National Department of Health

Title: Gram Stain test

ID: G_90_T_01_A

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Changes to the last authorized version:

Version	Date issued	Changes

1. Purpose and Scope

To describe the process of how to perform a Gram Stain. This technical SOP will cover detailed procedures including materials and equipment needed to complete a gram stain.

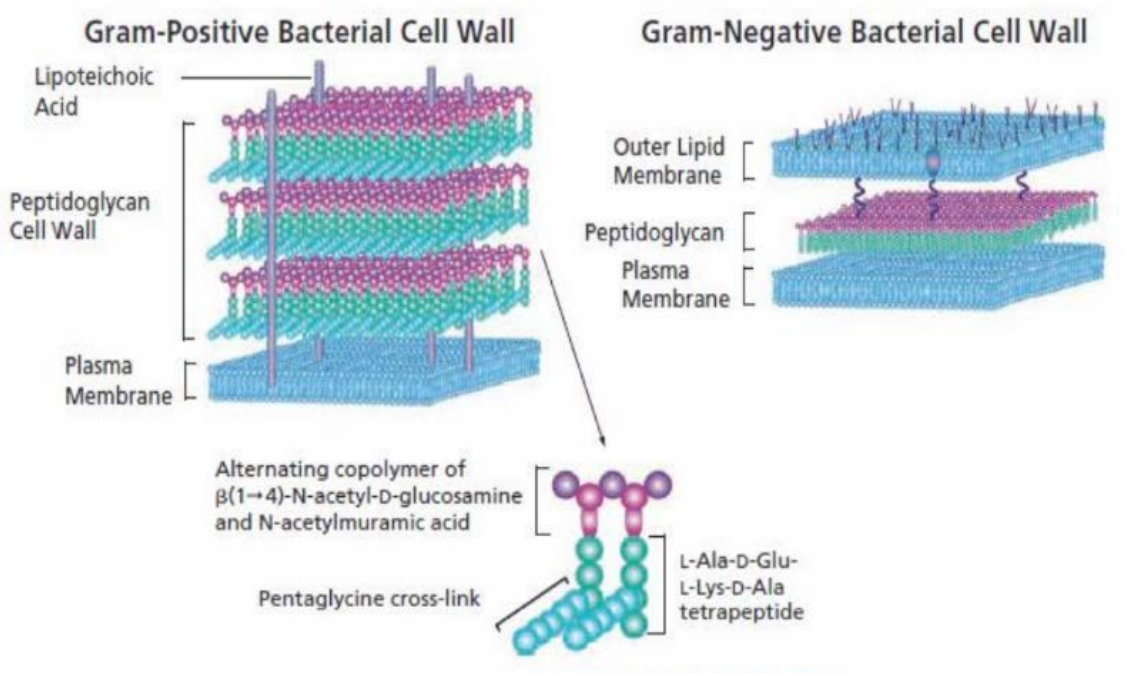


image from SigmAldrich.com

2. Principle/Clinical application

- The gram stain is the first step used in the identification and diagnosis of clinically significant bacteria
- The Gram stain differentiates bacteria into two groups, Gram positive bacteria and Gram negative bacteria, based on the properties of the bacterial cell wall. It also allows visualisation of bacterial morphology: size, shape and bacterial arrangement
- Bacteria stain Gram positive (purple) when they retain the crystal violet in the cell wall
- They stain Gram negative (pink) when they lose the crystal violet during the decolorization step

3. Responsibilities

- Staff performing gram stain require specific training and demonstrated competency.
- Staff performing Gram stain are responsible for the setup, reading and recording of the gram stain result.
- Staff are required to test and record Gram Positive and Gram Negative controls daily.

4. Specimens

- Direct specimen- swab or body fluid
- A colony from a culture plate
- Positive Blood culture broth

5. Safety

For safety aspects, please review this document G_10_Info_3_A Laboratory Biosafety.

6. Equipment and Materials

- Quality control slide: E.coli / S.aureus
- Glass Slides
- Saline
- Crystal violet
- Gram's Iodine
- Decoloriser-Alcohol/Acetone (50%/50%)
- Carbol Fuchsin or Safranin-counterstain
- Sink and Tap water for rinsing slides
- Metal staining rack to overlay the sink
- Forceps
- Microscope and Immersion oil
- Blotting paper
- Paper towel
- Timer
- Hot plate for fixing slides

7. Procedure

7.1 Slide Preparation:

- Label the slide with sample number, patient name and date
- Prepare a thin smear on a glass slide of:

<u>Direct specimen:</u>

- Use the specimen swab or sterile swab/sterile loop to transfer specimen to the glass slide, make a thin smear.
- If a specimen swab is used directly ensure that you inoculate agar plates before touching the glass slide.

