

# **SOP on Identification of Bacteria Using the Gram Staining Technique**

Department of Microbiology

Faculty of Medicine

UWUSL

# **Standard Operating Procedure (SOP)**

## **Identification of Bacteria Using the Gram Staining Technique**

### **Title:**

Identification of Bacteria Using the Gram Staining Technique

### **Issued By:**

Faculty of Medicine, Uva Wellassa University of Sri Lanka

### **1. Purpose**

To establish a standardized procedure for performing Gram staining in microbiology practical sessions to differentiate bacteria into Gram-positive and Gram-negative groups based on cell wall characteristics.

### **2. Scope**

This SOP applies to all medical students, academic staff, and laboratory personnel involved in microbiology practical sessions, including:

- Preparation of bacterial smears
- Performing Gram staining
- Microscopic identification of bacteria

### **3. Responsibilities**

#### **3.1 Students**

- Follow correct staining procedures
- Handle reagents and equipment safely
- Accurately observe and record findings

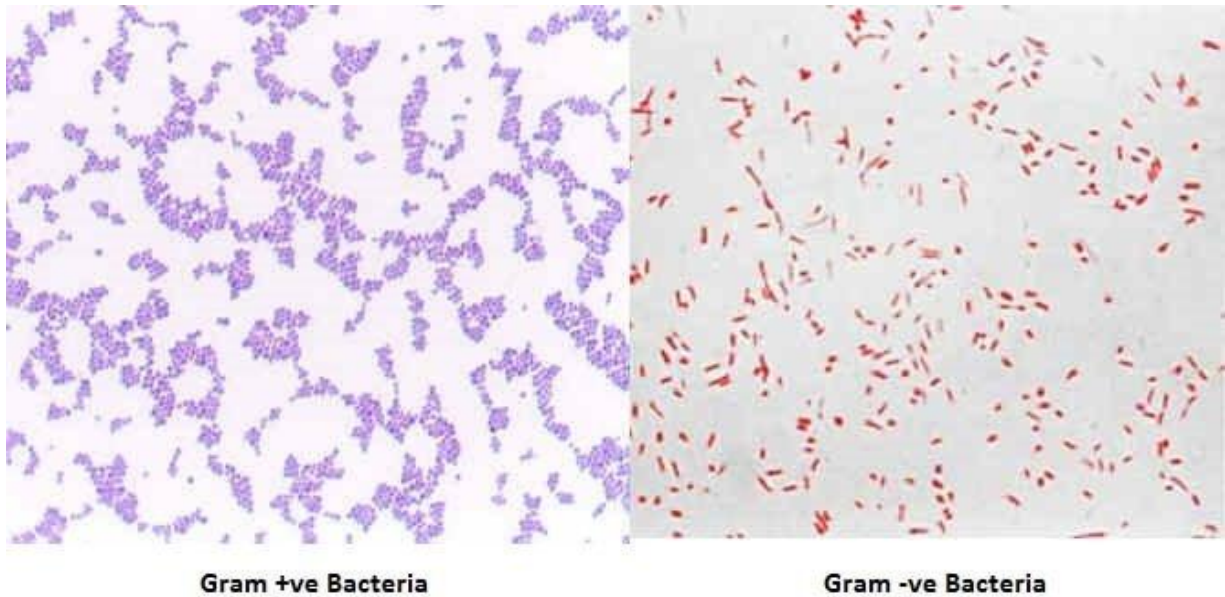
#### **3.2 Demonstrators / Lecturers**

- Supervise staining procedure
- Ensure correct technique is followed
- Assist in interpretation of results

#### **3.3 Laboratory Staff**

- Provide reagents and materials
- Ensure proper functioning of equipment
- Maintain laboratory safety standards

## 4. Principle



Gram staining is a differential staining technique where bacteria are stained with crystal violet and iodine, then decolorized and counterstained.

- Gram-positive bacteria retain the crystal violet–iodine complex and appear purple.
- Gram-negative bacteria lose the complex during decolorization and take up the counterstain, appearing pink.

