Acid fast bacteria (atypical mycobacteria) on a formalin fixed tissue slide by Fite's method
Wisconsin Veterinary Diagnostic Laboratory
Standard Operating Procedure

1 Introduction
The Fite’s acid fast method helps differentiate between Nocardia and Actinomyces. Because Nocardia spp. are weakly acid fast, and not alcohol fast, it is important to protect the waxy capsule surrounding the bacteria by using the xylene-peanut oil solution.

2 Specimen submission
2.1 Type
Paraffin embedded tissue block

2.2 Special requirements for collection -- NA

2.3 Handling conditions -- NA

2.4 Criteria for rejection of sample -- NA

3 Materials
3.1 Equipment & Instrumentation
1. Automatic stainer
2. Automatic coverslipper
3. Fume Hood
4. Slide dryer
5. Plastic funnel
6. Coplin jars with lids
7. Beakers
8. 100 ml graduated cylinders
9. Pipette filler
10. Shallow plastic tub
11. Slide racks and slide rack holders

3.2 Reagents & Media
1. Acid Alcohol 1% solution
2. Carbol – Fuchsin Ziehl – Neelsen solution
3. Ethanol 100% & 95%
4. Hydrochloric Acid 35% to 38%
5. Methylene blue stock solution
6. Peanut oil
7. Xylene

3.3 Supplies
1. Disposable Pasteur pipettes
2. Pipette bulbs
3. Permanent slide markers
4. Filter paper
5. 5 ml disposable serological pipettes
6. Paper towels or all purpose wipes

4 Safety Management

4.1 Required Safety Training:
- Chemical fume hood operation mandatory use when working with Carbol Fuchsin
- Hazardous chemical use and disposal training

4.2 Required personal protective equipment (PPE):
Minimum: ☑ Lab coat, Safety glasses and closed toe shoes– upon entry of lab
Gloves: ☑ Nitrile ☑ Chemical resistant when immersing fingers in xylene
Eyewear: ☑ Splash Goggles or face shield-when pouring liquid

4.3 Hazard Communication
A. Chemical: See SDS
   - Ethanol 100% & 95% (SDS-260 and 261)
     - Flammable liquid and vapor
   - Xylene (SDS-574)
     - Flammable liquid and vapor. Possible cancer hazard. May cause cancer based on animal data. Harmful if absorbed through skin or if inhaled. Causes eye, skin, and respiratory tract irritation. Inhalation may cause central nervous system effects. Aspiration hazard if swallowed - can enter lungs and cause damage.
   - Acid alcohol 1%
     - Hydrochloric acid 35 to 38% (MSDS-826)
     - Causes burns by all exposure routes. May be harmful if inhaled.
B. Biological: ☑ Biosafety Level 2
C. Physical: NA
D. Electrical: NA
E. Sharps: NA
F. Ergonomics: NA

4.4 Disposal of waste
- Xylene is discarded in a designated waste xylene carboy and recycled using the in-house xylene recycler.
- Ethanol, ethanol contaminated with xylene, histological stains, heavy metal stains, acids and the xylene / peanut oil mixture must be dumped into a designated waste carboy. Document the type and amount of each solution on the Chemical Inventory Analysis For Waste Solvents In Carboys. Once the carboy is full, transport the carboy with the completed documentation to room 1101 for safe storage until it can be picked up by UW-Safety.
- All broken glassware and/or slides are disposed in the broken glass box located in Room 1124.
5 Preparation for procedure

5.1 Equipment and instrumentation preparation
   1. Turn on automatic stainer and coverslipper.

5.2 Reagents and media preparation – See Section 12.1

5.3 Standards/controls preparation
   1. Use a validated Fites control slide cut at 5 µm (PSECTIONING).

5.4 Specimen preparation
   1. Cut paraffin sections at 5µm (PSECTIONING).

6 Performance of procedure

The following steps are applied to both the control and diagnostic slides:

DO NOT USE H2O Program on the Automatic Stainer!

