PRO-LAB DIAGNOSTICS

ACID FAST STAINS FOR MYCOBACTERIA

(for *in vitro* diagnostic use)

CE

INTENDED USE

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

SUMMARY AND EXPLANANTION

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 decolourisors. 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.7018	ZN Carbol Fuchsin	500 ml
PL.7019	ZN Carbol Fuchsin	1 litre
PL.7020	ZN Carbol Fuchsin	2 litres
PL.7021	Kinyoun Carbol Fuchsin	500 ml
PL.7022	Kinyoun Carbol Fuchsin	1 litre
PL.7024	ZN & Kinyoun CF Differentiator	500 ml
PL.7025	ZN & Kinyoun CF Differentiator	1 litre
PL.7026	ZN & Kinyoun CF Differentiator	2 litres
PL.7027	Methylene Blue	500 ml
PL.7028	Methylene Blue	1 litre
PL.7029	Methylene Blue	2 litres
PL.7030	Malachite Green	500 ml
PL.7031	Malachite Green	1 litre
PL.7032	Malachite Green	2 litres
PL.7033	Auramine Phenol	500 ml
PL.7034	Auramine Phenol	1 litre
PL.7035	Auramine Phenol	2 litres
PL.7036	Auramine Differentiator	500 ml
PL.7037	Auramine Differentiator	1 litre
PL.7038	Auramine Differentiator	2 litres
PL.7059	Thiazine Red	500 ml
PL.7060	Thiazine Red	1 litre

Concentrated Stains - Dilute to 1 litre with distilled water before use.

PL.8005	ZN Carbol Fuchsin	100 ml
PL.8006	Methylene Blue	100 ml
PL.8007	Malachite Green	100 ml
PL.8008	Auramine Phenol	100 ml
PL.8013	Potassium Permanganate	100 ml

Concentrated Stains - Dilute to 4 litres with distilled water before use.

PL.8005-4.0	ZN Carbol Fuchsin	400 ml
PL.8006-4.0	Methylene Blue	400 ml
PL.8007-4.0	Malachite Green	400 ml
PL.8008-4.0	Auramine Phenol	400 ml
PL.8013-4.0	Potassium Permanganate	400 ml

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

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 under test should be regarded as a potential biohazard.
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 sun light. 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.
9. View using oil immersion microscopy.

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.
9. View using oil immersion microscopy.

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 Thiazine Red, stand for 30 seconds.
8. Rinse well with water, gently blot dry or dry using gentle heat.
9. View using oil immersion fluorescent microscopy.

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.

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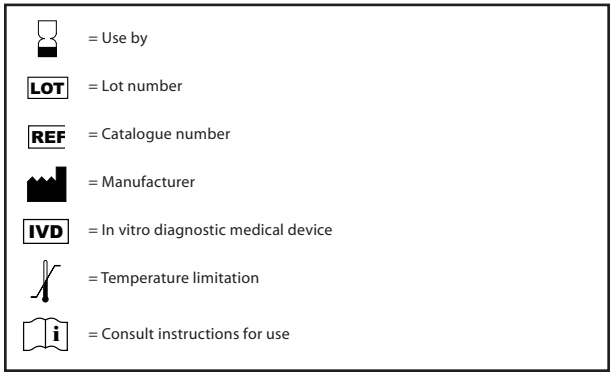
Cultural methods should also be employed for positive identification of *M. tuberculosis*.

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

1. Ziehl, F. 1882. Zur Farbung des Tuberkelbacillus. Dtsch. Med. Wochenschr. 8:451.
2. Neelson, F. 1883. Ein Casuistischer Beitrag zur Lehre von der Tuberkulose. Centraldl. Med. Wiss. 21:497-501.
3. Kinyoun, J. J. 1915. A note on Uhlenhuth's method for sputum examination for tubercle bacilli. Am. J. Clin. Pathol. 46:472-4.
4. Manual of Clinical Microbiology. Lennette.
5. The Practice of Medical Microbiology. 12 Edition. V2. R. Cruickshank, J. P. Duguid, B.P. Marmion, R. H. A. Swain.



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