Molecular assays in Tuberculosis

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INTRODUCTION

Burden of drug resistance

- Globally in 2016, an estimated **4.1%** (95% CI: 2.8–5.3%) of **new** cases and **19%** (95% CI: 9.8–27%) of **previously treated** cases had MDR/RR-TB.
- India - estimated % of TB cases with MDR/RR-TB **2.8%** (2–3.5) of **new** and **12%**

  (10–13) of **Previously treated**

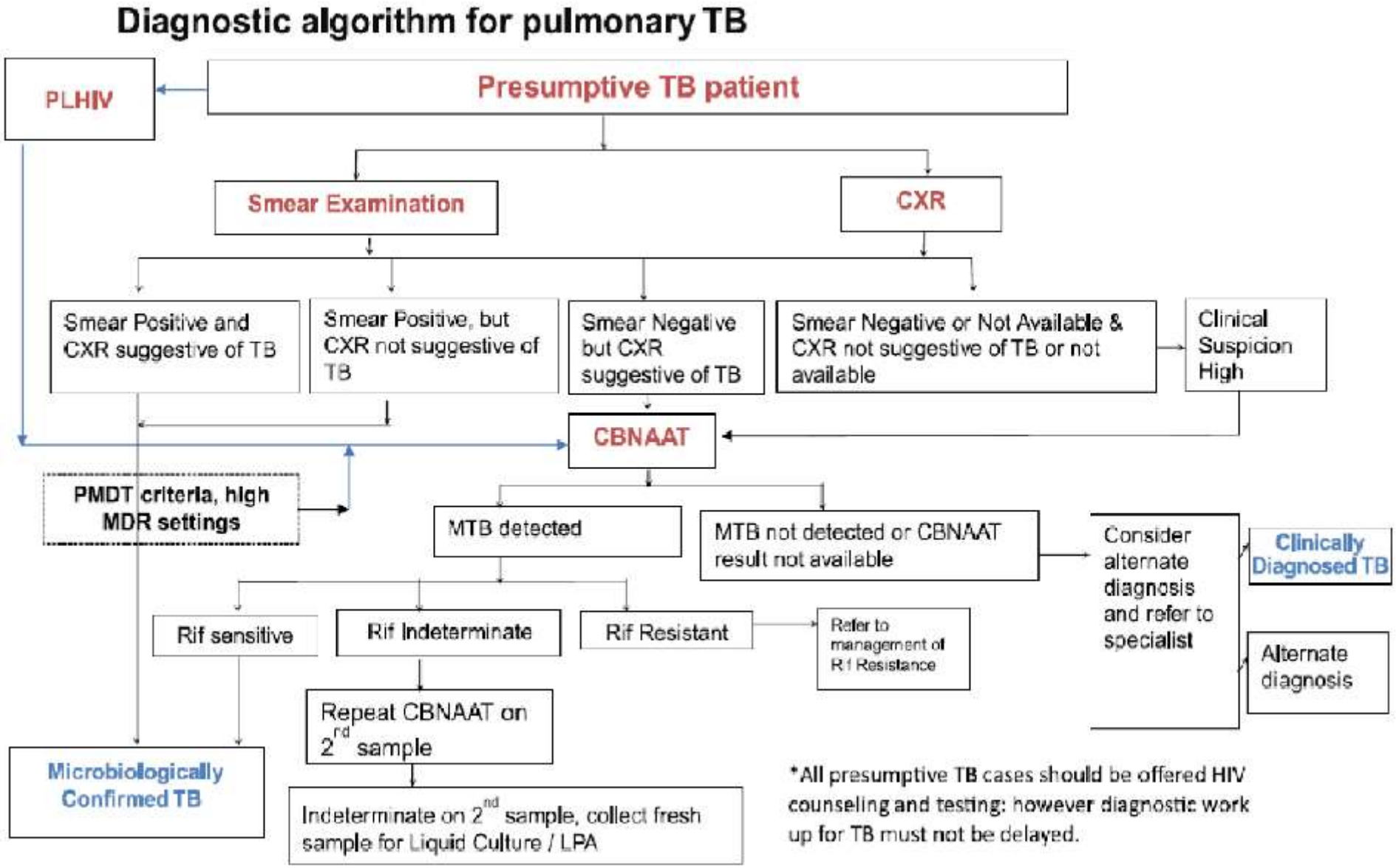
  cases had MDR/RR-TB

WHO global tuberculosis report 2017

Burden of drug resistance

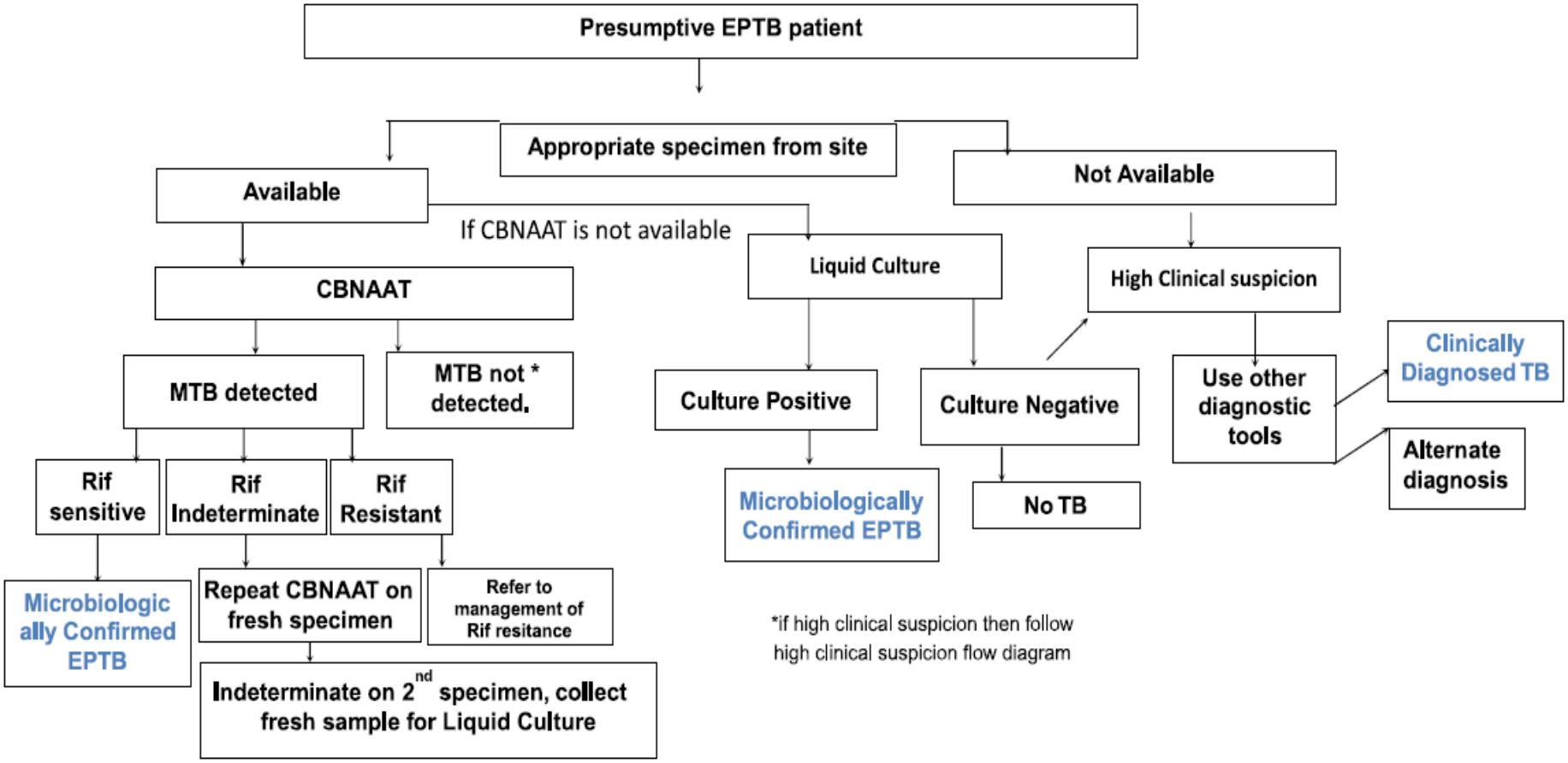
- In 2016, Average proportion of MDR-TB cases with XDR-TB was **6.2%** (95% CI: 3.6–9.5%), with the best estimate lower than those based on data available in previous years (9.5% in 2015, 9.7% in 2014

