ACID FAST STAINS FOR MYCOBACTERIA
(for in vitro diagnostic use)

INTENDED USE
Pro-Lab Acid Fast Stains are for use in staining smears prepared from specimens suspected of containing mycobacteria.

SUMMARY AND EXPLANATION
Mycobacteria possess unique acid fast characteristics that make the acid fast staining techniques valuable for detecting tubercle bacilli.

PRINCIPLE
The lipid content of the cell wall of Acid Fast Bacilli makes staining of the organisms difficult. In acid fast stains, the phenol allows the stain to penetrate, even after exposure to decolourisers. If an organism is to be termed Acid Fast, it must resist decolourisation by acid-alcohol. A counterstain is then used to emphasise the stained organism.

REAGENTS
Ready to use stains:
- PL.8013 Potassium Permanganate 100 ml
- PL.8008 Auramine Phenol 100 ml
- PL.8007 Malachite Green 100 ml
- PL.8006 Methylene Blue 100 ml
- PL.8005 ZN Carbol Fuchsin 100 ml

Concentrated Stains - Dilute to 1 litre with distilled water before use:
- PL.805 ZN Carbol Fuchsin
- PL.806 Methylene Blue
- PL.807 Malachite Green
- PL.808 Auramine Phenol
- PL.801 Potassium Permanganate

Concentrated Stains - Dilute to 5 litres with distilled water before use:
- PL.8005-5.0 ZN Carbol Fuchsin 500 ml
- PL.8006-5.0 Methylene Blue 500 ml
- PL.8007-5.0 Malachite Green 500 ml
- PL.8008-5.0 Auramine Phenol 500 ml
- PL.8013-5.0 Potassium Permanganate 500 ml

Staining Kits (Ready to use):
- PL.8060/25 TB Staining Kit- ZN Carbol Fuchsin 250 ml, ZN Differentiator 2 x 250 ml, Methylene Blue 250 ml.
- PL.8061/25 TB Staining Kit- ZN Carbol Fuchsin 250 ml, ZN Differentiator 2 x 250 ml, Malachite Green 250 ml.

Immersion Oil (Reduced hazard -DBP free)
- PL.396 Immersion Oil 50 ml

CONCENTRATED SOLUTIONS
- PL.7059 Thiazine Red 500 ml
- PL.7038 Auramine Differentiator 2 litres
- PL.7035 Auramine Phenol 2 litres
- PL.7034 Auramine Phenol 1 litre
- PL.7031 Malachite Green 1 litre
- PL.7029 Methylene Blue 2 litres
- PL.7027 Methylene Blue 500 ml
- PL.7026 ZN & Kinyoun CF Differentiator 2 litres
- PL.7021 Kinyoun Carbol Fuchsin 500 ml

QUALITY CONTROL
The age of the cultures and the pH of the medium in which the bacteria are grown can markedly affect their reaction to the stain. Use fresh cultures up to 24 hours old.

Recommended QC cultures:
- Mycobacterium tuberculosis HR37 Rv NCTC 7416
- Streptomyces griseus NCTC 7807

INTERPRETATION OF RESULTS

Ziehl-Neelson method:
Acid Fast Bacilli are stained red, other organisms are stained blue or green dependent on the counterstain used.

Kinyoun Carbol Fuchsin method:
Acid Fast Bacilli are stained red, other organisms are stained blue or green dependent on the counterstain used.

Auramine Phenol method:
Acid Fast Bacilli appear as bright luminous rods against a dark background.

LIMITATIONS
1. False staining results can be seen due to cellular debris being stained by the technique.
2. Positive staining reactions provide presumptive evidence of the presence of M. tuberculosis in the specimen only. Negative staining results do not necessarily indicate the specimen will be negative on culture.

SAFETY PRECAUTIONS
1. Acid Fast Stains from Pro-Lab Diagnostics are offered as an in vitro material and are in no way intended for a curative or prophylactic purpose.
2. During and after use, handle all materials in a manner conforming to Good Laboratory Practices and consider at all times that material and are in no way intended for a curative or prophylactic purpose.
3. The device poses no environmental hazard in excess of those posed by the clinical specimens used with the device. Safety precautions should be taken in handling, processing and discarding all clinical specimens as a pathogenic organism may be present. Environmental impact exists and is adequately addressed through proper disposal.

