Twort’s Gram Stain Set - Technical Memo

**SET INCLUDES:**

**Part 14034A**

| Solution A: Neutral Red Stain 1% | 250 ml |
| Solution B: Fast Green Stain 1% | 100 ml |

**Part 14034B**

| Solution A: Neutral Red Stain 1% | 500 ml |
| Solution B: Fast Green Stain 1% | 200 ml |

- **Additionally Needed For Gram Stain, Hucker-Twort:**
  - Gram, Multi-Tissue, Artificial Control Slides: Part 4256
  - Crystal Violet-Oxalate Stain, Alcoholic, Hucker-Twort: Part 10422
  - Iodine, Weigert & Lugol, Aqueous: Part 12092
  - Xylene, ACS: Part 1445
  - Alcohol, Ethyl Denatured, 100%: Part 10841
  - Alcohol, Ethyl Denatured, 95%: Part 10842
  - Acetone, ACS: Part 10014

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

**APPLICATION:**

Newcomer Supply Twort’s Gram Stain Set provides stain solutions for the Gram Stain, Hucker-Twort, a rapid and simple procedure that stains gram-positive and gram-negative bacteria without the use of picric acid. A Fast Green secondary counterstain provides the green background for clear detection of any red gram-negative bacteria present.

**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 Sets are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below. Some solutions in the set may contain extra volumes.

**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 Note #1.**
2. Stain in Crystal Violet-Oxalate Stain, Alcoholic, Hucker-Twort (10422) for 30 seconds.
3. Rinse quickly in distilled water.
4. Mordant in Iodine, Weigert & Lugol, Aqueous (12092) for 20 seconds.
5. Rinse quickly in distilled water.
6. Decolorize one slide at a time with Acetone, ACS (10014) until majority of the purple stain is removed, and tissue remains light gray. Approximately 2 quick dips.
7. Rinse quickly in distilled water.
8. Prepare fresh Twort Stain; combine and mix well. Use within 30 minutes of preparation:
   - **a. Solution A: Neutral Red Stain 1%, Alcoholic** 9 ml
   - **b. Solution B: Fast Green Stain 1%, Alcoholic** 3 ml
   - **c. Distilled Water** 30 ml
9. Stain in fresh Twort Stain for 2 minutes.
10. Rinse quickly in distilled water and carefully blot dry.
11. Agitate slides quickly in clean Acetone, ACS to dehydrate (do not use any alcohols).
   - **a. See Procedure Notes #2 and #3.**
12. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

**RESULTS:**

- Gram-positive bacteria: Dark blue
- Gram-negative bacteria: Red
- Cytoplasm and red blood cells: Shades of green
- Nuclei: Red

**PROCEDURE NOTES:**

1. Drain staining rack/slides after each step to prevent solution carry over.
2. To tone down excessive red staining, add extra dips in acetone to differentiate and dehydrate the section. Check microscopically to ensure that over-differentiation does not occur.
3. Do not use any alcohol dehydration steps. The Neutral Red will be removed with alcohol exposure.
4. If using a xylene substitute, closely follow the manufacturer’s recommendations for deparaffinization and clearing steps.

**REFERENCES:**

4. Modifications developed by Newcomer Supply Laboratory.