WHO global tuberculosis report 2017



Technical and operational guidelines for TB control in India, 2016

Diagnostic Algorithm for Extra Pulmonary TB



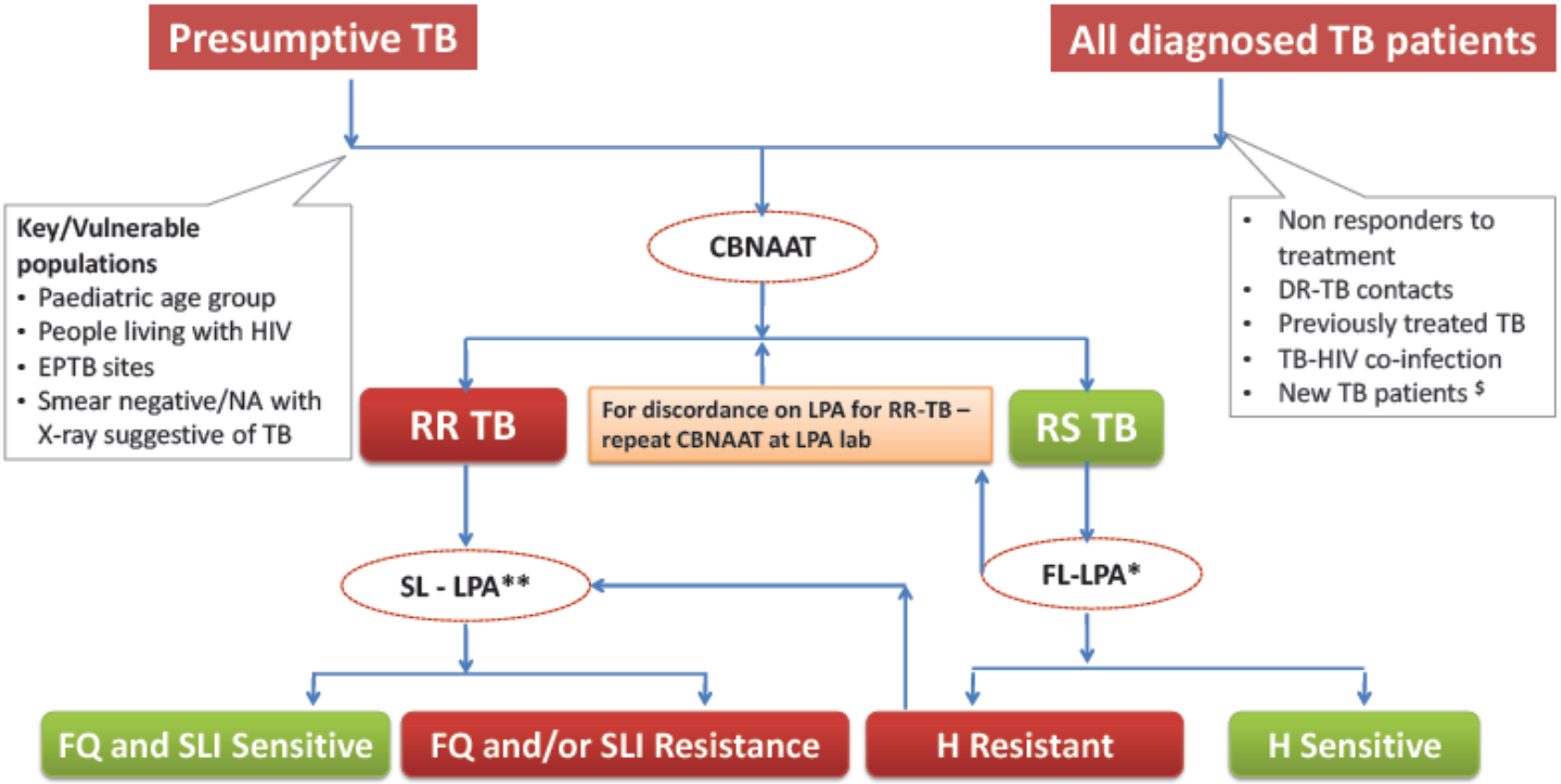
Technical and operational guidelines for TB control in India, 2016

CANDIDATES FOR DST

- Failed treatment with first line drugs
- Contacts of MDR-TB (or R resistance)
- Any Positive follow-up sputum smear test during 1^{st} line ATT
- Prior history of anti-TB treatment
- HIV co-infection
- All presumptive TB cases among PLHIV

Ref - Standards for TB Care in India, WHO 2014

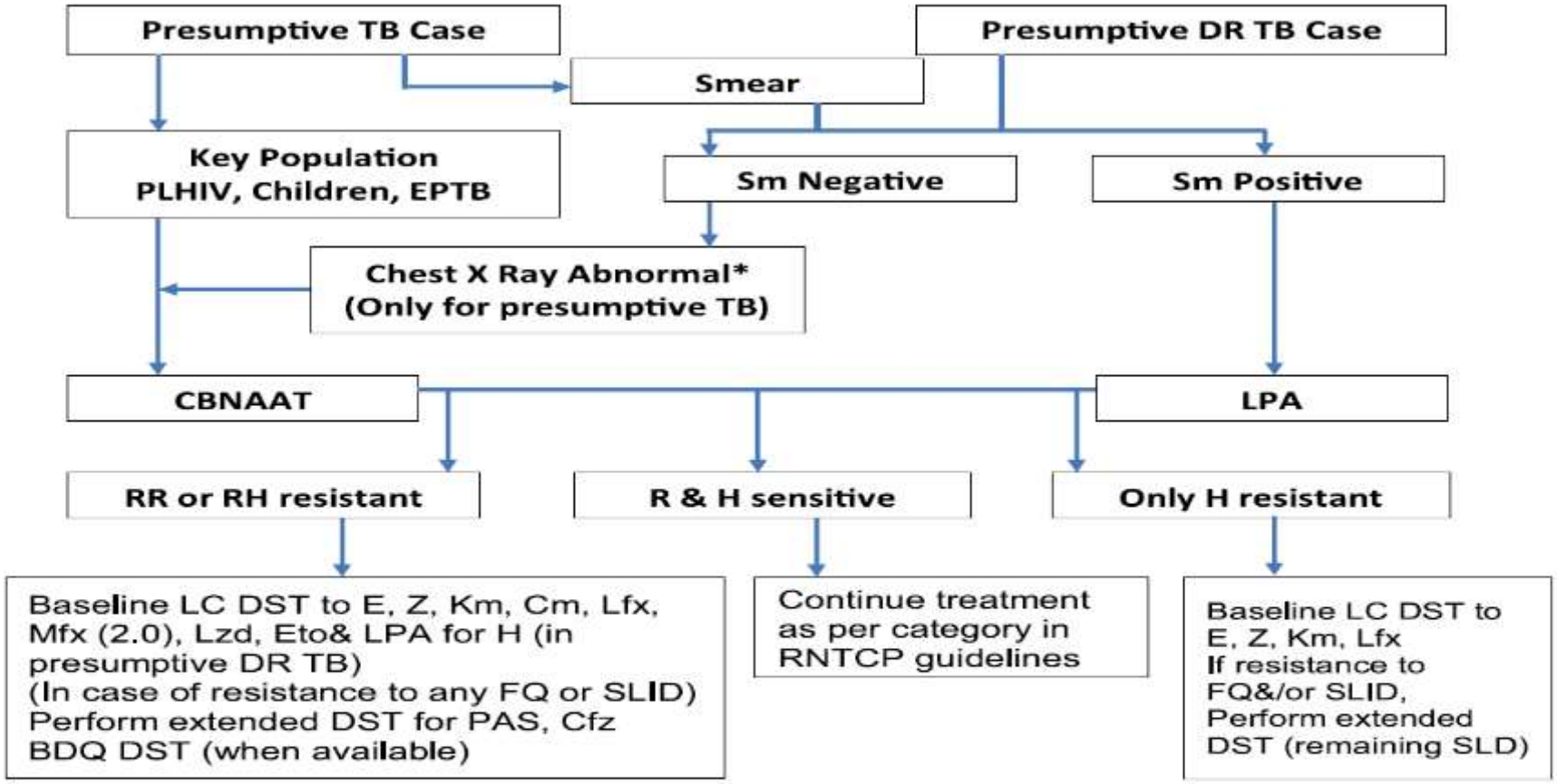
Figure 5.2 DR-TB Diagnostic Algorithm



*Offer molecular testing for H mono/poly resistance to TB patients prioritized by risk as per the available lab capacity
**LC DST (Mfx 2.0, Km, Cm, Lzd) will be done only for patients with any resistance on baseline SL-LPA. DST to Z, Cfz, Bdq & Dlm would be considered for policy in future, whenever available, standardized & WHO endorsed.
$ States to advance in phased manner as per PMDT Scale up plan for universal DST based on lab capacity and policy on use of diagnostics

PMDT 2017

Diagnostic Algorithm for Bedaquiline containing and optimized treatment regimen



- If RR by CBNAAT, in addition to other drugs, H resistance (by LPA) to be done and treatment modified accordingly.
- For samples reported by LPA – report must mention H- resistance by Kat G or INH A mutation.
- For new patients (those who do not fit in the definition of presumptive DR-TB case diagnosed as TB with RR by CBNAAT – a second CBNAAT test will be offered along with liquid culture DST

** Those who do not fit in the definition of presumptive DR-TB case*

Phenotypic vs Genotypic DST

Phenotypic DST

- Evaluation of growth in drug containing media
- Proportion method
- Turn around time:
  - Solid LJ media 84 days
  - Liquid Culture(MGIT) - 42 days

Genotypic DST

- Detect genetic mutations
- Amplification of specific target of RNA/DNA sequence using NA probe
- Targets- 16S RNA, IS6110
- Turnaround time:
  - LPA:72 hours
  - CB-NAAT: 2 hours

Phenotypic DST/Culture based tests

- Merits
  - Bacterial growth can be identified visually or by automated detection of its metabolism
  - Provides definitive diagnosis of TB
  - Provides necessary isolates for conventional DST
- Demerits
  - Takes longer time
  - Needs appropriate lab infrastructure and bio safety precautions

