
**A-106 BROWN-HOPPS METHOD FOR GRAM
POSITIVE AND GRAM NEGATIVE BACTERIA**

FIXATION: 10% Buffered Neutral Formalin (F-113)

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 Solution, 1% Aqueous** (A-106-1), for two minutes. Rinse in distilled water.
3. Mordant in **Gram's Iodine** (A-106-2), for five minutes. Rinse in distilled water.
4. Differentiate in **Cellosolve** (A-106-5), until blue color no longer streams away from the section (approximately 5-10 seconds). (See note)
5. Quickly rinse in distilled water. (See note)
6. Stain in **Basic Fuchsin Solution, 0.5%, Aqueous** (A-106-3), for five minutes. Rinse in distilled water.
7. **Gallego's Solution** (A-106-4), for five minutes. (Differentiates and "fixes" the Basic Fuchsin).
8. Rinse thoroughly in distilled water and blot lightly to remove excess water (not to dryness).
9. Stain for three seconds in **Tartrazine Solution** (A-106-6). Immediately blot away excess, but not to dryness).
10. **Cellosolve** (A-106-5), three changes, for six quick dips in each. (See note)
11. Xylene, three changes, for 10 dips each. Slides may remain in Xylene until ready for mounting.
12. Mount with **Permount** (M-18).

RESULTS:

Gram Negative Bacteria	Red
Gram Positive Bacteria	Blue
Background	Yellow

NOTES: Preferably, all stains and solutions are applied to the slide while it is in a horizontal position, except for steps 4, 10 and 11, where the slide should be dipped into the solutions contained in coplin jars.

Steps 4, 5, 9 and 10 are critical with regard to timing.

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

REFERENCES:

AFIP Manual of Histologic Staining Methods; ed. G. Luna, McGraw-Hill Publications, 3rd. ed.; New York, c. 1968, p.224.

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