Safranin O Stain 0.25%, Aqueous for Gram Staining of Smears

Technical Memo

**SOLUTION:**

- Safranin O Stain 0.25%, Aqueous
- Crystal Violet Stain 1%, Aqueous, Brown-Hopps
- Iodine, Gram, Aqueous
- Acetone, ACS

500 ml

Part 1360A

Part 1014

Part 1140

Part 10014

**APPLICATION:**

Newcomer Supply Safranin O Stain 0.25%, Aqueous provides the preferred counterstain for Gram staining of microbiology smears. The Gram Stain technique, is used for differential staining of gram-positive and gram-negative bacteria in both smears and tissue sections.

**METHOD:**

**Technique:** Flat staining rack method.

**Solutions:** All solutions are manufactured by Newcomer Supply, Inc.

**STAINING PROCEDURE:**

1. Prepare within an accepted time frame, a well-made smear(s) per laboratory protocol, with a focus on uniform distribution of material.
2. Allow slides to thoroughly air-dry before staining.
3. Fix slides according to laboratory protocol.
   a. See Procedure Note #1.
4. Place slides on flat staining rack suspended over sink.
   a. See Procedure Note #2.
5. Flood fixed smears with Crystal Violet Stain 1%, Aqueous, Brown-Hopps (1041) for 45-60 seconds.
6. Drain off Crystal Violet Stain and rinse well in distilled water.
7. Mordant smears in Iodine, Gram, Aqueous (1140) for 45-60 seconds.
8. Rinse well in running tap water to remove excess iodine.
9. Blot one slide at a time and individually decolorize in Acetone (10014) until color stops running off the smear; 5-10 seconds.
   a. See Procedure Note #3.
10. Quickly rinse in distilled water to remove excess acetone.
11. Counterstain in Safranin O Stain 0.25%, Aqueous for 30-60 seconds.
   a. See Procedure Note #4.
12. Wash well in distilled water.
13. Allow slides to air-dry.
14. Examine microscopically with oil immersion.

**RESULTS:**

- **Gram-positive bacteria**
  - Blue to blue/black
- **Gram-negative bacteria**
  - Pink to red
- **Background**
  - Yellow

**PROCEDURE NOTES:**

1. Gentle heat or methanol are both accepted methods of smear fixation.
2. Do not allow smears to dry out at any point during staining procedure.
3. To decolorize slower, dip slides in Alcohol, Ethyl Denatured, 95% (10842) in Coplin jar for 10-60 seconds or until color stops running off the smear.
4. For enhanced counterstaining:
   a. Safranin O Stain 0.25%, Aqueous 10 ml
   b. 95% Ethyl Alcohol (10842) 1 ml
   c. Combine; mix well and stain for 30-60 seconds.

**REFERENCES:**

5. Modifications developed by Newcomer Supply Laboratory.

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