vacuum pump or other suitable equipment to aid filtration.

Transfer of Membrane Filter:

a. Use sterile forceps to carefully transfer the membrane filter to a selective agar plate.
b. Ensure the filter is placed on the agar surface without trapping air bubbles.

Incubation:

a. Incubate the agar plates at a specified temperature (usually 35-37°C) for 24 hours.
b. For mFC agar, incubate in a dark environment.

Enumeration:

a. After incubation, count the colonies that exhibit typical fecal coliform characteristics.

b. Use a colony counter or a grid on the agar plate.

Calculation:

a. Calculate the fecal coliform concentration based on the number of colonies and the volume of water filtered.

Confirmation:

a. For regulatory purposes, confirm the identity of the isolates by performing confirmatory tests or subculturing onto suitable media.

Quality Control:

a. Run parallel samples with known concentrations of fecal coliforms as positive controls.

b. Monitor pH and temperature during the procedure.

Record Keeping:

a. Document all relevant information, including sample details, filtration volumes, incubation conditions, and colony counts.

Notes:

- Always follow laboratory safety protocols when handling potentially contaminated water samples.
- Regularly calibrate equipment such as pH meters and thermometers.
- Adapt the method to comply with regulatory requirements in your region.
- This method is effective for detecting fecal coliforms in water samples and is commonly used for environmental monitoring and water quality assessment.

- **Observation: Observation of Culture**
-

Date of Media Preparation	**Date of Culture**	**Observation recorded**

Objective 8: MPN method for enumeration of Microorganism

The Most Probable Number (MPN) method is a statistical method used for estimating the concentration of viable microorganisms in a sample, particularly in cases where direct enumeration is challenging. The MPN method is commonly employed in microbiology, especially for water quality testing and the detection of coliform bacteria. Here's a step-by-step guide on how to perform the MPN method:

Materials Needed:

- Multiple tubes or vessels with selective broth media (e.g., lactose broth for coliforms)
- Sterile pipettes or syringes
- Sample dilution blanks (sterile water or diluent)
- A sample of the material to be tested (e.g., water sample)

Incubator

Procedure:

Prepare Serial Dilutions:

a. Label a series of tubes or vessels with the appropriate dilution factor.
b. Add a known volume of the sample to the first tube (e.g., 10 mL).
c. Transfer a portion of the diluted sample to the next tube, and so on, creating a series of dilutions.

Inoculate the Dilutions:

a. Aseptically transfer a portion of each dilution into the corresponding tube with selective broth media.
b. Mix the contents of each tube thoroughly.

Incubate:

a. Incubate the tubes at the appropriate temperature for the specific microorganisms you are testing (e.g., 35-37°C for coliforms).
b. Incubate for a predetermined period (typically 24 hours).

Observe Growth:

a. After incubation, observe each tube for growth (e.g., gas production, color change) that indicates the presence of the target microorganism.
b. Tubes showing growth are considered positive for the microorganism.

MPN Calculation:

a. Use the MPN table or statistical software to determine the Most Probable Number based on the number of positive tubes at each dilution level.
b. The MPN is an estimate of the number of microorganisms per unit volume of the original sample.

Notes:

- The MPN table is a statistical tool that provides the most probable number of microorganisms based on the observed growth in the tubes at different dilutions.
- For statistical calculations, there are MPN calculators available online, or you can refer to standard statistical tables for MPN estimation.

- Quality control measures, such as positive and negative controls, should be included in each set of MPN tests.
- The MPN method is often used for testing water samples for the presence of coliform bacteria, which are indicative of fecal contamination.
- Performing the MPN method requires attention to detail and adherence to aseptic techniques. It provides a quantitative estimate of viable microorganisms in a sample and is particularly useful when direct enumeration is impractical.

Observation: Observation of Culture

Date of Media Preparation	**Date of Culture**	**Observation recorded**

Objective 9: Microbial examination of milk

Microbial examination of milk is essential for ensuring its safety and quality. It involves assessing the microbial content to detect any contamination that may affect the shelf life of the product or pose health risks. Here is a basic procedure for microbial examination of milk:

Materials Needed:

- Sterile containers for sample collection
- Sterile pipettes or syringes
- Petri dishes
- Agar media (e.g., Standard Plate Count Agar, MacConkey Agar)
- Incubator
- Sterile swabs or spreaders
- Distilled water
- Bunsen burner or alcohol lamp
- Microscope
- Staining reagents (optional, for differential staining)

Procedure:

Sample Collection:

a. Collect a representative sample of the milk in sterile containers.
b. Note the source, date, time, and any relevant information.

Serial Dilution:

a. Aseptically dilute the milk sample using a series of dilutions with sterile distilled water.
b. Transfer aliquots of each dilution onto agar plates using a sterile pipette or spreader.

Incubation:

a. Incubate the agar plates at the appropriate temperature for microbial growth (usually 35-37°C) for a specified period (typically 24-48 hours).