Genes involved in Drug resistance

Drug	Genes involved in resistance	Gene function
Isoniazid	inhA, katG, kasA	Enoyl ACP reductase
Rifampicin	rpoB gene	Mycobacterial RNA polymerase
Pyrazinamide	pncA	Pyrazinamidase
Ethambutol	embB	Arabinosyl transferase
Streptomycin	rpsL ,rrs, gidB	rRNA methyltransferase

Genes involved in drug resistance

Drug	Genes involved
Fluoroquinolones	gyrA/gyrB
Kanamycin/amikacin	rrs
Capreomycin	tlyA
Ethionamide	inhA
p-amino salicylic acid	thyA
PA-824 and OPC-67683	Rv3547 (hypothetical)
TMC207	atpE

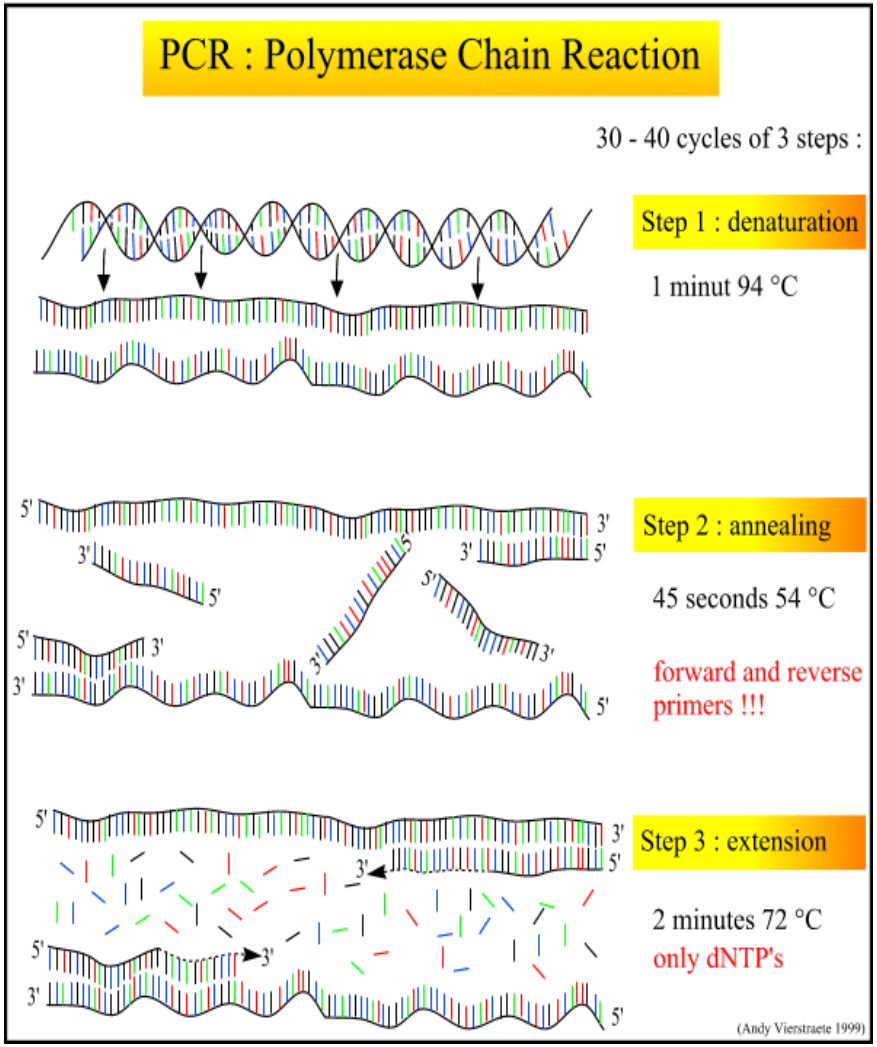
Genotype DST/PCR based tests

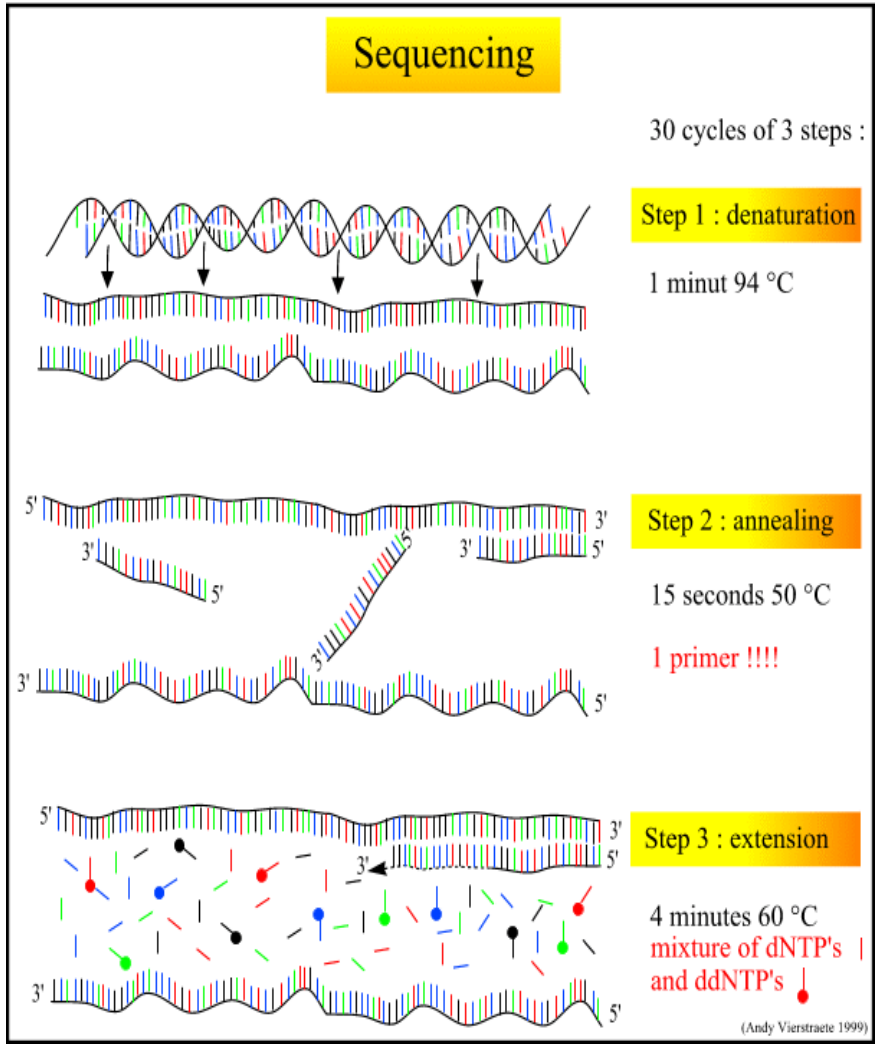
- Demerits
  - Detects DNA from **both viable and non-viable** bacteria.
    - Cannot be used for monitoring the progression or successful therapy.
  - Only screens the nucleic acid sequence and **not the amino acid sequence**.
    - Mutations in the probe region that don't cause amino acid exchange (silent mutations) will still produce the absence of one of the wild type bands.

Probe Based vs Sequence based tests

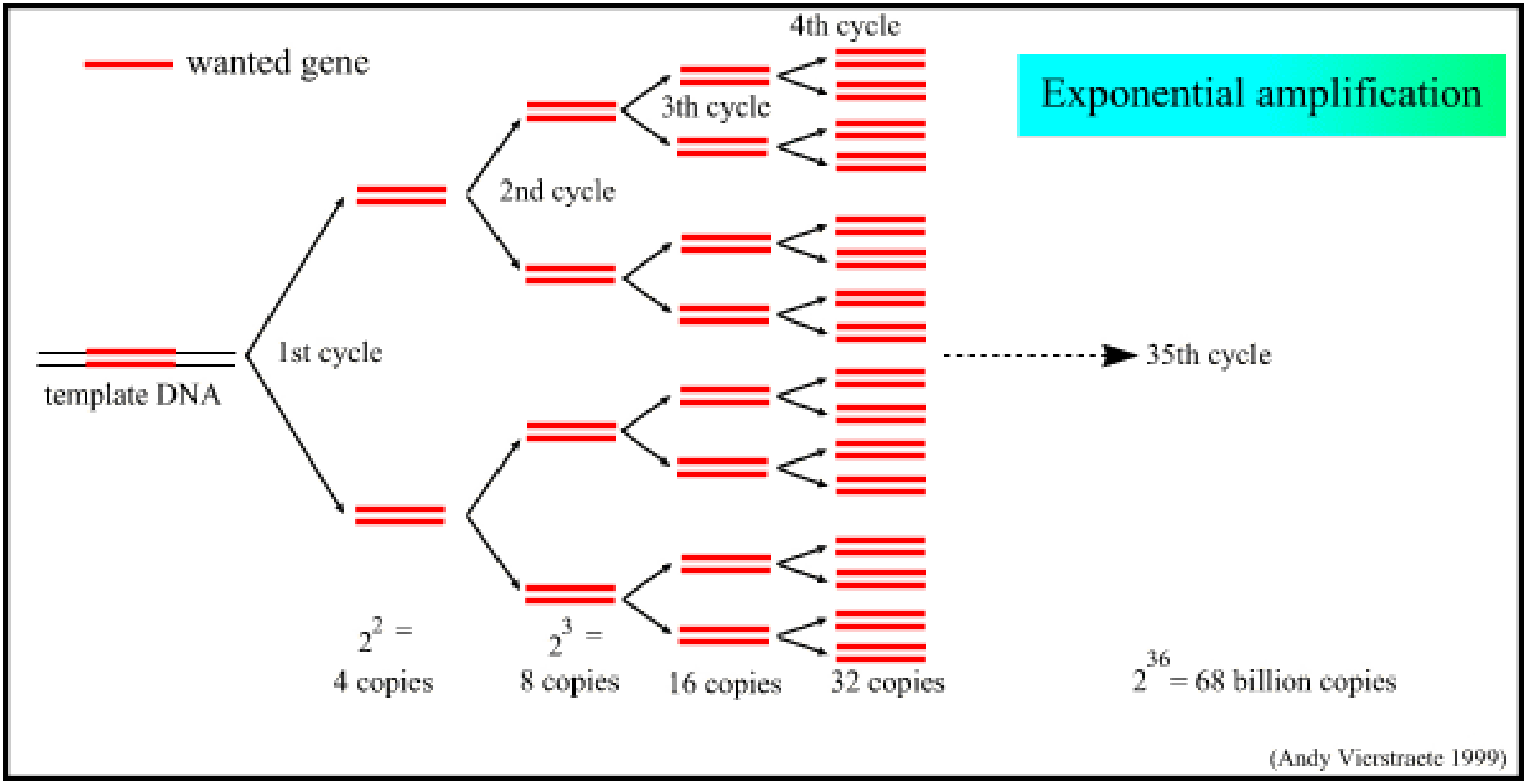
Probe based tests	Sequence based tests
Detect only if a mutation is present	Provide sequence information and nature of specific mutation
Also detect silent or missense mutations and signal drug resistance which do not confer drug resistance in culture	Hence, can predict drug resistance with greater accuracy
CB-NAAT LPA	Pyro sequencing Sanger sequencing Next generation sequencing
FDA approved	Not FDA approved

