GENETIC TESTING IN TUBERCULOSIS

Dr. Sharad Prasad Dahal

OUTLINE

- WHO endorsed testing
- ICMR approved and other moderate complexity NAAT
- Genome Analysis

INTRODUCTION

- Genetic testing are nucleic acid based techniques that detects *Mycobacterium tuberculosis* and associated drug-resistance mutations by identifying specific DNA sequences in patient sample
- Provides rapid diagnosis
- Can be done directly on specimens
- Identify drug resistance

However, Detects genetic materials not viable bacteria

Genetic Testing in tuberculosis

Endorsed by WHO

1. Xpert MTB/RIF
2. Xpert MTB/RIF Ultra
3. Truenat MTB
4. FL-LPA
5. SL-LPA
6. TB LAMP

Others commercially available

1. GeneXpert MTB/XDR
2. GeneXpert Omni
3 Quantiplus MTB FAST
4. PathoDetect™ MTB RIF & INH assay
5. FluoroType MTBDRs/Assay
6. BD Max MDR-TB
47 M2000 RealTime MTB System,

Genomic Testing

1. Whole Genome Sequencing
2. Targeted Next Generation Sequencing
3. Pyrosequencing

WHO ENDORSED TECHNOLOGIES

Molecular detection of TB and Drug Resistance

- Xpert MTB/RIF (Cepheid, Sunnyvale, USA)
- Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, USA)
- Truenat MTB, MTB Plus & MTB-RIF Dx assays (Molbio Diagnostics, Goa, India)
- FL-LPA- GenoType MTBDRs/Assay (Hain Life science, Germany)
- SL-LPA- GenoType MTBDRs/Assay (Hain Life science, Germany)
- TB LAMP (Eiken, Tokyo, Japan)

Fig. 2.1.1. The GeneXpert four-module instrument and the Xpert MTB/RIF test cartridge

XPERT MTB/RIF

Evidence Summary (27 studies, N= 9558) for Pulmonary TB		
	Detecting PTB	Detecting RR
Reference standard	Solid/Liquid Culture	Phenotypic DST
	Pooled Sensitivity	**Pooled Specificity**
Vs Smear Microscopy	88% (84-82%)	99% (98-99%)
Add on test following Negative smear Microscopy	68% (61-74%)	99% (98-99%)
For Smear Positive Culture- positive	98% (97-99%)	
For Smear Negative Culture- positive	68% (61-74%)	
PLHA	79% (70-86%)	
Without HIV	86% (76-92%)	
RR detection	95% (90-97%)	98% (97-99%)
TB vs NTM	1 +ve out of 180 specimen	(14 studies, 2626)

Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children: policy update WHO, 2014

XPERT MTB/RIF

Evidence Summary (15 studies, N= 5922) for Extra-Pulmonary TB		
	Pooled Sensitivity	**Pooled Specificity**
Lymph node tissue and Aspirate	84.9 (72-92)	92.5 (80-97)
Gastric Lavage & Aspirate	83.8 (66-93)	98.1 (92-100)
Other tissue samples	81.2 (68-90)	98.1 (92-100)
CSF	79.5 (62-90)	98.6 (96-100)
Pleural Fluid	43.7 (25-65)	98.1 (95-99)

Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children: policy update WHO, 2014

WORKING PRINCIPLE: CBNAAT

Polymerase Chain Reaction works by repeated heating and cooling called thermal cycles

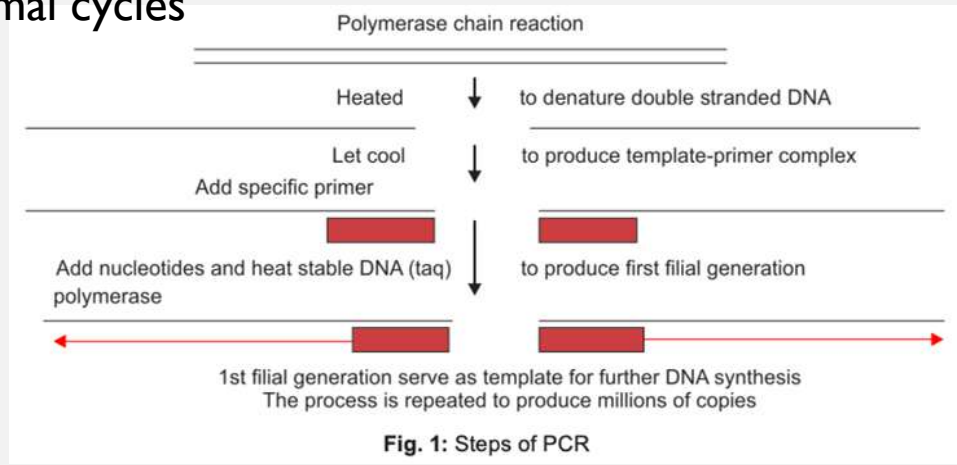
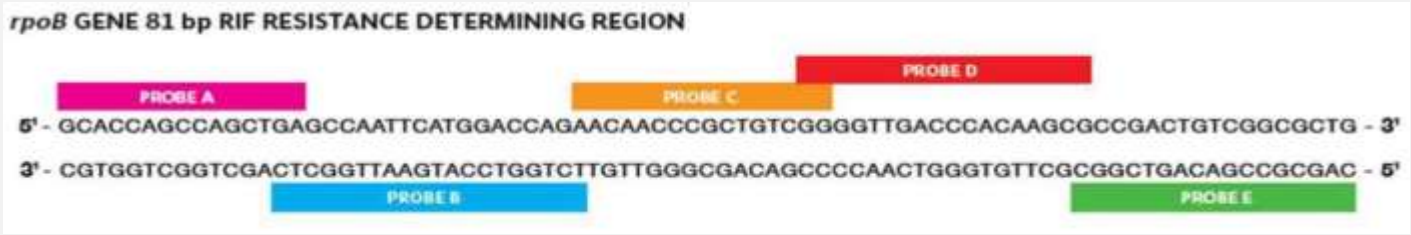


Fig. 1: Steps of PCR

Has three steps

- **Denaturation** (~95'c): Ds-DNA splits into two single strands
- **Annealing** (55-65'): Primers bind to the target DNA
- **Extension** (~72'c): DNA polymerase extends the primers & New DNA strand in synthesized

Primers are short DNA sequence specific to 81-base protein Rifampicin Resistance Determining Region of rpoB



WORKING PRINCIPLE: CBNAAT

During annealing phase:

- Primers bind and DNA amplifies

Beacons "scan" the amplified DNA

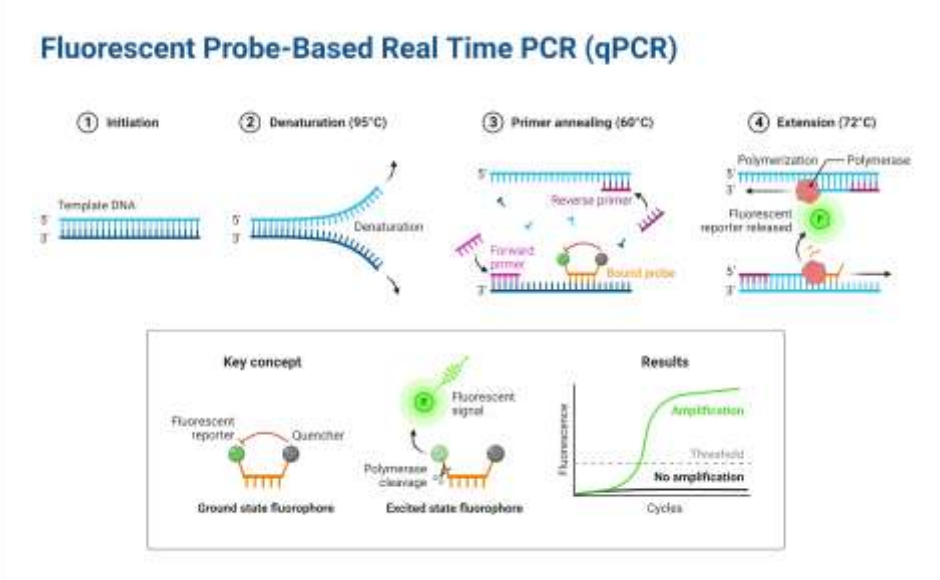
Fluorescent Molecular beacons:

- Pre-manufactured synthetic DNA probes that are already present inside the CBNAAT cartridge reagent
- fluorescent probes light up only when bound to the correct DNA sequence
- If target is present:

Beacon binds → hairpin opens → **fluorescence machine detects signal**

If mutation is present:

- Binding is weak or incomplete
- Signal reduced / delayed



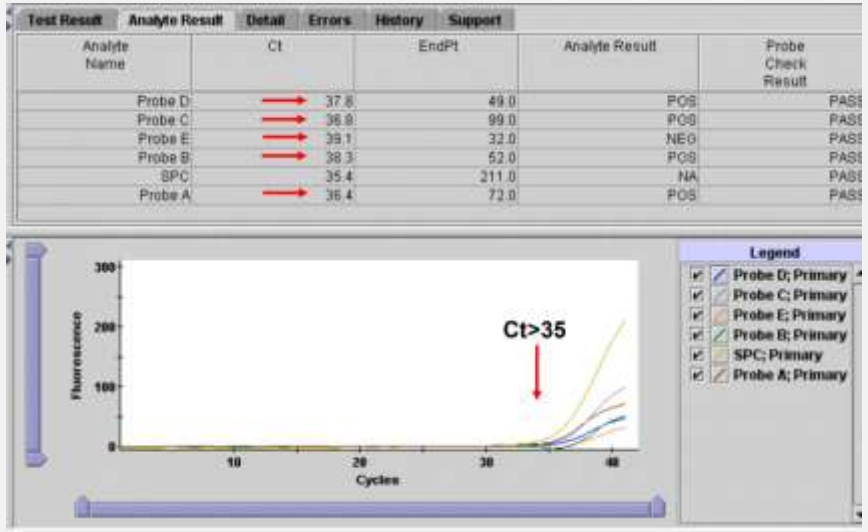
XPERT MTB/RIF

- **QC-1**: Cartridge & Reagent are working correctly. There was no contamination
- **QC-2**: Control for PCR amplification process confirms PCR reagent are functioning correctly
- **SPC**: Ensure sample processing & DNA extraction steps were successful

All three control should show signal for validation

Probes for RpoB

- **Probe A**: Codon 507-511: less common mutation
- **Probe B**: Codon 512-518: Mutation @516 (Asp516 val, Asp516 tyr)
- **Probe C:** Codon 519-523: Mutation impact binding of rifampicin
- **Probe D:** Codon 524-529:
- **Probe E:** Codon 530-533: Most common mutation



- All probe A-E shows signal: No mutations detected
- If one or more probe fails to Hybridize: Mutation is corresponding region of rpoB gene = Rifampicin Resistant
- >35 Threshold cycle in one or more probe: Indeterminate i.e. Bacillary load is very low

QC: quality control probes, SPC: Sample processing control

XPERT MTB/RIF

Limitation of Xpert MTB/RIF

- Less sensitive in tests of smear-negative sputum (Pooled sensitivity :67%)
- Even lower sensitivity in PLHA with smear negative (43%) & smear-negative TB patients from high-resource country with a low TB incidence
- Less sensitive in paucibacillary samples like in extrapulmonary samples
- Decreased capacity to detect rpoB C533G mutations & false detection of non functional rpoBF514F silent mutation as conferring RR

WHO ENDORSED TECHNOLOGIES

Molecular detection of TB and Drug Resistance

- Xpert MTB/RIF (Cepheid, Sunnyvale, USA)
- Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, USA)
- Truenat MTB, MTB Plus & MTB-RIF Dx assays (Molbio Diagnostics, Goa, India)
- FL-LPA- GenoType MTBDRs/Assay (Hain Life science, Germany)
- SL-LPA- GenoType MTBDRs/Assay (Hain Life science, Germany)
- TB LAMP (Eiken, Tokyo, Japan)

XPERT MTB/RIF ULTRA

- Uses 2 different multiple copy amplification targets (IS6110 & IS1081)
- Even **1–2 bacilli** give multiple DNA targets

Target	Copies per MTB cell
rpoB	1 copy
IS6110	~1–25 copies (strain dependent)
IS1081	~5–6 copies

