**PROCEDURE: AURAMINE STAIN**

1. **PRINCIPLE**

The unique acid-fast characteristic of mycobacteria is the basis of microscopic examination for these organisms. Whether the method used is the classic stain represented by the Ziehl-Neelsen procedure or the fluorochrome technique, the identical property is determined, bacterial retention of the primary dye after exposure to acid-alcohol. Fluorescence with the auramine technique is equivalent to acid-fastness. The advantages of fluorescence microscopy include the ease, speed, and completeness of observation. Other advantages are better contrast, minimal eye strain, and the relative unimportance of the color acuity of microscopists. Microscopy is used to detect new cases of mycobacterial infection, to determine the acid-fast characteristics of bacterial growth in cultures, to provide an indication of the progress of the disease in individual patients from whom a series of specimens are examined, and to provide a criterion for discharge from the hospital after the initiation of therapy.

1. **AVAILABILITY**

Auramine stains are done Monday through Friday (excluding holidays) and are performed once during the day. Results are available early afternoon.

1. **TEST CODE**

See appendix for Soft codes

1. **SPECIMEN COLLECTION AND PROCESSING**

See section **IV** of Mycobacterial Culture Manual

1. **MATERIALS**
   1. TB Auramine M BD BBL 212514
      1. Auramine O – 2.0 g
      2. Phenol, USP – 4.0 g
      3. Glycerine , USP – 100 mL
      4. Isopropanol – 250 mL
      5. Distilled water – 650 mL
   2. TB Decolorizer TM BD BBL 212512
      1. Hydrochloric Acid – 5.0 mL
      2. Isopropanol – 700.0 mL
      3. Distilled Water – 300.0 mL
   3. TB Potassium Permanganate BD BBL 212513
      1. Potassium Permanganate – 5.0 g
      2. Distilled water – 1000.0 mL
   4. Fluorescent microscopy
      1. Filter: Blue Excitation Filter
      2. Slides: Cell bond 2 well
   5. Quality Control Slides AlphaTec QC1 slides
2. **STORAGE AND HANDLING**
   1. All stains and QC slides are stored at room temperature and in the dark.
   2. All procedures related to the processing, culturing and preparation of stains for Mycobacteria species should be performed in a certified biological safety cabinet.
3. **QUALITY CONTROL**
   1. A control slide is performed with each test.
   2. POSITIVE – *Mycobacterium scrofulacium*
   3. Negative Control:  *E. coli* A control slide is performed with each test
   4. NEGATIVE – *E. coli*
   5. Expected results – positive (bright yellow –green fluorescent rods), negative( no fluorescence)
4. **TEST PROCEDURE**
   1. Preparation of slide
      1. Label a teflon-coated slide.
      2. Spread 1 to 2 drops of specimen on slide.
      3. Heat fix smears on a slide warmer for 2 hours at 65-75oC
   2. Staining procedure
      1. Shake Auramine M bottle.
      2. Flood slides with Auramine M stain and allow to stain at room temperature for 15 minutes
      3. Gently rinse with tap water and drain.
      4. Decolorize with TB Decolorizer TM for 30-60 seconds.
      5. Rinse gently with water.
      6. Counterstain with TB Potassium Permanganate for 2 min.
      7. Rinse gently with water, air dry, store stained slides in a cool dark place until ready to be read using a fluorescent microscope.
      8. Exam slides using the 40X objective. Acid fast organisms will appear as dainty, well-defined rods which emit a yellow fluorescence.
      9. Any positive or suspicious smears may be confirmed with the Kinyoun stain. The Kinyoun stain can be performed over the Auramine but renders the smear no longer satisfactory for fluorescent examination.
5. **INTERPRETATION**
   1. Positive: bright yellow-green fluorescing rods
   2. Negative: No fluorescence
   3. Quantitation will be performed according to the following protocol:

|  |  |  |
| --- | --- | --- |
| **X100 (low)** | **X400 (high dry)** | **REPORT** |
| 0 | 0 | No acid-fast bacilli seen |
| 1-9/10 fields | 2-18/50 fields | 1+ |
| 1-9/field | 4-36/10 fields | 2+ |
| 10-90/field | 4-36/field | 3+ |
| >90/field | >36/field | 4+ |

* 1. For reporting positive smears refer to *Notification Scheme for Test Results of Clinical Significance.*  All positive smears are reported to Infection Control as well. Positive smears are automatically faxed to the Rhode Island Department of Health.
  2. Save all smears 6 weeks.

1. **LIMITATIONS**
   1. Nonspecific fluorescence may be seen in smears. Morphology must be closely adhered to in interpreting smears.
   2. Stain cannot differentiate *Mycobacterium tuberculosis* from other *Mycobacteria species.*
2. **REFERENCES**
   1. Della-Latta, P. “Mycobacteriology and Antimycobacterial Susceptiblity Testing”. In Clinical Microbiology Procedures Handbook, 2nd Edition. Vol 2. Editor: Isenberg, H. 2004, pp. 7.0.1 – 7..8.8.3.
   2. Pfyffer, G., Brown-Elliott, B., Wallace, R. “*Mycobacterium*: General Characteristics, Isolation, and Staining Procedures”. In: Manual of Clinical Microbiology, 8th Edition. Editors: Murray, P., Baron, E., Jorgensen, J., Pfaller, M., Yolken, R. 2003, pp. 532-559.
   3. C. Truant, J.P., Brett, W.A., and Thomas, W. Henry Ford Hosp. Med. Bull. 10:287-296, 1962.
3. **REVISIONS**
   1. December 10, 2019 – Revised wording under the principle section. Updated reagents under materials section and changed the test procedure to reflect that. Revised reagent storage requirements.