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Part 10422
Revised April 2025

Crystal Violet-Oxalate Stain, Alcoholic for Brown-Brenn Gram Stain - Technical Memo

SOLUTION:	**250 ml**	**500 ml**
Crystal Violet-Oxalate Stain, Alcoholic	Part 10422A	Part 10422B

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			
Iodine, Gram, Aqueous	Part 1140			
Acetone-Alcohol 1:1	Part 10016			
Basic Fuchsin Stain 0.25%, Aqueous	Part 1011			
Tartrazine Stain 0.25%, Acetic Aqueous	Part 14016			
Acetone-Xylene 1:1	Part 10015			

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

APPLICATION:

Newcomer Supply Crystal Violet-Oxalate Stain, Alcoholic, a component of the Brown-Brenn Gram Stain, is used for differential staining of gram-positive and gram-negative bacteria in tissue sections, cultures and smears. Tartrazine provides the yellow background in this procedure, replacing picric acid-acetone counterstain.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 4 microns and cultures/smears.
a. *See Procedure Note #1.*
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.

PRESTAINING PREPARATION:

1. If necessary, heat dry tissue sections/slides in oven.
2. Filter 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 Notes #2 and #3.*
4. Stain in freshly filtered Crystal Violet-Oxalate Stain, Alcoholic for 1 minute.
5. Rinse in distilled water.
6. Mordant in Iodine, Gram, Aqueous (1140) for 1 minute.
7. Rinse well in distilled water; removing excess iodine.
8. Decolorize one slide at a time in Acetone-Alcohol 1:1 (10016) until blue stops running. Approximately 7-10 dips.
9. Rinse well in distilled water.
10. Place in Basic Fuchsin Stain 0.25%, Aqueous (1011); 90 seconds.
11. Rinse well in distilled water.
12. Dip once in Acetone-Alcohol 1:1.
13. Counterstain in Tartrazine Stain 0.25%, Acetic Aqueous (14016) for 5-15 seconds.
14. Rinse well in distilled water.
15. Dehydrate in two changes of 100% ethyl alcohol, 5 dips each. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.
    a. *Do not use 95% alcohol in the dehydration step.*

RESULTS:

Gram-positive bacteria	Blue
Gram-negative bacteria	Red
Nuclei	Red
Background tissue	Yellow

PROCEDURE NOTES:

1. For cultures/smears: Prepare a well-made culture/smear with a focus on uniform cell distribution.
   a. *Timings in this protocol are based on paraffin sections and may need to be altered for culture/smear staining.*
2. Drain slides after each step to prevent solution carry over.
3. Do not allow sections to dry out at any point during procedure.
4. If using a xylene substitute, follow manufacturer's recommendation for deparaffinization and clearing steps.

REFERENCES:

1. Bancroft, John D. and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 312-313.
2. Brown, J.H. and L. Brenn. "A Method for the Differential Staining of Gram Positive and Gram Negative Bacteria in Tissue Sections". *Bulletin of The Johns Hopkins* 48.2 (1931): 69-73.
3. Luna, Lee G. *Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. Gaitheresburg, MD: American Histolabs, 1992. 188-189.
4. Modifications developed by Newcomer Supply Laboratory.

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Part 10422
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Crystal Violet-Oxalate Stain, Alcoholic for Hucker-Twort Gram Stain - Technical Memo

SOLUTION:	**250 ml**	**500 ml**
Crystal Violet-Oxalate Stain, Alcoholic	Part 10422A	Part 10422B

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			
Iodine, Lugol's, Aqueous	Part 12092			
Acetone, ACS	Part 10014			
Twort's Gram Stain Set	Part 14034			
Solution A: Neutral Red Stain 1%, Alcoholic				
Solution B: Fast Green Stain 1%, Alcoholic				

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

APPLICATION:

Newcomer Supply Crystal Violet-Oxalate Stain, Alcoholic, a component of the Hucker-Twort Gram Stain that stains gram-positive bacteria. 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 (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.

PRESTAINING PREPARATION:

1. If necessary, heat dry tissue sections/slides in oven.
2. Filter 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 Crystal Violet-Oxalate Stain, Alcoholic for 30 seconds.
5. Rinse quickly in distilled water.
6. Mordant in Iodine, Weigert, Lugol's, Aqueous (12092); 20 seconds.
7. Rinse quickly in distilled water.
8. Decolorize individually with Acetone, ACS (10014); 2 quick dips.
9. Rinse quickly in distilled water.
10. Prepare fresh Twort Stain (14034); combine and mix well.
    a. *Neutral Red Stain 1%, Alcoholic 9 ml*
    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 Stain.*
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, follow manufacturer's recommendation 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 (including museum 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.
