Carbol Fuchsin Stain, Ziehl-Neelsen for AFB Stain, Ziehl-Neelsen -
Technical Memo

SOLUTION:  
Carbol Fuchsin Stain, Ziehl-Neelsen 250 ml Part 1030A 500 ml Part 1030B 1 Liter Part 1030C

Additionally Needed For AFB Stain, Ziehl-Neelsen:

<table>
<thead>
<tr>
<th>Acid Fast Bacteria (AFB) Control Slides</th>
<th>Part 4011</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Alcohol 1%</td>
<td>Part 1001</td>
</tr>
<tr>
<td>Methylene Blue Stain 1.4%, Alcoholic</td>
<td>Part 1240 or Methylene Blue Stain 0.14%, Alcoholic Part 12401</td>
</tr>
<tr>
<td>Xylene, ACS</td>
<td>Part 1445</td>
</tr>
<tr>
<td>Alcohol, Ethyl Denatured, 100%</td>
<td>Part 10841</td>
</tr>
<tr>
<td>Alcohol, Ethyl Denatured, 95%</td>
<td>Part 10842</td>
</tr>
</tbody>
</table>

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

APPLICATION:

Newcomer Supply Carbol Fuchsin Stain, Ziehl-Neelsen, a crucial element in the AFB Stain, Ziehl-Neelsen is used to demonstrate the presence of acid-fast mycobacteria in tissue sections. Phenol is employed in this solution to render the cell walls of bacteria permeable to the fuchsin stain. The use of weak acid for differentiation allows excess stain to be removed from tissues, but will not remove stain from the acid-fast organisms.

METHOD:

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

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below.

STAINING PROCEDURE:

1. Filter Carbol Fuchsin Stain, Ziehl-Neelsen before use.
2. 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.  
3. Stain in freshly filtered Carbol Fuchsin Stain, Ziehl-Neelsen for 60 minutes at room temperature. Keep solution covered.
4. Rinse in running tap water for 2 to 3 minutes.
5. Differentiate in Acid Alcohol 1% until color no longer runs off the slide and sections are pale pink; 3 to 10 rapid dips.
6. Wash in running tap water 3 to 5 minutes; rinse in distilled water.
7. Counterstain in Methylene Blue Stain 0.14%, Alcoholic (12401) or dilute 5 ml Methylene Blue Stain 1.4%, Alcoholic (1240) with 45 ml tap water to a 0.14% solution.
   a. Dip slides a few times in counterstain; rinse in tap water, followed by a distilled water rinse and check microscopically. Sections should be pale blue.  
   b. See Procedure Notes #3 and #4.
8. Wash in running tap water for 1 minute; rinse in distilled water.
9. 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:

Acid-fast bacilli Bright red
Background Pale blue

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. It is important not to over-counterstain, as the organisms may be masked. If section is over-stained, remove methylene blue with Acid Alcohol 1% (10011), rinse thoroughly, and repeat methylene blue step (Step #7).
4. If laboratory tap water is generally acidic, the methylene blue stain may be pale. Adjust staining time accordingly.
5. If using a xylene substitute, closely follow the manufacturer’s recommendations for deparaffinization and clearing steps.

REFERENCES:

3. Modifications developed by Newcomer Supply Laboratory.
Carbol Fuchsin Stain, Ziehl-Neelsen for AFB Stain, Fite - Technical Memo

**SOLUTION:**

<table>
<thead>
<tr>
<th>Carbol Fuchsin Stain, Ziehl-Neelsen</th>
<th>250 ml</th>
<th>500 ml</th>
<th>1 Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part 1030A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Part 1030B</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Part 1030C</td>
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</tbody>
</table>

**Additionally Needed For AFB Stain, Fite:**

- Fite Stain, *Nocardia Sp.* Control Slides: Part 4215
- Xylene/Peanut Oil, 2:1: Part 1449
- Sulfuric Acid 1%, Aqueous: Part 14012
- Methylene Blue Stain 0.5%, Aqueous: Part 12402
- Acid Alcohol 1%: Part 10011 (If staining for *Mycobacterium leprae* sp.)
- Xylene, ACS: Part 1445

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

**APPLICATION:**

Newcomer Supply Carbol Fuchsin Stain, Ziehl-Neelsen, a crucial element in the AFB Stain, Fite is used to detect the presence of either *Nocardia sp.* or *Mycobacterium leprae sp.* (causative agent of leprosy) in tissue sections with minor variations in the procedure.

**METHOD:**

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

**Technique:** Paraffin Sections cut at 5 microns

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

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below.

**STAINING PROCEDURE:**

1. Filter Carbol Fuchsin Stain, Ziehl-Neelsen.
2. Deparaffinize slides in Xylene/Peanut Oil, 2:1, two changes, 12 minutes each.
   a. See Procedure Note #1
3. Drain slides, wipe off excess oil, and blot to opacity taking care to remove residual oil.
   a. See Procedure Note #2.
4. Stain slides in freshly filtered Carbol Fuchsin Stain, Ziehl-Neelsen for 30 minutes at room temperature.
5. Wash in running tap water for 3 minutes.
6. Differentiation:
   a. If staining for *Nocardia sp.*, differentiate slides in Sulfuric Acid 1%, Aqueous (14012) for 3 minutes.
   b. If staining for *Mycobacterium leprae* sp., differentiate slides individually in Acid Alcohol 1% (10011) until sections are light pink; approximately 1 minute.
7. Wash in running tap water for 3 minutes.
8. Counterstain lightly with Methylene Blue Stain 0.5%, Aqueous, for 5-10 seconds.
   a. See Procedure Notes #3 and #4.
9. Rinse off excess Methylene Blue Stain 0.5%, Aqueous in running tap water. Background should be a light sky blue.
10. Blot excess water from slide and air-dry completely.
11. Dip dried slides in xylene and coverslip with a compatible mounting medium.

**RESULTS:**

- Acid-fast bacilli and *Mycobacterium leprae* sp. Red
- *Nocardia sp.* Red
- Red blood cells Yellow-orange
- Other tissue elements Pale blue

**PROCEDURE NOTES:**

1. Acid-fastness of the leprosy organisms is enhanced when the waxy capsule is protected by the mixture of xylene-peanut oil and the avoidance of dehydrating solutions.
2. It is important to blot well, residual oil may produce staining artifact.
3. If over-stained with methylene blue, organisms may be masked. Check microscopically before air drying. If over-stained, remove methylene blue with Acid Alcohol 1% (10011); rinse thoroughly; repeat Step #8 with a shorter timing.
4. If laboratory tap water is generally acidic, the methylene blue stain may be pale. Adjust staining time accordingly.
5. A small percentage of *Nocardia sp.* organisms may resist taking the red stain and remain blue due to the growth phase of the individual organism.
6. If using a xylene substitute, closely follow the manufacturer’s recommendations for coverslipping step.

**REFERENCES:**

5. Modifications developed by Newcomer Supply Laboratory.