STEINER STAIN KIT PROCEDURE

Item# KTSTEPT

(Revised 04/19/11)

<table>
<thead>
<tr>
<th>KIT COMPONENTS</th>
<th>Included</th>
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<tbody>
<tr>
<td>1 PINT OXIDIZER SOLUTION</td>
<td>1 PINT 10% ZINC FORMALIN</td>
</tr>
<tr>
<td>1 PINT 0.1% SILVER NITRATE</td>
<td>2 PINTS 2.5% GUM MASTIC</td>
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<tr>
<td>25 each HYDROQUINONE CAPSULES</td>
<td>30ml 1% SILVER NITRATE</td>
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</tbody>
</table>

PRINCIPLE: This kit demonstrates fungi, Helicobacter pylori, Legionella pneumophila, and spirochetes.

SPECIMEN: Any well fixed paraffin embedded tissue cut at 3 to 4 microns.

QUALITY CONTROL: American MasterTech Recommended Control Slides:
- Aspergillus, CSA0625P; Candida Albicans, CSC0525P; Helicobacter Pylori, CSH0125P; Legionnaires, CSL0125P; Spirochete, CSS0125P

Reducing Solution: Prepare at Step 11

Hydroquinone Solution ……………………. 25 ml
2.5% GUM MASTIC ……………………….. 15 ml
1% SILVER NITRATE …………………. 6 drops

Prepare reducing solution by adding the contents of one HYDROQUINONE CAPSULE to 25 ml of Distilled water and mix until the hydroquinone is completely dissolved. Add 15ml of 2.5% GUM MASTIC, mix thoroughly and then filter solution through medium filter paper. Add 6 drops of 1% SILVER NITRATE and mix thoroughly.

PROCEDURE:
1. Heat water bath to 70°C.
2. Deparaffinize slide using Xylene or a Xylene Substitute and hydrate through alcohols.
3. Rinse slide in Tap water.
4. Place slide in OXIDIZER SOLUTION for 20 minutes.
5. Rinse slide through 2 changes of Distilled water.
6. Place slide in 10% ZINC FORMALIN for 5 minutes. At this time place 20 ml of 0.1% SILVER NITRATE in the waterbath.
7. Rinse slide through 2 changes of Distilled water.
8. Place slide in 0.1% SILVER NITRATE for 5 minutes at 70°C; discard solution after this step.
9. Rinse slide through 2 changes of Distilled water.
10. Dehydrate slide through 1 change of fresh Absolute Alcohol.
11. Place slide in 2.5% GUM MASTIC for 3 minutes. (At this time prepare Reducing Solution and place in the water bath.)
12. Air dry slide for 1 minute or until the gum mastic is completely dry.
13. Place slide in Reducing Solution at 70°C for 10 to 15 minutes or until section is tan to brown; discard Reducing Solution after this step.
14. Dehydrate slide through 3 changes of fresh Absolute Alcohol.
15. Clear slide through 3 changes of fresh Xylene or Xylene Substitute.
16. Coverslip slide immediately using a permanent mounting media.

RESULTS:
Cat Scratch Fever, Legionella pneumophila,
Spirochetes, Helicobacter pylori, and Fungi: DARK BROWN, GRAY or BLACK
Background: YELLOW, TAN or BROWN


ALTERNATE PROCEDURE NOTE: MasterTech Steiner Stain Kit Microwave Procedure on opposite side.

Human Gastric Tissue
STEINER STAIN KIT MICROWAVE PROCEDURE

(Revised 04/19/11)

**PRINCIPLE:** This kit demonstrates fungi, Helicobacter pylori, Legionella pneumophilia and spirochetes.

**SPECIMEN:** Any well fixed paraffin embedded tissue cut at 3 to 4 microns.

**QUALITY CONTROL:** American MasterTech Recommended Control Slides:
- Aspergillus, CSA0625P
- Candida Albicans, CSC0525P
- Helicobacter Pylori, CSH0125P
- Legionnaires, CSL0125P
- Spirochete, CSS0125P

**REDUCING SOLUTION:** Prepare at Step 11

Hydroquinone Solution .................................. 25 ml
2.5% GUM MASTIC .................................. 15 ml
1% SILVER NITRATE ................................. 6 drops

Prepare reducing solution by adding the contents of one HYDROQUINONE CAPSULE to 25ml of Distilled water and mix until the hydroquinone is completely dissolved. Add 15ml of 2.5% GUM MASTIC, mix thoroughly and then filter solution through medium filter paper. Add 6 drops of 1% SILVER NITRATE and mix thoroughly.

**PROCEDURE:**
1. Deparaffinize slide using Xylene or Xylene Substitute and hydrate through alcohols.
2. Rinse slide in Tap Water.
3. Place slide in OXIDIZER SOLUTION for 20 minutes.
4. Rinse through two changes of Distilled water.
5. Place slide in 10% ZINC FORMALIN for 5 minutes.
6. Rinse slide through two changes of Distilled water and hold in Distilled water until Step 8.
7. In a loosely capped Slide Jar (available from A.M.T.S.) heat 20 ml of 0.1% SILVER NITRATE in a microwave oven for 10 seconds at full power. Repeat as needed until solution is hot, but do not allow solution to boil. Remove Slide Jar from microwave, tighten Slide Jar cap and agitate to equalize solution temperature.
8. Place slide into the hot 0.1% SILVER NITRATE, agitate thoroughly and incubate for 2 minutes.
9. Rinse slide through 2 changes of Distilled water.
10. Dehydrate slide through 2 changes of fresh Absolute Alcohol.
11. Place slide in 2.5% GUM MASTIC for 3 minutes. *(During this step, prepare Reducing Solution.)*
12. Air dry slide for 1 minute or until the gum mastic is completely dry.
13. In a loosely capped Slide Jar heat 20 ml of Reducing Solution in a microwave oven for 25 seconds at full power. Tightly cap Slide Jar, invert several times and place slide into the Reducing Solution. Loosely cap Slide Jar, return Slide Jar to the microwave and heat at full power until the Reducing Solution is hot, but do not allow solution to boil. Incubate slide for 3 minutes, then heat again at full power until the Reducing Solution is hot. Incubate slide for 2 to 3 minutes; section color should be tan to light brown.
14. Rinse slide quickly in Distilled water.
15. Dehydrate slide through 3 changes of fresh Absolute Alcohol.
16. Clear slide through 3 changes of fresh Xylene or Xylene Substitute.
17. Cover slip slide immediately using a permanent mounting media.

**RESULTS:**
- Cat Scratch Fever, Legionella pneumophilia, Spirochetes, Helicobacter pylori, and Fungi: DARK BROWN, GRAY or BLACK
- Background: YELLOW, TAN or BROWN


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