JHARKHAND Rai University
RANCHI

Bachelor of Science in Medical Laboratory Technology (BMLT)

MICROBIOLOGY LAB. - I

Semester: Third (IIIrd)

PRACTICAL LABORATORY MANUAL

INDEX

EXPERIMENT NO. - 01

AIM: - Introduction to the use of laboratory instruments and safety precautions.

A) Instruments for Inoculation and Incubation
B) Instruments for Sterilization

1. Safety Precautions

- Always wear a clean lab coat, gloves, and closed footwear.
- Tie back long hair and avoid loose clothing near flame sources.
- Disinfect work surfaces before and after each procedure.
- Use instruments only after proper sterilization.
- Do not touch sterile surfaces or instruments with bare hands.
- Dispose of biohazardous materials as per institutional guidelines.
- Report any accidents or injuries to the instructor immediately.

2. Instruments and Equipment

A) Instruments for Inoculation and Incubation

Instrument	Description	Use	Precautions
Inoculating Loop/Needle	Wire loop or straight needle attached to a handle, often made of Nichrome	Transferring microorganisms to culture media	Sterilize before and after use in a flame
Bunsen Burner/Spirit Lamp	Flame-producing device for sterilization	Flaming loops and necks of test tubes	Use in well-ventilated area, keep flammables away
Laminar Air Flow Hood	Enclosed bench to work in sterile conditions	Prevents contamination during inoculation	Clean with ethanol before and after use, turn on UV before use
Incubator	Device that maintains constant temperature (usually 37°C)	Growth of microorganisms	Do not overload; label cultures properly
Petri Dishes	Shallow cylindrical containers	Culturing microorganisms on agar	Use sterile ones or autoclave reusable ones
Test Tubes/Slants/Broths	Tubes containing liquid or solid media	Culture and maintain microbial strains	Always label clearly and handle with care

B) Instruments for Sterilization

Instrument	Description	Use	Precautions
Autoclave	High-pressure steam sterilizer (121°C, 15 psi)	Sterilizes media, glassware, and instruments	Ensure correct pressure & time; release pressure safely
Hot Air Oven	Dry heat sterilizer (160–180°C for 2 hrs)	Sterilizes glassware and metallic items	Allow items to cool before handling
Bead Sterilizer	Uses heated beads for rapid sterilization	Sterilizing inoculating loops/needles quickly	Insert only metal part; monitor temperature
Flame Sterilization (Bunsen burner)	Direct flame to kill microbes	Loops, needles, test tube rims	Avoid burns; keep flammable materials away
Chemical Disinfectants (e.g., Ethanol, Lysol)	Chemical agents for surface sterilization	Cleaning surfaces, hands, tools	Use appropriate concentration and avoid inhalation

3. Procedure

A) Inoculation and Incubation

1. Prepare the workstation by disinfecting surfaces and gathering sterile tools.
2. Sterilize inoculation loop using flame till red hot.
3. Cool the loop before collecting microbial sample.
4. Inoculate the sample onto culture media using aseptic technique.
5. Label and place the inoculated media in the incubator at specified temperature.
6. Incubate for 24–48 hours as required.

B) Sterilization Procedures

1. Glassware: Place in hot air oven (160°C for 2 hours) or autoclave (121°C, 15 psi, 15–20 min).
2. Media and Culture Tools: Sterilize in autoclave before use.
3. Inoculation tools: Flame sterilize or use bead sterilizer before and after inoculation.

4. Observations

- Observe the growth pattern in incubated cultures.
- Check for sterility in sterilized items.
- Record any contamination or procedural errors.

5. Precautions during Instrument Use

- Never leave burners unattended.
- Ensure all instruments are clean before sterilization.
- Avoid overheating instruments like loops as it may cause aerosol formation.
- Always check temperature and pressure gauges on autoclaves and ovens.

6. Conclusion

Understanding the correct use of instruments and maintaining aseptic conditions are crucial in microbiology laboratories. Proper sterilization ensures accurate results and maintains laboratory safety standards.