1. Place slides in the slide dryer for 10 – 20 minutes.
2. Place slides in a coplin jar filled with the xylene-peanut oil solution for 30 minutes.
3. Remove the slides from the coplin jar and hold vertically over a paper towel to allow the excess xylene-peanut oil solution to run off.
4. Remove the excess xylene-peanut oil solution from the slide:
   a. Lay slides flat on a piece of small filter paper.
   b. Lay another piece of small filter paper across the slide and gently blot the xylene-peanut oil solution off.
   c. Remove the piece of filter paper used for blotting.
   d. Remove the slides from the filter paper and lay them flat on an all purpose wipe in the fume hood.
   e. Allow slides to air dry for 15 minutes.
5. Under a fume hood, use a Pasteur pipette and bulb to flood slides with freshly filtered carbol-fuchsin and let sit for 30 minutes.
6. Rinse slides in a tub of running water for 3-5 minutes.
7. Decolorize the tissues by dipping one slide at a time in a glass container filled with 1% acid alcohol.
   a. This step of the procedure is subjective.
   b. Dip the slide until the tissue appears to be a light pink color.
   c. Decolorizing in acid alcohol works best by dipping slides in a container of acid alcohol and then rinsing in the running tap water, repeating as necessary, until a pale pink color is observed.
8. After each slide has been decolorized, stand it in the tub of running water until all slides are finished.
9. Place slides in a coplin jar filled with methylene blue working solution for 30 seconds.
10. Rinse slides by quickly dipping 5-10 times in running tap water.
11. Place slides in a slide rack.
12. Dehydrate and clear using the “specials kwik” program on the automatic stainer or manually dehydrate:
   a. station 12 (95% alcohol),
   b. stations 13, 19-18 (100% alcohol),
   c. stations 17-14 (xylene).
13. Remove the slides from xylene and coverslip (PGLASCOVERSLIP).
14. Pour the unused filtered carbol-fuchsin back in the bottle at.
15. Wash all glassware. Glassware that has been used with the carbol-fuchsin must first be rinsed with 1% acid alcohol before rinsing in water.

7 Interpretation of results
   1. Use the microscope to check the control slide.
   2. When stained properly, the control slide will look as follows:
      Mycobacterium leprosy and other acid-fast bacilli…………..Red
      Nocardia filaments………………………………………..Red
      Background………………………………………………..Blue
   3. If the control slide stained properly, the stain can be considered valid and the pathologist can determine the results of the specimen slide.
   4. If the control slide does not stain properly, a RAW-non-conformance must be created in QPulse.

8 Report of results
   1. Record the completion of the stain in the Histo Slides database.
   2. Place slides in a flat and place in the outbox of the appropriate pathologist.

9 Procedure notes
   9.1 Details and helpful hints
      1. When blotting the peanut oil-xylene off the slides, be careful not to damage tissue. Air drying allows the oil film to be more even across the tissue.
      2. Be careful not to rinse out too much of the counterstain (methylene blue working solution) in tap water, or the background will be understained.
      3. If the tissue does not seem to hold the counterstain well, fresh methylene blue may need to be made.
   9.2 Limitations of procedure
      If the slides are not left in the peanut oil-xylene solution for long enough, or if some of the xylene has evaporated from the solution, the paraffin may not be adequately removed from the tissue section. This can result in uneven staining with the carbol-fuchsin and make decolorizing difficult.
10 References


11 Summary of Current Revisions

1. Section 5: Use Fites control and not the AFB control.
2. Section 5.2: Moved reagent prep to 12.1.

12 Supplemental Information

12.1 Quick Procedure Reference – Fite’s Acid Fast

Xylene – Peanut oil solution
- Xylene…………………………………………………………..25ml
- Peanut Oil……………………………………………………...25ml
Stability: 6 months at room temperature in a closed coplin jar.

Methylene blue working solution
- Methylene blue stock solution………………………………..5.0 ml
- RO/DI H₂O……………………………………………………..45.0 ml
Stability: 6 months at room temperature in a closed coplin jar.

Fite’s Method for Acid Fast Staining

DO NOT USE H₂O Program on the Automatic Stainer!

1. Place slides in the slide dryer for 10 – 20 minutes.
2. Place slides in a coplin jar filled with xylene-peanut oil solution for 30 minutes.
3. Hold slides vertically over a wypall to allow excess xylene-peanut oil solution to run off.
4. Lay slides on a piece of filter paper and place another piece of filter paper on top and blot.
5. Allow slides to air dry for 15 minutes.
6. Flood slides with freshly filtered Carbol-Fuchsin for 30 minutes.
7. Rinse well in running tap water.
8. Decolorize with 1% acid alcohol until sections are pale pink.
9. Rinse thoroughly in running tap water.
10. Place slides in a coplin jar filled with methylene blue working solution for 30 seconds.
11. Dip 5-10 times in running tap water.
12. Dehydrate and clear using the “specials kwik” program on the automatic stainer.
13. Coverslip (PGLASCOVERSLIP).
14. Clean all glassware.

**Interpretation of results**

1. Use the microscope to check the control slide.
2. When stained properly, the control tissue will look as follows:
   - Mycobacterium leprosy and other acid-fast bacilli........Red
   - Nocardia filaments.....................................................Red
   - Background.................................................................Blue

12.2 Flow Diagram – NA

12.3 Manufacturer’s Information

Newcomer Supply
2505 Parview Rd., Middleton, WI 53562
608-831-7888

Catalog # 1030  Ziehl-Neelsen carbol-fuchsin solution
Catalog # 1240A  Methylene blue stock solution