NEWCOMER SUPPLY®
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Part 9125
Revised November 2019

Gram, Hucker-Twort Stain Kit - Technical Memo

KIT INCLUDES:	**Part 9125A**
Solution A: Crystal Violet-Oxalate Stain, Alcoholic	250 ml
Solution B: Iodine, Weigert & Lugol, Aqueous	250 ml
Solution C: Neutral Red Stain 1%, Alcoholic	125 ml
Solution D: Fast Green Stain 1%, Alcoholic	50 ml

COMPLIMENTARY POSITIVE CONTROL SLIDES: Enclosed with this kit are two complimentary unstained positive control slides to be used for the initial verification of staining techniques and reagents. Verification must be documented by running one Newcomer Supply complimentary positive control slide along with your current positive control slide for the first run. Retain the second complimentary control slide for further troubleshooting, if needed.

Individual stain solutions and additional control slides may be available for purchase under separate part numbers at www.newcomersupply.com.

Additionally Needed:

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 Gram, Hucker-Twort Stain Kit is a rapid and simple procedure that stains gram-positive and gram-negative bacteria without the use of picric acid. The Neutral Red Stain combined with a Fast Green counterstain provides clear detection of any red gram-negative bacteria present against a green background.

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

PRESTAINING PREPARATION:

1. If necessary, heat dry tissue sections/slides in oven.
2. Filter Solution A: Crystal Violet-Oxalate Stain, Alcoholic 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 Solution A: Crystal Violet-Oxalate Stain, Alcoholic (Step #2) for 30 seconds.
5. Rinse quickly in distilled water.
6. Mordant in Solution B: Iodine, Weigert & Lugol, Aqueous for 20 seconds.
7. Rinse quickly in distilled water.
8. Decolorize individually with Acetone (10014); 2 quick dips.
    a. *Or until majority of purple stain is removed and tissue remains light gray.*
9. Rinse quickly in distilled water.
10. Prepare fresh Twort Stain; combine and mix well.
    a. *Solution C: Neutral Red Stain 1%, Alcoholic 9 ml*
    b. *Solution D: 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 (10014) to dehydrate; do not use any alcohols.
    a. *See Procedure Notes #2 and #3.*
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 needed, add extra dips in acetone to further differentiate and dehydrate sections.
    a. *Check microscopically to avoid over-differentiation.*
3. The use of alcohol will remove Neutral Red staining.
4. 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.