<u>Colony from a culture plate:</u>

- Use a clean stick or toothpick to pick a small portion of an isolated colony and spread thinly on the slide.

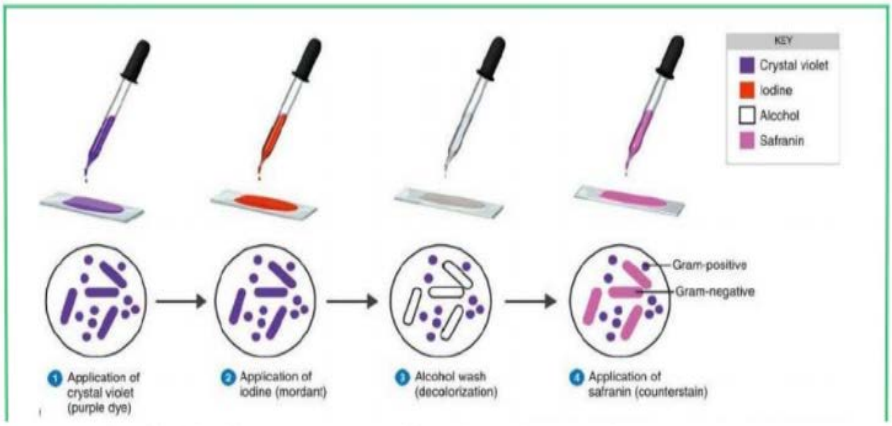
  OR
- Emulsify the colony in a drop of saline on the slide, mix well and spread to make a thin smear.
- You can mark the slide with a wax pencil or diamond pencil so that you can easily find the area under the microscope when adding a CSF or broth to slide.

- Fix the slide by allowing to air dry in the biosafety cabinet on a hotplate.

7.2 Gram stain procedure:

- Place slide on rack over sink
- Flood the smear with crystal violet, leave for 1 minute
- Rinse well with tap water
- Flood the smear with Gram's iodine, leave for 1 minute

• Rinse well with tap water
• Hold the slide with forceps, flood slide with acetone-alcohol, and quickly wash off within 5 sec
• Rinse well with water
• Flood the smear with carbol fuchsin or safranin, leave for 1 minute.
• Rinse well with water
• Stand slides to drain on paper towelling
• Allow to air dry



Heat fix slide before staining and wash with water between steps

7.3 Microscopy

• Place slide on microscope stage to view.
• Focus on the smear using a low power lens (x10)
• Search for a good representative area of material.
• Place a small amount of oil on the slide and change to the (x100) oil immersion lens.

Expected Values
• Gram positive bacteria are purple
• Gram negative bacteria are pink

Limitations and Variables
- Age of the culture (always use fresh cultures)
- Amount of decoloriser and the timing of decolorisation
- Age and condition of reagents
- Type of organism (acid-fast bacteria and spores do not stain well)
- Thickness of the smear, and general care of the technician

8. Results Recording

• Record all results onto paper worksheet with registered lab number and patient identification.
• Record results into LIMS

9. Interpretation

- Record the Gram stain reaction, the shape and arrangement of all types of bacteria present and quantity. i.e.:
- Gram positive cocci in pairs, chains or clusters
- Gram negative or Gram positive rods or Gram variable rods (describe shape, eg branching)
- Gram negative coccobacilli
- Gram negative cocci intracellular or extracellular
- Presence of Yeast and Hyphae

Gram Stain Reporting Guidelines

Grade	Cells per LPF (10x)	Bacteria perHPF (100x oil)
Occasional	<1	<1
1+	1-9	1-5
2+	10-25	6-30
3+	>25	>30

10. Quality Control

- Prepare a slide using a saline suspension of E. coli ATCC 25922 and S. aureus ATCC 25213
- Stain one QC slide each day a gram stain is performed
- Record the results on the Bench Reagent QC worksheet

11. Related Documents

For access, refer to https://path-png.org/microbiology-sops-fleming-fund/

- Bench Reagent QC Worksheet G_90_WS_1
- Gram Stain Procedure Job Aid G_90_J_15
- Common Blood Culture Gram stains and Implications G_90_Info_10
- Laboratory Biosafety Info Sheet G_10_Info_3

12. References

- Manual of Clinical Microbiology, 9th Edition. Patrick R Murray. Ellen Jo Baron, James H Jorgensen, Michael Pfaller, Robert H Yolkien.
- DMDP Gram Stain SOP- Joanne Letchford 2012