

Gram, Gram Positive & Gram Negative Bacteria, Artificial Control Slides Technical Memo

CONTROL SLIDES:	Part 4255A	Part 4255B
	10 Slide/Set	98 Slide/Set

PRODUCT SPECIFICATIONS:

Tissue: Positive 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.

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

PRODUCT DESCRIPTION:

The enclosed positive control slides are intended to verify histological techniques and reagent reactivity. The intended use is for the qualitative purpose of determining positive or negative results, and not intended for any quantitative purpose. *Escherichia coli* and *Staphylococcus aureus* purchased from Remel Microbiology Products is used to produce the positive rat lung control tissue and the negative control sections are produced from human surgical or autopsy tissues under carefully controlled conditions. Quality control measures are used to deliver control slides that are as consistent as possible.

CONTROL SLIDE VALIDATION:

With Gram, Brown-Hopps Stain Kit:

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

APPLICATION:

Newcomer Supply Gram, Gram Positive & Gram Negative Bacteria, Artificial Control Slides are for the positive histochemical staining of gram positive and gram negative bacteria in the same tissue section.

NEWCOMER SUPPLY VALIDATION PROCEDURE:

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

RESULTS:

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

PROCEDURE NOTES:

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

REFERENCES:

1. 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.
2. Carson, Freida L., and Christa Hladik Cappellano. *Histotechnology: A Self-instructional Text*. 4th ed. Chicago: ASCP Press, 2015. 222-224.
3. Modifications developed by Newcomer Supply Laboratory.

