Crystal Violet Stain 1%, Aqueous, Brown-Hopps for Gram Stain Technical Memo

SOLUTION:
Crystal Violet Stain 1%, Aqueous, Brown-Hopps 500 ml Part 1041A

Additionally Needed:
- Gram Multi-Tissue, Artificial Control Slides Part 4256 or Gram, Gram+ & Gram- Bacteria, Artificial Control Slides Part 4255
- Xylene, ACS Part 1445
- Alcohol, Ethyl Denatured, 100% Part 10841
- Alcohol, Ethyl Denatured, 95% Part 10842
- Iodine, Gram, Aqueous Part 1140
- Acetone, ACS Part 10014
- Basic Fuchsine Stain 0.25%, Aqueous Part 1011
- Gallego Solution Part 1098
- Picric Acid-Acetone 0.05% Part 13351
- Acetone-Xylene 1:1 Part 10015

For storage requirements and expiration date refer to individual product labels.

APPLICATION:
Newcomer Supply Gram Stain, Brown-Hopps, a modification of the original Gram Stain technique, is used for differential staining of gram-positive and gram-negative bacteria in tissue sections.

METHOD:
Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 5 microns
Solutions: All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below.

STAINING PROCEDURE:
1. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
   a. See Procedure Notes #1 and #2.
2. Stain slides in Crystal Violet Stain 1%, Aqueous, Brown-Hopps for 2 minutes.
3. Rinse well in distilled water to remove excess stain.
4. Mordant in Iodine, Gram, Aqueous (1140) for 5 minutes. Sections should turn black.
5. Rinse well in running tap water to remove excess iodine.
6. Blot one slide at a time and individually decolorize in Acetone (10014) until the blue color stops running, 1-2 dips. Sections should be very light gray in color.
7. Quickly rinse in running tap water to remove excess acetone.
8. Place in Basic Fuchsine Stain 0.25%, Aqueous (1011) for 5 minutes.
9. Rinse well in running tap water.
10. Differentiate sections in Gallego Solution (1098) for 5 minutes.
11. Rinse thoroughly in running tap water. Blot excess water off slide, but not to dryness.
   a. Proceed with Steps #12 to #15 one slide at a time.
13. Dip directly in Picric Acid-Acetone 0.05% (13351) for 3-10 dips.
15. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:
- Gram-positive bacteria Blue
- Gram-negative bacteria Red
- Nuclei Red
- Background tissue Yellow

PROCEDURE NOTES:
1. Drain staining rack/slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during staining procedure.
3. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:
4. Modifications developed by Newcomer Supply Laboratory.