7. Reference

Textbook of Microbiology by **C.P. Baveja** – Arya Publications, New Delhi.

EXPERIMENT NO.-02

AIM: - To prepare basic liquid media (broth) for routine cultivation of bacteria.

INTRODUCTION

Nutrient broth is a general-purpose liquid medium that supports the growth of a wide range of non-fastidious microorganisms. It contains basic nutrients like peptone**,** beef extract, and sodium chloride dissolved in distilled water.

COMPOSITION OF NUTRIENT BROTH

Ingredient	**Quantity per 1000 mL**
Peptone	5.0 g
Beef extract	3.0 g
Sodium chloride	5.0 g
Distilled water	Up to 1000 mL
Final pH	7.0 ± 0.2 at 25°C

SAFETY PRECAUTIONS

- Wear lab coat**,** gloves, and eye protection.
- Handle glassware carefully to avoid breakage.
- Do not inhale powdered media or touch with bare hands.
- Use autoclave only under supervision and check for proper pressure and seal.

REQUIREMENTS

Apparatus:

- Beaker (1000 mL)
- Measuring cylinder
- Conical flask (500–1000 mL)
- Magnetic stirrer or glass rod
- pH meter or pH strips
- Autoclave
- Test tubes
- Cotton plugs or screw caps

Chemicals:

- Peptone
- Beef extract
- Sodium chloride
- Distilled water

PROCEDURE

Step 1: Weighing and Mixing Components

1. Weigh the following using a digital balance:
    - Peptone: 5 g
    - Beef extract: 3 g
    - NaCl: 5 g
2. Pour 500 mL of distilled water into **a** beaker or conical flask.
3. Add the ingredients one by one into the flask.
4. Stir with a glass rod or magnetic stirrer until dissolved.

Step 2: Adjust Volume and pH

1. Once dissolved, make up the volume to 1000 mL with distilled water.
2. Check and adjust the pH to 7.0 ± 0.2 using 1N NaOH or 1N HCl.

Step 3: Dispensing and Plugging

1. Dispense 10–15 mL of the broth into clean test tubes or 100–250 mL into flasks.
2. Plug the tubes with cotton wool or use screw caps.

Step 4: Sterilization

1. Place the tubes or flasks in an autoclave**.**
2. Sterilize at 121°C, 15 psi for 15–20 minutes.
3. Allow to cool before removing from autoclave.

STORAGE

- Store sterilized nutrient broth at room temperature or **4°C**.
- Label each container with:
    - Media type
    - Preparation date
    - pH
    - Prepared by

APPLICATIONS

- Used for routine sub culturing of bacteria.
- Useful for biochemical tests**,** growth kinetics, and fermentation studies.
- Can be enriched with additional nutrients or indicators for specific bacterial groups.

OBSERVATIONS

Parameter	**Observation**
Color	Pale yellow
pH (after autoclaving)	~7.0
Clarity	Clear, no precipitation
Growth support	Supports bacterial growth

RESULT

Basic nutrient broth was successfully prepared, sterilized, and stored for use in the routine cultivation of bacteria**.**

CONCLUSION

Understanding the proper preparation of nutrient broth is essential for microbiological work. Following accurate measurements, aseptic techniques, and proper sterilization ensures contamination-free and effective culture media.

Reference: -

Textbook of Microbiology by **C.P. Baveja** – Arya Publications, New Delhi.

EXPERIMENT NO. - 03

AIM: - Preparation of basic solid media, Agar slants and Agar deep tubes for routine cultivation of microorganism.

INTRODUCTION

Solid media like Nutrient Agar provide a firm surface for microbial growth. Different tube orientations serve distinct purposes:

- **Agar Slants** – for stock culture maintenance.
- **Agar Deep Tubes** – for studying oxygen requirements or motility.

