

## 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 Part 4256 or  
Crystal Violet-Oxalate Stain, Alcoholic Part 10422  
Iodine, Lugol's, Aqueous Part 12092  
Xylene, ACS Part 1445  
Alcohol, Ethyl Denatured, 100% Part 10841  
Alcohol, Ethyl Denatured, 95% Part 10842  
Acetone, ACS Part 10014

Gram+ & Gram- Bacteria, Artificial Control Slides Part 4255

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

### APPLICATION:

Newcomer Supply Twort's Gram Stain Set provides stain solutions for the Gram Stain, Hucker-Twort, a simple, rapid procedure for staining gram-positive and gram-negative bacteria without picric acid. The Twort Stain combines Neutral Red and Fast Green, for clear detection of red gram-negative bacteria with a green counterstain.

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

### PRESTAINING PREPARATION:

1. If necessary, heat dry tissue sections/slides in oven.
2. Filter Crystal Violet-Oxalate Stain, Alcoholic (10422) with high quality filter paper.

### STAINING PROCEDURE:

3. 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.
4. Stain in freshly filtered Crystal Violet-Oxalate Stain, Alcoholic, (Step #2) for 30 seconds.
5. Rinse quickly in distilled water.
6. Mordant in Iodine, Lugol's, Aqueous (12092) for 20 seconds.
7. Rinse quickly in distilled water.
8. Decolorize individually with Acetone, ACS (10014); 2 quick dips.
  - a. Or until tissue remains light gray.
9. Rinse quickly in distilled water.
10. Prepare fresh Twort Stain; combine and mix well.
  - 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
  - d. Use within 30 minutes.
11. Stain in fresh Twort Stain for 2 minutes.
12. Rinse quickly in distilled water; carefully blot dry.
13. Agitate slides quickly in clean Acetone, ACS to remove excess stain and dehydrate (do not use any alcohols).
  - a. The use of alcohols will remove Neutral Red.
14. 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 slides after each step to prevent solution carry over.
2. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

### REFERENCES:

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