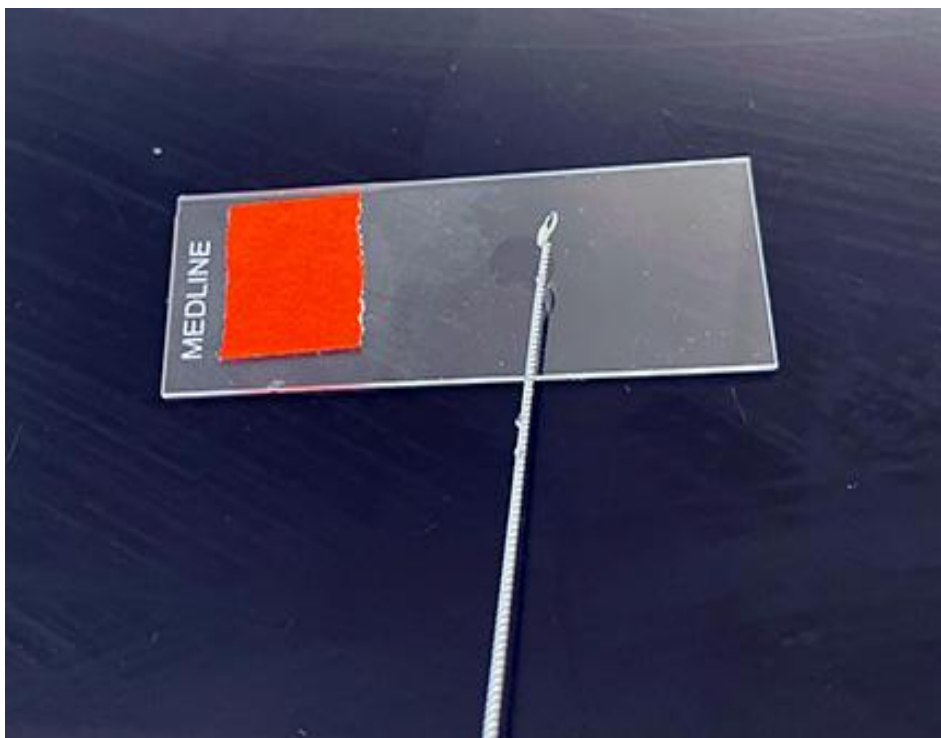
## 5. Equipment and Materials

- Sterile inoculating loop
- Crystal violet stain
- Gram's iodine
- Iodine Acetone or Acetone alcohol (decolorizer)
- Dilute Carbol Fuchsin (counterstain)
- Distilled water
- Bunsen burner
- Glass slides and cover slips
- Compound microscope

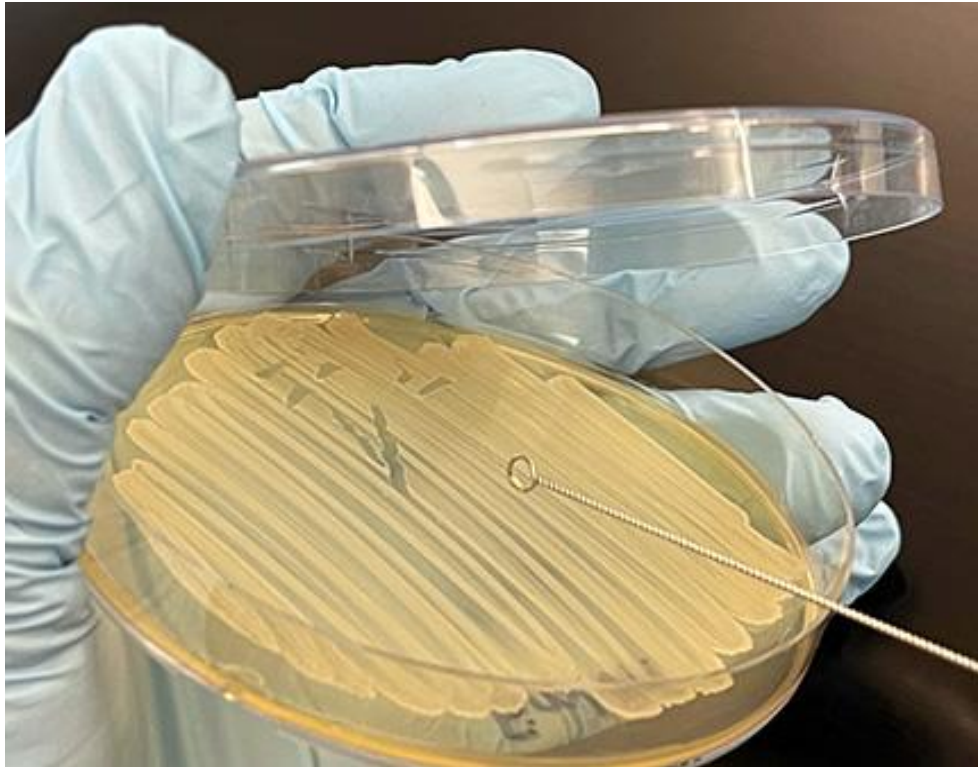
## 6. Procedure

### Step 1: Smear Preparation & Heat Fixing

Label one end of a clean, dry glass slide using a grease pencil to ensure proper identification and prevents loss of labeling during staining. Place a small drop of distilled water on a clean slide.



