

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

SOLUTION:	250 ml	500 ml	1 Liter
Carbol Fuchsin Stain, Ziehl-Neelsen	Part 1030A	Part 1030B	Part 1030C
<u>Additionally Needed For AFB Stain, Ziehl-Neelsen:</u>			
Acid Fast Bacteria (AFB) Control Slides	Part 4011		
Acid Alcohol 1%	Part 10011		
Methylene Blue Stain 1.4%, Alcoholic	Part 1240	or	Methylene Blue Stain 0.14%, Alcoholic
Xylene, ACS	Part 1445		Part 12401
Alcohol, Ethyl Denatured, 100%	Part 10841		
Alcohol, Ethyl Denatured, 95%	Part 10842		

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:

1. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 226-227.
2. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 236-237.
3. Modifications developed by Newcomer Supply Laboratory.

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

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

Additionally Needed For AFB Stain, Fite:

Fite Stain, <i>Nocardia</i> 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 <i>Mycobacterium leprae</i> 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 <i>Mycobacterium leprae</i> sp.	Red
<i>Nocardia</i> 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:

1. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 228-229.
2. Fite, George, P.J. Cambre and M.H. Turner. "Procedure for Demonstrating Lepra Bacilli in Paraffin Sections". *Archives of Pathology* 43 (1947). 624-625.
3. Luna, Lee G. *Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. Gaithersburg, MD: American Histolabs, 1992. 180-181
4. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 237.
5. Modifications developed by Newcomer Supply Laboratory.