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Part 1041
Revised March 2017

Crystal Violet Stain 1%, Aqueous, Brown-Hopps for Gram Stain Technical Memo

SOLUTION:	**500 ml**
Crystal Violet Stain 1%, Aqueous, Brown-Hopps	Part 1041A

Additionally Needed:

Gram, Multi-Tissue, Artificial Control Slides	Part 4256	or	Gram, 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, ACS	Part 10014			
Basic Fuchsin Stain 0.25%, Aqueous	Part 1011			
Gallego Solution	Part 1098			
Picric Acid-Acetone 0.05%	Part 13351			
Acetone-Xylene 1:1	Part 10015			

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

APPLICATION:

Newcomer Supply Gram Stain, Brown-Hopps, a modification of the original Gram Stain technique, is used for differential staining of gram-positive and gram-negative bacteria in tissue sections.

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 procedures are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below.

STAINING PROCEDURE:

1. 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 #1 and #2.*
2. Stain slides in Crystal Violet Stain 1%, Aqueous, Brown-Hopps for 2 minutes.
3. Rinse well in distilled water to remove excess stain.
4. Mordant in Iodine, Gram, Aqueous (1140) for 5 minutes. Sections should turn black.
5. Rinse well in running tap water to remove excess iodine.
6. Blot one slide at a time and individually decolorize in Acetone (10014) until the blue color stops running, 1-2 dips. Sections should be very light gray in color.
7. Quickly rinse in running tap water to remove excess acetone.
8. Place in Basic Fuchsin Stain 0.25%, Aqueous (1011) for 5 minutes.
9. Rinse well in running tap water.
10. Differentiate sections in Gallego Solution (1098) for 5 minutes.
11. Rinse thoroughly in running tap water. Blot excess water off slide, but not to dryness.
    a. *Proceed with Steps #12 to #15 one slide at a time.*
12. Dip quickly in Acetone, 1-2 dips.
13. Dip directly in Picric Acid-Acetone 0.05% (13351) for 3-10 dips.
14. Dip quickly in Acetone-Xylene 1:1 (10015) for 5 dips.
15. 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. Drain staining rack/slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during staining procedure.
3. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Carson, Freida L., and Christa Hladik Cappellano. Histotechnology: A Self-instructional Text. 4th ed. Chicago: ASCP Press, 2015. 222-224.
2. Chladny, M. Jane. "Batch Staining for Demonstration of Gram Positive and Gram Negative Bacteria in Tissue Sections." *The Journal of Histotechnology* 15.1 (1992): 49-50.
3. Luna, Lee G. *Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. Gaitheresburg, MD: American Histolabs, 1992. 194-195.
4. Modifications developed by Newcomer Supply Laboratory.