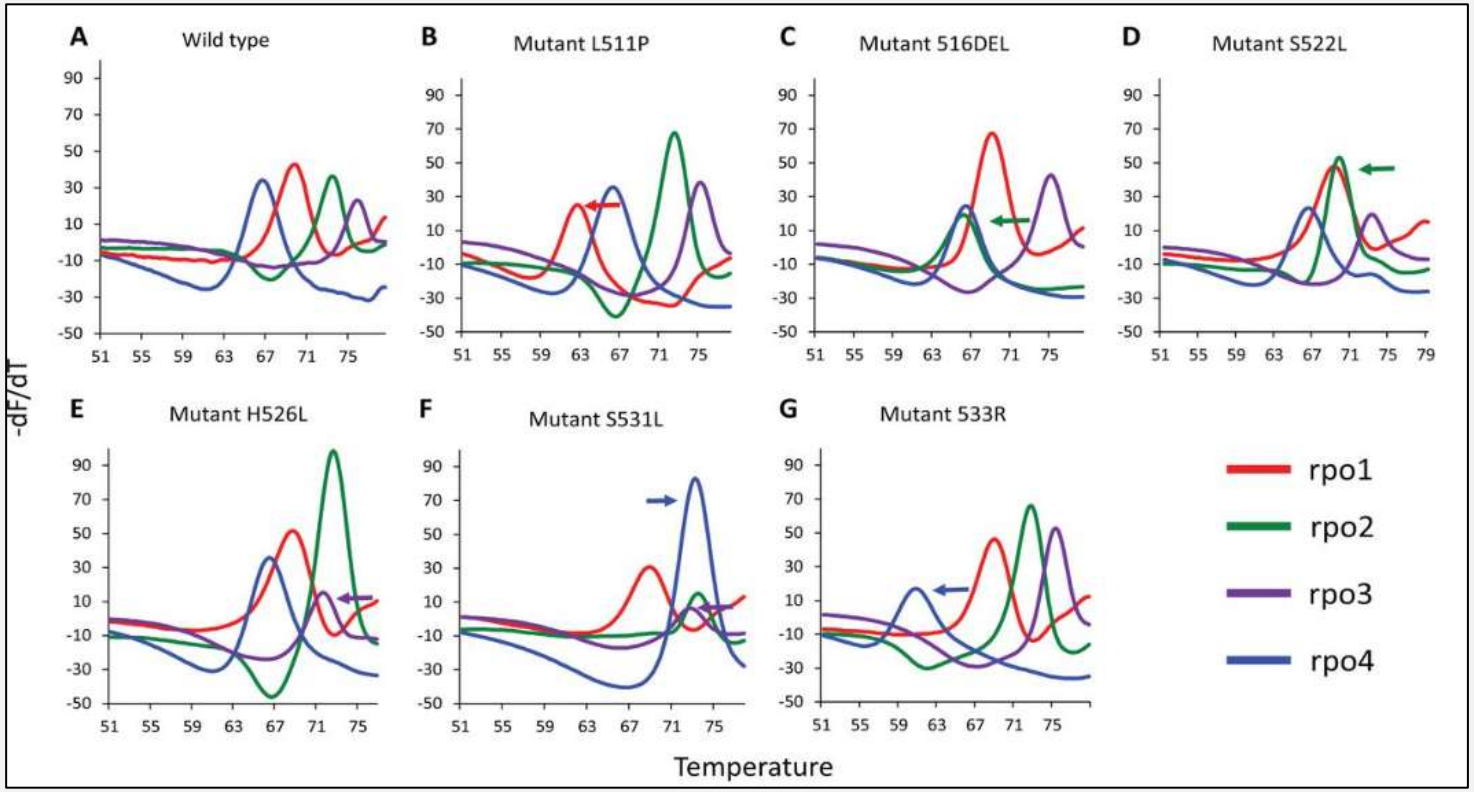
Xpert MTB/RIF	**Xpert MTB/RIF Ultra**
•Targets **single-copy rpoB** •Needs more bacilli to amplify signal	Targets: • rpoB (for rif resistance) • **IS6110 + IS1081** (for TB detection)

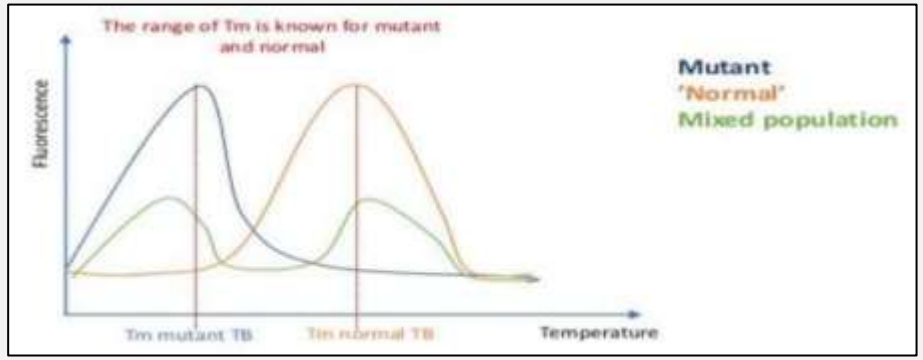
No signals generates from the two M. tuberculosis detection probes targeting the IS6110 and IS1081 genes "MTB not detected"

Chakravorty S, et al. mBio 2017

XPERT MTB/RIF ULTRA : DETECTION OF MUTATIONS

Melting Curve Analysis



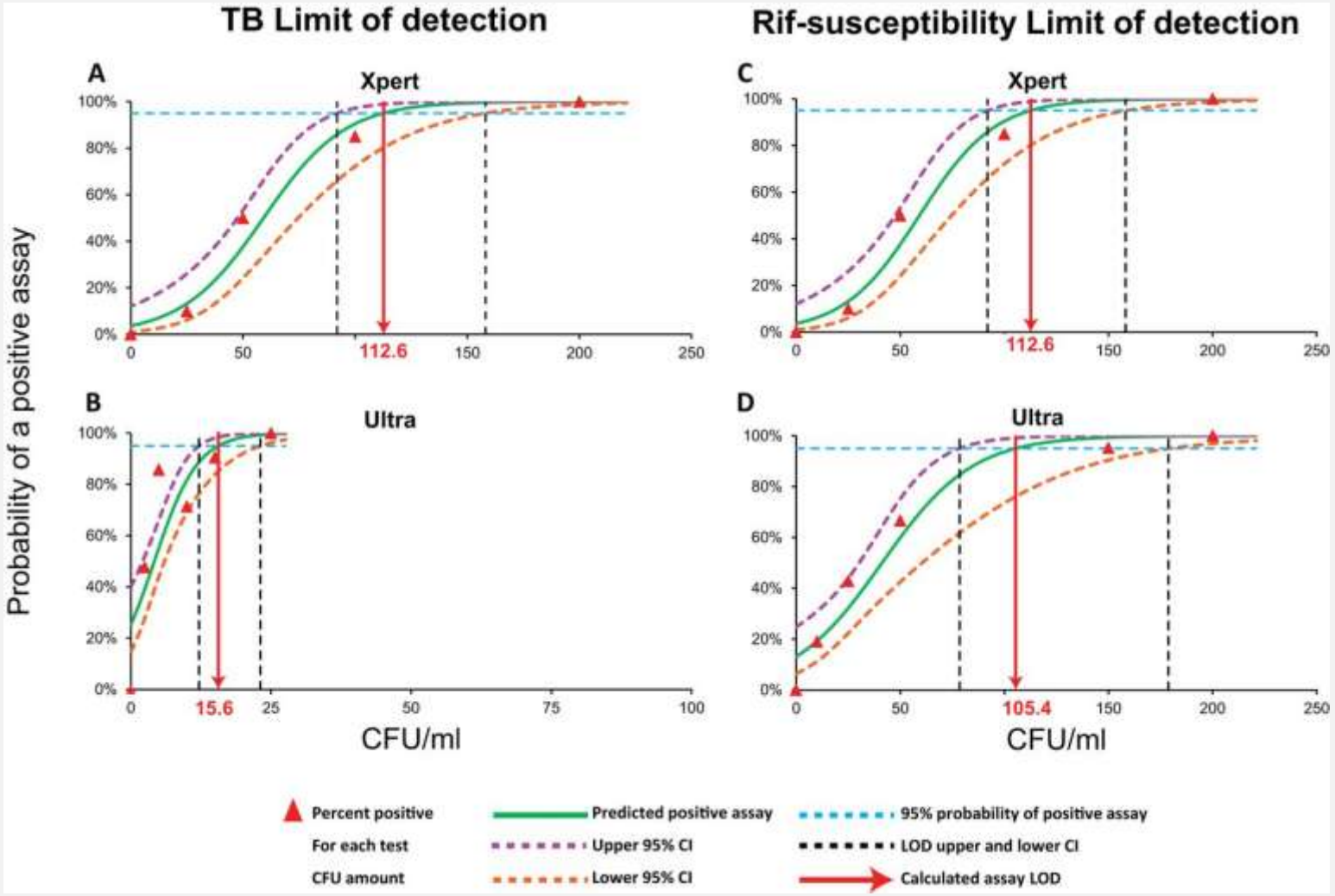


- Uses 4 probes to identify Rif-resistance mutations of rpoB gene
- Uses melting temperature based analysis instead of real-time PCR
- If a mutation is present, dsDNA dissociates sooner than normal DNA

- Detects subtle mutation: as probe dropout might miss weak binding
- Works in low bacillary load as well
- Detects heteroresistance: by showing both wild and mutant population
- Less dependence on signal intensity : not affected by poor amplification

Chakravorty S, et al. mBio 2017

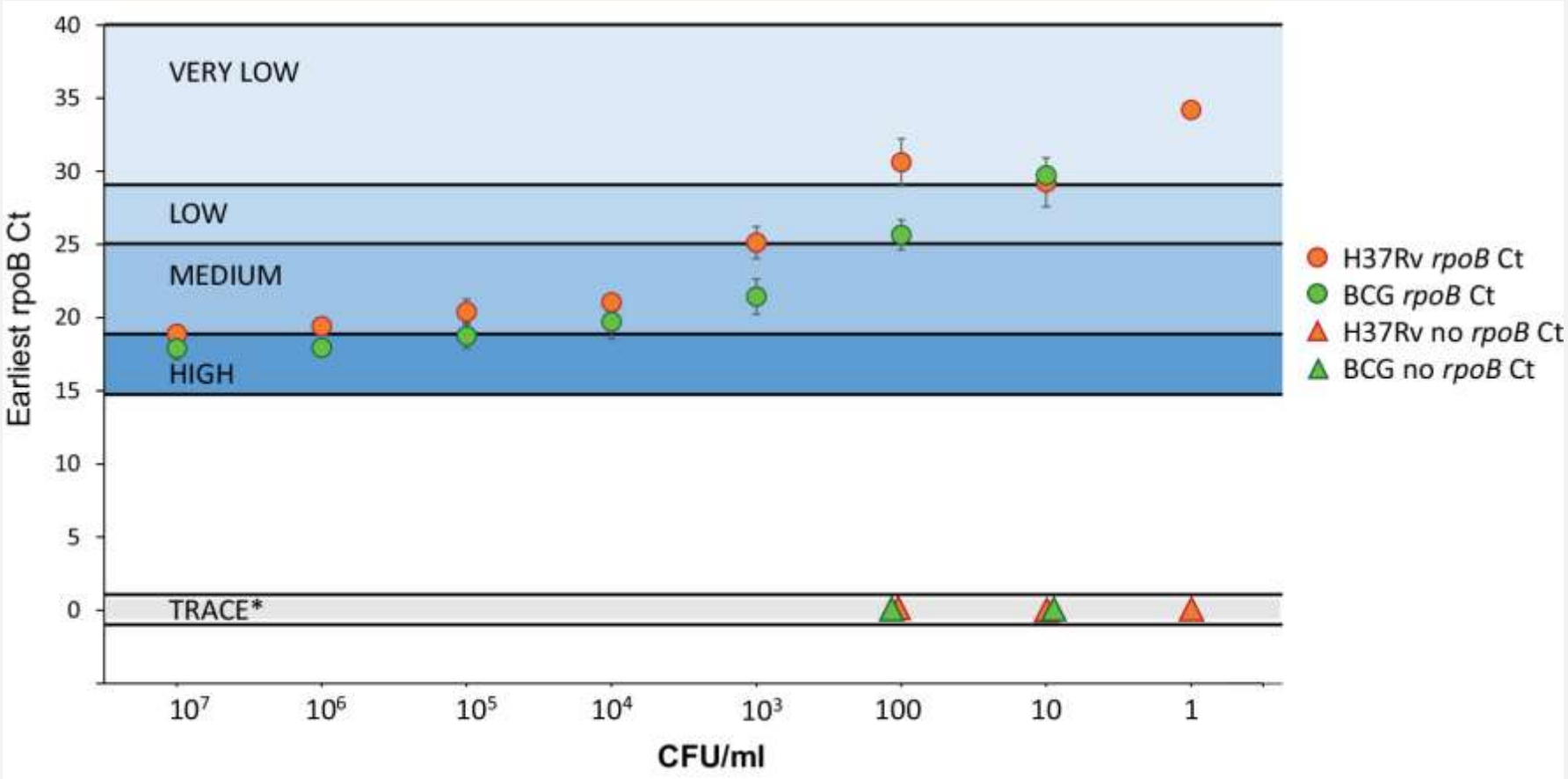
XPERT MTB/RIF ULTRA: IMPROVED LIMIT OF DETECTION



Limit of detection for M. tuberculosis H37Rv. The limit of detection of tuberculosis detection is shown for Xpert (A) versus Ultra (B).