COMPOSITION OF NUTRIENT AGAR

Ingredient	Quantity per 1000 mL
Peptone	5.0 g
Beef Extract	3.0 g
Sodium Chloride	5.0 g
Agar-Agar	15.0 g
Distilled Water	Up to 1000 mL
Final pH	7.0 ± 0.2 at 25°C

SAFETY PRECAUTIONS

- Wear lab coat, gloves, and goggles at all times.
- Handle hot agar cautiously to avoid burns.
- Sterilize all glassware and tools before and after use.
- Do not touch the inner part of tubes or stoppers to avoid contamination.

REQUIREMENTS

Apparatus:

- Beakers (1000 mL)
- Measuring cylinder
- Conical flask (1000 mL)
- Magnetic stirrer or glass rod
- pH meter or pH strips
- Test tubes (culture tubes)
- Test tube rack, slanting rack
- Autoclave
- Cotton plugs or screw caps

Reagents:

- Peptone, Beef Extract, Sodium Chloride, Agar
- Distilled water
- 1N NaOH / 1N HCl for pH adjustment

PROCEDURE

Step 1: Weigh and Mix Components

1. Measure:
    - Peptone – 5 g
    - Beef extract – 3 g
    - NaCl – 5 g
    - Agar – 15 g
2. Add to ~500 mL of distilled water in a conical flask.
3. Stir and heat gently until agar dissolves completely.

Step 2: Adjust Volume and pH

1. Add distilled water to make up the volume to **1000 mL**.
2. Check pH using a pH meter or strip and adjust to **7.0 ± 0.2**.

Step 3: Dispense into Tubes

1. Pour **5–10 mL** into sterile test tubes.
2. Plug tubes with **cotton** or **screw caps**.
3. Label tubes properly (e.g., Nutrient Agar, date, initials).

Step 4: Sterilization

1. Place tubes upright in a test tube rack and autoclave at:
    - **121°C**, **15 psi**, for **15–20 minutes**.
2. Allow to cool slightly before setting slants.

Step 5: Setting Slants and Deeps

- For **Agar Slants**: While still hot (~50–55°C), tilt tubes at an angle to solidify slanted.
- For **Agar Deeps**: Let tubes solidify in the **upright position**.

STORAGE

- Store slants and deeps at **4°C** or room temperature if used within a few days.
- Label each tube with:
    - Media type
    - Preparation date
    - Prepared by

APPLICATIONS

Form	Use
Solid Agar Plate	For streaking and isolation of colonies
Agar Slants	For stock culture maintenance
Agar Deep Tubes	For motility and oxygen requirement studies

OBSERVATIONS

Parameter	Observation
Clarity	Clear, no turbidity
Color	Pale yellow
Slant uniformity	Proper angle and smooth surface
Deep tubes	Evenly filled and solidified upright

RESULT

Basic solid media were successfully prepared in the form of **slants and deeps**, sterilized, and stored for routine microbial cultivation.

CONCLUSION

Solid media like **nutrient agar slants and deeps** are essential tools for the isolation, cultivation, and maintenance of microbial cultures. This practical enhances understanding of media preparation, sterilization, and aseptic techniques.

Reference: -

Textbook of Microbiology by **C.P. Baveja** – Arya Publications, New Delhi.

EXPERIMENT NO.- 04

AIM: - To obtain pure culture of bacteria by streak plate method.

INTRODUCTION

The streak plate method is a microbiological technique used to isolate individual colonies of a bacterium on an agar surface. It relies on diluting the bacterial load with each streak, allowing well-isolated colonies to grow in the final streaks.

PRINCIPLE

A small amount of mixed culture is spread across an agar plate using a sterile loop in a pattern that thins out the bacteria. By the third or fourth quadrant, the bacteria are sufficiently diluted to grow into isolated colonies.

SAFETY PRECAUTIONS

- Wear a lab coat, gloves, and goggles.
- Disinfect your workspace before and after the experiment.
- Always flame the **loop** before and after each use.
- Do not open the plate unnecessarily after streaking.

