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A-105 BROWN AND BRENN METHOD FOR GRAM+ AND GRAM- BACTERIA

FIXATION: **Formalin, 10% Buffered Neutral** (F-113)
TECHNIQUE: Cut **paraffin** at 6 microns
*Recommended technique includes a control slide.

STAINING PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. Mix 1.0ml (20 drops) **Crystal Violet, 1% Aq.** (A-105-1), with 5 drops **Sodium Bicarbonate, 5% Aq.** (A-105-2); pour onto slides held in a staining rack. Agitate gently to cover section. Stain slides for 1 min. Rinse in distilled water.
3. Flood with **Gram's Iodine** (A-105-4), 1 min. Rinse with water and carefully blot with filter paper to complete dryness.
4. Decolorize with **Acetone-Alcohol, 1:1** (A-105-5) by dropping onto the slide until no more color runs off.
5. Stain in the **Basic Fuchsin Working** (A-105-3A) or (dilute one vol. **Basic Fuchsin Stock, 0.25%, Aq.,** (A-105-3) with 10 vol. distilled water), 1 minute; wash in water, blot carefully but not to complete dryness as in step #3.
6. Differentiate in **Acetone,** (A-105-7), one quick dip, then transfer immediately to the **Picric Acid – Acetone Solution, 0.1%** (A-105-6) to complete. Differentiate until sections show yellowish-pink.
7. Rinse quickly in **Acetone;** then **Acetone-Xylene,** (A-105-8).
8. Clear in 3-4 changes **Xylene,** (C-120).
9. Mount with **Permount** (M-18).

RESULTS:

Gram+ Bacteria, Nocardia and Actinomyces Filaments	blue
Gram- Bacteria, Nuclei	red
Additional tissue elements	yellow

Note: See also the Taylor modification of the Brown and Brenn+/- technique noted for the varying differentiation available. Over-differentiation in the B&B step #6 is a problem with some sections; run the control slides at varying rates to determine the amount for the specific organism.

REFERENCES:

Brown, J.H. and Brenn, L. Bull. Johns Hopkins Hosp., 48:69 (1931).
AFIP Manual of Histologic Staining Techniques: 3rd. ed., ed. G. Luna; New York: McGraw-Hill Publications, c. 1968, p. 222.