Chakravorty S, et al. mBio 2017

XPERT MTB/RIF ULTRA : SEMIQUANTITATIVE MEASURES



"Trace" : Paucibacillary samples with IS6110/IS1081 positive but rpoB negative
But RR cannot be determined

Chakravorty S, et al. mBio 2017

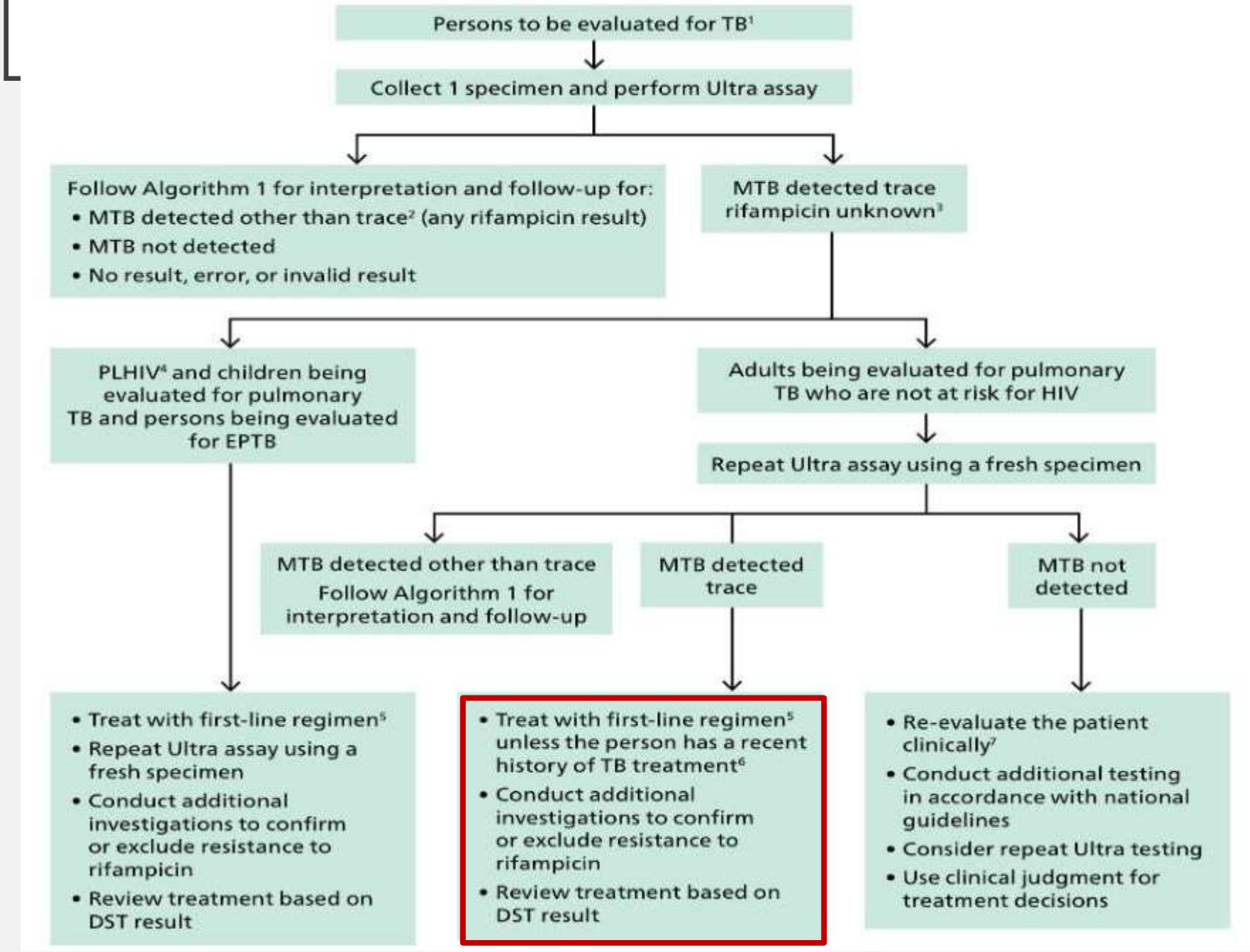
XPERT MTB/RIF ULTRA : SEMIQUANTITATIVE MEASURES

MTB detected: Trace Calls

- Among persons with **HIV, children and extra pulmonary specimens** → Considered to be true positive results for use in clinical decisions and patient follow-up
- Among persons not at risk for HIV → initial "trace call" → a fresh specimen repeat testing & result of the second Ultra test to be used for clinical decisions and patient follow-up
- A second "trace call" positive → diagnosis of pulmonary TB (unless there is a recent history of TB)

WHO Meeting Report, 2017

URES



WHO Meeting Report, 2017

XPERT MTB/RIF ULTRA : IMPROVED SENSITIVITY

- Has Larger DNA reaction chamber than Xpert MTB/RIF
- 50 microlitre PCR reaction in Ultra versus 25 microlitre in Xpert MTB/RIF
- Melting temperature-based analysis - better differentiates silent mutations (such as Q513Q or F514F) from resistance conferring mutations

	Xpert MTB/RIF	XPERT MTB/RIF Ultra
Amplification for TB detection	Single target : rpoB core region	Multi-copy target : rpoB core region Insertion sequence : IS6110, IS1081
Resistance detection	Real-Time PCR 5 probes bind to rpoB gene	Melting curve analysis 4 probes bind to rpoB gene
PCR reaction	25ul	50ul
Assay TAT	112 min	65-87min
LOD	131 cfu/ml	16cfu/ml

Chakravorty S, et al. mBio 2017

XPERT MTB/RIF ULTRA : IMPROVED SENSITIVITY

Non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF

- Method: Multi-Centre, non inferiority diagnostic accuracy study
- Xpert MTB/RIF and Ultra were performed from the **same sputum specimen**
- Accuracy was determined with cultures as the reference standard for TB
- **Phenotypic drug-susceptibility** + sequencing → for rifampicin resistance detection
- N= 1,520 (with signs and symptoms of PTB)

WHO Meeting Report, 2017

XPERT MTB/RIF ULTRA : IMPROVED SENSITIVITY

Non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF

Assay	Tuberculosis detection			Rifampicin resistance detection	
	% sensitivity (95% CI)				
	All culture-positive specimens (*n* = 200)	Smear-negative specimens (*n* = 109)	% specificity (95% CI) (*n* = 77)	% sensitivity (95% CI) (*n* = 41)	% specificity (95% CI) (*n* = 98)
Xpert	81.0 (74.9-86.2)	66.1 (56.4- 74.9)	98.7 (93.0- 100)	92.7 (80.1- 98.5)	99.0 (94.4- 100)
Ultra	87.5 (82.1-91.7)	78.9 (70.0- 86.1)	98.7 (93.0- 100)	92.7 (80.1- 98.5)	98.0 (92.8- 99.9)

EPTB

- Ultra 95% vs 45% GeneXpert (CSF)
- More beneficial due to the "trace call"

In epidemiological,

- Ultra could detect 2 to 9 additional TB cases per 1000 individuals evaluated for presumptive TB
- Prevent one additional TB death per 700 to 30,000 individuals evaluated

WHO Meeting Report, 2017

WHO ENDORSED TECHNOLOGIES

Molecular detection of TB and Drug Resistance

- Xpert MTB/RIF (Cepheid, Sunnyvale, USA)
- Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, USA)
- Truenat MTB, MTB Plus & MTB-RIF Dx assays (Molbio Diagnostics, Goa, India)
- FL-LPA- GenoType MTBDRs/Assay (Hain Life science, Germany)
- SL-LPA- GenoType MTBDRs/Assay (Hain Life science, Germany)
- TB LAMP (Eiken, Tokyo, Japan)

TRUENAT MTB

- Indigenously developed in India
- Chip based real time micro - PCR assay
- Automated battery operated devices for separate extraction and amplification
- Two step detection for MTB (Trunat MTB, MTB plus) and RIF resistance (MTB – RIF Dx)
- Advantages
  - Minimal infrastructure involved – suitable for peripheral laboratories
  - DNA extracted can be used for multiple tests

TAT – 1+1 hour

Sensitivity – Truenat MTB – 83%, MTB - Rif Dx 93%
Specificity - Truenat MTB – 99% MTB - Rif Dx 95%

WHO Module 3, 2025

TRUENAT MTB: PERFORMANCE

Rapid Diagnosis of *Mycobacterium tuberculosis* with Truenat MTB: A Near-Care Approach

Chaitali Nikam[1], Manjula Jagannath[2], Manoj Mulakkapurath Narayanan[2], Vinaya Ramanabhiraman[2], Mubin Kazi[1], Anjali Shetty[1], Camilla Rodrigues[1]*

1 Department of Microbiology, P. D. Hinduja Hospital and Medical Research Centre, Mahim, Mumbai, India, 2 bigtecLabs, bigtec Pvt.Ltd, Rajajinagar, Bangalore, India

N= 274, Suspected PTB
Sputum samples

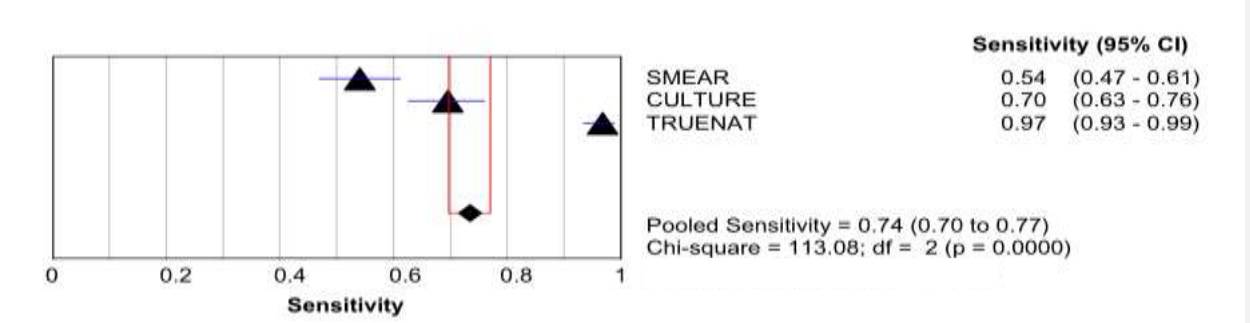


Table 1 – Performance (% of cases detected) of molecular tests in various specimen categories.

Test	S+ (*n* = 108)	C+ (*n* = 151)	S+C+ (*n* = 93)	S−C+ (*n* = 58)
Xpert MTB/RIF	100 [96.5–100.00]	96.02 [89.09–98.63]	100 [96.5–100.00]	90.14 [88.71–94.35]
TrueNAT MTB	99.07 [94.2–99.95]	92.71 [88.65–97.06]	98.92 [94.2–99.95]	86.21 [74.07–93.44]

Nikam et. al. IJoM, 2016

TRUENAT MTB: LITERATURE

Rapid, point-of-care diagnosis of tuberculosis with novel Truenat assay: Cost-effectiveness analysis for India's public sector

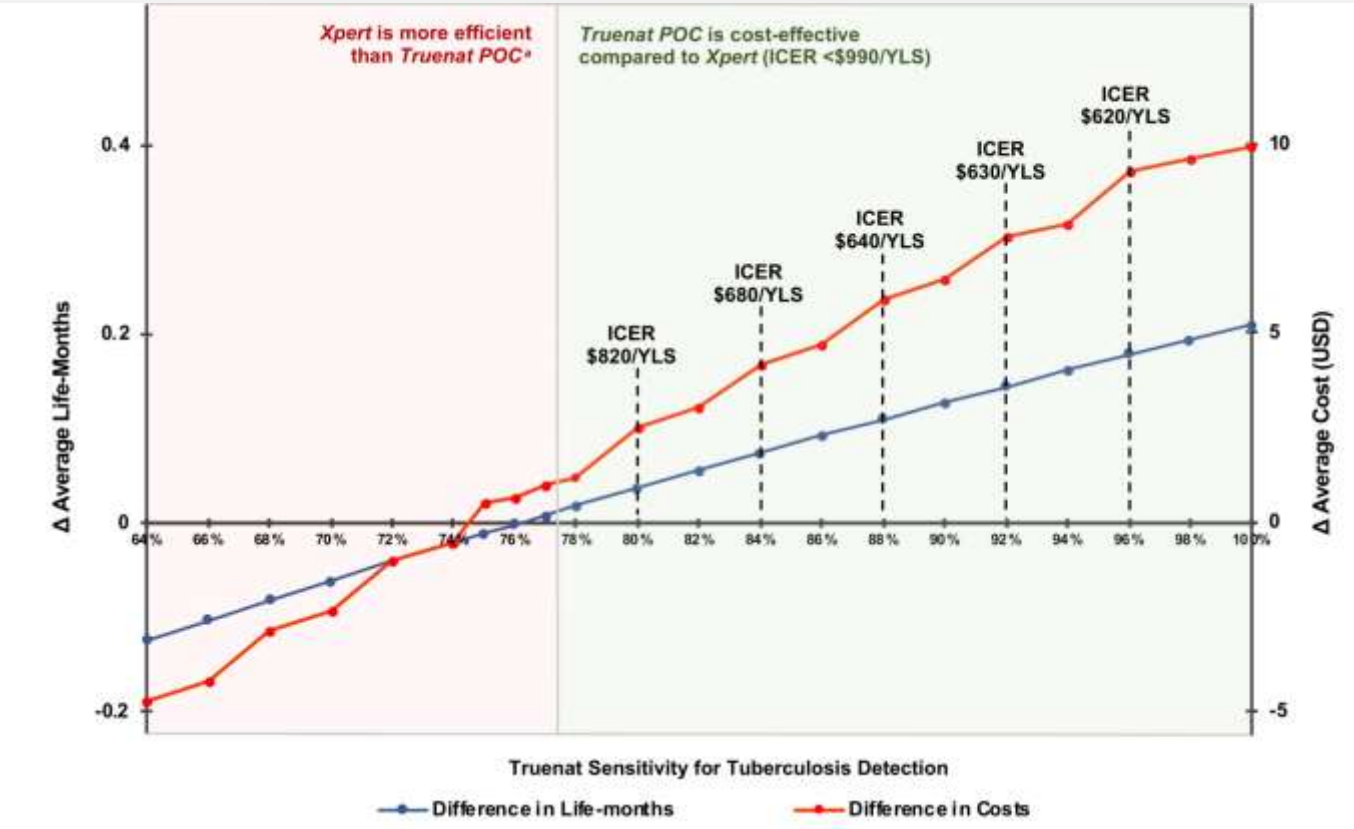
- Compared 3 TB diagnostic strategies for HIV-negative adults with presumptive TB:
  - sputum smear microscopy
  - Xpert MTB/RIF (Xpert)
  - Truenat for point-of-care testing in primary healthcare

- Outcome measures: life expectancy, costs, incremental cost-effectiveness ratios (ICERs), and 5-year budget impact of deploying Truenat POC in India's public sector

Lee et al, 2019 Plos One

TRUENAT MTB: LITERATURE

Rapid, point-of-care diagnosis of tuberculosis with novel Truenat assay: Cost-effectiveness analysis for India's public sector



Findings:

- Compared to SSM, Truenat POC increased life expectancy by 0.39 years and was cost-effective (ICER $210/YLS)
- Compared to Xpert, Truenat POC increased life expectancy by 0.08 years due to improved linkage-to-care and was cost-effective (ICER $120/YLS)

Lee et al, 2019 Plos One

WHO ENDORSED TECHNOLOGIES

Molecular detection of TB and Drug Resistance

- Xpert MTB/RIF (Cepheid, Sunnyvale, USA)
- Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, USA)
- Truenat MTB, MTB Plus & MTB-RIF Dx assays (Molbio Diagnostics, Goa, India)
- FL-LPA- GenoType MTBDRs/Assay (Hain Life science, Germany)
- SL-LPA- GenoType MTBDRs/Assay (Hain Life science, Germany)
- TB LAMP (Eiken, Tokyo, Japan)

