

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.

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:

	Part 9124A	Individual Stain Solution
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 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. *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 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 positive bacteria	Blue/violet
Gram negative bacteria	Red
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.

