

LAB DIAGNOSIS OF TB:

1. Specimen collection: PTB: 2 samples
 → One sputum sample
 or 2 sputum sample one hour apart. → Early morning sample
 ↓ collected in empty
 stomach after rinsing
 mouth with soap & water.

Smear deeply (2-3 times) & cough out deep from within the day.

Quality: 3-5ml, thick & persistent. Salivary specimens - rejected.

2. Digestion, Decomposition & Staining: → for staining & culture
 hot for molecular method.

i) Molecular Petroff's method (4% NaOH) = Sputum + 4% NaOH centrifuged & sediment is neutralised with phosphate buffer. (Recommended for LT culture)

ii) NALC (N-acetyl L-cysteine) + 2% NaOH = NALC liquefies sputum & NaOH kills normal flora.

3. Direct Microscopy by Acid fast =

ZN technique: Positive: long, slender beaded, less uniformly stained, red colored

AFB. Staining:

No. of AFB seen	OT to be screened	Methods	Result
1-9 / 100 IF	100	Scanty	Positive
10-99 / 100 IF	100	Scanty	Positive
1-10 / 01F	50	+	Positive
>10 / 01F	20	++	Positive

(iii) Fluorescence staining - auramine prourel 8% (7-10min) → 0.5% acid alcohol (amin x 2),

Di 10% potassium permanganate (for 30sec) Examined under UV. Bacteria - brilliant yellow.

4.) Culture Methods:

Conventional Solid media (LT medium)

Composed of unregulated Rum's egg minimal salt, apoptogenic malnutrition

Colours - Range, High, long columns

Automated liquid culture -

Liquid broth - Middlebrook 7H9 medium + enriched growth media + antibiotics
 BACTEC NIT / Mycobacteria Growth Indicator Tube)

Positively flagged automated culture bottles → Acid fast → positive staining

MPT 64ng, MARDITOF-VITEK

5.) Molecular: PCR: Nested PCR targeting IS6110 gene. DHMS → mpt64 gene, 38kDa genes, ksrA.

Auto induced Real time PCR - 1) CBNAAT -

Gene Xpert ⇒ detect MTB DNA

Recommended for EPTB Rifampicin resistance

2) Chip Based NAAT (Imenat)
↳ PRC

3) Line Probe Assay:- Use - Identification
of MEB complex specimens,
detect of resistance

Resistance to ATTDS-

Type - 1^o - Detection of individual
by drug resistant strain.

Acquired - Infective strain -
initially sensitive becomes
resistant later.

Mechanism - point mutation
which occurs at a rate of 10^{-8} cell
division.