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Part 9123
Revised June 2016

Gram, Brown-Brenn Modified Stain Kit - Technical Memo

KIT INCLUDES:	**Part 9123A**
Solution A: Crystal Violet-Oxalate Stain, Alcoholic	250 ml
Solution B: Iodine, Gram, Aqueous	250 ml
Solution C: Acetone-Alcohol 1:1	250 ml
Solution D: Basic Fuchsin Stain 0.25%, Aqueous	250 ml
Solution E: Picric Acid-Acetone 0.1%	250 ml
Solution F: Acetone-Xylene 1:1	250 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, Brown-Brenn Modified Stain Kit procedure 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 5 microns and cultures/smears
  a. *See Procedure Note #1.*
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 staining procedure provided below. Some solutions in the kit may contain extra volumes.

STAINING PROCEDURE:

1. Filter Solution A: Crystal Violet-Oxalate Stain, Alcoholic with high quality filter paper.
2. 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.*
3. Stain slides in freshly filtered Solution A: Crystal Violet-Oxalate Stain, Alcoholic for 30 seconds.
4. Rinse well in several changes of distilled water, ensuring excess Crystal Violet-Oxalate Stain is removed.
5. Mordant in Solution B: Iodine, Gram, Aqueous for 1 minute.
6. Rinse well in distilled water, ensuring excess iodine is removed. Blot excess water from slide, but not from the tissue section.
7. Decolorize one slide at a time by dipping in Solution C: Acetone-Alcohol 1:1 until blue color stops running. Approximately 1-3 dips.
8. Counterstain in Solution D: Basic Fuchsin Stain 0.25%, Aqueous for 3 minutes.
9. Rinse in distilled water and blot excess water from slide, but not from the tissue section.
   a. *Proceed with Steps #10 to #13 one slide at a time.*
10. Dip once in Acetone (10014).
11. Dip in Solution E: Picric Acid-Acetone 0.1% until sections have a yellowish-pink color, 3-10 dips. Agitate slides until desired intensity is achieved.
12. Dip in Solution F: Acetone-Xylene 1:1, 5-10 dips. Check the control microscopically for proper differentiation.
   a. *Repeat Step #11 if additional differentiation is needed.*
13. 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 your laboratories protocol with a focus on uniform cell distribution. The timings offered in this protocol are based on paraffin sections and may need to be altered for optimal culture/smear staining.
2. Drain staining rack/slides after each step to prevent solution carry over.
3. Do not allow sections to dry out at any point during staining 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.
