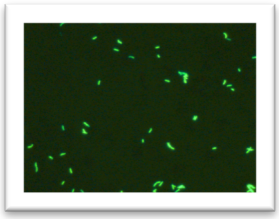
Fluorescence Staining of Mycobacteria



1. Principle

RAL Auramine Staining Kit is one of the kits that allows screening of the slide using a quick fluorescence detection of mycobacteria or Acid Fast Bacilli (AFB).
For the fluorochrome stain, the principle of stain, decolorizer and counterstain is the same as for Ziehl-Neelsen staining. With auramine O stain, organisms fluoresce bright yellow, non-specific debris stains pale yellow, and the background is almost black.
Fluorochrome stain is more sensitive than Ziehl-Neelsen because the smear can be examined under a lower power, thus more fields can be read in the same amount of time, and the bacilli stand out brightly.

2. Equipment, materials and reagents

2.1 Equipments

Fluorescence Microscope with Auramine filter set

2.2 Materials

- Tuberculocidal disinfectant
- Waste receptacles (including splash proof receptacle for liquids)
- Discard bucket with biohazard bag insert, containing appropriate disinfectant
- Paper towel soaked in appropriate disinfectant
- Microscope slides, frosted at one end, new and clean
- Pencil for labeling slides
- Hot plate or slide warmer
- Bunsen burner (or spirit lamp)
- Sterile, transfer pipettes (individually wrapped)
- Sterile loop or disposable applicator stick
- Staining sink or bucket
- Staining rack
- Forceps
- Timer
- Vortex mixer

2.3 Reagents

2.3.1. Non specific reagents

- Tap water wash bottle
- Absolute alcohol

2.3.2. RAL Auramine Staining KIT description (Ref.: 359500-0000)

- Carbolic Auramine (Fluorescence Technique) 1 x 1000 mL
- Discolouring Solution (Fluorescence Technique) 1 x 1000 mL
- Methylene Blue 0.3% (Fluorescence Technique) 1 x 1000 mL

The kit allows staining between 600 and 800 slides.
Processing Time: 24 minutes

2.3.3. Available Refills

- Carbolic Auramine (Fluorescence Technique) Ref. 361435-1000 mL
- Discolouring Solution (Fluorescence Technique) Ref. 320810- 1000 mL
- Methylene Blue 0.3% (Fluorescence Technique) Ref. 365350- 1000 mL

2.3.4. Recommendations and/or Note of use:

For professional, In vitro use only.
The collection and processing of chemical biological waste must be conducted by specialized and registered companies
Auramine toxicity : acute oral and dermal toxicity, carcinogenicity (safety data RAL 310220)

Storage: 15 – 25 °C away from light

3. Specimen preparation

Specimen must be treated in accordance with procedures available in the laboratory and promulgated by national authorities.

3.1. Preparation of Smears from processed sputum

1. Label the frosted end of the slide in pencil with the laboratory accession number, screening ID number and/or subject ID number, visit number, sputum specimen number (#1 or #2, unless specimen is from V2 or V3), and date
2. Draw a circle in the center of the slide with a diamond tip
3. Use a transfer pipette to place ~100 µl (2 drops) from the specimen (10 µl for the workshop) onto the slide, spreading over an area approximately 1 x 2 cm. Air-dry the smear.
4. Place the slides on a hot plate or slide warmer at a temperature between 65°C to 75°C until the slide is dry. Do not expose slides to UV light.
5. Work systematically through the samples with slides on one side and the discard bucket in close proximity. Remember to open only one specimen tube at a time. Dispose of the transfer pipette into the biohazard discard bucket.

3.2. Staining procedure

- Wear gloves
- Place the slide on a staining rack with fixed smear on top, at least 1 cm apart
- Flood the slide with absolute alcohol during 10 minutes or heat fix on a hot plate or slide warmer at a temperature between 65°C to 75°C for at least 2 hours (longer time is preferable)
- Stain by flooding the slide with Carbolic Auramine (Bottle n) and let stand during 20 minutes
- Rinse the stain away with tap water and tilt slide to drain. Water must be Auramine free.
- Discolour by flooding the slide with Discolouring Solution (Bottle o) during 3 minutes
- Wash off the Discolouring Solution with tap water
- Counterstain by flooding the slide with Methylene Blue 0.3% (Bottle p) during 1 minute
- Wash off the Methylene Blue with tap water
- Air dry and keep away from light
- Protect smears from light and examine immediately using the fluorescent microscope. If unable to read right away, place slides in covered box

4. Results

The microscopic examination is usually performed with x20 and x40 dry objectives, without coverslip, using a Fluorescence Microscope with Auramine filter set.

Acid-Alcohol Fast Bacilli (AFB) : fluorescent green-yellow
Background of the preparation: black-blue

Each time fluorescent organisms are detected, it is necessary to confirm the presence of Acid Fast Bacilli (AFB) by a ZN staining. In practice, it is possible to stain the same slide for both stainings.

5. References

TECHNICAL GUIDE Sputum Examination for Tuberculosis by Direct Microscopy in Low Income Countries. Fifth ed. 2000. International Union Against Tuberculosis and Lung Disease
http://www.uphs.upenn.edu/bugdrug/antibiotic_manual/IUATLD_afb%20microscopy_guide.pdf

Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. K R Steingart, M Henry, V Ng, P C Hopewell, A Ramsay, J Cunningham, R Urbanczik, M Perkins, M Abdel Aziz, M Pai, The Lancet Infectious Diseases. 2006; 6, 570–581