Brown-Hopps Modified Gram Stain with Tartrazine - Technical Memo

**SOLUTION:**

<table>
<thead>
<tr>
<th>500 ml</th>
<th>1 Liter</th>
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<tr>
<td>Tartrazine Stain 1.5%, Aqueous</td>
<td>Part 14015A</td>
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Additionally Needed:

| Gram, Multi-Tissue, Artificial Control Slides | Part 4256 | or Gram+ & Gram- Bacteria, Artificial Control Slides | Part 4255 |
| Xylene, ACS | Part 1445 |
| Alcohol, Ethyl Denatured, 100% | Part 10841 |
| Alcohol, Ethyl Denatured, 95% | Part 10842 |
| Crystal Violet Stain 1%, Aqueous, Brown-Hopps | Part 1041 |
| Iodine, Gram, Aqueous | Part 1140 |
| Acetone, ACS | Part 10014 |
| Basic Fuchsin Stain 0.25%, Aqueous | Part 1011 |
| Gallego Solution | Part 1098 |
| Acetone-Xylene 1:1 | Part 10015 |

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

**APPLICATION:**

Newcomer Supply Brown-Hopps Modified Gram Stain with Tartrazine is a modification of the original Gram stain technique. Tartrazine, a synthetic water soluble, lemon yellow colored azo dye, provides a safe alternative to picric acid stains. Tartrazine Stain 1.5%, Aqueous provides yellow background staining, replacing the picric acid-acetone counterstain and the disposable issues associated with picric acid.

**METHOD:**

**Fixation:** Formalin 10%, Phosphate Buffered (Part 1090)

**Technique:** Paraffin sections cut at 4 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 provided staining procedure.

**STAINING PROCEDURE:**

1. If necessary, heat dry tissue sections/slides in oven
2. 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.
3. Stain in Crystal Violet Stain 1%, Aqueous, Brown-Hopps (1041) for 2 minutes.
4. Rinse well in distilled water.
5. Mordant in Iodine, Gram, Aqueous (1140) for 5 minutes.
6. Rinse well in distilled water.
7. Blot excess water from slide; decolorize one slide at a time in Acetone (10014) until the blue stops running, 1-2 dips. 
   a. Sections should be very light gray in color.
8. Quickly rinse in running tap water.
9. Place in Basic Fuchsin Stain 0.25% Aqueous (1011) for 5 minutes.
10. Rinse well in running tap water.
11. Differentiate sections in Gallego Solution (1098) for 5 minutes.
12. Rinse in running tap water. Blot water off slide(s) but not to dryness. 
   a. Proceed with Steps #13 to #16 one slide at a time.
13. Place in Tartrazine Stain 1.5%, Aqueous for 1 minute.
14. Rinse well in running tap water.
17. 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 slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during procedure.
3. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

**REFERENCES:**

5. Modifications developed by Newcomer Supply Laboratory.

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