MATERIALS REQUIRED

Apparatus / Reagents	Description
Nutrient Agar Plates	Solid media for bacterial growth
Inoculating Loop	For transferring bacteria
Bunsen Burner	For flame sterilization
Bacterial Culture	Mixed bacterial sample
Marker Pen	For labeling plates
Disinfectant	70% Ethanol or Lysol

PROCEDURE

Step 1: Preparation

1. Label the bottom of the Petri dish with:
    - Name of sample
    - Date
    - Initials
2. Sterilize the inoculating loop by flaming it in a Bunsen burner until red hot.

Step 2: Inoculation

1. Cool the loop briefly, then take a small amount of bacterial culture.
2. Lift the lid of the agar plate slightly, and streak the culture in quadrant 1 using a zig-zag motion.

Step 3: Dilution by Streaking

1. Flame the loop again and allow it to cool.
2. Rotate the plate slightly and streak quadrant 2 by dragging the loop from the edge of quadrant 1.
3. Repeat for quadrant 3 and 4, flaming the loop in between.

Step 4: Incubation

1. Close the plate and invert it (lid down).
2. Incubate at 37°C for 24–48 hours.

OBSERVATIONS

Quadrant	Growth Observed	Colony Characteristics
Q1	Heavy growth	Overlapping colonies
Q2	Moderate growth	Partially isolated colonies
Q3	Sparse growth	Well-separated colonies
Q4	Isolated colonies	Pure colonies (round, distinct)

RESULT

Well-isolated, distinct bacterial colonies were obtained in the final streaks, indicating successful isolation of a pure culture**.**

CONCLUSION

The streak plate method is a fundamental technique for obtaining pure cultures. Proper sterilization and streaking patterns ensure the separation of individual bacteria into discrete colonies, allowing for identification and further study.

Reference: -

Textbook of Microbiology by C.P. Baveja – Arya Publications, New Delhi

EXPERIMENT NO. - 05

AIM: - To obtain pure culture of bacteria by spread plate method.

INTRODUCTION

The spread plate technique is a quantitative method used to isolate pure colonies of microorganisms. A small volume of a diluted microbial sample is spread uniformly across the surface of an agar plate using a sterile glass or metal spreader**.**

PRINCIPLE

A known volume of a diluted sample is placed on an agar plate and spread evenly using a sterile spreader. The dilution ensures that individual cells are well separated, which, after incubation, form discrete and countable colonies.

SAFETY PRECAUTIONS

- Always wear lab coat**,** gloves, and safety goggles.
- Work near a Bunsen burner to maintain aseptic conditions.
- Sterilize spreader with alcohol and flame before each use.
- Dispose of all microbial waste in a biohazard container.

MATERIALS REQUIRED

Item	Description
Nutrient Agar Plates	Solid media for bacterial growth
Bacterial Culture	Diluted microbial suspension
Sterile Pipettes	For transferring precise volumes
Alcohol (70% ethanol)	For flaming the spreader
Glass or L-shaped Spreader	Used to evenly spread culture on agar
Bunsen Burner	For maintaining aseptic environment
Marker Pen	For labeling Petri plates

PROCEDURE

Step 1: Label and Prepare Plates

- Label the bottom of each agar plate with:
    - Sample ID
    - Dilution level (if any)
    - Date
    - Initials

Step 2: Pipette the Sample

- Using a sterile pipette, transfer 0.1 mL of the diluted bacterial sample onto the center of the agar plate.

Step 3: Spreading the Sample

1. Dip the L-shaped spreader in 70% alcohol.
2. Pass the spreader quickly through a flame to ignite the alcohol (let flame go out).
3. Allow the spreader to cool (5–10 seconds).
4. Gently spread the sample over the agar surface in a circular motion.

Step 4: Incubation

- Close the plate lid and **invert** the plate (lid side down).
- Incubate at 37°C for 24–48 hours.

