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CHALLENGE TB

Description of different diagnostic tests for drug-resistant tuberculosis

Method	Turnaround time of result (TAT)	Principle	What is detected	Indication	Detect living bacilli	Advantages	Disadvantages
Microscopy	Method: less than 1 hour Result in 24 hours	Staining	Acid-Fast Bacilli (AFB)	(Initial test if Xpert MTB/ RIF is not available) Monitoring treatment response	No	Fast Low cost Widely available	Low sensitivity- Bacterial load > 10 000 cfu/ml Usually negative in HIV+, Children and EPTB samples No speciation Does not detect resistance
Xpert MTB/ RIF	Method: 2 hours Result in 24-48 hours	Molecular genotypic test/ nucleic acid amplification test (NAAT). Real time PCR based	Detects MTB, and resistance to rifampicin	Initial test of choice for all presumptive TB patients	No	Fast (2 hours), High sensitivity (Xpert 131 cfu/ml/ Ultra 16 cfu/ml) High specificity	Relatively expensive Annual calibration and maintenance Electricity-Logistic supply Only detect resistance to RIF Cannot detect all rpoB mutations
LPA **FL:** Hain MTBDR plus **SL:** Hain MTBDRslv2	Method: 48 hours Result in 48-72 hours	Molecular genotypic test/ NAAT. Line probe assay (PCR + hybridization)	Detects MTB (and NTM) Detects resistance to RIF and INH (FL) and FQ and SLI (SL)	FL-LPA: detection of resistance to RIF and INH; use for RIF sensitive, MTB positive Xpert results (sputum smear + specimens or culture isolates) SL-LPA: use for SL resistance testing in all patients diagnosed with RR on Xpert (sputum specimens irrespective of the smear result or culture isolates)	No	Relatively fast (1 day), confirms MTB and detects resistance to FLD and SLD by recognition of mutations	Relatively expensive Biosafety level 2 & 3 Highly skilled staff Cross contamination (open tubes format) Relatively low sensitivity Can give negative or invalid result in smear negative/scanty samples

Culture **Liquid:** MGIT **Solid:** Lowenstein Jensen (LJ)	Culture positive results: MGIT: from 4 days (70% in 2 weeks) LJ: from 2-3 weeks Culture negative results: MGIT: 42 days LJ: 60 days	Incubation of bacteria on liquid (MGIT) or solid (LJ) media	Detects MTB (and NTM)	Test for all presumptive and RR positive DR-TB patients Monitoring treatment	Yes	Detects live mycobacteria, high sensitivity, relatively fast on liquid media (70% culture positive between 4-14 days)	Relatively expensive Biosafety level 3 Highly skilled staff Risk of contamination (2-4% in LJ, around 8% in MGIT) Long TAT of results Can give negative results if there are delays in sample transportation or bad quality samples
Phenotypic DST	After positive culture MGIT: 14 days (Z 21 days) LJ: 30 days	Phenotypic test, based on incubation of mycobacteria in presence of antibiotics	Detects resistance to FLD: R, H (EZ); SLD: FQ/SLID (+/- Eto, Cs, Cfz, Lzd, PAS, Bdq, Dlm)	Baseline for all DR-TB patients; during treatment if suspect failure or smear or culture positive after ≥ 4 months	Yes	Can catch resistant strains overlooked by genotypic methods	Relatively expensive Long TAT: require culture to be completed and confirmed MGIT can miss some Rif resistance close to the MIC (possible false negative result and discordance with molecular tests)
Urine LF-LAM TB	Method: minutes Result in 24 hours	Immunologic lateral flow (LF) strip-based immunodiagnostic test for the detection of LAM antigen in urine	Detects Lipoarabinomannan antigen (LAM) in urine	For HIV positive patients with CD4< 100 cells/µL, or seriously ill regardless the CD4 count	No	Easy to perform Result in minutes Urine samples Low cost	Only for HIV+ patients with CD4<100 cells/µL or seriously ill Only urine samples Rule in test (if negative still can be TB) Does not detect resistance
Next generation sequencing (NGS), including whole genome sequencing (WGS), and targeted sequencing (tNGS)	Method and result: Sample preparation & sequencing: between around 7 and 48 hrs, depending on instrument and test specifics	Nucleic acid amplification test (NAAT). Detects genes by sequencing WGS: determines the whole genome sequence by NGS, targeted NGS: targets specific regions in a genome using NGS, usually it targets genes known to be associated with drug resistance	Detects MTB (and NTM) Detects heteroresistance Detects resistance to R, H, Z, E, FQ, Inj. Detects resistance to new/ newly repurposed drugs, including Bdq and Dlm, but scientific knowledge is currently limited and more research evidence is needed	In research studies/ high resources settings. For genotyping of drug resistance and high resolution molecular epidemiology of TB.	No	NGS is becoming more affordable Probable future gold standard in case of discrepancies Different NGS platforms can use sputum samples and the use of PCR assays can target resistant genotypes to both first and second line drugs within a single test. Possibility of genotype drug resistance	Expensive Biosafety level 3 Qualified lab staff and substantial bioinformatics required to interpret the results. WGS requires enough DNA (cannot be reliably performed without culture to provide sufficient DNA), tNGS can be performed directly from sputum on higher bacillary load specimen. *NB: NGS is not yet endorsed by WHO for routine diagnostic purposes*