

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

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

Gram, Multi-Tissue, Artificial Control Slides contain sections of gram positive staining rat lung, gram negative staining rat lung and a section of human lung nonreactive to organisms.

PRODUCT DESCRIPTION:

The enclosed positive control slides are intended to be used to verify histological techniques and reagent reactivity. These slides are to be used for the qualitative purpose of determining positive or negative results, and are not intended to be used for any quantitative purpose. The first serial section within the control box is stained and provided for your reference. **Before using the unstained slides, review the enclosed stained slide with your pathologist to ensure that this tissue source is acceptable. Newcomer Supply will not accept a return with missing slides in the series. Newcomer Supply guarantees reactivity of these control slides for one year from the date of receipt. Revalidate after one year to verify continued reactivity. Store at 15-30°C in a light deprived and humidity controlled environment.**

These Gram, Multi-Tissue, Artificial Control Slides were produced at the Newcomer Supply Laboratory under carefully controlled conditions. The positive control sections are not human tissue. The microorganisms for the gram positive and gram negative sections were grown in pure culture, harvested, formalized and each introduced into individual freshly harvested rat lungs. No infective process occurred. *Escherichia coli* and *Staphylococcus aureus* were used to produce these control slides, and were purchased from Remel Microbiology Products (R4601997 ATCC® 11229™ and R4607010 ATCC® 25923™). Reasonable measures are used to deliver quality control slides that are as consistent as possible. However, characteristics of quality control slides may be dissimilar due to variations in the reagents, stains, techniques, laboratory conditions, and tissue sources used. Newcomer Supply Laboratory uses a manual method of performing quality control procedures, specifically avoiding automation, in order to provide reactive control slides for even less aggressive methods of staining that our customers may be using.

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

For storage requirements and expiration date refer to individual product labels.

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.

METHOD:

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

Technique: Paraffin sections cut at 4 microns on Superfrost® Plus

Solutions: All solutions are manufactured by Newcomer Supply, Inc.

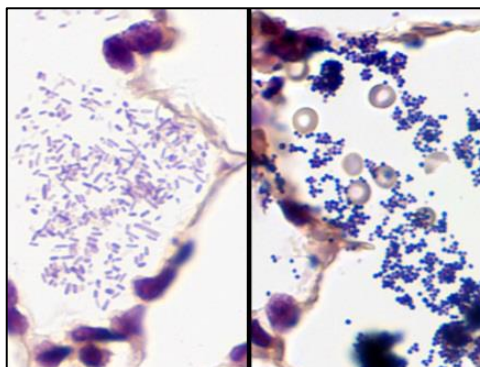
NEWCOMER SUPPLY VALIDATION PROCEDURE:

1. 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 (page 2).
2. Stain slides in Solution A: Crystal Violet Stain 1%, Aqueous, Brown-Hopps for 2 minutes.
3. Rinse well in distilled water, ensuring excess stain is removed.
4. Mordant in Solution B: Iodine, Gram, Aqueous for 5 minutes.
 - a. Sections should turn black.
5. Rinse well in running tap water, ensuring excess iodine is removed.
6. 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.
7. Quickly rinse in running tap water to remove excess Acetone.
8. Place in Solution C: Basic Fuchsin Stain 0.25%, Aqueous for 5 minutes.
9. Rinse well in running tap water.
10. Differentiate sections in Solution D: Gallego Solution for 5 minutes.

11. Rinse thoroughly in running tap water. Blot excess water off slide, but not to dryness.
 - a. Proceed with Steps #12 to #15 **one slide at a time**.
12. Dip quickly in Acetone, ACS (10014); 1-2 dips.
13. Dip directly in Solution E: Picric Acid-Acetone 0.05%; 3-10 dips.
14. Dip quickly in Solution F: Acetone-Xylene 1:1; 5 dips.
15. 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:

1. Drain staining rack/slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during staining 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. Luna, Lee G. *Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. Gaithersburg, MD: American Histolabs, 1992. 194-195.
4. Modifications developed by Newcomer Supply Laboratory.