OBSERVATIONS

Plate	Dilution Factor	Colony Appearance	Number of Colonies	Remarks
1	10^{-1}	Dense growth	TNTC (Too Numerous)	Overlapping
2	10^{-3}	Medium growth	50–100 colonies	Some separation
3	10^{-5}	Sparse growth	30–50 colonies	Well-isolated

RESULT

- Discrete and countable bacterial colonies were obtained on agar plates at suitable dilutions.
- A pure culture was successfully isolated using the spread plate method.

CONCLUSION

The spread plate method is an effective microbiological technique for isolating and quantifying viable bacteria in a sample. Proper dilution and aseptic technique are critical for obtaining pure, well-separated colonies suitable for further study.

Reference: -

Textbook of Microbiology by C.P. Baveja – Arya Publications, New Delhi

EXPERIMENT NO. - 06

AIM: - To obtain pure culture of bacteria by pour plate method.

INTRODUCTION

The pour plate method is widely used in microbiology laboratories to isolate and quantify microorganisms from a sample. In this technique, a diluted sample is mixed with molten agar and poured into a Petri dish. After solidification and incubation, well-isolated colonies appear both on the surface and within the agar.

PRINCIPLE

The diluted bacterial suspension is mixed with sterile molten agar (cooled to ~45°C) and poured into sterile Petri dishes. As the agar solidifies, individual bacteria become trapped and grow into discrete colonies inside or on the surface of the medium.

SAFETY PRECAUTIONS

- Wear lab coat, gloves, and goggles**.**
- Use autoclaved agar and sterile pipettes.
- Handle molten agar at safe temperatures (~45°C) to avoid burns.
- Flame all tools and work near a Bunsen burner or inside a laminar flow hood**.**

MATERIALS REQUIRED

Materials	**Description**
Nutrient Agar (molten)	Sterile agar maintained at 45–50°C
Bacterial Culture	Diluted bacterial suspension
Sterile Pipettes	For transferring bacterial suspension
Petri Dishes	Sterile disposable or reusable glass plates
Bunsen Burner	For aseptic handling
Alcohol & Cotton	For disinfection and flaming tools
Incubator	Set to 37°C

PROCEDURE

Step 1: Label the Petri Plates

Label the bottom **o**f each sterile Petri plate with:

- Sample ID
- Dilution Factor (e.g., 10^{-3}, 10^{-4})
- Date and Initials

Step 2: Pipette the Sample

1. Use a sterile pipette to transfer 1 mL of the diluted bacterial culture into the **c**enter of a sterile Petri dish.

Step 3: Add Molten Agar

2. Pour 15–20 mL of molten nutrient agar (cooled to ~45°C) into the plate containing the inoculum.
3. Gently rotate the plate in a circular motion to mix the sample and agar evenly.

Step 4: Allow to Solidify

- Let the plates stand at room temperature until the agar solidifies (5–10 minutes).

Step 5: Incubation

- Invert the plates (agar side up) and incubate at 37°C for 24–48 hours.

OBSERVATIONS

Plate	Dilution Factor	Colony Type	Count	Remarks
1	10^{-1}	Confluent growth	TNTC	Too many to count
2	10^{-3}	Some separation	50–100	Good colony separation
3	10^{-5}	Isolated colonies	20–50	Suitable for pure culture

RESULT

- Well-isolated colonies were obtained using the pour plate technique.
- Colonies were embedded within and present on the surface of the agar.

CONCLUSION

The pour plate method is a simple and effective technique to obtain pure bacterial cultures and perform colony counts. It allows for the growth of both aerobic (on the surface) and facultative anaerobic (inside the agar) microorganisms.

Reference

Textbook of Microbiology by C.P. Baveja – Arya Publications, New Delhi.

EXPERIMENT NO.- 07

AIM: - Physical agents of control by moist heat and dry heat.

