

Part 4256 **Revised February 2024**

Gram, Multi-Tissue, Artificial Control Slides – Technical Memo

CONTROL SLIDES:

Part 4256A 10 Slide/Set Part 4256B

98 Slide/Set

PRODUCT SPECIFICATIONS:

Tissue: Gram positive staining rat lung and gram negative staining rat lung.

Fixation: Formalin 10%, Phosphate Buffered (Part 1090).

Section/Glass: Paraffin sections cut at 4 microns on Superfrost™ Plus slides.

Quality Control Stain: Brown-Brenn quality control stained slide(s) included.

Reactivity: Guaranteed product specific reactivity for one year from date of receipt. Revalidate after one year to verify continued reactivity.

Storage: 15-30°C in a light deprived and humidity controlled environment.

Intended Use: To verify histological techniques and reagent reactivity.

Before using unstained control slides, review the enclosed stained slide(s) to ensure that this tissue source is acceptable for testing needs.

CONTROL SLIDE VALIDATION:

With Gram, Brown-Brenn, Stain Kit:	Part 9123A	Individual Stain Solution
Solution A: Crystal Violet-Oxalate Stain, Alcoholic	250 ml	Part 10422
Solution B: Iodine, Gram, Aqueous	250 ml	Part 1140
Solution C: Acetone-Alcohol 1:1	250 ml	Part 10016
Solution D: Basic Fuchsin Stain 0.25%, Aqueous	250 ml	Part 1011
Solution E: Tartrazine Stain 0.25%. Acetic Aqueous	250 ml	Part 14016

APPLICATION:

Newcomer Supply Gram, Multi-Tissue, Artificial Control Slides are for the positive histochemical staining of gram positive and gram negative bacteria in separate tissue sections. Escherichia coli and Staphylococcus aureus are used to produce the positive controls.

PRESTAINING PREPARATION:

- Heat dry sections in oven according to your laboratory protocol. 1.
- 2. Filter Solution A: Crystal Violet-Oxalate Stain, Alcoholic.

STAINING PROCEDURE:

- Deparaffinize sections thoroughly in three changes of xylene, 3 З. minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water. See Procedure Notes #1 and #2. a.
- 4. Stain in freshly filtered Solution A: Crystal Violet-Oxalate Stain, Alcoholic (Step #2) for 1 minute.
- 5 Rinse well in distilled water.
- Mordant in Solution B: Iodine, Gram, Aqueous for 1 minute. 6.
- 7. Rinse well in distilled water, removing excess iodine.
- Decolorize in Solution C: Acetone-Alcohol 1:1 until blue stops 8. running; 7-10 dips.
- 9 Rinse well in distilled water.
- Place in Solution D: Basic Fuchsin Stain 0.25%, Aqueous for 90 10. seconds.
- Rinse well in distilled water. 11
- 12. Dip once in Solution C: Acetone-Alcohol 1:1.
- 13. Counterstain in Solution E: Tartrazine Stain 0.25%, Acetic Aqueous for 5-15 seconds.
- Rinse well in distilled water. 14.
- Dehydrate in two changes of 100% ethyl alcohol, 5 dips each. Clear 15. in three changes of xylene, 10 dips each; coverslip with compatible mounting medium. а.
 - Do not use 95% alcohol in the dehydration step.

RESULTS:

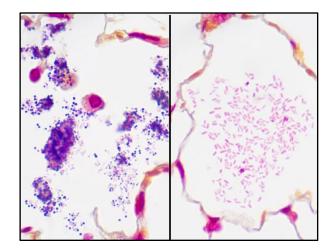
Gram negative bacteria Red Gram positive bacteria Blue/violet Background tissue Yellow

PROCEDURE NOTES:

- 1. Drain slides after each step to prevent solution carry over.
- Do not allow sections to dry out at any point during procedure. 2.
- If using a xylene substitute, follow manufacturer's recommendation 3. for deparaffinization and clearing steps.

REFERENCES:

- Bancroft, John D., and Marilyn Gamble. Theory and Practice of 1. Histological Techniques. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 312-313.
- Brown, J.H., and L. Brenn. "A Method for the Differential Staining 2. 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.
- Modifications developed by Newcomer Supply Laboratory. 4



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