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# AFB Stain, Ziehl-Neelsen - Technical Memo

| SOLUTIONS: | 250 ml | 500 ml | 1 Liter | 1 Gallon |
|---|---|---|---|---|
| Carbol Fuchsin Stain Solution | Part 1030A | Part 1030B | Part 1030C | |
| Acid Alcohol, 1% Solution | | Part 10011A | Part 10011B | Part 10011C |
| Methylene Blue Stain, 0.14% Working | Part 12401A | | | |

| Additionally Needed: | |
|---|---|
| Acid Fast Bacteria (AFB) Control Slides | Part 4011 |
| Xylene, ACS | Part 1445 |
| Alcohol, Ethyl Denatured, 100% | Part 10841 |
| Alcohol, Ethyl Denatured, 95% | Part 10842 |

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

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## APPLICATION:

Newcomer Supply AFB Stain, Ziehl-Neelsen is used to demonstrate the presence of acid-fast mycobacteria in tissue sections. Phenol is employed 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:** 10% Phosphate Buffered Formalin (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 Solution 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 Solution 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% Solution 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% Working. Sections should be pale blue.
    a. *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. Dip slides a few times in Methylene Blue Stain, 0.14% Working; rinse in tap water, followed by a distilled water rinse and check microscopically. It is important not to over-counterstain, as the organisms may be masked. If section is over-stained, remove methylene blue with acid alcohol, 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.
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.

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