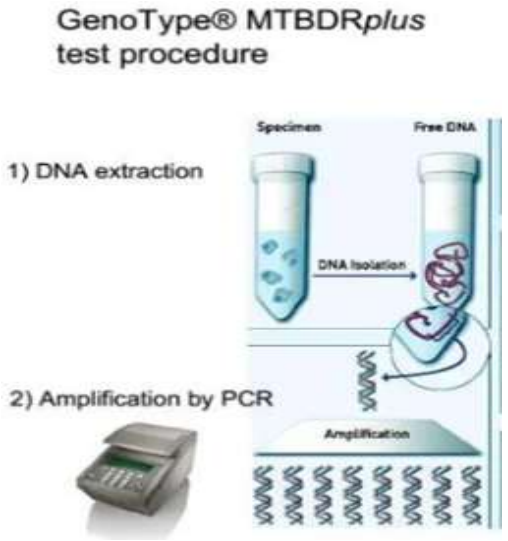
LINE PROBE ASSAY

- WHO endorsement for the use of GenoType MTBDRplus V1 → 2008
- Used for testing of
  - Culture isolates→ Indirect LPA
  - Acid-fast bacilli (AFB) smear microscopy positive specimens → Direct testing

LINE PROBE ASSAY: WORKING PRINCIPLE

Hybridization

- All PCRs will be usually followed by hybridization



- DNA extraction & PCR amplification of target genes
  - rpoB → Rifampicin
  - katG, inhA → Isoniazid
  - (MTBDRsl: gyrA/B, rrs → FQ & injectables)
- Step 2: Hybridization
  - Amplified DNA binds to immobilized probes on nitrocellulose strip
  - These strips contains Immobilized DNA probes in fixed positions : Either WT probe or MUT probe

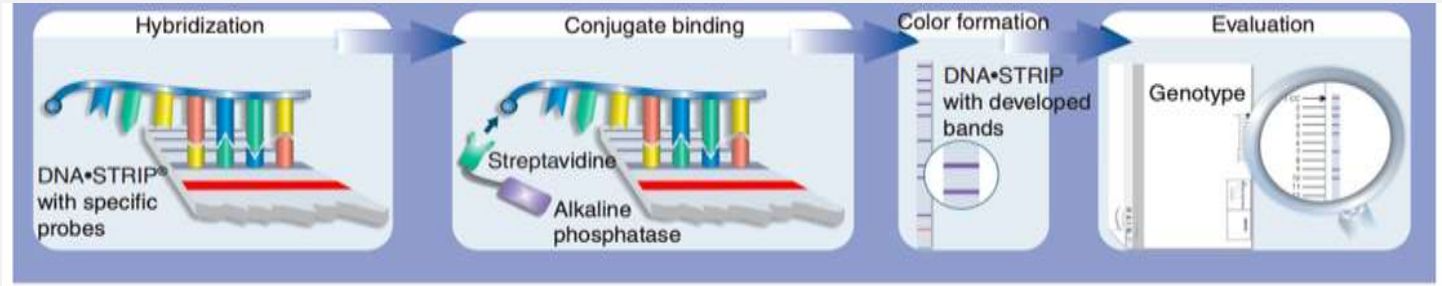
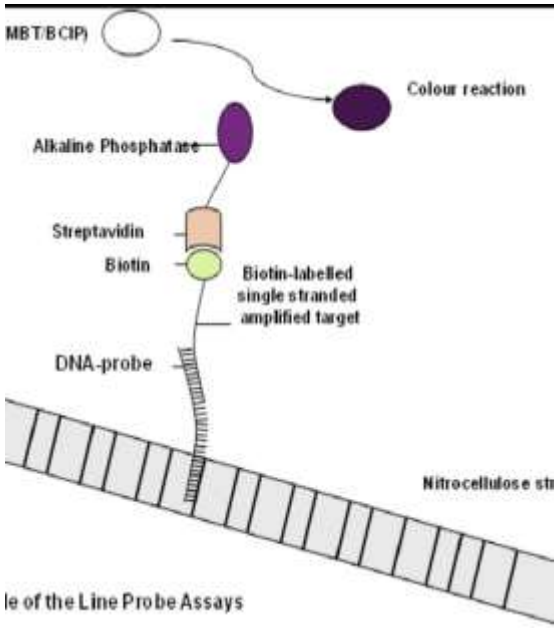


Figure 2. Overview of the GenoType® MTBDR*plus* assay.

LINE PROBE ASSAY: WORKING PRINCIPLE

Hybridization



- All PCRs will be usually followed by hybridization

Step 3: Conjugate binding

- Streptavidin-alkaline phosphate conjugate is added
- During PCR, TB DNA is biotin labeled → streptavidin binds to biotin with very high affinity
- Probe-DNA-biotin-streptavidin-enzyme complex is formed

Step 4: Color formation

- A substrate (colorless BCIP/Nitro-blue tetrazolium) is added → ALP cleaves the BCIP which generates electron and reduces NBT → if DNA is bound to the hybridized part → will develop colour

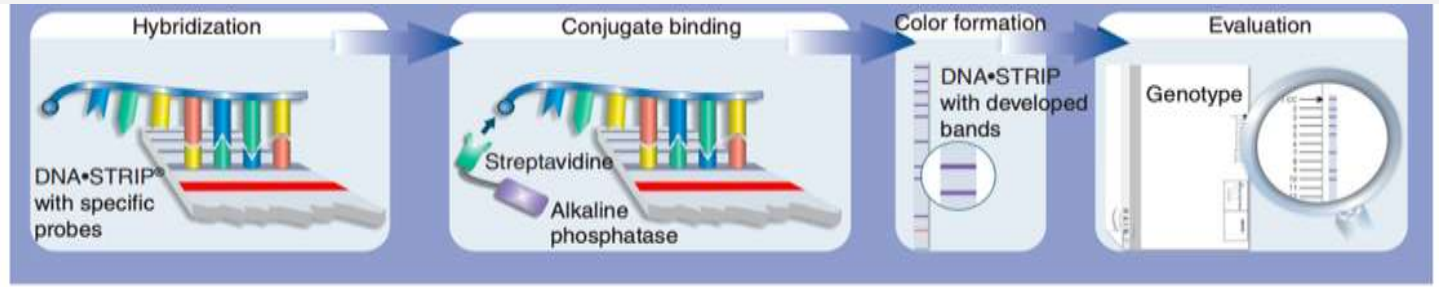
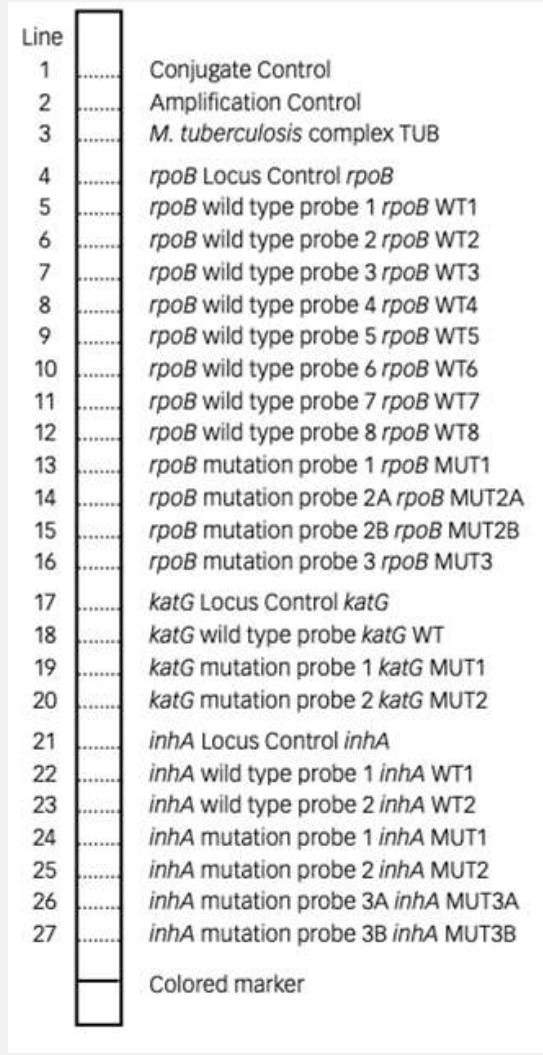


Figure 2. Overview of the GenoType® MTBDR*plus* assay.

FIRST LINE: LINE PROBE ASSAY

- First line LPA can detect MTB, and resistance to INH and RIF
- Drug resistance is detected by the **absence of colored band formation with wild-type probe** and by the **presence of color band formation with commonly occurring specific mutant-type probes**

		Sensitivity	**Specificity**
RR (direct)	Hain Ver 1 Hain Ver 2	97.1% 98.2%	97.1% 97.8
RR (indirect)	Hain Ver 1 Hain Ver 2	91.3% 91.3%	97.1% 97.1%
INH R (direct)	Hain Ver 1 Hain Ver 2	94.4% 96.4%	97.1% 97.1%
INH R (indirect)	Hain Ver 1 Hain Ver 2	89.4% 89.4%	98.9% 98.9%

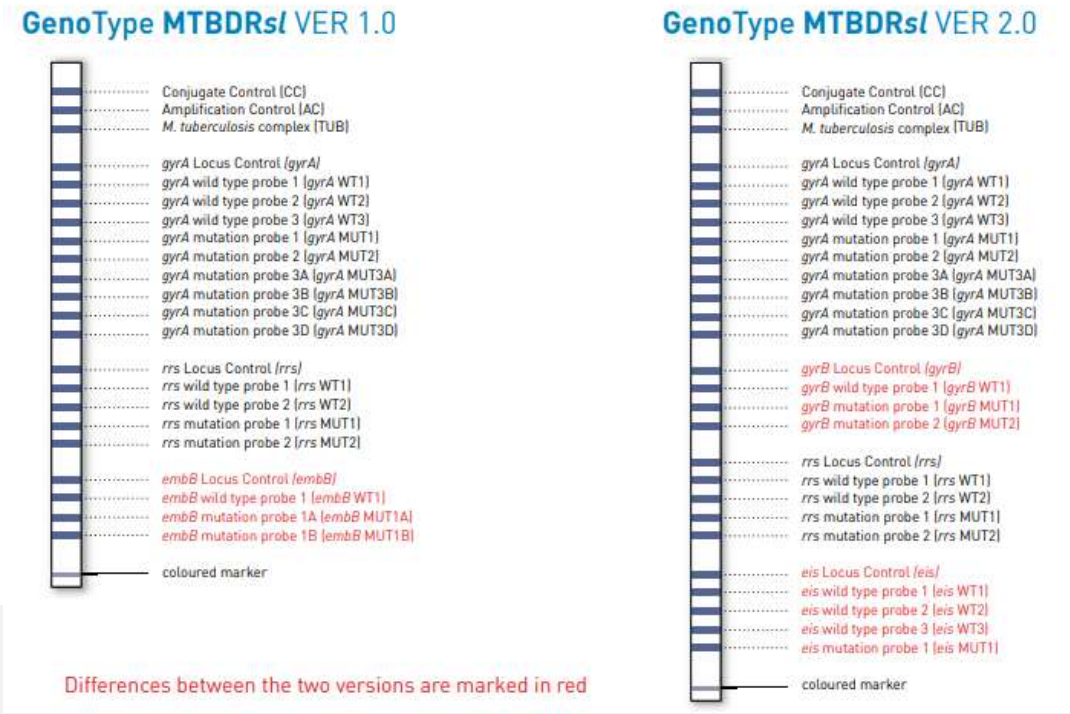


WHO module 3, 2025

SECOND LINE: LINE PROBE ASSAY

- DNA-based test that identifies genetic mutations for resistant to fluoroquinolones (FQs) and second line injectable drugs (SLIDs).

	Genotype MTBDRsl ver 1.0	**Genotype MTBDRsl ver 2.0**
Detection of	MTBC, resistance to FQ, Aminoglycosides and **ETHAMBUTOL**	MTBC, resistance to FQ, Aminoglycosides
Sample material	Smear **+** pulmonary & cultivated samples	Smear **+ and -** pulmonary/EP & cultivated samples
Ethambutol resistance	Detects emb gene mutation	-
FQ resistance	gyrA	Detects gyrA and **gyrB** gene mutations
SLID resistance	rrs	Detects rrs and **eis** gene mutation



Genoscholar™ PZA-TB II (Nipro): for Pyrazinamide Resistance

WHO module 3, 2025

SECOND LINE: LINE PROBE ASSAY

Table 2. Accuracy of MTBDRsl (version 1.0) for fluoroquinolone and second-line injectable drug resistance and XDR-TB, indirect and direct testing (smear-positive specimens), phenotypic culture-based DST reference standard

Pooled sensitivity (95% CI)	Pooled specificity (95% CI)	Pooled sensitivity (95% CI)	Pooled specificity (95% CI)
Fluoroquinolones, indirect testing (19 studies, 2 223 participants)		**Fluoroquinolones, direct testing** (9 studies, 1 771 participants)	
85.6% (79.2 to 90.4)	98.5% (95.7 to 99.5)	86.2% (74.6 to 93.0)	98.6% (96.9 to 99.4)
Second-line injectable drugs, indirect testing (16 studies, 1 921 participants)		**Second-line injectable drugs, direct testing** (8 studies, 1 639 participants)	
76.5% (63.3 to 86.0)	99.1% (97.3 to 99.7)	87.0% (38.1 to 98.6)	99.5% (93.6 to 100.0)
XDR-TB, indirect testing (8 studies, 880 participants)		**XDR-TB, direct testing** (6 studies, 1 420 participants)	
70.9% (42.9 to 88.8)	98.8% (96.1 to 99.6)	69.4% (38.8 to 89.0)	99.4% (95.0 to 99.3)

[1] Likelihood ratio test for evidence of a significant difference between accuracy estimates.

