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Part 91013
Revised June 2016

AFB, Fite Stain Kit - Technical Memo

KIT INCLUDES:	**Part 91013A**
Solution A: Xylene/Peanut Oil, 2:1	500 ml
Solution B: Carbol Fuchsin Stain, Ziehl-Neelsen	250 ml
Solution C: Sulfuric Acid 1%, Aqueous	250 ml
Solution D: Methylene Blue Stain 0.5%, Aqueous	250 ml

COMPLIMENTARY POSITIVE CONTROL SLIDES: Enclosed with this kit are two complimentary unstained positive control slides to be used for the initial verification of staining techniques and reagents. Verification must be documented by running one Newcomer Supply complimentary positive control slide along with your current positive control slide for the first run. Retain the second complimentary control slide for further troubleshooting, if needed.

Individual stain solutions and additional control slides may be available for purchase under separate part numbers at www.newcomersupply.com.

Additionally Needed:

Acid Alcohol 1%	Part 10011 (If staining for *Mycobacterium leprae sp.)*
Xylene, ACS	Part 1445

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

APPLICATION:

Newcomer Supply AFB, Fite Stain Kit 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 Kits are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below. Some solutions in the kit may contain extra volumes.

STAINING PROCEDURE:

1. Filter Solution B: Carbol Fuchsin Stain, Ziehl-Neelsen with high quality filter paper.
2. Deparaffinize slides in Solution A: Xylene/Peanut Oil, 2:1, two changes for 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 Solution B: 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 Solution C: Sulfuric Acid 1%, Aqueous 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 Solution D: Methylene Blue Stain 0.5%, Aqueous for 5-10 seconds.
    a. *See Procedure Notes #3 and #4.*
9. Rinse off excess Methylene Blue Stain 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:

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.
