**Pneumocystis sp., Animal Control Slides – Technical Memo**

**CONTROL SLIDES:**

<table>
<thead>
<tr>
<th></th>
<th>Part 4556A</th>
<th>Part 4556B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fixation</td>
<td>Formalin 10%, Phosphate Buffered (Part 1090).</td>
<td></td>
</tr>
<tr>
<td>Section/Glass</td>
<td>Paraffin sections cut at 4 microns on Superfrost™ Plus slides.</td>
<td></td>
</tr>
<tr>
<td>Quality Control</td>
<td>Grocott Methenamine Silver quality control stained slide(s) included.</td>
<td></td>
</tr>
<tr>
<td>Reactivity</td>
<td>Guaranteed product specific reactivity for one year from date of receipt. Revalidate after one year to verify continued reactivity.</td>
<td></td>
</tr>
<tr>
<td>Storage</td>
<td>15-30°C in a light deprived and humidity controlled environment.</td>
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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. These positive control sections are produced from animal tissue and the negative control sections 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.

**APPLICATION:**
Newcomer Supply *Pneumocystis sp.*, Animal Control Slides are for the positive histochemical staining of fungal organisms. The morphology of the organisms is consistent with *Pneumocystis sp.*

**PRESTAINING PREPARATION:**

1. Heat dry sections in oven according to your laboratory protocol.
2. All glassware/plasticware must be acid cleaned prior to use.
   a. See Procedure Notes #1 and #2 (page 2).
3. Prepare Silver-Methenamine Working Solution and mix well:
   a. Solution C: Silver Nitrate 125/250 ml
   b. Solution D: Methenamine Borate 125/250 ml
4. Preheat Silver-Methenamine Working Solution to 45°C - 60°C.
   a. See Procedure Notes #3 and #4 (page 2).

**NEWCOMER SUPPLY VALIDATION PROCEDURE:**

6. 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 #5 and #6 (page 2).
7. Oxidize in Solution A: Chronic Acid 5%. Aqueous for 1 hour.
   a. Oxidize slides in a plastic Coplin jar containing Solution A: Chronic Acid 5%, Aqueous and microwave for 1 minute and 20 seconds at 60°C.
8. Wash well in running tap water; rinse in distilled water.
9. Place in Solution B: Sodium Bisulfite 1%, Aqueous for 1 minute.
10. Wash for 5 minutes in running tap water; rinse well in distilled water.

11. Incubate slides in preheated Silver-Methenamine Working Solution (Step #4) at 45°C-60°C or at room temperature, for 12-18 minutes until sections appear paper-bag brown.
   a. Periodically remove control, rinse in warm distilled water, check microscopically for adequate silver impregnation. Fungi should be dark brown.
   b. If organisms are not sufficiently dark, return slides to warm silver solution. Recheck at 2-3 minutes intervals until desired intensity is achieved.
   c. Pneumocystis may take longer to stain than other fungus.
   d. Staining at room temperature will require an overall longer incubation time.

12. **Microwave Modification:**
   a. Incubate slides in a plastic Coplin jar containing Silver-Methenamine Working Solution and microwave for 1 minute at 70°C.
   b. Check microscopically for adequate development.
   c. If additional incubation is required, return slides to warm Silver-Methenamine Working Solution. Recheck at 2-3 minute intervals.

13. Rinse in three to four changes of distilled water.
   a. Never use tap water at this step.
14. Tone in Solution E: Gold Chloride 0.1%, Aqueous until sections turn gray; 20 seconds to 1 minute.
15. Rinse well in distilled water.
16. Remove unreduced silver in Solution F: Sodium Thiosulfate 2%, Aqueous for 2 minutes.
17. Wash in running tap water for 5 minutes; rinse in distilled water.
18. Counterstain in Solution G: Light Green SF Yellowish 0.02%, Aqueous for 2 minutes.
   a. Over counterstaining could mask organisms.
19. Dehydrate quickly in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

**PRODUCT SPECIFICATIONS:**

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<th>Tissue</th>
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RESULTS:

**Pneumocystis sp.**  Sharply outlined in black

**Background**  Pale green

**Negative lung**  Negative for fungal organisms

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**PROCEDURE NOTES:**

1. Acid clean all glassware/plasticware (12086) and rinse thoroughly in several changes of distilled water.
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. Preheating Silver-Methenamine Working Solution to 45°C-60°C prior to incubation is suggested for timely silver development. A water bath or oven can be used. Begin preheating the silver solution approximately 20-30 minutes before use.
4. Staining slides at higher temperatures will cause the development reaction to happen faster, but may also cause precipitate to form in the working silver solution and deposit on the slides. Maintaining the silver solution between 45°C-60°C will help to minimize precipitate.
5. Drain slides after each step to prevent solution carry over.
6. Do not allow sections to dry out at any point during procedure.
7. The suggested microwave procedure has been tested at Newcomer Supply. This procedure is a guideline and techniques should be developed for use in your laboratory.
8. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

**REFERENCES:**

5. Modifications developed by Newcomer Supply Laboratory.