

## Twort's Gram Stain Set - Technical Memo

### SET INCLUDES:

Solution A: Neutral Red Stain 1%, Alcoholic  
Solution B: Fast Green Stain 1%, Alcoholic

### Part 14034A

250 ml  
100 ml

### Part 14034B

500 ml  
200 ml

### Additionally Needed For Gram Stain, Hucker-Twort:

Gram, Multi-Tissue, Artificial Control Slides  
Crystal Violet-Oxalate Stain, Alcoholic, Hucker-Twort  
Iodine, Weigert & Lugol, Aqueous  
Xylene, ACS  
Alcohol, Ethyl Denatured, 100%  
Alcohol, Ethyl Denatured, 95%  
Acetone, ACS

Part 4256  
Part 10422  
Part 12092  
Part 1445  
Part 10841  
Part 10842  
Part 10014

or

Gram, Gram+ & Gram- Bacteria, Artificial Control Slides

Part 4255

**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:

- 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.
  - See Procedure Note #1.
- Stain in Crystal Violet-Oxalate Stain, Alcoholic, Hucker-Twort (10422) for 30 seconds.
- Rinse quickly in distilled water.
- Mordant in Iodine, Weigert & Lugol, Aqueous (12092) for 20 seconds.
- Rinse quickly in distilled water.
- 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.
- Rinse quickly in distilled water.
- Prepare fresh Twort Stain; combine and mix well. Use within 30 minutes of preparation:
  - Solution A: Neutral Red Stain 1%, Alcoholic 9 ml
  - Solution B: Fast Green Stain 1%, Alcoholic 3 ml
  - Distilled Water 30 ml
- Stain in fresh Twort Stain for 2 minutes.
- Rinse quickly in distilled water and carefully blot dry.
- Agitate slides quickly in clean Acetone, ACS to dehydrate (do not use any alcohols).
  - See Procedure Notes #2 and #3.
- 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:

- Drain staining rack/slides after each step to prevent solution carry over.
- 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.
- Do not use any alcohol dehydration steps. The Neutral Red will be removed with alcohol exposure.
- If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

### REFERENCES:

- Bancroft, John D., and Alan Stevens. *Theory and Practice of Histological Techniques*. 3rd ed. Edinburgh: Churchill Livingstone, 1990. 290-292.
- Culling, C.F.A. *Handbook of Histopathological and Histochemical Techniques (including museum techniques)*. 3rd ed. London: Butterworth, 1974. 393-395.
- Twort, F.W., "An Improved Neutral Red, Light Green Double Staining for Animal Parasites, Microorganisms and Tissues". *Journal of State Medicine* 32. (1924). 351.
- Modifications developed by Newcomer Supply Laboratory.