2. Enumeration of Total Viable Count (TVC):

- Perform serial dilutions of the milk sample using sterile diluent.
- Plate appropriate dilutions on Standard Plate Count Agar or similar media.
- Incubate the plates at an appropriate temperature (typically 30-37°C) for 48 hours.
- Count the colonies and calculate the TVC per milliliter of milk.

3. Enumeration of Coliforms:

- Perform serial dilutions of the milk sample.
- Plate the dilutions on selective media like MacConkey Agar.
- Incubate the plates at 35-37°C for 24-48 hours.
- Count characteristic colonies and calculate coliform counts**.**

4. Yeast and Mold Enumeration:

- Use Yeast and Mold Agar for the enumeration of yeast and molds.
- Incubate the plates at an appropriate temperature (usually 25°C) for 5-7 days.
- Count the colonies and report yeast and mold counts.

5. Microscopic Examination:

- Prepare a smear of the milk sample.
- Perform Gram staining for bacterial identification.
- Observe under a microscope for the presence of bacterial cells.

6. pH Measurement:

a. Measure the pH of the milk sample using a calibrated pH meter.

7. Incubation Conditions:

- Ensure proper incubation conditions for each type of microorganism, considering temperature and duration.

8. Record Keeping:

- Document all observations, counts, and measurements.
- Maintain a log of procedures followed and any deviations.

Notes:

- Strict aseptic techniques must be followed throughout the process to avoid contamination.
- Consider including positive and negative controls in the analysis.
- Interpret results based on established standards and regulations for microbial counts in milk.
- Rapid methods, such as ATP bioluminescence or molecular techniques, may also be employed for quick assessments.
- Regular microbial examination of milk is vital for ensuring its safety, quality, and adherence to regulatory standards. The specific methods used can vary based on the objectives of the examination and the microbial parameters of interest.

Observation: Observation of Culture

Date of Media Preparation	**Date of Culture**	**Observation recorded**

Objective 10: Microbial examination in fruits and vegetables

Microbial counts in fruits and vegetables are essential for assessing their safety and quality. The microbial content of these foods can be influenced by various factors, including the agricultural practices, handling, storage conditions, and processing methods. Here's a general guide on how to perform microbial counts in fruits and vegetables:

Materials Needed:

- Sterile containers for sample collection
- Sterile sampling tools (e.g., swabs, pipettes, or scoops)
- Diluents (e.g., sterile water or buffered peptone water)
- Agar plates with suitable culture media (e.g., Standard Plate Count Agar, Violet Red Bile Agar)
- Incubator
- Microscope
- Staining reagents (e.g., Gram stain for bacterial identification)
- Colony counter
- pH meter
- Blender or homogenizer (for processing solid samples)
- Bunsen burner or alcohol lamp

Procedure:

- Sample Collection:
    - Collect samples of fruits and vegetables aseptically.
    - Use sterile sampling tools to avoid contamination.

2. **Homogenization (for solid samples):**
    - Homogenize or blend solid samples to create a representative composite sample.
    - If necessary, dilute the homogenized sample with sterile diluent.

3. **Total Aerobic Plate Count (TAPC):**
    - Perform serial dilutions of the sample using sterile diluent.
    - Plate appropriate dilutions on Standard Plate Count Agar or similar media.
    - Incubate the plates at an appropriate temperature (typically 30-37°C) for 48 hours.
    - Count the colonies and calculate the TAPC per gram or milliliter of the sample.

4. **Coliform Enumeration:**
    - Perform serial dilutions of the sample.
    - Plate dilutions on selective media such as Violet Red Bile Agar.
    - Incubate the plates at 35-37°C for 24-48 hours.
    - Count characteristic colonies and calculate coliform counts.

5. **Yeast and Mold Enumeration:**
    - Use suitable media (e.g., Potato Dextrose Agar) for the enumeration of yeast and molds.
    - Incubate the plates at an appropriate temperature (usually 25°C) for 5-7 days.
    - Count the colonies and report yeast and mold counts.

6. **Microscopic Examination:**
   - Prepare a smear of the sample.
   - Perform Gram staining for bacterial identification.
   - Observe under a microscope for the presence of bacterial cells.

7. ***pH Measurement:***
   - Measure the pH of the sample using a calibrated pH meter.

8. **Incubation Conditions:**
   - Ensure proper incubation conditions for each type of microorganism, considering temperature and duration.

9. **Record Keeping:**
   - Document all observations, counts, and measurements.
   - Maintain a log of procedures followed and any deviations.

Notes:
- Strict aseptic techniques must be followed throughout the process to avoid contamination.
- Consider including positive and negative controls in the analysis.
- Interpret results based on established standards and regulations for microbial counts in fruits and vegetables.
- Microbial counts in fruits and vegetables are crucial for ensuring food safety and quality, especially for fresh produce that may be consumed without further processing. The specific methods used can vary based on the type of produce and the microbial parameters of interest.

Observation: Observation of Culture

Date of Media Preparation	**Date of Culture**	**Observation recorded**