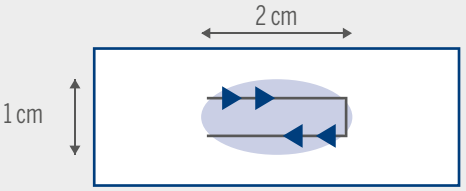
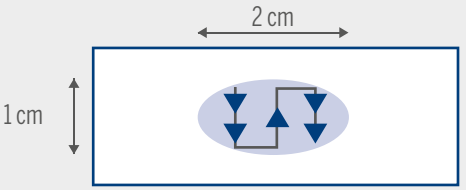
DIAGNOSIS OF MYCOBACTERIA

Fluorescence microscopy observation with Fluo-RAL *Methylene Blue*



HORIZONTAL SCANNING



VERTICAL SCANNING

NB: use appropriate microscope for LED*-based Fluorescence Microscopy (WHO recommendations)

(*) Light Emitting Diode

(**) Laboratory diagnosis of tuberculosis by Sputum Microscopy. The hand book. A publication of the global laboratory initiative, a working group of the stopTB partnership. Global edition. , 2013
(***) AFB: Acid-Fast Bacilli
(****) Confirmation required by another technician, or prepare another smear, stain and read.

Result to report The Handbook/ Stop TB Partnership(**)	Observed 200-250 x magnification 1 length = 30 fields	Observed 400 x magnification 1 length = 40 to 60 fields	Reading
No AFB(***) observed	No AFB / 1 length	No AFB / 1 length	
Confirmation required(****)	1-4 AFB / 1 length	1-2 AFB / 1 length	
Scanty	5-49 AFB / 1 length	3-24 AFB / 1 length	
1+	3-24 AFB / 1 field	1-6 AFB / 1 field	
2+	25-250 AFB / 1 field	7-60 AFB / 1 field	
3+	>250 AFB / 1 field	>60 AFB / 1 field	

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TROUBLESHOOTING

Problem	Possible cause	Remedy
Artefacts	• Sample integrity • Glass slide scratched	• Transport & store sample according to WHO &/or local guidelines • Learn to recognize glass artefacts
Unreadable slide	• Poor quality or insufficient amount of specimen	• New sample
Positive slide reported negative	• Incomplete slide reading • Slide exposed to daylight for too long • Smear too thick, washed off during staining/rising	• Read suggested number of fields • Store slides away from light • Prepare new smear less thick and respect the protocol of fixation >20 min 80°C on heating plate
Low positive or Pale AFB	• Too long an interval between staining and reading • Stains kept in the light • Outdated kit lifespan • Auramine staining time less than 10 min • Overdecolourised	• Keep smears covered and examine within 24 hours of staining • Store away from light • Change kit • Stain for a minimum of 10 min • Do not exceed the maximum time (1 min)
Negative slide reported positive	• Insufficient discolouration time • Small artefacts mistaken for AFB • Cross contamination • Smear size too large	• Increase discolouration time • Cf Artefacts • Respect fixation protocol > 20 min 80 °C on heating plate + fixative solution time for a minimum 5 min • WHO recommendation: 1 cm x 2 cm-sized smears
QC negative spot is positive	• Contaminated stain prepared with water containing environmental mycobacteria • Possible artefacts	• Use distilled water • Ready-to use QC slides recommended
Background too dark	• Counterstained too long (or) decolourised too long • Smear too thick	• Do not exceed 1 min for both steps • Prepare new smear
Too much fluorescence	• Insufficient decolourisation • Smear too thick	• Check decolourisation time • Prepare new smear

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