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AFB Stain, Fite - Technical Memo

SOLUTIONS:	**250 ml**	**500 ml**	**1 Liter**
Xylene/Peanut Oil 2:1	Part 1449A	Part 1449B	Part 1449C
Carbol Fuchsin Stain Solution, Ziehl-Neelsen	Part 1030A	Part 1030B	Part 1030C
Sulfuric Acid, 1% Aqueous Solution	Part 14012A	Part 14012B	Part 14012C
Methylene Blue, 0.5% Working Stain Solution	Part 12402A		

Additionally Needed:	
Fite Stain, *Nocardia Sp*. Control Slides	Part 4215
Acid Alcohol, 1% Solution	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 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: 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, 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 Solution 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 Solution for 3 minutes.*
    b. *If staining for Mycobacterium leprae sp., differentiate slides individually in Acid Alcohol, 1% Solution (10011) until sections are light pink; approximately 1 minute.*
7. Wash in running tap water for 3 minutes.
8. Counterstain lightly with Methylene Blue, 0.5% Working Stain Solution, for 5-10 seconds.
    a. *See Procedure Notes #3 and #4.*
9. Rinse off excess Methylene Blue, 0.5% Working Stain Solution 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% Solution (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*. Gaitheresburg, 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.
