

Cryptococcus sp. Control Slides – Technical Memo

CONTROL SLIDES: **Part 4135A** **Part 4135B**
 10 Slide/Set 98 Slide/Set

Cryptococcus sp. Control Slides contain sections of positive staining lung and negative staining lung.

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 positive control slides were produced from human surgical or autopsy tissues under carefully controlled conditions. 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 Mucin, Mayer Mucicarmine Stain Kit:	Part 9151A/B	Individual Stain Solution
Solution A: Ferric Chloride, Aqueous	125/250 ml	Part 1409
Solution B: Hematoxylin 1%, Alcoholic	125/250 ml	Part 1409
Solution C: Mucicarmine Stock Stain, Mayer	125/125 ml	Part 1250
Solution D: Metanil Yellow Stain, Aqueous	250/500 ml	Part 12235

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

APPLICATION:

Newcomer Supply *Cryptococcus sp.*, Control Slides are for the positive histochemical staining of fungal organisms. The morphology of the organism is consistent with *Cryptococcus sp.*

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 5 microns on Superfrost® Plus
Solutions: All solutions are manufactured by Newcomer Supply, Inc.

NEWCOMER SUPPLY VALIDATION PROCEDURE:

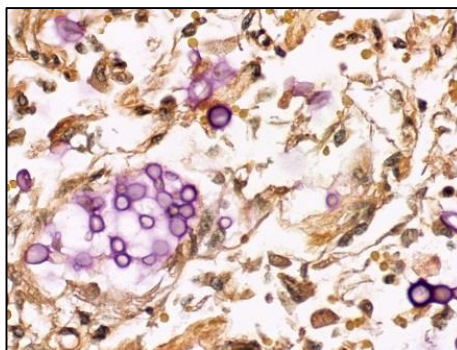
1. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
 - a. See Procedure Notes #1 and #2.
2. Prepare **fresh** Weigert Iron Hematoxylin Working Solution directly before use; combine and mix well:
 - a. Solution A: Ferric Chloride, Aqueous 20 ml
 - b. Solution B: Hematoxylin 1%, Alcoholic 20 ml
3. Stain in **fresh** Weigert Iron Hematoxylin Working Solution for 7 minutes.
4. Rinse in running tap water for 10 minutes.
5. Prepare **fresh** Mayer Mucicarmine Working Solution; combine and mix well:
 - a. Solution C: Mucicarmine Stock Stain, Mayer 10 ml
 - b. Tap Water (do not use distilled water) 30 ml
6. Stain slides in **fresh** Mayer Mucicarmine Working Solution for 60 minutes or longer if a more intense stain is desired.

Microwave Modification: See Procedure Note #3.

 - a. Place slides in a plastic Coplin jar containing **fresh** Mayer Mucicarmine Working Solution and microwave at 70°C for 10 minutes.
7. Rinse in several changes of tap water.
8. Counterstain in Solution D: Metanil Yellow Stain, Aqueous for 30 seconds to 1 minute.
9. Dehydrate quickly through 95% and 100% ethyl alcohols. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

Capsule of <i>Cryptococcus sp.</i>	Deep rose to red
Nuclei	Black
Other tissue elements	Yellow
Negative lung	Negative for fungus



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. The suggested microwave procedure has been tested at Newcomer Supply using an “EB Sciences”, 850 watt microwave oven with temperature probe and agitation tubes. This procedure is reproducible in our laboratory. It is nonetheless a guideline and techniques should be developed for your laboratory which meet the requirements of your situation. Microwave devices should be placed in a fume hood or vented into a fume hood, according to manufacturer’s instructions, to prevent exposure to chemical vapors.
4. If using a xylene substitute, closely follow the manufacturer’s recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 174-175.
2. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 142-144.
3. Luna, Lee G. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. 3rd ed. New York: Blakiston Division, McGraw-Hill, 1968. 161-162.
4. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 168-169.
5. Modifications developed by Newcomer Supply Laboratory.