Probe Based vs Sequence based tests

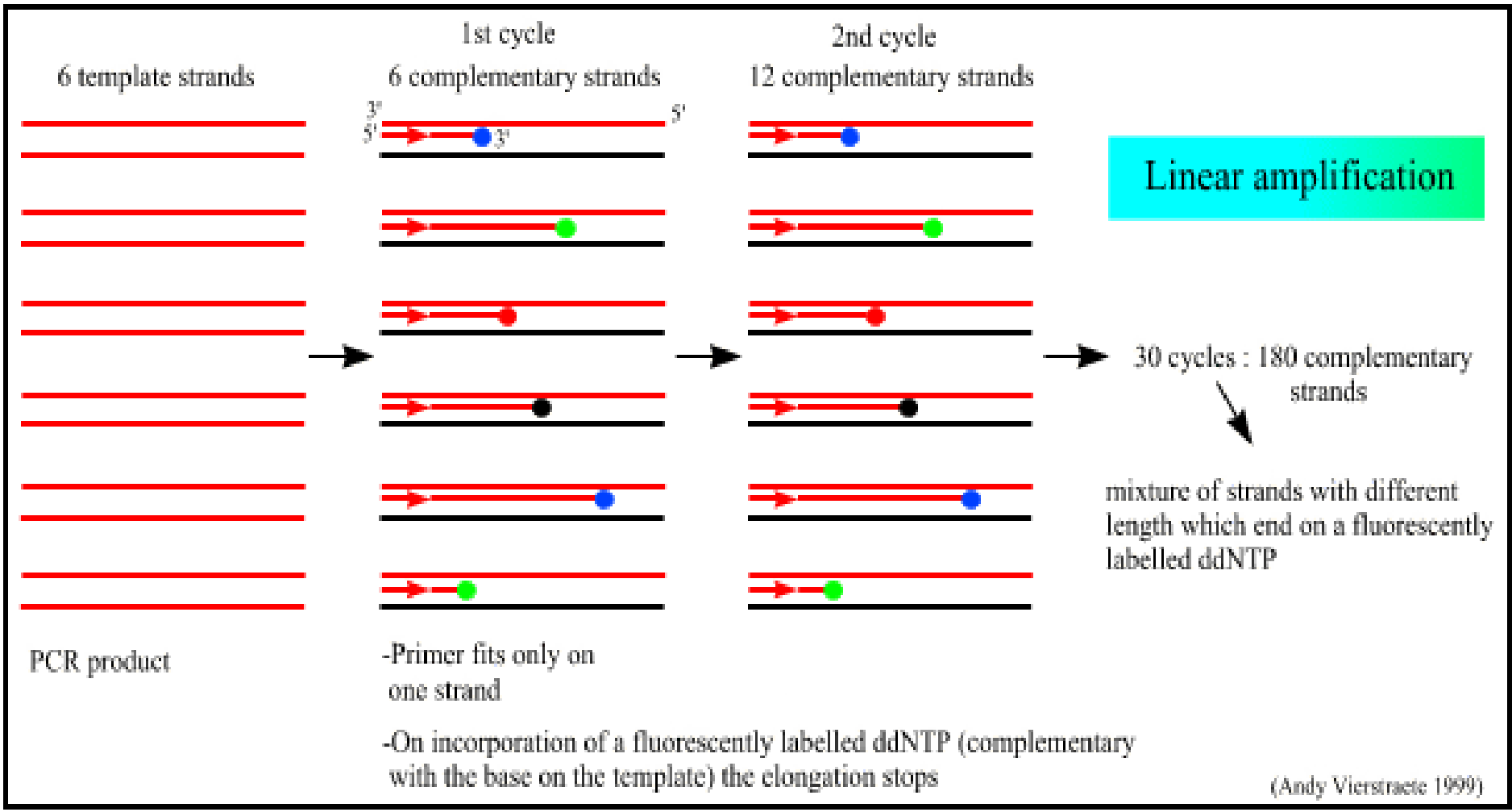




Probe based: Exponential Amplification



Sequencing: Linear amplification



TB PCR

TB PCR

- Multistep in-house PCR
- Amplifies IS 986 or IS 6110 repetitive element specific to M. tuberculosis
- Sensitivity 84 -96 % (Smear positive 96-100%,

  Smear negative 50-92%)
- Specificity 70-100%
- Provides result in 24-48 hrs

1.Amplified M. tuberculosis Direct Test (MTD)	AFB smear positive respiratory specimens
2.Amplicor M. tuberculosis Test	AFB smear positive respiratory specimens
3.Enhanced MTD test	AFB smear negative respiratory specimens

Ref : PCR For diagnosis of tuberculosis : where are we now ? Ind. J Tub. 2000, 47. 79

LINE PROBE ASSAY

LPA vs CB NAAT

	LPA	CB-NAAT
WHO endorsed	2008	2010
Diagnosis	Not used	Used
Resistance	INH and RIF	RIF alone
Specimens	Smear positive only	Smear positive/negative
Turnaround time	72 hours	2 hours
Steps	Separate steps DNA extraction-PCR amplification- Colorimetric detection	Single cartridge for sample processing, amplification and detection
Cross contamination and operator dependence	yes	No

LPA: Commercially available types

1. INNO-LiPA Rif.TB (Innogenetics, Ghent, Belgium)
2. Genotype MTBDR (Hain LifeScience, Germany)
3. Genotype MTBDRplus (Hain LifeScience, Germany)
4. Genotype MTBDRsl (Hain LifeScience, Germany)

Mutations detected

	Mutations detected	Drug
1. INNO-LiPA Rif.TB	rpoB	Rifampicin
2. Genotype MTBDR	rpoB katG	Rifampicin Izoniazid
3. Genotype MTBDRplus	rpoB katG inhA	Rifampicin Izoniazid
4. Genotype MTBDRsl version 1	rrs gyrA EMB	SLID Fluroquinolones Ethambutol
5. Genotype MTBDRsl version 2	rrs,eis gyrA,gyrB	SLID FQ