INTRODUCTION

Physical methods of microbial control are used to sterilize or disinfect materials in medical, pharmaceutical, and research settings. Among these, moist heat and dry heat are two widely used thermal methods:

- Moist Heat (e.g., autoclaving) uses steam under pressure.
- Dry Heat (e.g., hot air oven) uses high temperature without moisture.

PRINCIPLE

Method	Mechanism	Example
Moist Heat	Protein coagulation and enzyme denaturation via steam	**Autoclave**
Dry Heat	Oxidative damage and protein denaturation by hot air	**Hot Air Oven**

Microbial cells, especially spores, are more susceptible to moist heat because water conducts heat more efficiently.

SAFETY PRECAUTIONS

- Do not open autoclave or oven immediately after use – allow pressure/heat to normalize.
- Use heat-resistant gloves and face shield when handling hot equipment.
- Ensure proper sealing of autoclave lid.
- Always check temperature gauges and pressure indicators.

MATERIALS REQUIRED

Equipment	Use
Autoclave	For sterilization using moist heat (121°C, 15 psi)
Hot Air Oven	For sterilization using dry heat (160–180°C)
Test Tubes with Media	To test sterilization efficiency
Spore Strips / Bacillus spores	Biological indicator
Thermometer/Gauge	Temperature verification
Heat-resistant gloves	For handling hot materials

PROCEDURE

A) MOIST HEAT – AUTOCLAVE METHOD

Step 1: Load the Autoclave

- Place test tubes**,** media, or spore strips into the autoclave.

Step 2: Set Temperature and Pressure

- Set the autoclave to 121°C at 15 psi for 15–20 minutes**.**

Step 3: Sterilization Cycle

- Allow the cycle to run completely.

Step 4: Cooling and Unloading

- After pressure drops to 0, open the autoclave carefully and remove materials.

B) DRY HEAT – HOT AIR OVEN METHOD

Step 1: Prepare the Oven

- Place glassware**,** metal instruments, or spore strips inside the oven.

Step 2: Set Temperature and Time

- Heat to 160°C for 2 hours or 180°C for 1 hour**.**

Step 3: Allow Cooling

- Switch off and let cool before removing contents.

OBSERVATION TABLE

Method	Temperature	Time	Result (Growth/No Growth)	Remarks
Moist Heat	121°C	15 min	No Growth	Effective sterilization
Dry Heat	160°C	2 hours	No Growth	Longer time needed
Control	Room Temp	–	Growth	No sterilization

RESULT

- Moist heat (autoclave) was effective at killing all microbes, including spores, in shorter time.
- Dry heat required higher temperature and longer duration for similar effects.

CONCLUSION

- Both moist and dry heat are effective physical methods of microbial control.
- Moist heat is more efficient, especially for heat- and moisture-stable items like media and surgical dressings.
- Dry heat is preferred for glassware, metal tools, and oils that can't be autoclaved.

Reference Book:

C.P. Baveja – Textbook of Microbiology (Arya Publications, New Delhi)

EXPERIMENT NO. - 08

AIM: - Mechanical agents of control UV radiation.

INTRODUCTION

Ultraviolet (UV) radiation is a type of non-ionizing radiation in the electromagnetic spectrum ranging from 200–400 nm. Among UV types, UV-C (100–280 nm) has the strongest germicidal effect, especially at 254 nm. It is used as a mechanical agent of microbial control in hospitals, biosafety cabinets, water treatment, and laboratories.

PRINCIPLE

UV radiation penetrates microbial cells and causes formation of thymine dimers in DNA, leading to:

- DNA replication errors
- Enzyme inactivation
- Cell death

UV effectiveness depends on:

- Wavelength (254 nm is ideal)
- Exposure time
- Distance from the UV source
- Type of microorganism
- Presence of barriers (e.g., glass, plastic)

SAFETY PRECAUTIONS

- Never look directly at UV light; it can damage eyes and skin.
- Wear UV-blocking goggles and lab coats.
- Limit exposure time.
- Use UV radiation in biosafety cabinets or closed chambers**.**