MTBDRsl version 2.0

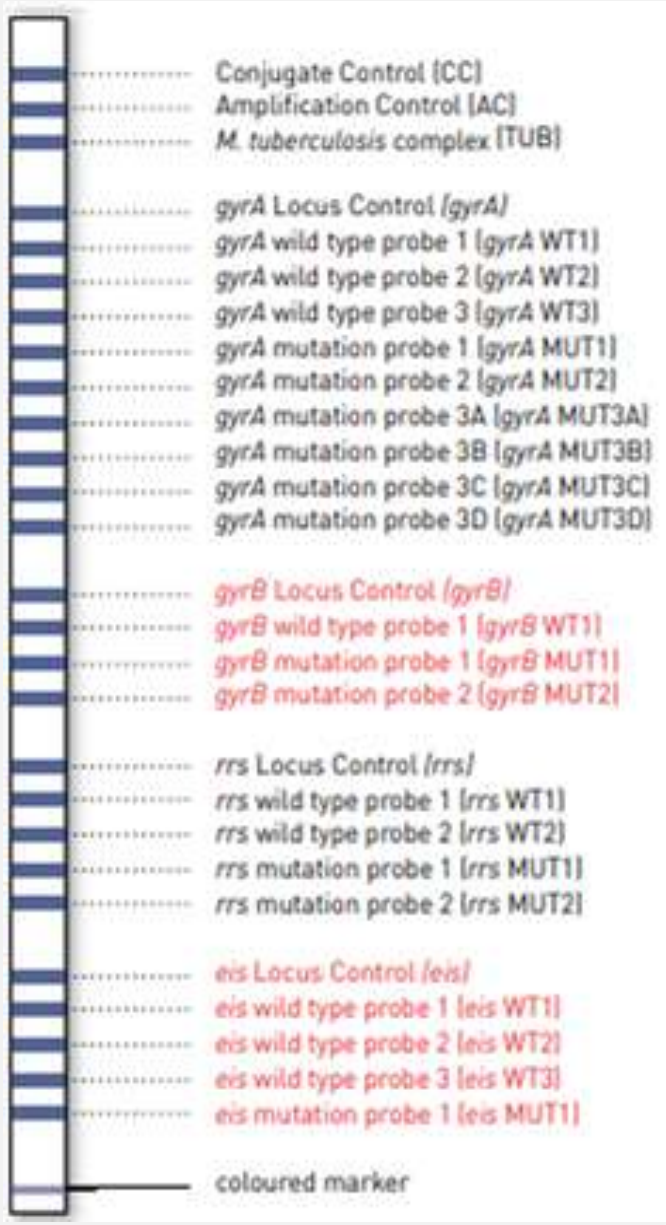
Direct testing		sensitivity	specificity
FQ resistance	**Smear +**	**100%**	**100%**
	Smear -	**100%**	**90%**
SLID resistance	**Smear+**	**62%**	**91%**
	smear -	**83%**	**78%**

Indirect testing	sensitivity	specificity
FQ resistance	84-100%	99-100%
SLID resistance	72-89%	90-99%

LINE PROBE ASSAY: INTERPRETATION

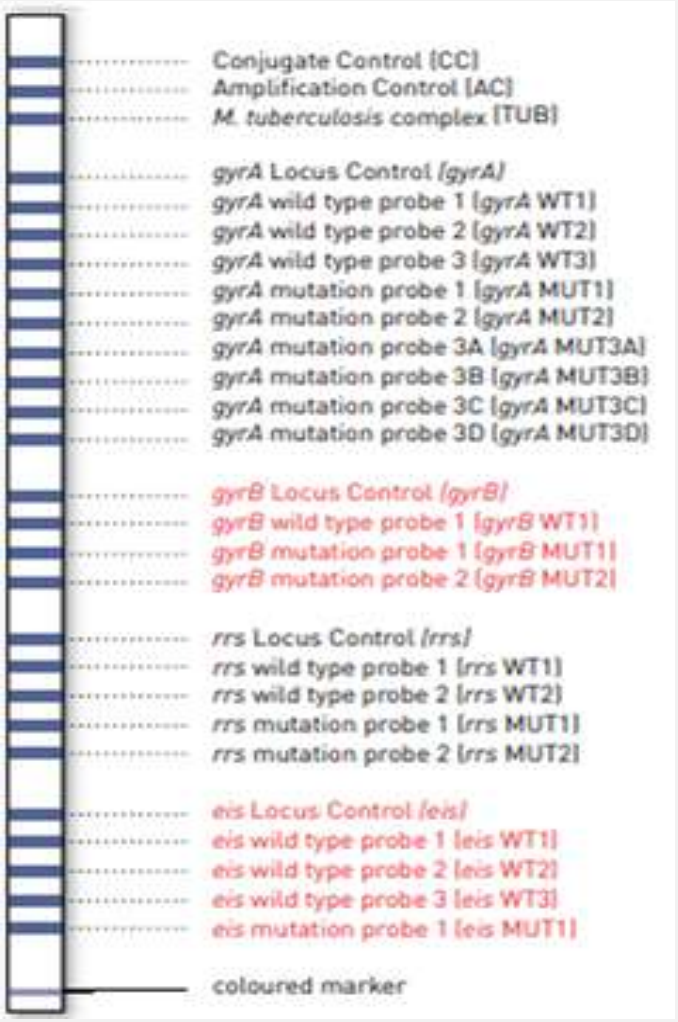
The LPA has two internal controls on the strip:

- the Conjugate Control (line 1), and the Amplification Control (line 2)
    - The Conjugate Control line should always be visible to document the efficiency of conjugate binding and substrate reaction
    - The Amplification Control serves as reference for the interpretation of WT and MUT probes
        - only those bands whose intensities are about as strong as or stronger than that of the Amplification Control band are to be considered
        - In case of a positive test result, the signal of the Amplification Control zone can be weak or even vanish totally
        - **In case of a negative test result, both Conjugate Control and Amplification Control bands should always be visible (i.e. valid negative result)**



LINE PROBE ASSAY: INTERPRETATION

Wild Type band	Mutant Bands	Interpretation
All developed	None developed	Resistance not detected
One or more not developed	None Developed	Resistance inferred
	One or more developed	Resistance detected



LINE PROBE ASSAY VS GENEXPERT

- N=145 sputum samples from suspected DRTB patients
- MDR :25.8%, Mono Rif : 22.3% Mono H ; 29 10.3%, Pansensitive : 41.5%

TABLE 1 Comparison of LPA, Xpert MTB/RIF, and MGIT-DST results on sputum samples

	No. (%) of samples with indicated result in:					
	Xpert MTB/RIF (n = 145)			MGIT-DST (n = 25)[a]		
LPA results (n = 145) (no. of samples)	Resistant	Susceptible	Error[b]	Resistant	Susceptible	Contaminated
LPA RIF^r (62)	38 (64.4)	21[a] (35.5)	3	20 (100)	0	1
LPA RIF^s (83)	4[a] (5.4)	74 (94.5)	5	0	3 (100)	1

[a] Discrepant results between LPA and Xpert MTB/RIF.
[b] Not included in the further analysis.

MGIT 960 100% agreement with LPA, only 64.4% agreement with Xpert MTB/Rif

Sequencing analysis 91.3% concordance with LPA, only 8.7% concordance with Xpert MTB/RIF assay

Rufai et al. Journal of clinical microbiology 2014

LINE PROBE ASSAY: INDIAN DATA

Comparison of line probe assay with liquid culture for rapid detection of multi-drug resistance in *Mycobacterium tuberculosis*

Table I. Comparison of LPA result with MGIT 960 for RIF and INH resistance (n=118)

	MGIT 960					
LPA	Resistant	Susceptible	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
RIF			97.6	94.4	97.6	94.4
Resistant	80	2				
Susceptible	2	34				
INH			83.3	93.80	98.80	46.90
Resistant	85	1				
Susceptible	17	15				

LPA, line probe assay; RIF, rifampicin; INH, isoniazid

- N=120 over 5 months
- Rapid, sensitive and specific test for routine drug susceptibility testing for diagnosis of RIF resistance which is more crucial for management of MDR-TB

Singhal et al, Indian J Med Res, 2012

WHO ENDORSED TECHNOLOGIES

Molecular detection of TB and Drug Resistance

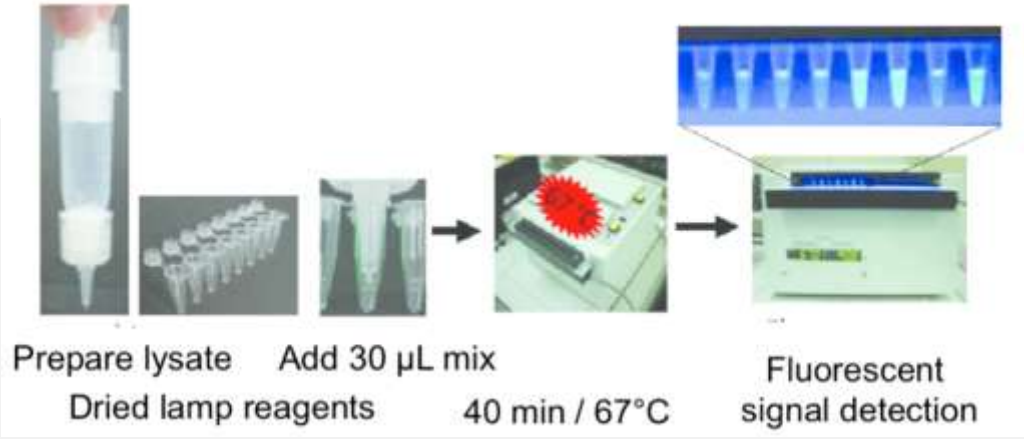
- Xpert MTB/RIF (Cepheid, Sunnyvale, USA)
- Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, USA)
- Truenat MTB, MTB Plus & MTB-RIF Dx assays (Molbio Diagnostics, Goa, India)
- FL-LPA- GenoType MTBDRs/Assay (Hain Life science, Germany)
- SL-LPA- GenoType MTBDRs/Assay (Hain Life science, Germany)
- TB LAMP (Eiken, Tokyo, Japan)

TB LAMP

- TB loop mediated isothermal amplification → Manual NAAT
- Requires less than one hour

Sample preparation (60 microliter of sputum) → mixed with extraction solution & heated 65'C → incubated for amplification → Detection of fluorescent light

	Pooled sensitivity	Pooled specificity
TB-LAMP	78.0 (66.6-86.4)	98.9 (97.4-99.6)
Xpert MTB/RIFb	81.1 (70.6-88.5)	98.2 (95.9-99.2)



Usage recommendations

- As a replacement test / follow on test to sputum-smear microscopy for diagnosing PTB in symptomatic adults

WHO Policy guidelines- LAMP, 2025

TB LAMP

Evaluation of the TB-LAMP assay for the rapid diagnosis of pulmonary tuberculosis in Northern India

R. Yadav,* N. Sharma,* R. Khaneja,† P. Agarwal,†‡ A. Kanga,§ D. Behera,¶ S. Sethi*

*Department of Medical Microbiology, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, †State TB Cell, Chandigarh, ‡World Health Organization Country Office of India, New Delhi, §Indira Gandhi Medical College, Shimla, ¶Pulmonary Medicine, PGIMER, Chandigarh, India

N= 530 (453 culture positive)	Study population: Pts with PTB Symptoms
	Outcome measures: Xpert vs TB-LAMP

	Sensitivity	Specificity	Concordance
TB-LAMP	100	99.2	0.75
Xpert	82.6	94.9	

The TB-LAMP assay showed high sensitivity and specificity with limited requirement of testing infrastructure, and is thus a promising diagnostic tool for TB diagnosis in resource-poor settings

Yadav et al. Ingenta, 2017

OTHERS

Molecular detection of TB and Drug Resistance

- GeneXpert MTB/XDR (Cepheid, USA)
- GeneXpert Omni (Cepheid, Sunnyvale, USA)
- Quantiplus MTB FAST (Huwel lifesciences, India)
- PathoDetect™ MTB RIF & INH assay (Mylab, Maharastra, India)
- FluoroType MTBDRs/Assay (Hain Life science, Germany)
- BD Max MDR-TB (Becton Dickinson, USA)
- M2000 RealTime MTB System (Abbott, USA)

GENEXPERT MTB/XDR

- Can detect resistance to Isoniazid, Ethionamide, Fluoroquinolones & Aminoglycosides

Isoniazid	inhA KatG fabG1 oxyR-ahpC
Ethionamide	inhA
Fluoroquinolones	gyrA gyrB
Aminoglycoside	rrs eis

GENEXPERT MTB/XDR

Cochrane Library

Cochrane Database of Systematic Reviews

Xpert MTB/XDR for detection of pulmonary tuberculosis and resistance to isoniazid, fluoroquinolones, ethionamide, and amikacin (Review)

Pillay S, Steingart KR, Davies GR, Chaplin M, De Vos M, Schumacher SG, Warren R, Theron G

6 studies, n= 1228	Patients with PTB	47.9% had RR
Detection of PTB	Isoniazid Resistant	FQ Resistant
Sensitivity: 98.3% (96.1-99.5) Specificity: 100% (86.3-100)	Sensitivity: 94.2% (87.5-97.4) Specificity: 98.5% (92.6-99.7)	Sensitivity: 93.2% (88.1-96.2) Specificity: 98% (90.8-99.6)
Ethionamide Resistance	Amikacin Resistance	
Sensitivity: 98% (74.2-99.9) Specificity: 99.7% (83.5-100)	Sensitivity: 86.1% (75-92.7) Specificity: 98.9% (93-99.8)	

OTHERS

Molecular detection of TB and Drug Resistance

- GeneXpert MTB/XDR (Cepheid, USA)
- GeneXpert Omni (Cepheid, Sunnyvale, USA)
- Quantiplus MTB FAST (Huwel lifesciences, India)
- PathoDetect™ MTB RIF & INH assay (Mylab, Maharastra, India)
- FluoroType MTBDRs/Assay (Hain Life science, Germany)
- BD Max MDR-TB (Becton Dickinson, USA)
- M2000 RealTime MTB System (Abbott, USA)

GENEXPERT OMNI

- Small, mobile phone operated, portable, automatic secured cloud-based connectivity
- Integrate battery, low power consumption
- Can use both Xpert MTB/RIF and Ultra cartridges
- Expands diagnostic testing to disseminated locations
- But, Processes only one sample at at time

GENEXPERT OMNI

RESEARCH ARTICLE

Equivalence of the GeneXpert System and GeneXpert Omni System for tuberculosis and rifampicin resistance detection

Sophia B. Georghiou[1], Riccardo Alagna[2], Daniela M. Cirillo[2], Sergio Carmona[1], Morten Ruhwald[1]*, Samuel G. Schumacher[1]

Table 2. Resistance detection results by Xpert MTB/RIF Ultra on Omni versus GeneXpert devices for rifampicin-resistant specimens.