Using a sterile loop, transfer a small amount of bacterial culture, and mix to form a thin smear then allow it to air dry completely.

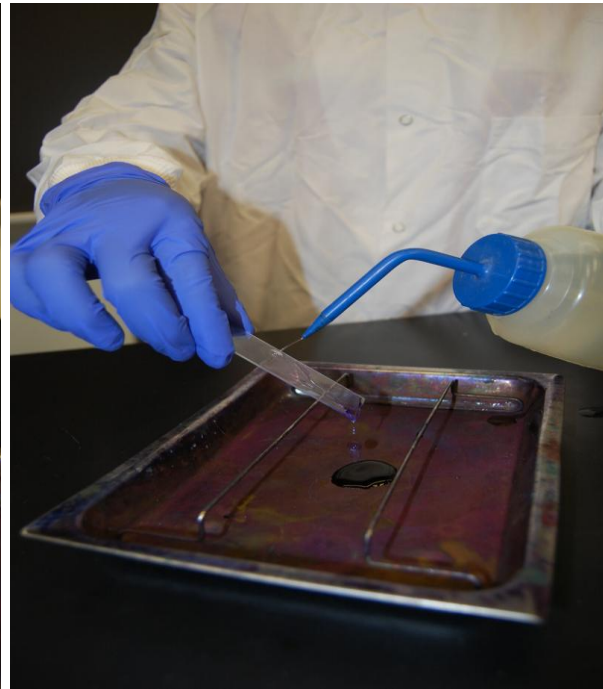
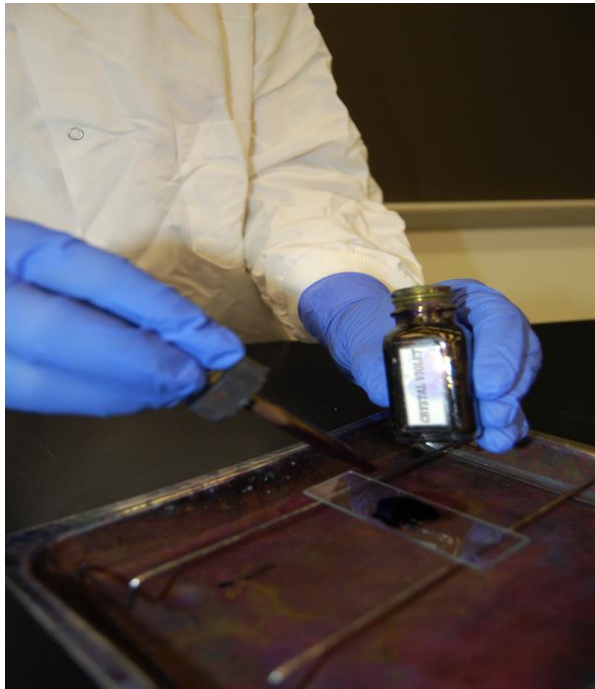


Pass the slide briefly through a flame 2–3 times to heat-fix.



Explanation: Heat fixing kills bacteria, adheres them to the slide, and preserves cell morphology for staining.

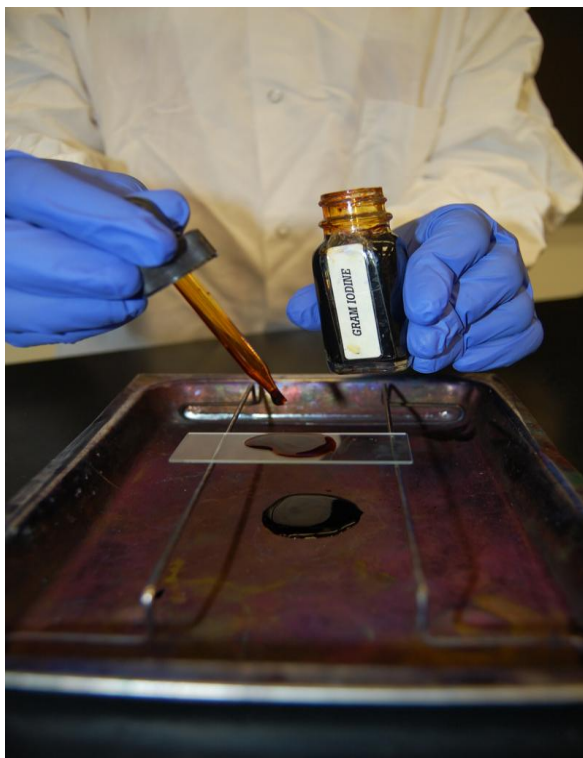
## Step 2: Crystal Violet (Primary Stain)



Flood the smear with crystal violet for 30 seconds, then rinse gently with water.