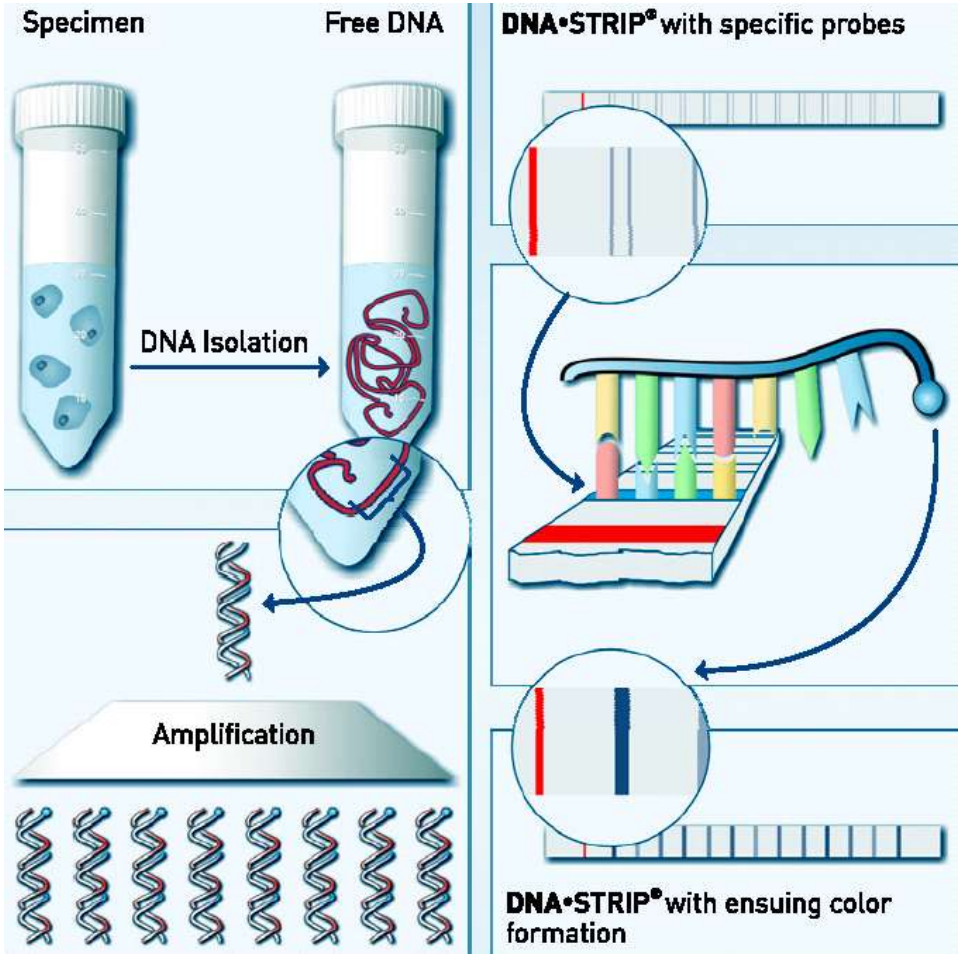
Madhavi Latha B, Anil K Bilolikar. Hain's test- a rapid aid for identification and sensitivity testing of multidrug resistant and extended drug resistant tuberculosis. J Med Sci Res. 2013; 1(3): 145-149.

Principle and procedure

1. DNA **extraction** from the clinical specimens (pulmonary, decontaminated) or the cultured material (solid/liquid medium)
2. Multiplex **amplification** with biotinylated primers
3. .**Reverse hybridization**.
   - Membrane strips are coated with specific probes complementary to the amplified nucleic acids.
   - After chemical denaturation, the single stranded amplicons bind to the probes (hybridization)

Madhavi Latha B, Anil K Bilolikar. Hain's test- a rapid aid for identification and sensitivity testing of multidrug resistant and extended drug resistant tuberculosis. J Med Sci Res. 2013; 1(3): 145-149.

Principle and procedure



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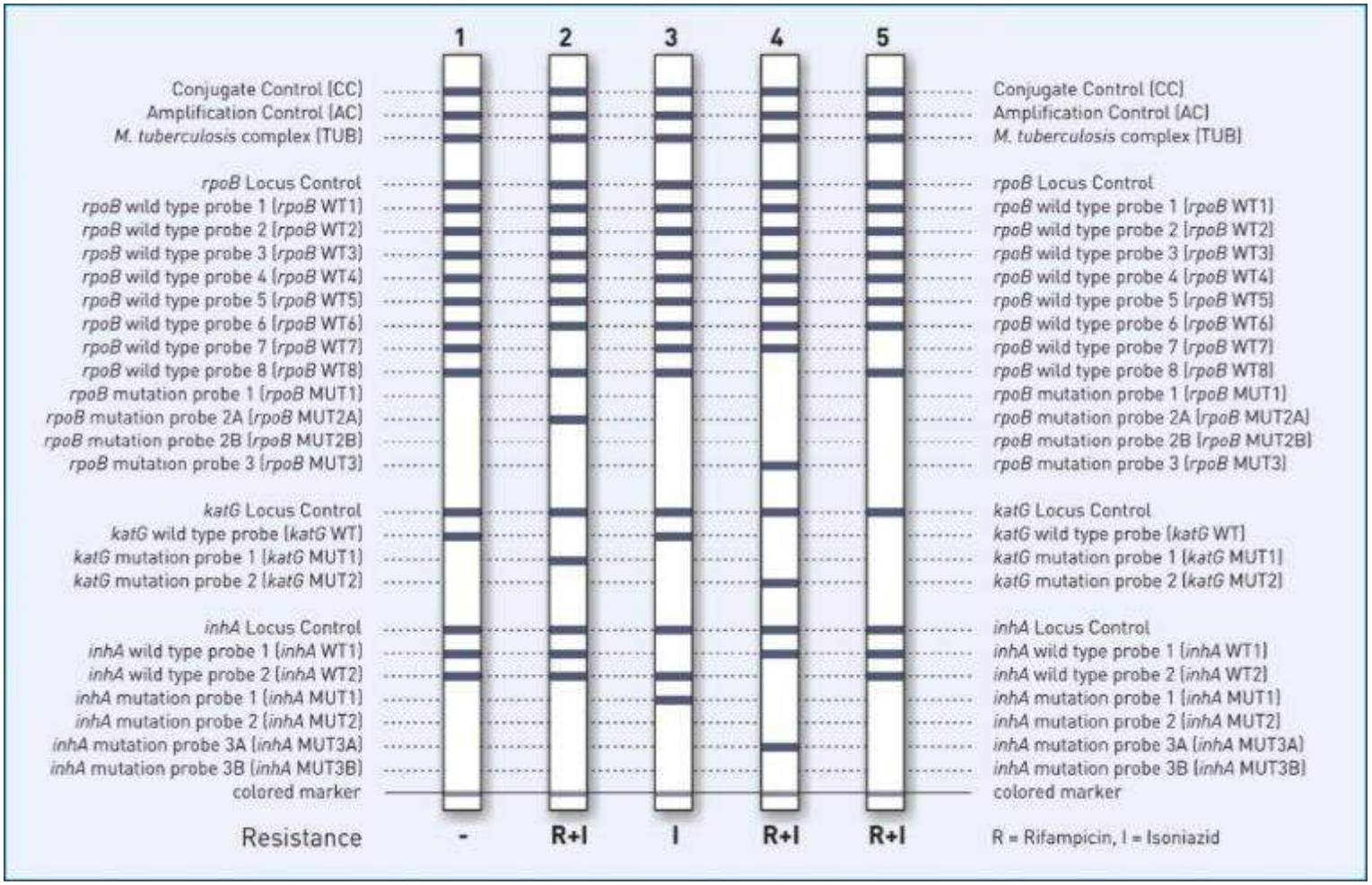
GenoType® MTBDRplus for MDR TB



Madhavi Latha B, Anil K Bilolikar. Hain's test- a rapid aid for identification and sensitivity testing of multidrug resistant and extended drug resistant tuberculosis. J Med Sci Res. 2013; 1(3): 145-149.

Specimen requirements

- Pulmonary smear positive specimens such as sputum (induction or expectorated), bronchial material (bronchoalveolar lavage) or aspirates (pleural aspirates).
- Cultivated samples (solid/ liquid medium).

Madhavi Latha B, Anil K Bilolikar. Hain's test- a rapid aid for identification and sensitivity testing of multidrug resistant and extended drug resistant tuberculosis. J Med Sci Res. 2013; 1(3): 145-149.

Storage and transport

1. Collected in a sterile container and stored at a temperature of 2-8 ^{o}C.
2. The transport of specimens at room temperature has to be carried out as soon as possible and should be done within 1-2 days.
3. Specimens used for decontamination should not be older than 4 days.
4. After decontamination & subsequent resuspension of the bacteria pellet with phosphate buffer, the samples can be stored at -20 ^{o}C or -80 ^{o}C for a maximum of 5 days until DNA extraction is performed.

Madhavi Latha B, Anil K Bilolikar. Hain's test- a rapid aid for identification and sensitivity testing of multidrug resistant and extended drug resistant tuberculosis. J Med Sci Res. 2013; 1(3): 145-149.

LPA Performance- WHO 2016

		Sensitivity	Specificity
Rifampicin resistance (Direct-sputum specimens)	V1 V2	97.1 (93.3–99.0) (166/171) 98.2 (95.0–99.6)(168/171))	97.1 (94.3–98.7) (267/275) 97.8 (95.3–99.2) (269/275
Rifampicin resistance (Indirect-culture isolates)	V1 V2	91.3 (86.0–95.0) (157/172) 91.3 (86.0–95.0) (157/172)	97.1 (94.3–98.7) (267/275) 97.1 (94.3–98.7) (267/275)
INH resistance (Direct testing)	V1 V2	94.4 (90.2–97.2) (186/197) 96.4 (93.2–98.3) (240/249)	95.4 (91.5–97.9) (188/197) 98.8 (96.5–99.8) (246/249)
INH resistance (Indirect testing)	V1 V2	89.4 (84.3–93.3) (178/199) 89.4 (84.3–93. 3) (178/199)	98.9 (96.0–99.9) (175/177) 98.9 (96.0–99.9) (175/177)

V1:MTBDRTB plus version 1(Hain version 1)
V2:MTBDRTB plus version 2(Hain version 2)

World Health Organization. *The use of molecular line probe assays for the detection of resistance to isoniazid and rifampicin.* WHO/HTM/TB/2016.12

LPA Performance- WHO 2016

- Patients with signs and symptoms consistent with TB and a positive LPA result can be treated with confidence.
- Strong correlation with Phenotypic resistance
- Similar diagnostic accuracy for direct or indirect tests