	Result of test	Omni			
		RIF-R	RIF-S	Indeterminate	Total (%)
GeneXpert	RIF-R	145 (92.9%)	0 (0.0%)	2 (1.3%)	**147 (93.1%)**
	RIF-S	1[a] (0.6%)	1[b] (0.6%)	1 (0.6%)	**3 (1.9%)**
	Indeterminate	2 (1.3%)	2 (1.3%)	2 (1.3%)	**6 (3.8%)**
	Total	**148 (94.9%)**	**3 (1.9%)**	**5 (3.2%)**	**156 (100%)**

Laboratory validation study N= 156	Sputum sample from patients with PTB
High concordance 99.5% agreement	RR detection 92.9% agreement
No loss of performance compared with Genxpert at 90% relative humidity and 35'c temperature	

Georghiou S et.al, Plos One, 2021

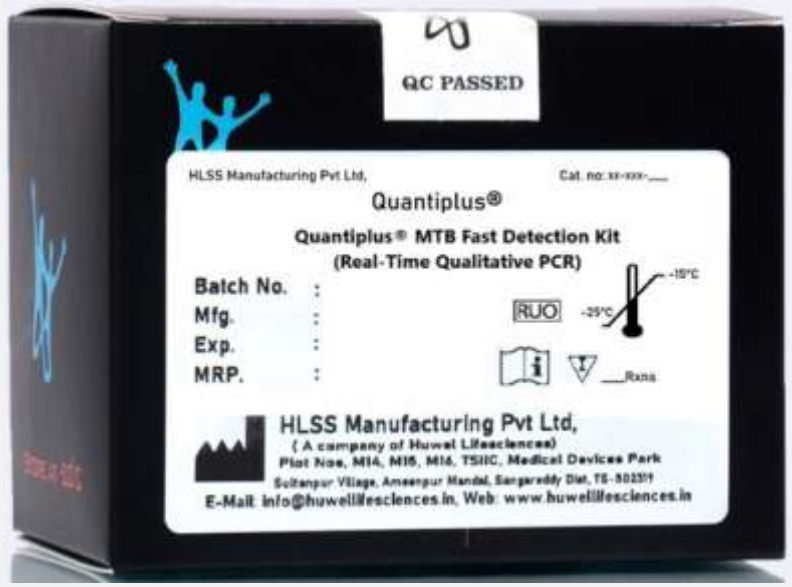
OTHERS: ICMR APPROVED

Molecular detection of TB and Drug Resistance

- GeneXpert MTB/XDR (Cepheid, USA)
- GeneXpert Omni (Cepheid, Sunnyvale, USA)
- Quantiplus MTB FAST (Huwel lifesciences, India)
- PathoDetect™ MTB RIF & INH assay (Mylab, Maharastra, India)
- FluoroType MTBDRs/Assay (Hain Life science, Germany)
- BD Max MDR-TB (Becton Dickinson, USA)
- M2000 RealTime MTB System (Abbott, USA)

QUANTIPLUS MTB FAST

- Targets IS1081, MPT64 and IS110 for MTB detection



QUANTIPLUS MTB FAST

Diagnostic accuracy of real-time PCR assay 'Quantiplus® MTB FAST' for detection of adult pulmonary tuberculosis (PTB): A multi-centric study

Madhumathi Jayaprakasam[1,†], Joy Sarojini Michael[2,†], Parul Jain[4,†], Shaoli Basu[5,†], Kanchan Ajbani[5,†], Siva Kumar Shanmugam[3,†], Marilyn Mary Ninan[2], Hansraj Choudhary[1], Amita Jain[4], Ravindra Mohan Pandey[4], Research Group* & Nivedita Gupta[1]

- Prospective
- 3 centres
- Jan 2024 to Dec 2024

- 37.4 % (241/644) →culture-positive for MTB

Reference standard:
- MGIT liquid culture as reference standard
- Genxpert MTB/Rif assay as comparator

- Sputum sample of presumptive PTB patients
- n= 657

- Already on ATT for > 4 days were excluded

	Detection of M tb			
	Overall	Smear +ve	Smear –ve	GeneXpert
Sensitivity	85.9 (81-90)	96.4 (92-99)	61.6 (50-73)	98.3 (96-99)
Specificity	96.3 (94-98)	91.7 (78-98)	96.7 (94-98)	96.3 (94-98)
PPV	93.2 (89-96)	98.2 (95-99)	78.9 (68-87)	94.1 (91-96)
NPV	91.9 (89-94)	84.6 (71-92)	92.3 (91-94)	98.9 (97-99)
Accuracy	92.4 (90-94)	95.6 (92-98)	90.9 (88-93)	97.1 (95-98)

Jayaprakasam et. Al. IJMR, 2025

OTHERS: ICMR APPROVED

Molecular detection of TB and Drug Resistance

- GeneXpert MTB/XDR (Cepheid, USA)
- GeneXpert Omni (Cepheid, Sunnyvale, USA)
- Quantiplus MTB FAST (Huwel lifesciences, India)
- PathoDetect™ MTB RIF & INH assay (Mylab, Maharastra, India)
- FluoroType MTBDRs/Assay (Hain Life science, Germany)
- BD Max MDR-TB (Becton Dickinson, USA)
- M2000 RealTime MTB System (Abbott, USA)

PATHODETECT™ MTB RIF & INH ASSAY

- Targets IS6110 and rrs for MTB detection
- Targets inhA and katG for HR & rifA, rifC and rifE hotspots of rpoB gene for RR
- Can simultaneously process 32 tests at once

PATHODETECT™ MTB RIF & INH ASSAY

Multicentric validation of the PathoDetect™ MTB RIF & INH assay for simultaneous detection of *Mycobacterium tuberculosis*, & drug resistance to rifampicin & isoniazid in presumptive pulmonary tuberculosis & drug-resistant TB patients

Hansraj Choudhary[1,#], Garima Malik[2,#], Devendra Singh Chauhan[5], Manpreet Bhalla[4], Azger Dusthackeer[6], Prabha Desikan[8], Sidhartha Giri[10], Sandeep Kumar[9], Madhumathi Jayaprakasam[1], Ajay Vir Singh[5], Prabhpreet Sethi[4], Md Shakir Reza[4], V. Mythily[6], V. Thiyagarajan[6], Nikita Panwalkar[8], Jyotismita Tripathy[10], Devdatt Mani[9], Diksha Singh[9], P. M. Ramesh[7], Manjeet Singh Chalga[3], Rajni Rani[2], Nivedita Gupta[1], Ravindra Mohan Pandey[#] & Manjula Singh[2]

- Cross sectional, Multicentric study
- March 2022 to Oct 2022
- 6 sites in India

- Sputum sample of presumptive PTB patients
- n=718
- Pregnant, immunosuppressed, already on ATT for > 7 days were excluded

Reference standard:
- MGIT liquid culture as reference for diagnosis
- DST and LPA for Resistance detection

- 50.4 % (362/718) →culture-positive for MTB,
- 9% (26/289) →RR
- 11.1% (32/289) →HR

Detection of Mtb (vs MGIT)

% Sensitivity (95% CI)	98.1 (96.1-99.2)
% Specificity (95% CI)	94.2 (91-96.5)
% PPV (95% CI)	94.9 (92.2-96.9)
% NPV (95% CI)	97.8 (95.5-99.1)

Detection of RR & HR (vs LPA)

% Sensitivity (95% CI)	86.5 (80.2-91.5)	93.3 (89.6-96)
% Specificity (95% CI)	91.6 (88.2-94.3)	95.8 (93-97.8)
% PPV (95% CI)	82.3 (75.6-87.8)	95.1 (91.4-97.4)
% NPV (95% CI)	93.8 (90.7-96.1)	94.3 (91.1-96.6)

Chaudhary et. Al. IJMR, 2025

OTHERS: MODERATE COMPLEXITY NAAT

Molecular detection of TB and Drug Resistance

- GeneXpert MTB/XDR (Cepheid, USA)
- GeneXpert Omni (Cepheid, Sunnyvale, USA)
- Quantiplus MTB FAST (Huwel lifesciences, India)
- PathoDetect™ MTB RIF & INH assay (Mylab, Maharastra, India)
- FluoroType MTBDRs/Assay (Hain Life science, Germany)
- BD Max MDR-TB (Becton Dickinson, USA)
- M2000 RealTime MTB System (Abbott, USA)

Real Time MTB and MTB-RIF/INH (Abbott)

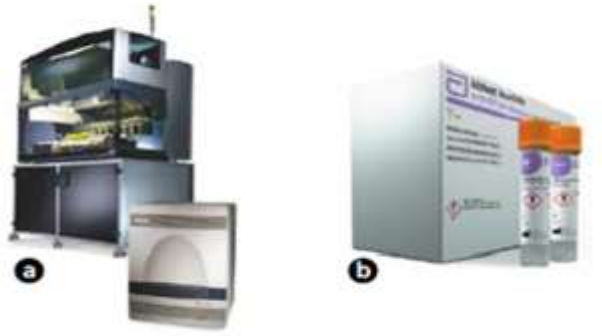
BD MAX™ MDR TB (Becton Dickinson)

Flourotype MTB DR ver 2.0 (Hain)

ABBOTT REALTIME MTB TESTS

- Based on the **IS6110 genetic element and the pab gene targets.**
- Eight dye-labelled probes to detect mutations.
- Automated DNA extraction and real-time polymerase chain reaction

Fig. 2.1.4. Abbott equipment: (a) m2000sp RealTime system and (b) RealTime MTB Amplification Reagent Kit



WHO: Module 3, 2025

BD MAX MDR-TB TEST

- Multiplexed RT PCR-NAAT. : Detects both RR and HR
- Targets IS6110 and IS1081, as well as a single copy genomic target.
- Targets the RIF-resistance determining region codons 507–533 of the rpoB gene;
- Targets both the inhA promoter region and the 315 codon of the katG gene
- BD Max platform, in which the DNA is automatically extracted and real-time PCR is performed

Fig. A2.1. BD MAX System and BD MAX PCR Cartridges

WHO: Module 3, 2025

FLUOROTYPE MTB

- Detection of IS6110 element
- Based on PCR and FluoroType technology
- Mycobacterial DNA is extracted from the patient specimen and specifically amplified via PCR.
- Then fluorescence-labelled probes are bound to single stranded amplicons.
- Changes in fluorescence intensity are measured and displayed.

