PURPOSE: The Gram stain is a differential staining method for staining bacteria from cultures or patient specimens. It classifies bacteria on the basis of their cell wall structure and allows observations of their size and cellular morphology. Bacteria can stain as Gram- positive, Gram- negative or Gram- variable.

SAMPLE INFORMATION:

Type	• Patient specimens requiring Gram stain. Refer to MIC10230 – Microbiology Specimen Processing. • Culture organisms requiring Gram stain for identification.

REAGENTS INFORMATION:

Type	BD™ Gram Crystal Violet, 3.8 L, B4312526 BD™ Gram Iodine (Stabilized), 3.8 L, B4312543 BD™ Gram Decolorizer, 3.8 L, B4312528 BD™ Gram Safranin, 3.8 L, B4312531
Source	Fisher Scientific Canada
Storage	Store at 15° to 30°
Stability	As per expiry date listed on bottle

SUPPLIES:

- Ringed cytology microscope slide
- Frosted end glass microscope slide
- QC slide
- Methanol, absolute
- Immersion oil
- Microscope
- Slide storage tray

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potential infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used when there is a known or potential risk of exposure of splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes and other sharp objects should be strictly limited.

All patient specimens are assumed to be potentially infectious. Universal precautions must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

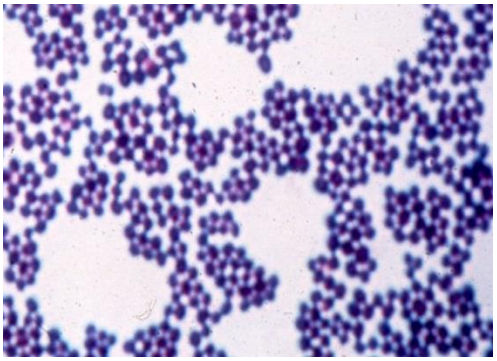
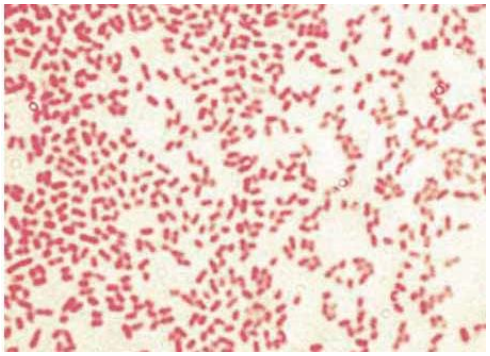
QUALITY CONTROL:

- Refer to MIC60060 – Microbiology Stain Quality Control.

PROCEDURE INSTRUCTIONS:

Step	Action
1	Prepare a smear of the specimen to be stained. Refer to MIC10220 – Specimen Handling for slide preparation instructions.
2	Air dry slides on the slide warmer in the BSC until dry.
3	Once dry, fix smears with methanol for 1 minute. After 1 minute, drain off remaining methanol without rinsing, and allow the slide to air dry again.
4	Flood the fixed smear with crystal violet and allow stain to remain for 1 minute. Decant crystal violet and rinse slide gently with running tap water. **NOTE:** Excessive rinsing in this step can cause crystal violet to be washed from Gram-positive cells.
5	Flood the smear with Gram's iodine and allow stain to remain for 1 minute. Rinse slide gently with running tap water.
6	Decolourize by letting the decolourizing reagent flow over the smear while the slide is held at an angle. Stop when the runoff becomes clear. Adjust decolourization time to thickness of smear. Remove excess decolorizer with gentle flow of tap water.
7	Flood smear with safranin and allow stain to remain for 1 minute. Remove excess counterstain with a gentle flow of tap water.
8	Drain slide and air dry in an upright position. Slides may be gently blotted with filter paper to remove excess water but care should be taken to avoid wiping the stained material from the slide.

INTERPRETATION OF RESULTS:

Step	Action
1	• Deep violet = Gram-positive organism. • Pink or red = Gram-negative organism. • Both Gram-positive and Gram-negative cells with same morphology = Gram variable organism.  
2	If unable to differentiate organisms or cellular debris or for positive blood cultures with no bacteria seen, perform acridine orange stain. Refer to MIC20100 – Acridine Orange Stain.

REPORTING OF RESULTS:

Refer to:

- MIC20200 – Gram stain resulting in LIS – Routine Samples
- MIC20300 – Gram stain resulting in LIS – Respiratory Cultures
- MIC20400 – Gram stain resulting in LIS – Sterile Fluids (except Blood Cultures)
- MIC20500 – Gram stain resulting in LIS – Blood Cultures
- MIC20600 – Gram stain resulting in LIS – Bacterial Vaginosis Screen

LIMITATIONS:

1. Use an 18-24 hour culture as they have a greater affinity for the dyes than do old cells.
2. Microorganisms that are physically disrupted by excess heat fixation will not react to the Gram staining as expected.
3. Gram stain results, including organism morphology, can be affected by the age of the isolate, autolytic enzymes, cultures transferred to media containing antibiotics, as well as specimens collected from patients on antibiotics.
4. Precipitate from crystal-violet stain can appear as irregular coccoid shapes or asters resembling fungal hyphae.

REFERENCES:

- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016.
- BD Gram Stain Kits and Reagents package insert. 2016-01.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0		Initial Release	L. Steven