Spirochete, Artificial Control Slides – Technical Memo

CONTROL SLIDES:  
Part 4655A  
10 Slide/Set  
Part 4655B  
98 Slide/Set

Spirochete, Artificial Control Slides contain sections of positive staining rat lung and negative staining human 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 Spirochete, Artificial Control Slides were produced at the Newcomer Supply Laboratory under carefully controlled conditions. The positive control sections are not human tissue. The microorganisms were grown in pure culture, harvested, formalized and introduced in a freshly harvested rat lung. No infective process occurred. Brachyspira hydrosyntenterae was used to produce these control slides, and was purchased from the American Type Culture Collection (ATCC® 27164™). 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 Laboratories 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 Steiner-Steiner Modified Silver Stain Kit:  
Solution A: Uranyl Nitrate 1%, Aqueous  
Solution B: Silver Nitrate 1%, Aqueous  
Solution C: Gum Mastic 2.5%, Alcoholic  
Ingredient D: Hydroquinone, Powder  

Individual Stain Solution  
Part 9171A  
Solution A: 250 ml Part 14036  
Solution B: 250 ml Part 13804  
Solution C: 350 ml Part 1145  
Ingredient D: 5 grams Part 12089

APPLICATION:
Newcomer Supply Spirochete, Artificial Control Slides are for the positive histochemical staining of spirochetes, the causative agent of a variety of diseases such as; syphilis, bejel, pinta, yaws and lyme.

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. All glassware/plasticware must be acid cleaned prior to use.  
a. See Procedure Notes #1 and #2 (page 2).
2. Preheat Solution A: Uranyl Nitrate 1%, Aqueous and Solution B: Silver Nitrate 1%, Aqueous to 60°C in a water bath.  
a. Skip Step #2 if using Microwave Modification.
3. 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 Note #3 (page 2).
4. Sensitize slides in preheated Solution A: Uranyl Nitrate 1%, Aqueous for 5 minutes in a 60°C water bath. Agitate solution to evenly distribute heat.
   a. See Procedure Note #4 (page 2).
   Microwave Modification: See Procedure Note #5 (page 2).  
a. Place slides in a plastic Coplin jar containing Solution A: Uranyl Nitrate 1%, Aqueous and microwave at 60°C for 5 minutes.
5. Rinse well in several changes of distilled water.
6. Prepare Hydroquinone Working Solution; combine and mix well.  
a. Ingredient D: Hydroquinone, Powder 0.5 gm  
b. Distilled Water 25 ml  
c. Save for use in Step #12.
7. Place slides in preheated Solution B: Silver Nitrate 1%, Aqueous and incubate in a 60°C water bath for 30 minutes.
   Microwave Modification:  
a. Place slides in a plastic Coplin jar containing Solution B: Silver Nitrate 1%, Aqueous and microwave at 70°C for 5 minutes.
8. Rinse well in several changes of distilled water.  
a. Excessive rinsing may cause nucleus to pick up silver.
9. Dip briefly in 2 changes each of 95% and 100% ethyl alcohols.
10. Place slides in Solution C: Gum Mastic 2.5%, Alcoholic for 5 minutes.
11. Air-dry for 1-5 minutes until slides are milky white.
12. Prepare fresh Reducing Solution by combining:  
a. Solution C: Gum Mastic 2.5%, Alcoholic 15 ml  
b. Hydroquinone Working Solution (Step #6) 25 ml  
c. Filter, then add and mix well.
   d. Solution B: Silver Nitrate 1%, Aqueous 0.3 ml
13. Preheat fresh Reducing Solution in a 45°C water bath. Place slides in preheated solution and incubate in 45°C water bath for 10-30 minutes with frequent agitation; examine microscopically at 10 minutes.
   a. Check staining progress at timed intervals. Tissue will turn tan in color; continue to check staining progress at timed intervals. Bacteria will be black when the tissue reaches a golden brown color.
   b. Dip in warm distilled water before and after examination.
   Microwave Modification: See Procedure Note #6 (page 2).  
a. Heat slides in a plastic Coplin jar containing Reducing Solution at 45°C for 30 seconds.
   b. Remove from microwave. Continue to incubate slides in the warm solution for an additional 2 minutes.
14. Wash for 3 minutes in running tap water; rinse in distilled water.
15. Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.
RESULTS:

<table>
<thead>
<tr>
<th>Spirochetes</th>
<th>Dark brown to black</th>
</tr>
</thead>
<tbody>
<tr>
<td>Background</td>
<td>Golden brown</td>
</tr>
<tr>
<td>Negative lung</td>
<td>Negative for spirochetes</td>
</tr>
</tbody>
</table>

REFERENCES:

8. Modifications developed by Newcomer Supply Laboratory.

PROCEDURE NOTES:

1. Acid clean all glassware/plasticware (12086) and rinse thoroughly in several changes of distilled water. Cleaning glassware with bleach is not equivalent to acid washing.
2. Plastic (5500), plastic-tipped or paraffin coated metal forceps must be used with any silver solution to prevent precipitation of silver salts. No metals of any kind should be in contact with any silver solution. Only glass thermometers should be used.
3. Drain staining rack/slides after each step to prevent solution carry over.
4. Dispose of Uranyl Nitrate as hazardous waste and/or according to local and state regulations. Refer to SDS for personal protective measures and handling information.
5. 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.
6. The Reducing Solution contains alcohol and will reduce its boiling point. To avoid boiling solution, adjust microwave times and power levels accordingly.
7. The use of some xylene substitutes have resulted in diminished spirochete staining. If using a xylene substitute exercise caution and closely follow the manufacturer’s recommendation for deparaffinization and clearing steps.