WHO: Module 3, 2025

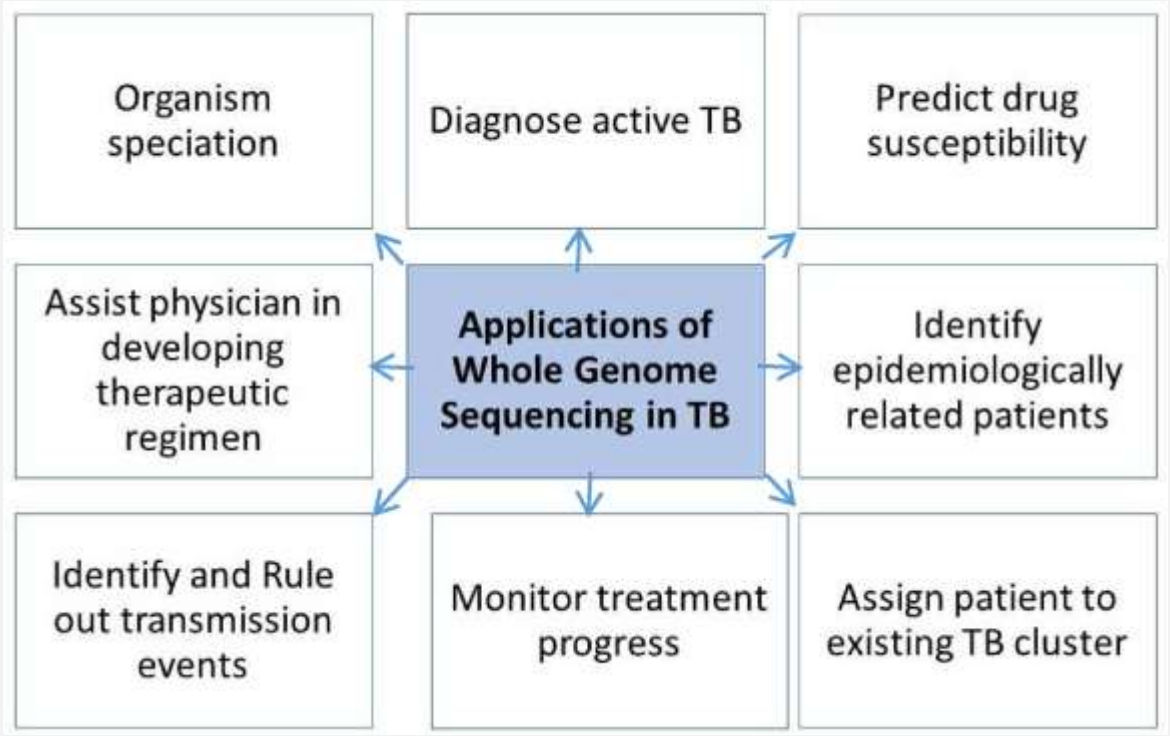
GENOME SEQUENCING

- Whole Genome Sequencing
- Targeted Next Generation Sequencing
- Pyrosequencing

WGS

WGS

1. Full genome sequenced
2. No pre specified targets needed
3. Obtains more information
4. Comprehensive solution

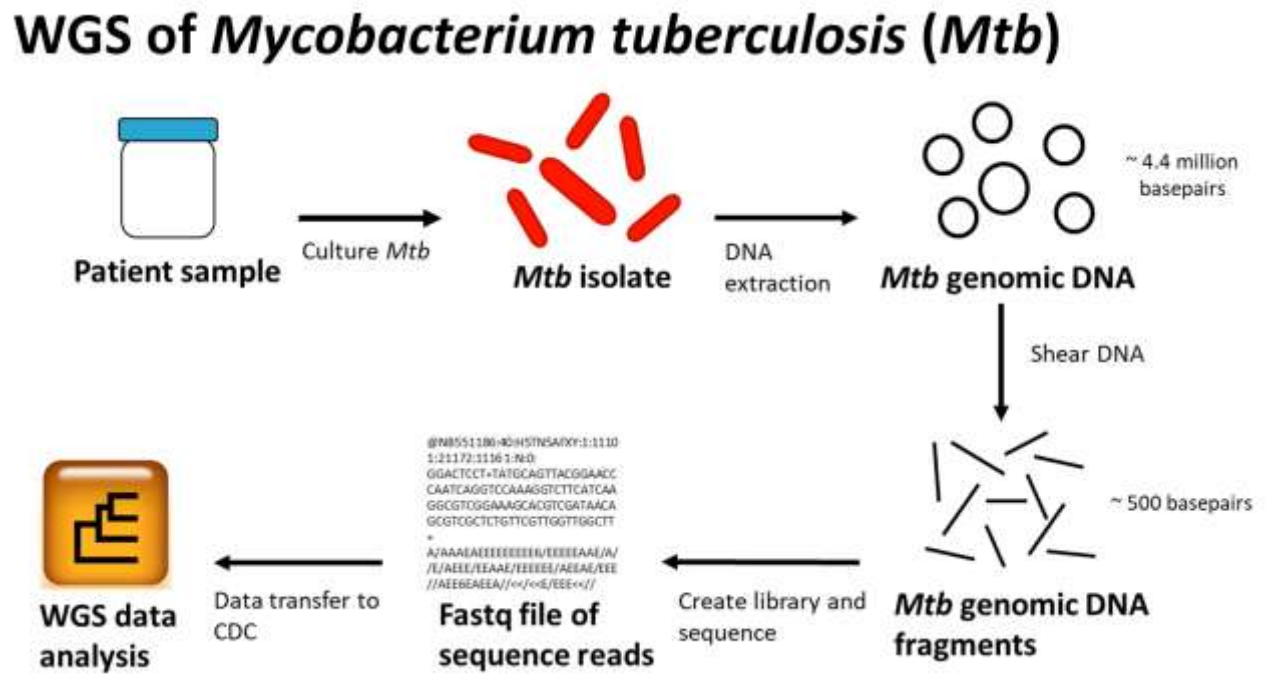


Benefits

- MDR-TB & XDR TB
- Relapse or Re-infection
- Inconclusive or Indeterminate results
- Helpful when phenotypic DST is not available for drugs like bedaquiline and delamanid
- Detect disease transmission—> Identify cluster and strain
- Provides drug susceptibility profile and mutation information.

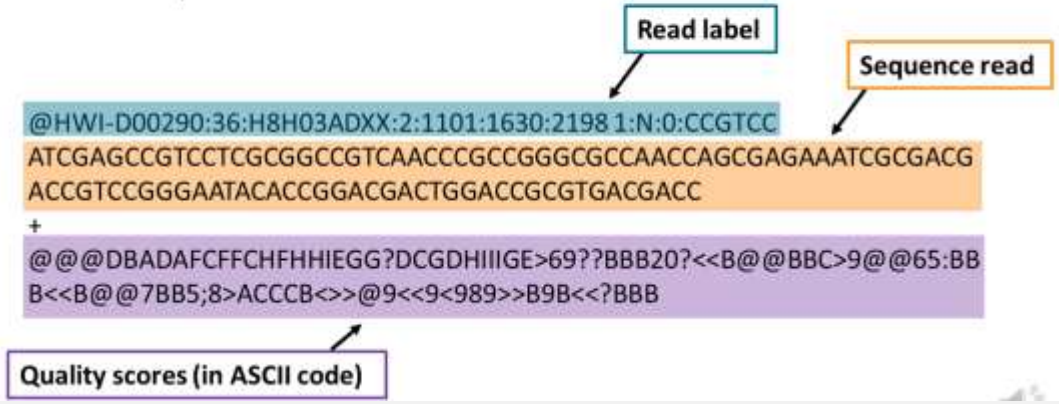
Arora et al Indian journal of Tuberculosis 2023

WHOLE GENOME SEQUENCING: PROCESS



Fastq file

A text file with sequence reads and quality scores for each base call in the sequence read



CDC, 2018

WHOLE GENOME SEQUENCING

Cons:

- Not been shown to be reproducible for direct-from-sample detection of drug resistance
- Application is limited to paucibacillary sample
- Even though detects >500 mutation across >35 genes: still cannot provide conclusive prediction of sensitivity

WHOLE GENOME SEQUENCING

RESEARCH ARTICLE

Transcriptome analysis of mycobacteria in sputum samples of pulmonary tuberculosis patients

Sumedha Sharma[1], Michelle B. Ryndak[2], Ashutosh N. Aggarwal[3], Rakesh Yadav[4], Sunil Sethi[4], Shet Masih[5], Suman Laal[2,6], Indu Verma[1]*

A whole genome microarray of Mycobacterium tuberculosis was used to evaluate the transcriptional profile of mycobacteria in the sputum samples of smear positive pulmonary tuberculosis patients

When compared to in-vitro grown M. tb Sputum M. tb bacilli are:

1. Metabolically inactive
2. Slow-growing or non replicating
3. Physiologically stressed

Because of downregulation of genes involved in

- ATP synthesis
- Virulence: Phthiocerol dimycocerosate (PDIM) & Phenolic glycolipids (PGL) → Cell wall lipids that helps in immune invasion, Macrophage survival and pathogenicity
- Aerobic respiration
- Translational machinery

Upregulated→ stress related genes of unknown function

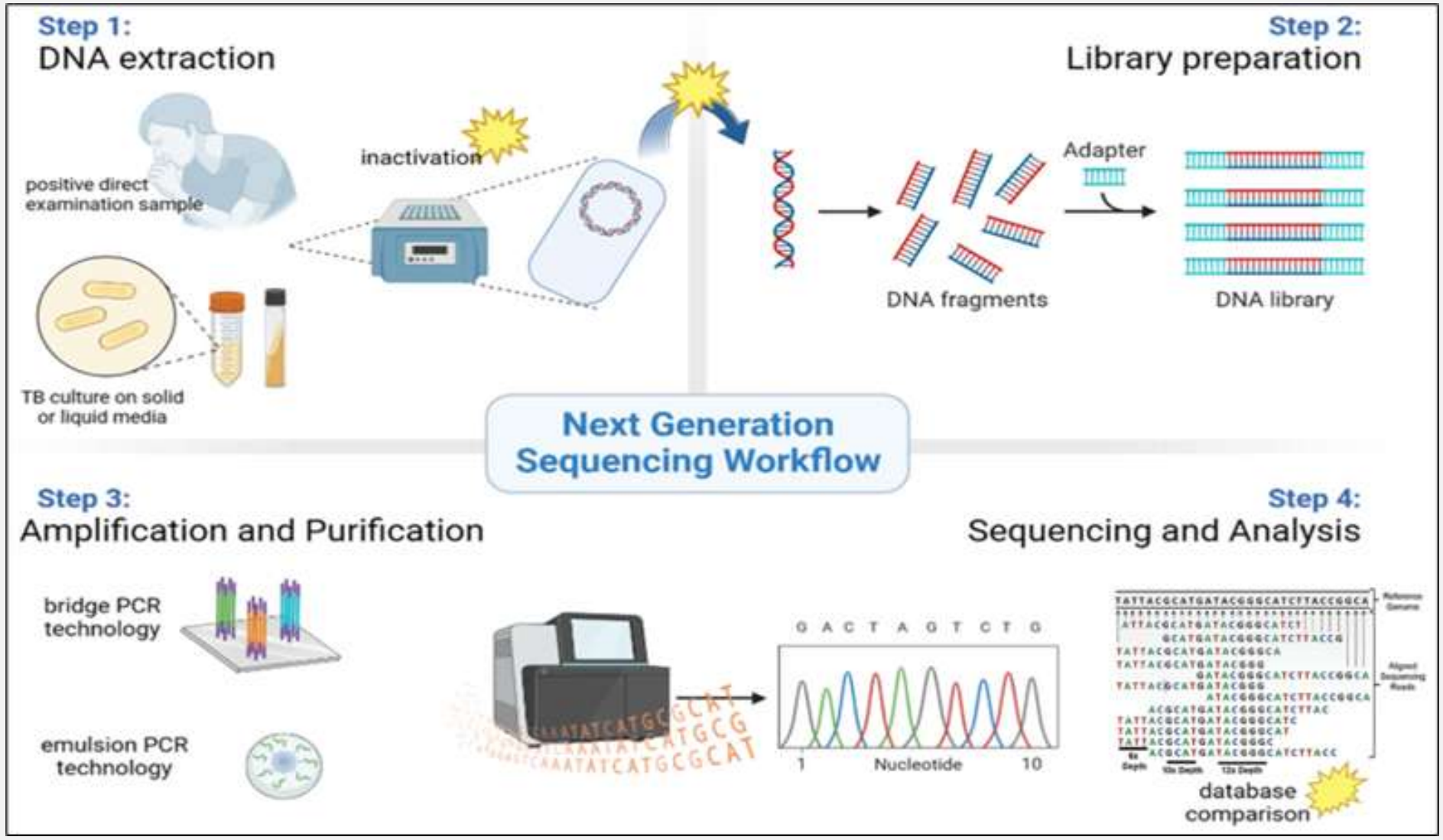
Sharma s. Plos one, 2017

TARGETED NEXT GENERATION SEQUENCING

- "Targeted" - map specific sections of the TB genome with known/novel mutations associated with drug resistance
- Relatively fast, with a TAT of one to two days,
- Can be run directly on clinical samples (sputum, stool, etc.)
- Detects resistance to up to 15 drugs
- High accuracy (Sen>99%, Spec>98%)

ILLUMINA NGS PLATFORM

TARGETED NEXT GENERATION SEQUENCING



NEXT GENERATION SEQUENCING

Targeted next generation sequencing directly from sputum for comprehensive genetic information on drug resistant *Mycobacterium tuberculosis*

Priti Kambli [a], Kanchan Ajbani [a], Mubin Kazi [a], Meeta Sadani [a], Swapna Naik [a], Anjali Shetty [a], Jeffrey A. Tornheim [b], Harpreet Singh [c], Camilla Rodrigues [a,*]

- Next generation sequencing (tNGS) - 40 uncultured sputum samples.
- Resistance profiles from tNGS were compared with profiles from Xpert MTB/RIF, line probe assay (LPA), pyrosequencing (PSQ), and phenotypic testing.
- tNGS provided results for 39 of 40 samples (97.5%) with **faster turnaround than phenotypic testing p = 0.0068**.
- Most samples were isoniazid and rifampicin resistant (N = 31, 79.5%), 21 (53.8%) were fluoroquinolone resistant, and 3 (7.7%) were also resistant to Kanamycin.
- **Agreement between tNGS and existing assays was excellent for isoniazid, (100) rifampicin(100), and SLDs, (100) very good for levofloxacin(90-95%), and good for moxifloxacin (75-85%).**

Kambli P, et. Tuberculosis (Edinb). 2021

NGS: RECOMMENDATION

17. **In people with bacteriologically confirmed pulmonary TB disease, targeted next-generation sequencing technologies may be used on respiratory samples to diagnose resistance to rifampicin, isoniazid, fluoroquinolones, pyrazinamide and ethambutol rather than culture-based phenotypic drug susceptibility testing.**

(Conditional recommendation, certainty of evidence moderate [isoniazid and pyrazinamide],low [rifampicin, fluoroquinolones and ethambutol])

18. **In people with bacteriologically confirmed rifampicin-resistant pulmonary TB disease, targeted NGS technologies may be used on respiratory samples to diagnose resistance to isoniazid, fluoroquinolones, bedaquiline, linezolid, clofazimine, pyrazinamide, ethambutol, amikacin and streptomycin rather than culture-based phenotypic drug susceptibility testing.**

(Conditional recommendation, certainty of evidence high [isoniazid, fluoroquinolones and pyrazinamide], moderate [ethambutol], low [bedaquiline, linezolid, clofazimine and streptomycin], very low [amikacin])