World Health Organization. *The use of molecular line probe assays for the detection of resistance to isoniazid and rifampicin.* WHO/HTM/TB/2016.12

LPA – WHO recommendations 2016

- Sputum smear-positive specimens (direct testing)
- Cultured isolates of MTBC (indirect testing) from both pulmonary and extra pulmonary sites
- Not recommended for the direct testing of sputum smear-negative specimens
- Do not eliminate the need for conventional culture-based DST

World Health Organization. *The use of molecular line probe assays for the detection of resistance to isoniazid and rifampicin.* WHO/HTM/TB/2016.12

LPA – WHO recommendations 2016

- Culture based DST will still be necessary in addition to LPA
  - to determine resistance to other anti-TB agents
  - to monitor the emergence of additional drug resistance
  - to detect INH resistance, when the LPA result is negative but high pre test probability present
  - to identify false positives from LPA (dead bacilli)
- Applied to the use of LPA in children based on the generalization of data from adults

World Health Organization. *The use of molecular line probe assays for the detection of resistance to isoniazid and rifampicin.* WHO/HTM/TB/2016.12

LPA

- Merits :
  - Lower contamination rates than culture
  - Detects resistance genes for Rifampicin and INH
  - Results available in 48-72 hours

- Demerits : Requires
  - Skilled man power (*Training)*
  - Specialized equipment
  - Dedicated space to avoid cross-contamination between specimens
  - Manual processing of specimen
  - Complexity & no. of steps preclude use in peripheral settings
  - Do not perform well on pauci-bacillary specimen

Second line LPA

GENOTYPE MTBDR*sL* ® TEST

GenoType MTBDR*sl* ®

- Endorsed by the WHO.
- Field validation of the MTBDR*sl* assay in smear-positive patients completed in India.
- To detect additional resistance to second line drugs in confirmed MDR-TB/RR-TB

New grouping of drugs			
A. Fluoroquinolones		Levofloxacin	Lfx
		Moxifloxacin	Mfx
		Gatifloxacin	Gfx
B. Second-line injectable agents		Amikacin	Am
		Capreomycin	Cm
		Kanamycin	Km
		(Streptomycin)	(S)
C. Other second-line agents		Ethionamide / Prothionamide	Eto/Pto
		Cycloserine / Terizidone	Cs/Trd
		Linezolid	Lzd
		Clofazimine	Cfz
D. Add-on agents (not part of the core MDR-TB regimen)	D1	Pyrazinamide	Z
		Ethambutol	E
		High-dose isoniazid	H^h
	D2	Bedaquiline	Bdq
		Delamanid	Dlm
	D3	p-aminosalicylic acid	PAS
		Imipenem-cilastatin	Ipm / Cls
		Meropenem	Mpm
		Amoxicillin-clavulanate	Amx-Clv
		(Thioacetazone)	(T)

Mutations detected

	Mutations detected	Drug
1. INNO-LiPA Rif.TB	rpoB	Rifampicin
2. Genotype MTBDR	rpoB katG	Rifampicin Izoniazid
3. Genotype MTBDRplus	rpoB katG inhA	Rifampicin Izoniazid
4. Genotype MTBDRsl version 1	rrs gyrA EMB	Fluroquinolones Ethambutol
5. Genotype MTBDRsl version 2	rrs,eis gyrA,gyrB	SLID FQ

Madhavi Latha B, Anil K Bilolikar. Hain's test- a rapid aid for identification and sensitivity testing of multidrug resistant and extended drug resistant tuberculosis. J Med Sci Res. 2013; 1(3): 145-149.

Sl LPA: Commercially available types

Table 1. Characteristics of Genotype MTBDR*sl* versions 1.0 and 2.0 as per manufacturer

Detection	Version 1	Version 2
Detects resistance to	FQ, SLID, Ethambutol	FQ,SLID
Samples	Smear +, cultures	Smear- and smear +,cultures
FQ	gyrA	gyrA , gyrB
SLID	rrs	rrs, eis
Ethambutol	embB	Not detected

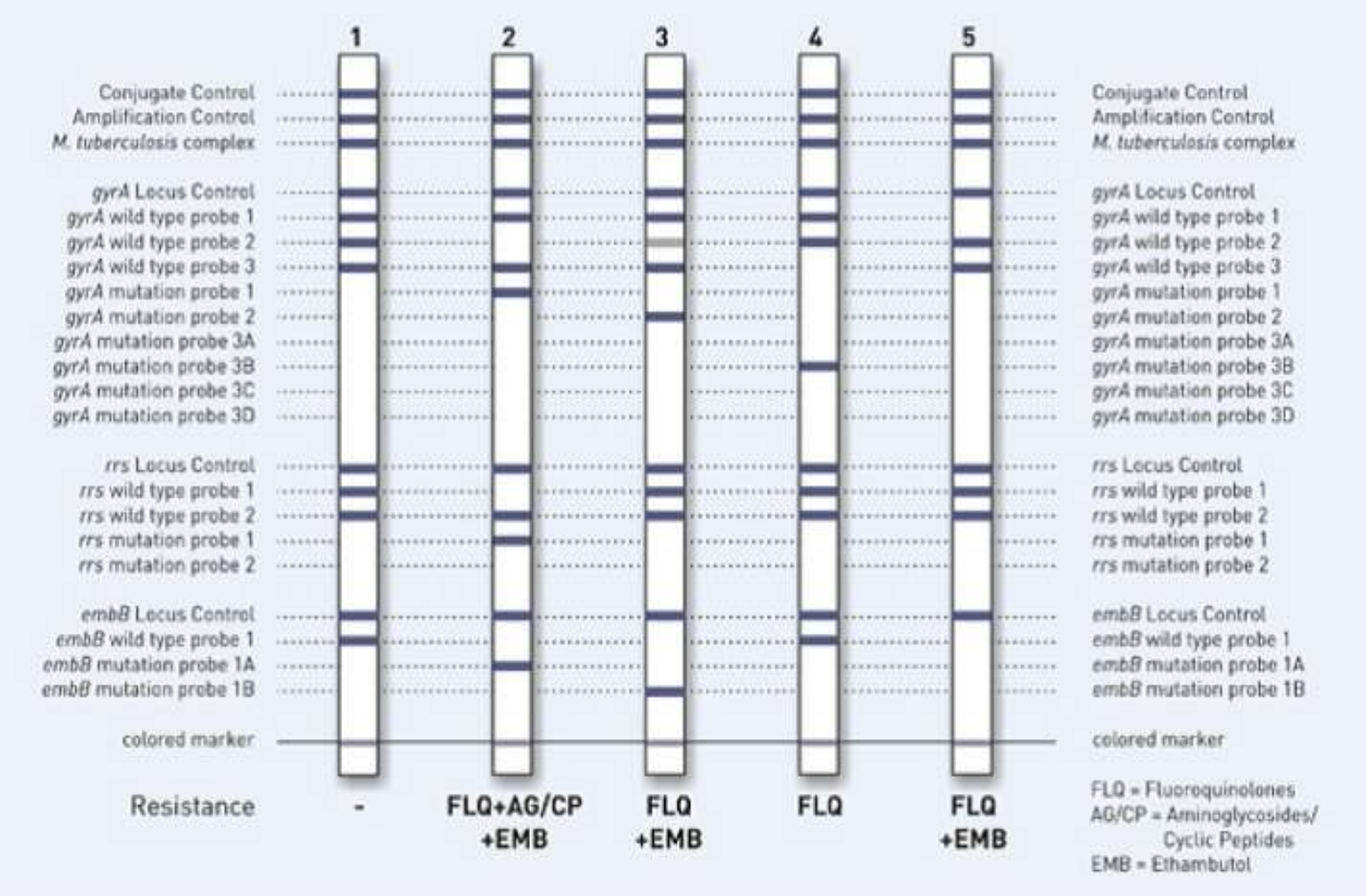
World Health Organization. *The use of molecular line probe assays for the detection of resistance to second-line anti-tuberculosis drugs: Policy guidance* WHO/HTM/TB/2016.07

Procedure

- Decontamination (e.g. with sodium hydroxide) and concentration of a sputum specimen by centrifugation
- Isolation and amplification of DNA
- Detection of the amplification products by reverse hybridization
- Visualization using a streptavidin-conjugated alkaline phosphatase colour reaction

World Health Organization. *The use of molecular line probe assays for the detection of resistance to second-line anti-tuberculosis drugs: Policy guidance* WHO/HTM/TB/2016.07

