

ZIEHL-NEELSEN STAIN FOR AFB

Adapted from:

- 1 Bancroft & Stevens, 2nd Edition 1982
- 2 Amer J Clin Pathol, Vol 21, 1951
- 3 Fremantle Hospital, Department of Histopathology, Western Australia

PRINCIPLE:

As the causative agents for Leprosy (*Mycobacterium leprae*) and Nocardiasis (*Nocardia asteroides*) are much less acid and alcohol fast than *Mycobacterium tuberculosis* bacilli, a more gentle dewaxing and minimal exposure to organic solvents is required for adequate staining.

CAUTION: HOT SOLUTIONS, POTENTIALLY TOXIC

METHOD:

- (1) Heat slides in slide dryer to facilitate dewaxing.
- (2) Take sections to water.
- (3) Drain slide and place in a slide mailer with 15 ml of Soft Carbol Fuchsin.
- (4) Fill Slide Mailer (~ 15 ml volume), so that fluid comes up to slide frosting.
Place Mailer into microwave in a beaker, in case of spillage, leave cap of mailer open.

Microwave on FULL POWER,

First time 10 seconds FULL POWER

Second Time 7 seconds FULL POWER

(Check slides each time. CAUTION – halt if solution boils.)

- (5) Allow to stand for 2 - 5 minutes.
- (6) Wash excess stain from slide.
- (7) Wash well in running water, wipe away excess stain 5 min
- (8) Differentiate individually with **0.5% Acid Alcohol**, control microscopically 1 - 3 min
(Use 5% Sulphuric acid for Atypical AFB)
- (9) Wash in running tap water. 5 min
- (10) Filter on **Working Methylene Blue** 20 secs
Avoid overstaining as some weak staining bacterial staining can be replaced by methylene blue.
- (11) Wash with water.
- (12) Drain and air dry thoroughly.
- (13) Dip slides in xylene and mount in DPX.

RESULTS:

Acid Fast Bacilli	RED	(Lepae bacilli are short rods)
Nocardia	RED	(Nocardia organisms are long, thin, and filamentous)
Erythrocytes	PALE PINK	
Background	BLUE	

REAGENTS:

(1) CARBOL SOFT FUCHSIN

New Fuchsin (CI42520)	1.5 gm
Absolute alcohol	10 ml
Distiller water	80 ml
Glycerol	10 ml
Triton X100	0.75 ml

Add the New fuchsin to the alcohol in a 100 ml flask and mix, on a magnetic stirrer for 30 minutes. Add the glycerol and the Triton X 100 to the water and mix till dissolved. Add to the New Fuchsin mixture and continue mixing on the stirrer overnight. Filter and store in a brown glass bottle.

(2) STOCK METHYLENE BLUE

Methylene Blue (CI52015)	2 gm
Using a magnetic stirrer dissolve in	100 ml distilled water
Then add absolute alcohol	100 ml

(4) WORKING METHYLENE BLUE

Stock Methylene Blue	40 ml
Distilled water	60 ml
Glacial acetic acid	0.5 ml

(5) 0.5% ACID ALCOHOL

Distiller water	700 ml
Absolute alcohol	300 ml
Hydrochloric acid	5 ml

(6) 5% SULPHURIC ACID

Distilled water	475 ml
Sulphuric acid	25 ml