MATERIALS REQUIRED

Materials	**Description**
UV Lamp (254 nm)	Germicidal wavelength
Bacterial culture (e.g., *E. coli*)	Sensitive test organism
Nutrient agar plates	For bacterial growth
Sterile swabs	For spreading culture
Aluminum foil or cardboard	To act as UV shields (partial covers)
Timer	To control exposure time
Ruler	To measure distance from UV source

PROCEDURE

Step 1: Prepare the Agar Plates

1. Label 4 nutrient agar plates as:
    - Control (no UV)
    - 30 sec exposure
    - 1 min exposure
    - 3 min exposure
2. Swab *E. coli* culture evenly across the surface of all plates.

Step 2: UV Exposure

3. Remove the lid of each test plate and expose to UV light at a distance of 30 cm from the lamp.
4. Cover half of each plate with cardboard to demonstrate shielding effect.
5. Expose each plate for their respective time durations.
6. After exposure, replace lids and incubate at 37°C for 24 hours**.**

OBSERVATIONS

Plate Label	Exposure Time	Zone of Bacterial Inhibition	Growth	Remarks
Control	0 min	None	Full growth	No UV effect
Test Plate 1	30 sec	Partial	Reduced growth	UV inhibits growth
Test Plate 2	1 min	Moderate	Few colonies	Increased bacterial death
Test Plate 3	3 min	Large clear zone	No growth	Complete inhibition (uncovered area)

RESULT

- UV radiation showed a significant germicidal effect proportional to exposure time.
- Shielded areas showed bacterial growth, confirming mechanical blocking of UV.
- No bacterial growth in 3-min exposure plate (uncovered region).

CONCLUSION

- UV radiation is an effective mechanical agent of microbial control, especially for air and surfaces.
- Its efficiency depends on exposure time**,** wavelength, and distance**.**
- It is widely used in biosafety cabinets**,** water purifiers, and operating rooms in India.

Reference Textbook:

C.P. Baveja – Textbook of Microbiology (Arya Publications, New Delhi)

EXPERIMENT NO. - 09

AIM: - Preparation of bacterial smears.

INTRODUCTION

Bacterial smears are thin layers of bacteria placed on a slide for microscopic examination. Proper smear preparation is crucial for:

- Observing cell morphology.
- Performing differential staining like Gram staining.
- Ensuring accurate interpretation of microbial features.

A well-prepared smear should:

- Be thin and even**.**
- Be well-fixed (heat-fixed).
- Not wash off during staining.

PRINCIPLE

The procedure involves:

1. Spreading a bacterial suspension over the slide.
2. Air drying to remove moisture.
3. Heat fixing to kill and fix the bacteria onto the glass surface.
4. The smear is then ready for staining and microscopy.

SAFETY PRECAUTIONS

- Handle all bacterial cultures as potential pathogens**.**
- Use gloves, lab coat, and goggles.
- Dispose of contaminated materials in biohazard bins.
- Avoid inhaling aerosols during heat fixing.

MATERIALS REQUIRED

Materials	**Use**
Clean glass slides	Smear preparation
Inoculating loop	Transfer of bacterial culture
Bunsen burner or spirit lamp	Heat fixing the smear
Distilled water	For emulsifying solid cultures
Bacterial culture (broth or colony)	Smear material
Marker pen	To label the slide
Blotting paper	To dry the slide after staining

PROCEDURE

A. FROM BROTH CULTURE

1. Take a clean glass slide, label it with a marker.
2. Flame the inoculating loop, cool it, and collect a loopful of broth**.**
3. Gently spread it in a circular motion on the center of the slide.
4. Allow it to air dry completely.
5. Pass the slide over the flame 2–3 times (heat fixing).

B. FROM SOLID CULTURE (AGAR PLATE/SLANT)

1. Place a drop of distilled water in the center of a clean slide.
2. Flame the loop and touch a colony with the sterile loop.
3. Mix the loopful of bacteria with the water to form an even thin smear.
4. Air dry and heat fix as above.

