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Policy Area:	*Lab / Micro*
Standards:	
Applicability:	*Saints and Highline*

Gram Stain Preparing and Staining, R-W-MB-707-01

PURPOSE

To provide instructions for the preparation and staining of a gram stain slide from microbiology specimens.

RELATED DOCUMENTS

R-W-MB-701	Gram Stain Interpretation
R-W-MB-404	Sputum Adequacy

SUPPLIES

1. Crystal Violet
2. Gram's iodine
3. Decolorizer, can be purchased commercially or prepared by making a 50:50 preparation of Acetone and Ethanol. Reagent is good for one month from time of preparation.
   Label container.
4. Basic Fuchsin
5. All gram stain reagents must be stored in the dark at room temperature prior to use at the bench.

STEPS

1. Preparation of slide:
   - Always inoculate culture media prior to making the slide
   - Label the slide with the patient's accession number.
   - For direct smears from the original specimen, carefully roll the swab over the surface of the slide.
   - For CSF's and clear body fluid specimens:
     - Clean microscope slides with alcohol and allow to dry or use pre-cleaned slides.
     - Cytocentrifuge the sample (per your specific cytocentrifuge model's instructions) at 1,000 RPM for 4 minutes.
     - Use a sterile pipette to add the patient's sample to the chamber. Sample volume can be 0.1-0.3

mls. Samples with
less than 0.1 mls, may have increased cell loss onto the slide. The maximum amount of sample that can be added
to the chamber is 0.5 mls. Do not add albumin.

- For other body fluid specimens, centrifuge first, then place a drop on a slide and spread with a wooden stick being careful to no make the slide too thick.
- For urine specimens, do not centrifuge the specimen. Place a drop of urine directly onto the slide.
- For stains from colonies on a plate, touch the colony with a tip of a wooden stick or sterile loop and emulsify in a drop of sterile saline.
- Allow slides to air dry or place on a warming block.
- The slide is fixed using Methanol. Squirt the methanol over the specimen on the slide and allow the slide to dry prior to staining.

2. Staining the slide:
   - Place the slide on the stain rack in the sink
   - Flood the stain with crystal violet. Allow the stain to remain on the slide for 30 seconds to one minute. Rinse with tap water into the bucket in the sink.
   - Flood the slide with gram's iodine. Allow the stain to remain on the slide for 30 seconds to one minute. Rinse with tap water into the bucket in the sink.
   - Using forceps, hold the slide at an angle and gently squirt the decolorizer onto the slide until no more color runs off. The reaction can be very quick or may take a few squirts. Rinse with tap water.
   - Flood the slide with Basic Fuchsin. Allow the stain to remain on the slide for 30 seconds to one minute. Rinse with tap water into the bucket in the sink.
   - Blot the slide on a paper towel to remove excess water and allow to air dry.

QUALITY CONTROL

1. QC will be performed weekly using a QC slide prepared from known QC organisms. QC must also be performed when a new lot of reagent is opened before reporting patient samples.
2. A positive organism (gram pos cocci) which stains purple and a gram negative organism (gram neg rods) which stains pink-red will be used as the control organisms.
3. Record results on weekly QC log or in the LIS. Log lot numbers and expiration dates of stains.
4. If QC fails, repeat with a fresh slide or stain reagents. Check lot numbers expiration dates and if stain was stored properly.
5. Do not report patient results if QC does not pass.

LIMITATIONS

1. All smears must be stained properly for correct identification of bacteria. If smears are made or stained incorrectly, erroneous results may occur.
2. The decolorizing step is critical to a good stain. If too much is used the Crystal Violet will be washed away and the smear will appear all gram negative (pink-red). If not enough is used everything will appear purple.

REFERENCES

Murray, P, et al, Manual of Clinical Microbiology, 10th ed. American Society of Microbiology, 2011

Attachments:

No Attachments

Approval Signatures

Approver	Date
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Applicability

Highline Medical Center, St. Anthony Hospital, St. Clare Hospital, St. Elizabeth Hospital, St. Francis Hospital, St. Joseph Medical Center