GenoType MTBDRsl®



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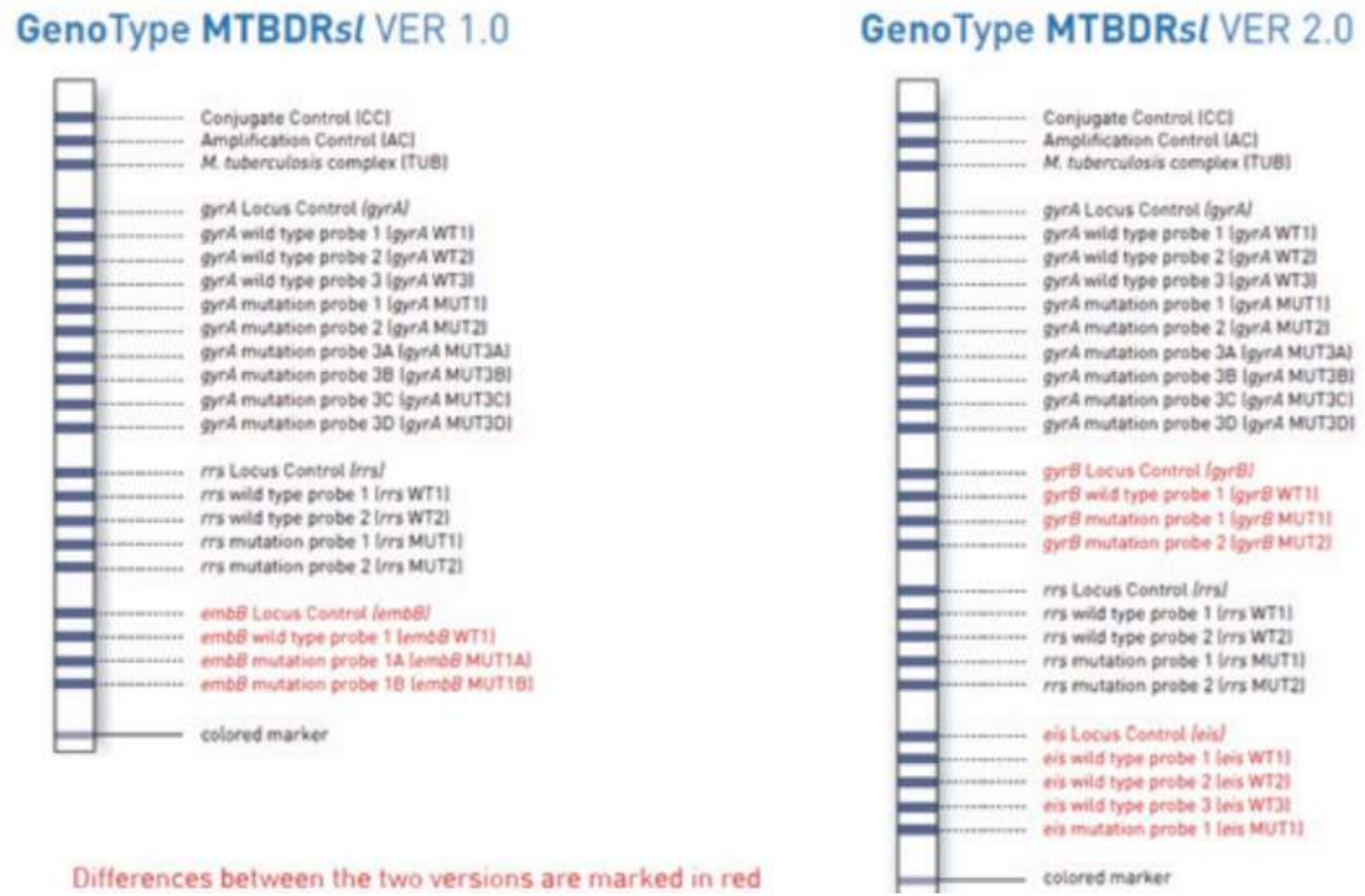
GenoType MTBDRsl®



World Health Organization. *The use of molecular line probe assays for the detection of resistance to second-line anti-tuberculosis drugs: Policy guidance* WHO/HTM/TB/2016.07

GenoType MTBDR*s*l ®

- Turn around time: 24-48 hours
- Allows quick triage of MDR-TB patients into either the shorter MDR-TB regimen or the conventional longer regimen.
- If Positive SL-LPA is treated with shorter regimen
  - treatment outcome jeopardised
  - risk of development of XDR-TB
- XDR-TB + by the SL-LPA : carefully designed individual regimen

Accuracy of MTBDRsl

Sr. No	Name of Drug	Sensitivity	Specificity
1	Fluoroquinolone	97%	98%
2	SLID (Second line injectable dug)	89%	90%
3	XDR TB	79%	97%

Cochrane Database of Systematic Reviews 2016, Issue 9. Art. No.: CD010705.

Accuracy of MTBDRsl

Pooled sensitivity (95% CI)	Pooled specificity (95% CI)	Pooled sensitivity (95% CI)	Pooled specificity (95% CI)	Pooled sensitivity P value[1]	Pooled specificity P value[1]
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)	0.932	0.333
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)	0.547	0.664
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)	0.888	0.855

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

World Health Organization. *The use of molecular line probe assays for the detection of resistance to second-line anti-tuberculosis drugs: Policy guidance* WHO/HTM/TB/2016.07

WHO Recommendation 2016

WHO recommends the use of the **SL-LPA for patients with MDR-TB** (or RR-TB) as the initial test to detect **resistance to fluoroquinolones and the second-line injectable drugs**, instead of phenotypic culture-based drug-susceptibility testing (DST).

(conditional recommendation)

World Health Organization. *The use of molecular line probe assays for the detection of resistance to second-line anti-tuberculosis drugs: Policy guidance* WHO/HTM/TB/2016.07

WHO Recommendation 2016

- Both direct as well as indirect testing
- Both pulmonary and extra pulmonary samples
- For **second-line injectable results**, resistance conferring mutations detected by SL-LPA are **highly correlated** with culture-based phenotypic resistance.
- For **fluoroquinolone**s, ofloxacin/levofloxacin **better correlated** than moxifloxacin
  - Inclusion of moxifloxacin in a RR or MDR-TB regimen: best guided by phenotypic testing
- Need phenotypic DST

World Health Organization. *The use of molecular line probe assays for the detection of resistance to second-line anti-tuberculosis drugs: Policy guidance* WHO/HTM/TB/2016.07

XPERT MTB/RIF

LPA vs CB NAAT

	LPA	CB-NAAT
WHO endorsed	2008	2010
Diagnosis	Not used	Used
Resistance	INH and RIF	RIF alone
Specimens	Smear positive only	Smear positive/negative
Turnaround time	72 hours	2 hours
Steps	Separate steps DNA extraction-PCR amplification- Calorimetric detection	Single cartridge for sample processing, amplification and detection
Cross contamination and operator dependence	yes	No

Xpert MTB/RIF (GeneXpert)

- Single-use disposable Cartridge containing all necessary elements
  - Automated sample preparation, amplification & detection
- Provides results from unprocessed sputum samples
- Limit of detection (LOD) of

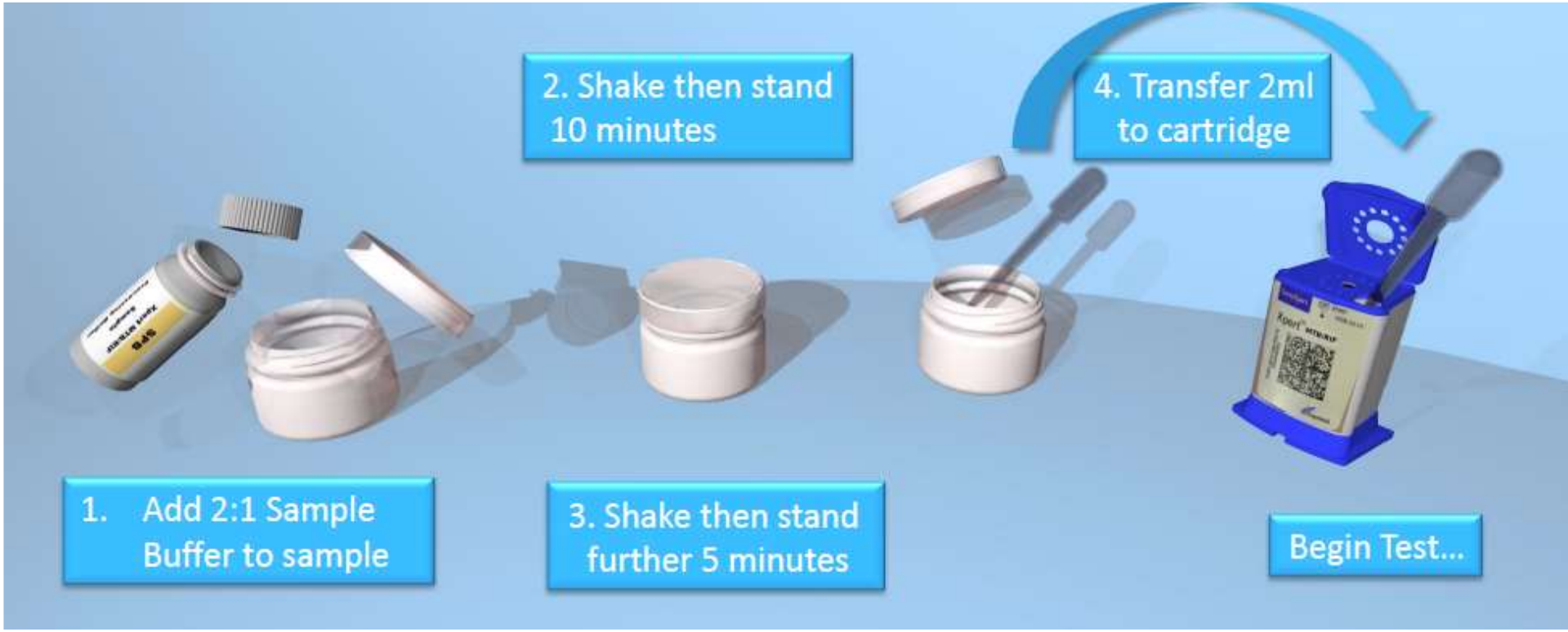
  133 CFU/ml sputum
- Digital read outs within 2 hours
- Minimal specimen handling