OBSERVATIONS

Parameter	Observation
Smear thickness	Thin / Too thick
Fixation quality	Adhered / Washed off
Morphological clarity	Clear / Distorted

A properly prepared smear will appear as a thin, translucent film under reflected light before staining.

RESULT

The smear was successfully prepared and adhered well to the slide. It was ready for further staining such as Gram staining.

CONCLUSION

- A good bacterial smear is essential for observing cellular morphology and arrangement.
- Smear preparation varies slightly for broth and solid cultures.
- Proper fixation is critical to prevent washing off during staining.

Reference Book

C.P. Baveja – Textbook of Microbiology, Arya Publications, New Delhi

EXPERIMENT NO. - 10

AIM: - To perform gram staining of bacteria**.**

INTRODUCTION

Gram staining is a differential staining technique developed by Hans Christian Gram in 1884. It is one of the most important and widely used staining techniques in microbiology to classify bacteria into two major groups:

- **Gram-positive bacteria**: Retain crystal violet stain (purple)
- **Gram-negative bacteria**: Do not retain crystal violet and take up safranin (pink/red)

PRINCIPLE

The difference in staining is due to the cell wall structure:

- Gram-positive bacteria have a thick peptidoglycan layer, which retains crystal violet–iodine complex.
- Gram-negative bacteria have a thin peptidoglycan layer and an outer lipid membrane, which allows the stain to wash out with alcohol and take up the counterstain (safranin).

SAFETY PRECAUTIONS

- Handle cultures using aseptic technique.
- Use gloves**,** lab coat, and eye protection.
- Properly dispose of all used slides and materials in biohazard containers.
- Avoid skin contact with stains and reagents.

MATERIALS REQUIRED

Materials	**Purpose**
Clean glass slides	For smear preparation
Inoculating loop	For transferring bacterial culture
Bunsen burner or spirit lamp	For heat fixation
Bacterial culture (*E. coli*, *S. aureus*)	Test organisms
Gram stain reagents:	
– Crystal violet (primary stain)	Stains all cells purple
– Gram's iodine (mordant)	Forms CV-I complex
– Alcohol or acetone (decolorizer)	Removes CV-I from Gram-negative
– Safranin (counterstain)	Stains Gram-negative pink
Microscope	For observation
Blotting paper	Drying slides

PROCEDURE

A. Smear Preparation

1. Prepare a thin bacterial smear from broth or colony.
2. Air dry and heat fix the slide.

B. Staining Steps

Step	**Reagent Used**	**Duration**	**Action**
1	Crystal violet	1 minute	Stains all bacteria purple
2	Gram's iodine	1 minute	Forms complex with crystal violet
3	Alcohol/Acetone	Few seconds	Decolorizes Gram-negative cells
4	Safranin	1 minute	Counterstains Gram-negative pink
5	Wash with water	As required	After each staining step

Step	Reagent Used	Duration	Action
6	Blot dry	Final step	Prepare for microscopy

DIAGRAMS / FIGURES

Figure 1: Gram Staining Steps

Figure 2: Microscopic Appearance

- **Purple (Gram-positive)**: e.g., *Staphylococcus aureus*
- **Pink (Gram-negative)**: e.g., *Escherichia coli*

OBSERVATIONS

Bacterial Sample	Stain Color	Gram Reaction	Morphology
Staphylococcus aureus	Purple	Gram-positive	Cocci in clusters
Escherichia coli	Pink	Gram-negative	Rods (bacilli)

RESULT

- The bacteria were successfully stained.
- Differentiation between Gram-positive and Gram-negative organisms was achieved based on color and shape.

CONCLUSION

- Gram staining is a quick and essential diagnostic tool.
- It helps in:
    - Classifying bacteria
    - Choosing appropriate antibiotics
    - Preliminary identification in clinical microbiology

Reference Book:-

C.P. Baveja – Textbook of Microbiology, Arya Publications, New Delhi