## **PROCEDURE: KINYOUN**

1. **PRINCIPLE**

Mycobacteria have the unique property of retaining dye after exposure to acid alcohol. This property is attributed to a lipid substance in the membrane. This ability to resist decolorization, referred to as acid-fastness, allows mycobacteria to be differentiated from other bacteria.

Note: All procedures related to the processing, culturing and preparation of stains for *Mycobacteria* species should be done in a biological safety cabinet.

1. **AVAILABILITY**

Stat direct smears requested (when clinically indicated) are available 7 days a week from 7:30 to 3:00pm.

1. **TEST CODE**

DAFST

1. **SPECIMEN**
   1. Slides prepared from unconcentrated clinical specimen suspected of containing mycobacteria.
   2. A positive or suspicious AFB culture.
   3. A positive or suspicious fluorochrome smear.
2. **MATERIALS**
   1. Materials
      1. TB Carbolfuchsin KF : BBL 212518
         1. Basic Fuchsin – 15 g
         2. Phenol, USP – 45 g
         3. Isopropanol – 200 mL
         4. Ethanol – 50 mL
         5. Distilled water – 750 mL
      2. TB Decolorizer BBL 212517
         1. Hydrochloric Acid - 30mls
         2. Ethanol/Methanol – 970.0mls
      3. TB Brilliant Green K BBL 212523
         1. Brilliant Green – 2 g
         2. Sodium Hydroxide – 0.02 g
         3. Distilled Water – 1000 mL
      4. Quality Control Slides AlphaTec QC1 slides
3. **STORAGE AND HANDLING**
   1. All stains and QC slides are stored at room temperature
4. **QUALITY CONTROL**
   1. A control slide is performed with each test
   2. POSITIVE – Mycobacterium scrofulacium
   3. NEGATIVE- E. coli
   4. Expected results – positive (red staining rods), negative( blue-green bacteria)
5. **TEST PROCEDURE**
   1. Preparation and staining of slide.
      1. Heat fix slide until adequately dried.
      2. Flood the smear with TB Carbolfuchsin KF and let stand for 4 min.
      3. Wash gently with tap water.
      4. Decolorize with TB Decolorizer for 3-5 seconds.
      5. Wash gently with tap water.
      6. Counterstain with TB Brilliant Green K for 30 seconds.
      7. Wash gently with tap water and dry over gentle heat.
6. **INTERPRETATION**
   1. Quantitation will be performed according to the following protocol

|  |  |
| --- | --- |
| ***x1000 (OIL IMMERSION)*** | ***REPORT*** |
| **0** | **No acid-fast bacilli seen** |
| **1-9/100 fields** | **1+** |
| **1-9/10 fields** | **2+** |
| **1-9/field** | **3+** |
| **>9/field** | **4+** |

* 1. Positive: red, sometimes beaded rods
  2. Negative : blue-green bacteria

1. **NOTES**
   1. A modified or partial acid-fast Kinyoun stain is performed when *Nocardia* is suspected.
   2. Insufficient decolorization or thick specimen smears may be a source of error.
   3. Transfer of acid-fast bacilli from one slide to another may occur if stained in a common container or if the oil objective is not cleaned between smears.
   4. Care should be taken to separate positive control from other slides to avoid splattering.
   5. Blotting smears after staining may transfer organisms. It is best to put stained smears back in the hood to air dry.
2. **LIMITATIONS OF TEST**
   1. Acid fast stain is not specific for Mycobacteria species. Other organisms may exhibit varying degrees of acid fastness (Nocardia, Cryptosporidium, Legionella, Rhodococcus etc.).
   2. Some rapidly growing AFB may be less acid fast than the other groups and may therefore appear to be negative.
3. **REFERENCES**
   1. Della-Latta, P. “Mycobacteriology and Antimycobacterial Susceptibility 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.