STABILITY AND STORAGE
Room Temperature. Away from sources of ignition. Away from direct sunlight. Stored under these conditions, reagents may be used up to the date of expiry on the label.

SPECIMEN COLLECTION AND PREPARATION OF CULTURES
Refer to a standard microbiology text.

MATERIALS REQUIRED BUT NOT PROVIDED
Clean glass slides, sterile loop, flame / hot air, staining rack, tap water, immersion oil, microscope, blotting paper or equivalent substitute.

PROCEDURE

Classical Ziehl-Neelson Method.
1. Prepare a thin, uniform smear and air dry.
2. Heat fix and allow to cool.
3. Flood the slide with ZN Carbol Fuchsin and heat gently (do not boil). Allow to stand for 10 minutes applying heat again after 5 minutes.
4. Rinse with water.
5. Flood the slide with Differentiator for 10 minutes, applying a change of Differentiator at 5 minutes.
6. Rinse with water.
7. Flood the slide with counterstain (Methylene Blue or Malachite Green), stand for 1 minute.
8. Rinse well with water, gently blot dry or dry using gentle heat.

Kinyoun Carbol Fuchsin Method.
1. Prepare a thin, uniform smear and air dry.
2. Heat fix and allow to cool.
3. Flood the slide with Kinyoun Carbol Fuchsin, stand for 10 minutes.
4. Rinse with water.
5. Flood the slide with Differentiator for 10 minutes, applying a change of Differentiator at 5 minutes.
6. Rinse with water.
7. Flood the slide with counterstain (Methylene Blue or Malachite Green), stand for 1 minute.
8. Rinse well with water, gently blot dry or dry using gentle heat.

Auramine Phenol Staining Method.
1. Prepare a thin, uniform smear and air dry.
2. Heat fix and allow to cool.
3. Flood the slide with Auramine Phenol, stand for 10 minutes.
4. Rinse with water.
5. Flood the slide with Differentiator for 10 minutes, applying a change of Differentiator at 5 minutes.
6. Rinse with water.
7. Flood the slide with Potassium Permanganate or Thiazone Red, stand for 30 seconds.
8. Rinse well with water, gently blot dry or dry using gentle heat.

MATERIALS REQUIRED BUT NOT PROVIDED
Clean glass slides, sterile loop, flame / hot air, staining rack, tap water, immersion oil, microscope, blotting paper or equivalent substitute.

PROCEDURE

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Kinyoun Carbol Fuchsin Method.
1. Prepare a thin, uniform smear and air dry.
2. Heat fix and allow to cool.
3. Flood the slide with Kinyoun Carbol Fuchsin, stand for 10 minutes.
4. Rinse with water.
5. Flood the slide with Differentiator for 10 minutes, applying a change of Differentiator at 5 minutes.
6. Rinse with water.
7. Flood the slide with counterstain (Methylene Blue or Malachite Green), stand for 1 minute.
8. Rinse well with water, gently blot dry or dry using gentle heat.

Auramine Phenol Staining Method.
1. Prepare a thin, uniform smear and air dry.
2. Heat fix and allow to cool.
3. Flood the slide with Auramine Phenol, stand for 10 minutes.
4. Rinse with water.
5. Flood the slide with Differentiator for 10 minutes, applying a change of Differentiator at 5 minutes.
6. Rinse with water.
7. Flood the slide with Potassium Permanganate or Thiazone Red, stand for 30 seconds.
8. Rinse well with water, gently blot dry or dry using gentle heat.

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Cultural methods should also be employed for positive identification of *M. tuberculosis*.

3. Organisms other than mycobacteria may display varying degrees of acid fastness. E.g. *Rhodococcus* spp., *Cryptosporidium* spp. and *Isopora* spp.

4. It is difficult to over-decolorize acid-fast organism. Ensure thorough decolorization.

5. Timing is important with the counter-staining step using Potassium Permanganate to avoid quenching the fluorescent bacilli.

6. Read prepared slides immediately, or store in the dark at 2-8°C to avoid fading of the fluorescence.

REFERENCES


