

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

<b>CONTROL SLIDES:</b>	<b>Part 4256A</b>	<b>Part 4256B</b>
	10 Slide/Set	98 Slide/Set

### PRODUCT SPECIFICATIONS:

**Tissue:** Gram positive staining rat lung, gram negative staining rat lung and negative staining human lung.

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

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

**Quality Control Stain:** Brown-Hopps 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-Hopps, Stain Kit:

	<b>Part 9124A</b>	<b>Individual Stain Solution</b>
Solution A: Crystal Violet Stain 1%, Aqueous, Brown-Hopps	250 ml	Part 1041
Solution B: Iodine, Gram, Aqueous	250 ml	Part 1140
Solution C: Basic Fuchsin Stain 0.25%, Aqueous	250 ml	Part 1011
Solution D: Gallego Solution	250 ml	Part 1098
Solution E: Picric Acid-Acetone 0.05%	250 ml	Part 13351
Solution F: Acetone-Xylene 1:1	250 ml	Part 10015
Acetone, ACS		Part 10014

### 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* purchased from Remel Microbiology Products is used to produce the positive control tissue.

### NEWCOMER SUPPLY VALIDATION PROCEDURE:

- Heat dry sections in oven according to your laboratory protocol.
- 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.
  - See Procedure Notes #1 and #2.
- Stain in Solution A: Crystal Violet Stain 1%, Aqueous, Brown-Hopps for 2 minutes.
- Rinse well in distilled water, ensuring excess stain is removed.
- Mordant in Solution B: Iodine, Gram, Aqueous for 5 minutes.
  - Sections should turn black.
- Rinse well in running tap water, ensuring excess iodine is removed.
- Blot excess water from slide; decolorize one slide at a time in Acetone, ACS (10014) until blue color stops running; 1-2 dips.
  - Sections should be very light gray in color.
- Quickly rinse in running tap water to remove excess Acetone.
- Stain in Solution C: Basic Fuchsin Stain 0.25%, Aqueous; 5 minutes.
- Rinse well in running tap water.
- Differentiate sections in Solution D: Gallego Solution for 5 minutes.
- Rinse in running tap water. Blot water off slide(s), but not to dryness.
  - Proceed with Steps #13 to #16 one slide at a time.
- Dip quickly in Acetone, ACS (10014); 1-2 dips.
- Dip directly in Solution E: Picric Acid-Acetone 0.05%; 3-10 dips.
- Dip quickly in Solution F: Acetone-Xylene 1:1; 5 dips.
- Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

### RESULTS:

Gram negative bacteria	Red
Gram positive bacteria	Blue/violet
Nuclei	Red
Background tissue	Yellow
Nonreactive lung	Negative for gram positive/negative bacteria

### PROCEDURE NOTES:

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

### REFERENCES:

- Brown, Robert C., and Howard C. Hopps. "Staining of Bacteria in Tissue Sections: A Reliable Gram Stain Method." *American Journal of Clinical Pathology* 60.2 (1973): 234-240.
- Carson, Freida L., and Christa Hladik Cappellano. *Histotechnology: A Self-instructional Text*. 4th ed. Chicago: ASCP Press, 2015. 222-224.
- Modifications developed by Newcomer Supply Laboratory.