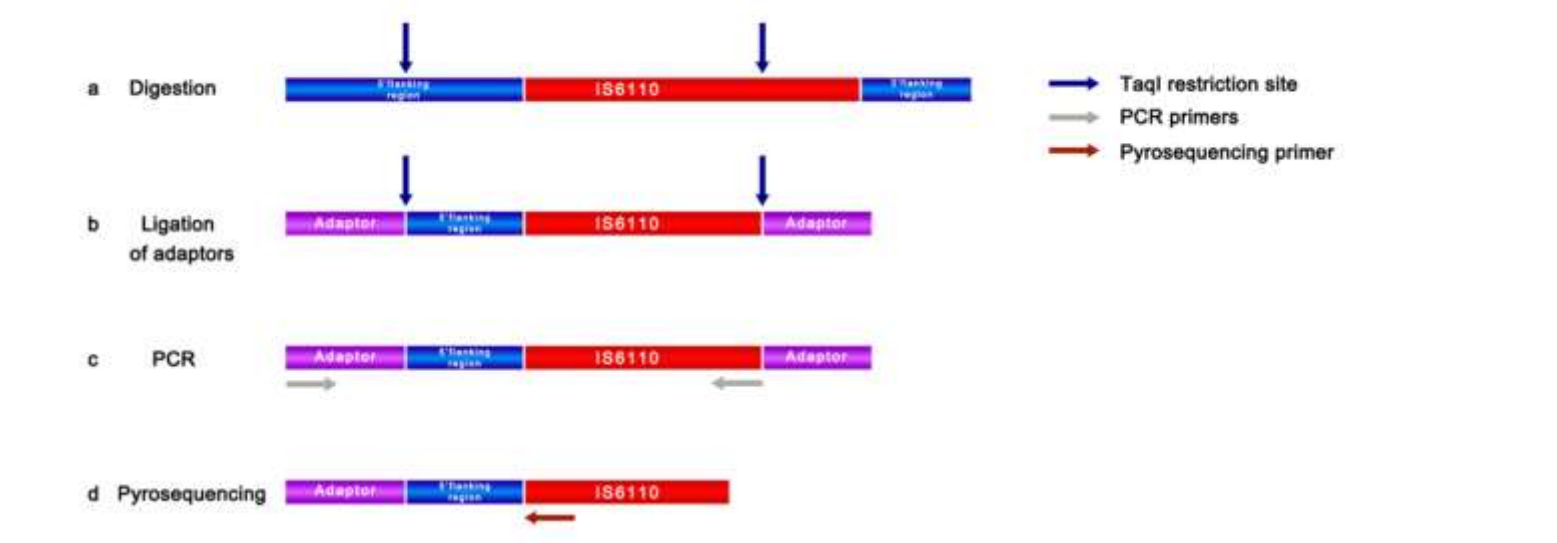
India PMDT

Implementation of NGS-based DST is to be focused, at least initially, on capacity- building at the National TB Reference Laboratories and at well-performing Intermediate TB Reference Laboratories.

WHO module 3, 2025

PYROSEQUENCING

- Real time diagnostic DNA sequencing by synthesis
- Based on actual short segment sequencing (<100 bp)
- DNA synthesis is monitored in real time by detecting light emitted upon nucleotide incorporation
- Results <6 hrs
- Disadvantage- expensive



Moya et al, Nature, 2016

PYROSEQUENCING

INH

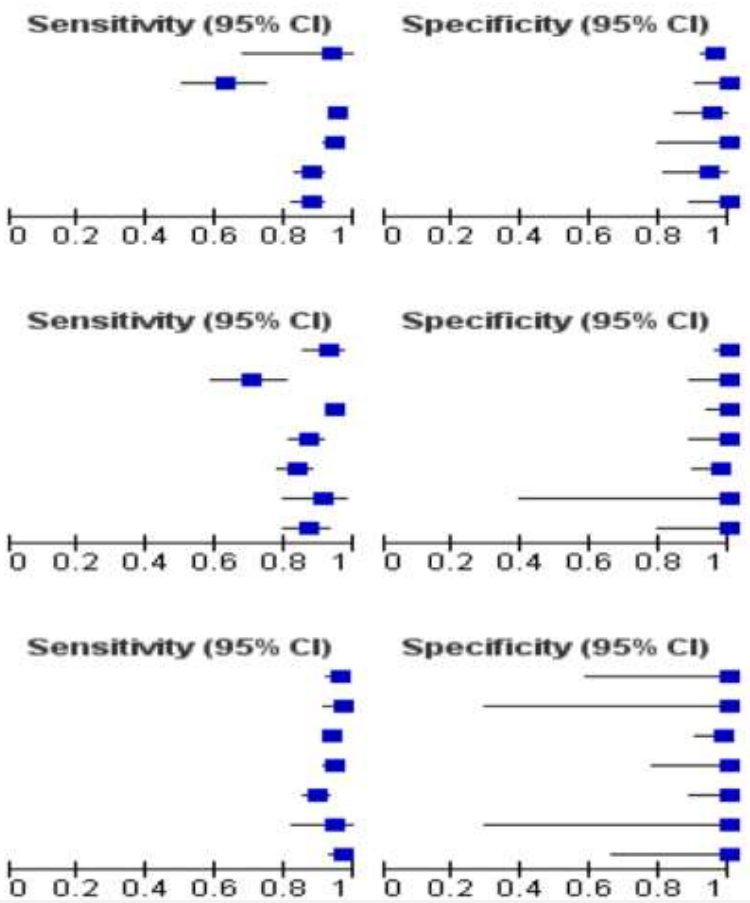
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Ajbani 2014	14	7	1	165	0.93 [0.68, 1.00]	0.96 [0.92, 0.98]
Bravo 2009	41	0	24	37	0.63 [0.50, 0.75]	1.00 [0.91, 1.00]
Catanzaro 2015	822	2	41	42	0.95 [0.94, 0.97]	0.95 [0.85, 0.99]
Engström 2012	258	0	15	16	0.95 [0.91, 0.97]	1.00 [0.79, 1.00]
Georghiou 2016	234	2	33	34	0.88 [0.83, 0.91]	0.94 [0.81, 0.99]
Lin 2014	188	0	27	30	0.87 [0.82, 0.92]	1.00 [0.88, 1.00]

FQ

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Ajbani 2014	87	0	7	93	0.93 [0.85, 0.97]	1.00 [0.96, 1.00]
Bravo 2009	50	0	21	31	0.70 [0.58, 0.81]	1.00 [0.89, 1.00]
Catanzaro 2015	802	0	51	54	0.94 [0.92, 0.96]	1.00 [0.93, 1.00]
Engström 2012	186	0	28	32	0.87 [0.82, 0.91]	1.00 [0.89, 1.00]
Georghiou 2016	212	1	42	50	0.83 [0.78, 0.88]	0.98 [0.90, 1.00]
Govindaswamy 2018	42	0	4	4	0.91 [0.79, 0.98]	1.00 [0.40, 1.00]
Lin 2014	95	0	14	16	0.87 [0.79, 0.93]	1.00 [0.79, 1.00]

RIF

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Ajbani 2014	167	0	7	7	0.96 [0.92, 0.98]	1.00 [0.59, 1.00]
Bravo 2009	96	0	3	3	0.97 [0.91, 0.99]	1.00 [0.29, 1.00]
Catanzaro 2015	798	1	53	55	0.94 [0.92, 0.95]	0.98 [0.90, 1.00]
Engström 2012	251	0	14	15	0.95 [0.91, 0.97]	1.00 [0.78, 1.00]
Georghiou 2016	246	0	28	31	0.90 [0.86, 0.93]	1.00 [0.89, 1.00]
Govindaswamy 2018	35	0	2	3	0.95 [0.82, 0.99]	1.00 [0.29, 1.00]
Lin 2014	220	0	8	9	0.96 [0.93, 0.98]	1.00 [0.66, 1.00]



Getachew E, et al. JoTB&MD, 2021

PYROSEQUENCING

AMK

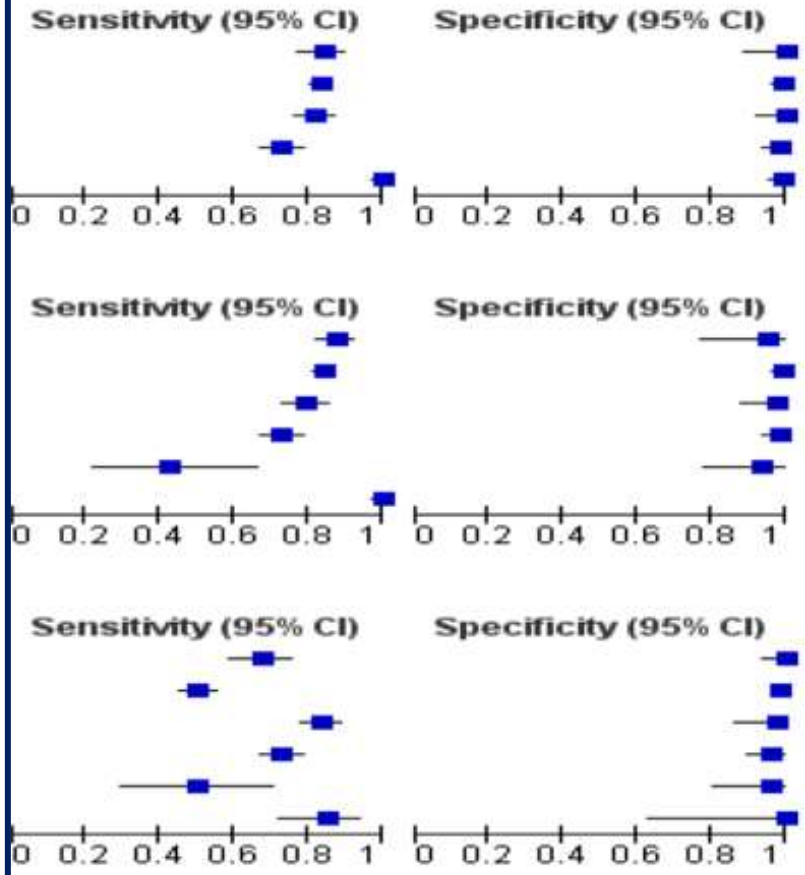
Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Ajbani 2014	132	0	25	30	0.84 [0.77, 0.89]	1.00 [0.88, 1.00]
Catanzaro 2015	632	1	125	149	0.83 [0.81, 0.86]	0.99 [0.96, 1.00]
Engström 2012	161	0	35	43	0.82 [0.76, 0.87]	1.00 [0.92, 1.00]
Georghiou 2016	163	1	60	81	0.73 [0.67, 0.79]	0.99 [0.93, 1.00]
Lin 2014	120	1	0	119	1.00 [0.97, 1.00]	0.99 [0.95, 1.00]

CAP

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Ajbani 2014	145	1	20	21	0.88 [0.82, 0.92]	0.95 [0.77, 1.00]
Catanzaro 2015	643	1	121	142	0.84 [0.81, 0.87]	0.99 [0.96, 1.00]
Engström 2012	140	1	36	44	0.80 [0.73, 0.85]	0.98 [0.88, 1.00]
Georghiou 2016	163	1	60	81	0.73 [0.67, 0.79]	0.99 [0.93, 1.00]
Govindaswamy 2018	9	2	12	28	0.43 [0.22, 0.66]	0.93 [0.78, 0.99]
Lin 2014	119	0	0	0	1.00 [0.97, 1.00]	Not estimable

KAN

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Ajbani 2014	86	0	41	60	0.68 [0.59, 0.76]	1.00 [0.94, 1.00]
Catanzaro 2015	230	5	227	446	0.50 [0.46, 0.55]	0.99 [0.97, 1.00]
Engström 2012	158	1	31	36	0.84 [0.78, 0.89]	0.97 [0.86, 1.00]
Georghiou 2016	163	3	60	79	0.73 [0.67, 0.79]	0.96 [0.90, 0.99]
Govindaswamy 2018	13	1	13	25	0.50 [0.30, 0.70]	0.96 [0.80, 1.00]
Lin 2014	40	0	7	8	0.85 [0.72, 0.94]	1.00 [0.63, 1.00]



Getachew E, et al. JoTB&MD, 2021

IN CONCLUSION

WHO 2025

Technology class	Products included in the evaluation
	Xpert® MTB/RIF and Xpert® MTB/RIF Ultra (Cepheid)*
	Truenat™ (Molbio) *;
Moderate complexity automated NAATs for detection of TB and resistance to rifampicin and isoniazid	Abbott RealTime MTB and Abbott RealTime MTB RIF/INH (Abbott) BD MAX™ MDR-TB (Becton Dickinson) cobas® MTB and cobas MTB-RIF/INH (Roche) FluoroType® MTBDR and FluoroType® MTB (Hain Lifescience/Bruker)
	TB-LAMP (Eiken) *
Antigen detection in a lateral flow format (biomarker-based detection)	Alere Determine™ TB LAM Ag (Alere)
Low complexity automated NAATs for the detection of resistance to isoniazid and second-line anti-TB agents	Xpert® MTB/XDR (Cepheid)
Line probe assays (LPAs)	GenoType® MTBDR*plus* v1 and v2; GenoType® MTBDR*sl*, (Hain Lifescience/Bruker), Genoscholar™ NTM+MDRTB II; Genoscholar™ PZA-TB II (Nipro)

*These recommendations are currently product specific but will be changed to class-based to align with the other recommendations.

- Moderate complexity automated NAATs for the detection of TB and resistance to rifampicin and isoniazid
- Low complexity automated NAATs for the detection of resistance to isoniazid and second-line anti-TB agents
- High complexity reverse hybridization-based NAATs for the detection of pyrazinamide resistance.

IN CONCLUSION

1. **Xpert MTB/RIF Ultra** offers superior sensitivity, especially in **paucibacillary disease**, with robust rifampicin resistance detection
2. **Truenat** provides an **indigenous, point-of-care solution**, improving access, linkage to care, and cost-effectiveness in resource-limited and peripheral laboratories
3. GeneXpert XDR, when readily available, shifts the dependency from LPAs for Second line resistance testing.
4. TB-LAMP may be used as a replacement test for SSM to diagnose PTB in adults with signs and symptoms consistent with TB
5. Quantiplus MTB Fast and Pathodetect Assay are being incorporated into NTEP
6. **tNGS/WGS** enables comprehensive resistance prediction, outbreak tracking, and management of complex MDR/XDR-TB, though currently limited to reference laboratories

THANK YOU