
AB-100 GRAM MICRO-ORGANISM STAINING PROTOCOL

FIXATION: 10% Buffered Neutral Formalin

SECTION: Cut Paraffin sections at 6 microns.

STAINING PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. Place slides on staining rack and pour on **Crystal Violet Gram** (AB-100-1), for two minutes.
3. Rinse in distilled water.
4. Mordant in **Gram's Iodine** (SO-347), for five minutes.
5. Rinse in distilled water.
6. Differentiate in **Grams Decolorizer** (AB-100-4), until blue color no longer streams away from the section (approximately 5-10 seconds).
7. Quickly rinse in distilled water.
8. **NOTE: If the section appears too blue repeat steps 6 and 7.**
9. Stain for 15 seconds in **Safranin O Counterstain** (AB-100-3). Immediately blot away excess, but not to dryness).
10. Dehydrate absolute alcohol and clear.
11. Mount with **Permount** (M-18).

RESULTS:

Gram Negative Bacteria	Red
Gram Positive Bacteria	Blue/black
Nuclei	Red

NOTES: Preferably, all stains and solutions are applied to the slide while it is in a horizontal Position, except for steps 4 and 9 where the slide should be dipped into the solutions contained in coplin jars.

This procedure does not work well on Bouin's fixed tissues.

REFERENCES: IHCWORLD Life Science Information Network.