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Part 10422
Revised November 2019

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			
Acetone, ACS	Part 10014			
Picric Acid-Acetone 0.1%	Part 1335			
Acetone-Xylene 1:1	Part 10015			

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

APPLICATION:

Newcomer Supply Gram Stain, Brown-Brenn is the traditional method used for differential staining of gram-positive and gram-negative bacteria in tissue sections, cultures and smears.

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 30 seconds.
5. Rinse well in several changes of distilled water.
6. Mordant in Iodine, Gram, Aqueous (1140) for 1 minute.
7. Rinse in distilled water; blot excess water from slide, but not from the tissue section.
8. Decolorize one slide at a time by dipping in Acetone-Alcohol 1:1 (10016) until blue color stops running. Approximately 1-3 dips.
9. Counterstain in Basic Fuchsin Stain 0.25%, Aqueous (1011) for 3 minutes.
10. Rinse in distilled water; blot excess water from slide, but not from the tissue section.
    a. Proceed with Steps #11 to #14 one slide at a time.
11. Dip once in Acetone (10014).
12. Dip in Picric Acid-Acetone 0.1% (1335) until sections have a yellowish-pink color, 3-10 dips. Agitate slides until desired intensity is achieved.
13. Dip in Acetone-Xylene 1:1 (10015), 5-10 dips.
    a. Check control microscopically for proper differentiation.
    b. Repeat Step #12 if additional differentiation is needed.
14. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

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

PROCEDURE NOTES:

1. For cultures/smears: Prepare within an accepted time frame a well-made culture/smear per laboratory protocol with a focus on uniform cell distribution. Timings offered in this protocol are based on paraffin sections and may need to be altered for optimal 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, closely follow the manufacturer's recommendations 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, Weigert & Lugol, 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 Gram Stain, Hucker-Twort is a rapid and simple procedure that stains gram-positive and gram-negative bacteria without the use of picric acid. The 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 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, Aqueous (12092); 20 seconds.
7. Rinse quickly in distilled water.
8. Decolorize one slide at a time with Acetone (10014) until majority of the purple stain is removed, and tissue remains light gray. Approximately 2 quick dips.
9. Rinse quickly in distilled water.
10. Prepare fresh Twort Stain (14034); combine and mix well. Use within 30 minutes of preparation:
    a. *Neutral Red Stain 1%, Alcoholic 9 ml*
    b. *Fast Green Stain 1%, Alcoholic 3 ml*
    c. *Distilled Water 30 ml*
11. Stain in fresh Twort Stain for 2 minutes.
12. Rinse quickly in distilled water and carefully blot dry.
13. Agitate slides quickly in clean Acetone to dehydrate; do not use any alcohols.
    b. *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. To tone down excessive red staining, add extra dips in acetone to differentiate and dehydrate the section.
    a. *Check microscopically to ensure that over-differentiation does not occur.*
3. Do not use alcohol in 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:

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.