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  (Leprae 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

3. **WORKING METHYLENE BLUE**
   - Stock Methylene Blue  40 ml
   - Distilled water  60 ml
   - Glacial acetic acid  0.5 ml

4. **0.5% ACID ALCOHOL**
   - Distiller water  700 ml
   - Absolute alcohol  300 ml
   - Hydrochloric acid  5 ml

5. **5% SULPHURIC ACID**
   - Distilled water  475 ml
   - Sulphuric acid  25 ml

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