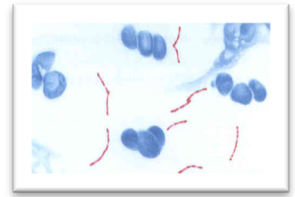


Cold Ziehl-Neelsen Staining of Mycobacteria



1. Principle

The Ziehl-Neelsen method uses a carbol fuchsin stain, acid alcohol decolorizer, and methylene blue counterstain. Acid-fast organisms stain red, while the background of debris stains blue. The ZN stain confirms the acid-fast property of mycobacteria.

QUIK-TB Kit is a Ziehl-Armand staining that is a variation of the Ziehl-Neelsen technique of mycobacteria or Acid Fast Bacilli (AFB) staining. The use of Armand Solution offers the advantage that all bacteria are discoloured, except the mycobacteria, and the background of the coloration is colored at the same time. It is possible to stain the same slide for both stainings (first Auramine Staining and then stained with ZN).

2. Equipment, materials and reagents

2.1 Equipments

Optic Microscope

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 KIT QUICK-TB description (Ref.: 361560-0000)

- Carbolic Fuschin RAL 1 x 125 mL (bottle 1)
- Armand Solution 1 x 125 mL (bottle 2)

The kit allows staining between 125-200 slides.

Processing Time: 7 minutes

For professional, In vitro use only.

The collection and processing of chemical biological waste must be conducted by specialized and registered companies

Fushine toxicity : acute oral toxicity, skin corrosion, serious eye damage, germ cell mutagenicity

Physical hazards : Flammable liquids

Storage: 15 – 25 °C

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

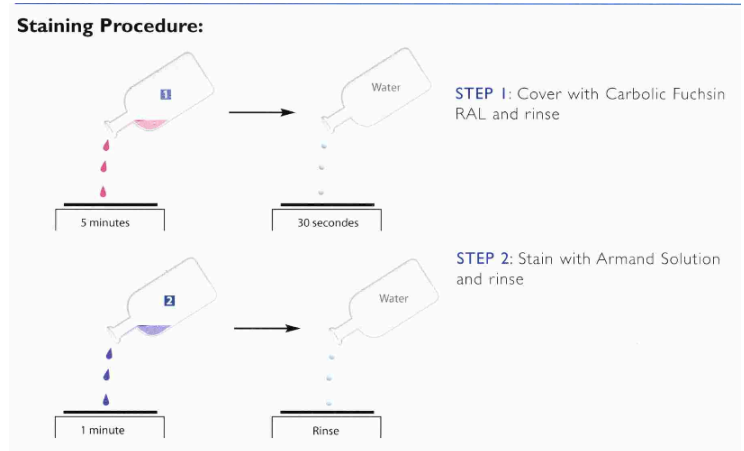
1. Label the frosted end of the slide in pencil with the laboratory accession number, screening ID number and/or subject ID number, visit number, sputum specimen number (#1 or #2, unless specimen is from V2 or V3), and date
2. Draw a circle in the center of the slide with a diamond tip
3. Use a transfer pipette to place ~100 µl (2 drops) (10 µl for the workshop) from the specimen 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 Preparation of Smears from Positive Cultures

1. Label frosted end of slide in pencil with laboratory accession number, screening and/or subject ID number, sputum specimen number (if applicable), and date.
2. Work in a BSC as described in GLI Mycobacteriology Laboratory Manual, 11/04/2014
3. If examining a positive MGIT culture: vortex tube well, unscrew MGIT tube cap and sample an aliquot of broth using a disposable pipette. Place ~100 µl (2 drops) of broth onto the slide, spreading it to cover an area approximately 1 x 2 cm. Dispose of transfer pipette into the biohazard discard bucket.
4. If examining colonies on solid medium, transfer 2 to 3 colonies to the slide, using a sterile loop or disposable applicator stick. Dispose of applicator stick into the biohazard discard bucket.
5. Air-dry smear.

4. 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 Fuchsin RAL (bottle 1) during 30 seconds
- Rinse the stain away with tap water and tilt slide to drain. Water must be Fuchsin free.
- Cover the slide with Armand Solution (bottle 2) during 1 minutes
- Wash off the Armand Solution with tap water
- Air dry



5. Results

The microscopic examination is usually performed with x100 oil objectives, without coverslip.

Acid-Alcohol Fast Bacilli (AFB) : pink
Background of the preparation: black-blue

A negative result should follow from reading 200 (or 100) microscopic fields.

A positive result must provide quantitative data (table 1).

The observation of a single bacillus on a given slide is a dubious result and should always lead to a new investigation of another sample.

Table 1. IUATLD-recommended grading of sputum smear microscopy results	
AFB counts	Recording/reporting
No AFB in at least 100 fields	0/negative
1 to 9 AFB in 100 fields*	Actual AFB counts**
10 to 99 AFB in 100 fields***	+
1 to 10 AFB per field in at least 50 fields**	++
>10 AFB per field in at least 20 fields***	+++
*A finding of 1 to 3 bacilli in 100 fields does not correlate well with culture positivity. The interpretation of the significance of this result should be left to the NTP and not to the microscopist. it is recommended that a new smear be prepared from the same sputum and re-examined	
** the reporting of actual AFB counts is recommended to allow a competent authority to determine whether the number fits the TB case definition of the NTP	
*** in practice most microscopists read a few fields and confirm the finding by a quick visual scan of the remaining fields	

6. References

- TECHNICAL GUIDE Sputum Examination for Tuberculosis by Direct Microscopy in Low Income Countries. IUATLD Fifth ed. 2000
http://www.uphs.upenn.edu/bugdrug/antibiotic_manual/IUATLD_afb%20microscopy_guide.pdf
- Mycobacteriology Laboratory Manual, First Edition, April 2014
A publication of the Global Laboratory Initiative a Working Group of the Stop TB Partnership
<http://www.who.int/tb/laboratory/mycobacteriology-laboratory-manual.pdf>
- AUBERT E., "Cold" Stain for Acid-Fast Bacteria, Canad. J. Public Health, N°41, 1950, p. 31-32.
- CLARK G., Staining Procedures, Williams and Wilkins, 4th ed., 1981, p. 384-385.