Explanation: Crystal violet, a basic dye, penetrates the bacterial cell wall and cytoplasm, staining all bacteria purple.

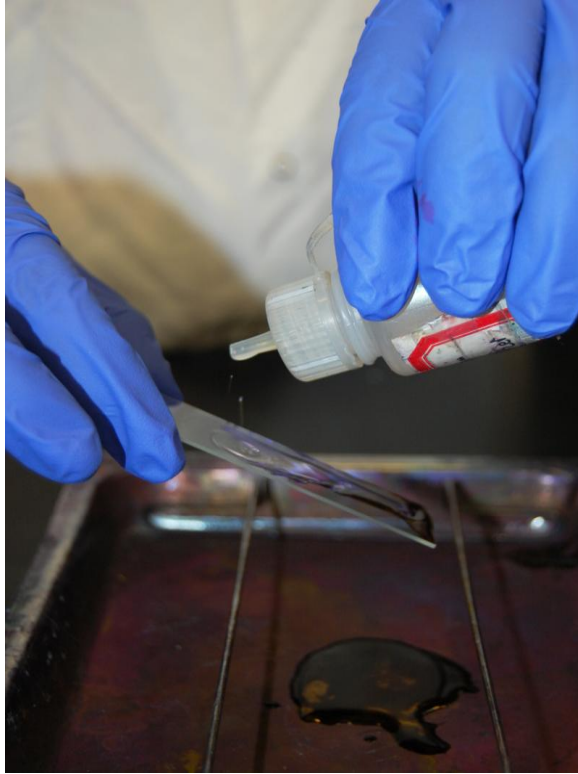
## Step 3: Gram's Iodine



Flood the smear with Gram's iodine for 1 minute, then rinse with water.

Explanation: Iodine forms a crystal violet–iodine complex, making the dye less soluble and more firmly retained in Gram-positive bacteria.

**Step 4: Decolorization (Iodine Acetone or Acetone Alcohol)**



Apply decolorizer dropwise for 1-5 seconds (until the runoff becomes clear), then immediately rinse with water.

Explanation: Acetone-based agents act almost instantly compared to ethyl alcohol. They dissolve the outer membrane of Gram-negative bacteria to remove the dye complex.

### **Step 5: Dilute Carbol Fuchsin (Counterstain)**



Flood the smear with Dilute Carbol Fuchsin for 30 seconds to 1 minute, then rinse and air dry.

Explanation: In Sri Lanka, Dilute Carbol Fuchsin is the preferred counterstain because it provides a much more intense contrast than Safranin, making it easier to identify Gram-negative organisms.

### **Step 6: Microscopic Observation**

1. Place the stained slide on the microscope stage.
2. Focus under the low power (10x) to locate the smear.
3. Switch to high power (40x) and observe.
4. Add a drop of immersion oil and observe under oil immersion (100x)
5. Observe and record:
  - Shape (cocci, bacilli)
  - Arrangement (chains, clusters)
  - Gram reaction (purple/pink)

## **7. Results Interpretation**

Gram-positive bacteria → Purple

Gram-negative bacteria → Pink

## **8. Precautions**

- Use thin smear
- Do not over-decolorize ( Acetone acts very rapidly )
- Heat-fix gently (avoid overheating)
- Use clean slides and fresh reagents
- Follow aseptic technique
- Handle flame carefully

## **9. Post Practical Procedure**

- Dispose of slides safely
- Clean microscope stage
- Turn off light source
- Clean work area

## **10. Records and Documentation**

- Practical number and title
- Date of experiment
- Observations (shape, arrangement, Gram reaction)
- Student name and signature
- Demonstrator verification

**Reference for images:**

BioLibreTexts. Gram Staining Procedure. Microbiology Labs II. Available at: [https://bio.libretexts.org/Learning\\_Objects/Laboratory\\_Experiments/Microbiology\\_Labs/Microbiology\\_Labs\\_II/06%3AGram\\_Stain\\_and\\_Capsule\\_Stain/6.02%3AGram\\_Staining\\_Procedure](https://bio.libretexts.org/Learning_Objects/Laboratory_Experiments/Microbiology_Labs/Microbiology_Labs_II/06%3AGram_Stain_and_Capsule_Stain/6.02%3AGram_Staining_Procedure) (Accessed April 2026).

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