## Center for MusculoSkeletal Research



## Brown – Brenn Modified Gram Stain

Using Gram, Brown-Brenn Modified Stain Kit, Newcomer Supply cat#9123A

## **Procedure:**

- 1. Deparaffinize and rehydrate slides to distilled water.
- 2. Stain in freshly filtered **Crystal Violet (solution A)** for **30 seconds**.
- 3. Rinse quickly until clear in distilled water, 4 dips.
- 4. Stain slides in Gram Iodine Solution (solution B) for 1 minute.
- 5. Rinse quickly until clear in distilled water, **4 dips**.
- 6. Decolorize samples in Acetone-Alcohol 1:1 (solution C) for 3 dips.
- 7. Counterstain in Basic Fuchsin (solution D), agitate slides and stain for 3 minutes.
- 8. Rinse quickly until clear in distilled water, **4 dips**.
- 9. Dip once in **Acetone**.
- 10. Dip in Picric Acid Acetone (solution E) 4 dips.
- 11. Hold in Acetone Xylene (Solution F) during differentiation.
- 12. Clear slides in 3 changes of xylene and cover slip.

Results: Gram positive bacteria: blue Gram negative bacteria: red Nuclei: red Background tissue: yellow

8/2018



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