  & bio-safety requirements
- Technicians trained in 2-3 hrs
- In-built quality control

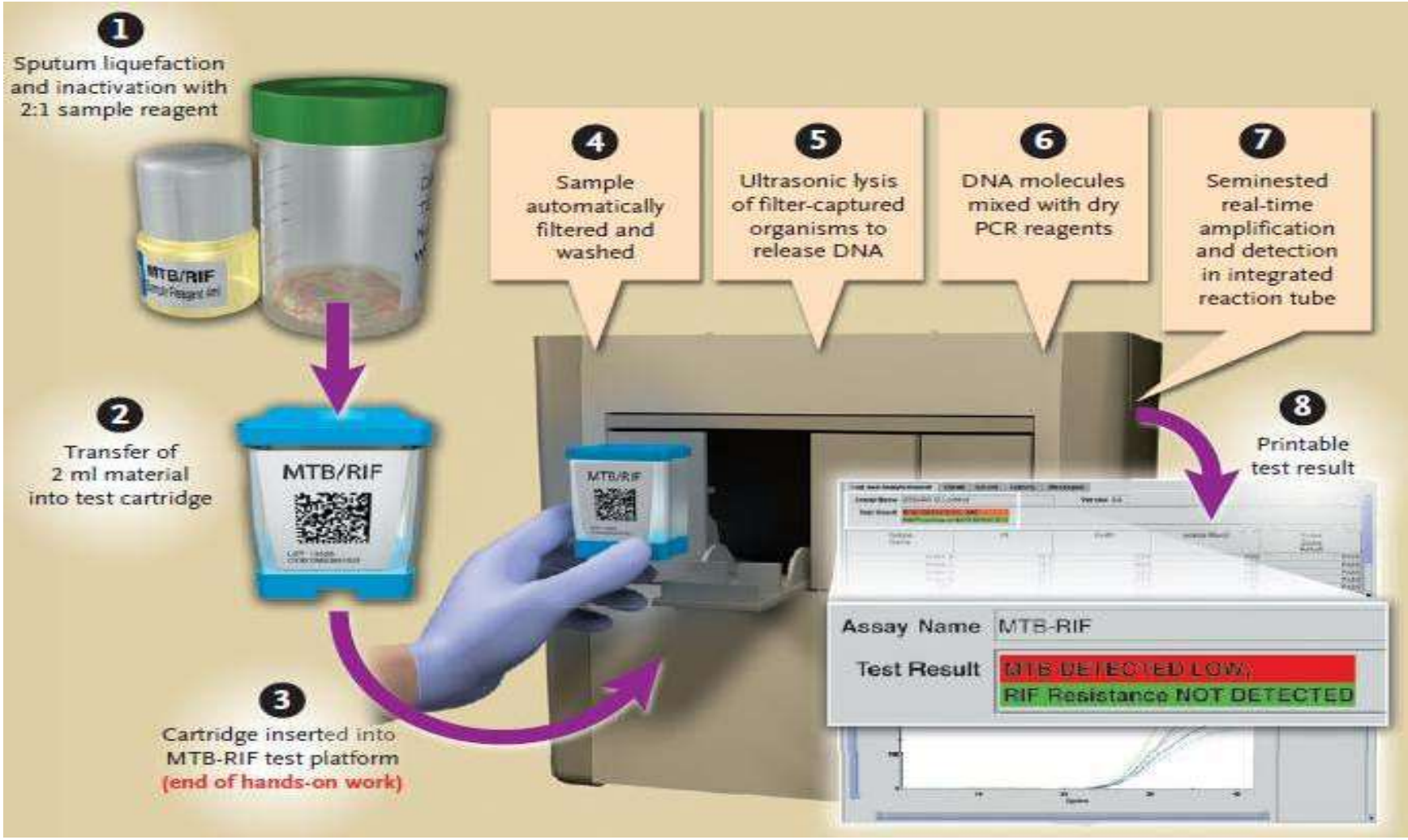
GeneXpert Dx System Components

- Catridge
  - Self contained
  - Disposable
- Computer system
  - Software
  - Barcode scanner
- Optional accessories
  - Printer
  - UPS
- Modules
  - Thermal and optical system

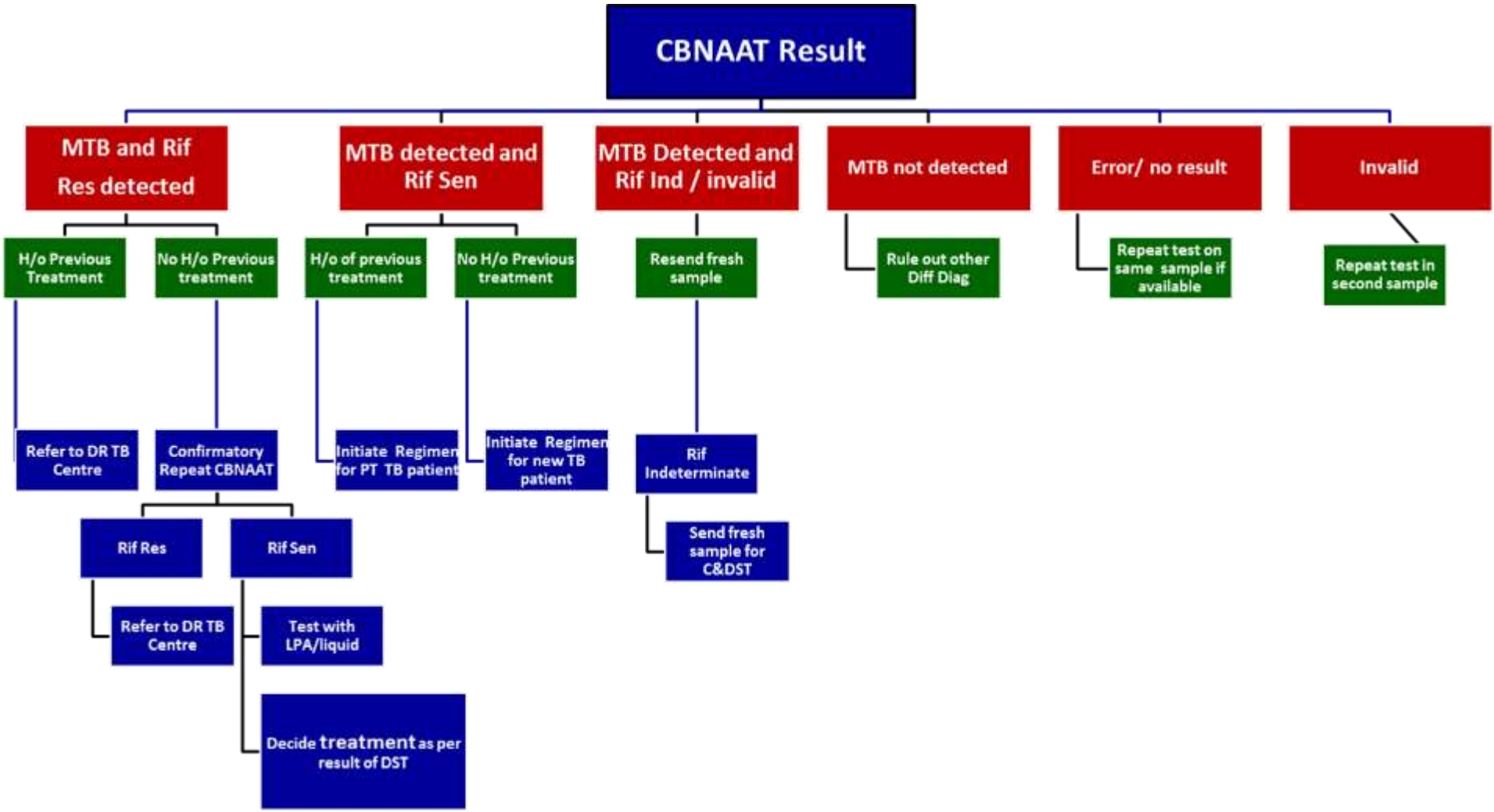
GeneXpert- Procedure



GeneXpert- Procedure



CBNAAT Result algorythm



(Guidance document for use of CB-NAAT under RNTCP, Sept 2013)

GeneXpert Performance for pulmonary samples

Sensitivity % (95% CI)			
N = 1437	All Culture positive	Smear negative-culture positive	Smear positive-culture positive
	95.7 (93.4–97.2)	77.7 (66.9–85.8)	99.2 (97.6–99.7)

Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
99.6 (98.9–99.8)	99.0 (97.6–99.6)	98.1 (97.0–98.7)

S K Sharma et al. *Evaluating the Diagnostic Accuracy of Xpert MTB/RIF Assay in Pulmonary Tuberculosis.* PLoS One. 2015; 10(10): e0141011.

Diagnostic performance of Xpert MTB/RIF assay in different respiratory samples

Sample type	Expectorated sputum [n = 1092]	Endotracheal tube aspirate [n = 143]	Bronchoalveolar lavage [n = 127]
Sensitivity %	96.9 (94.7–98.2)	87.5 (63.9–96.5)	90.0 (69.9–97.2)
Specificity %	99.8 (99.2–99.9)	98.4 (94.4–99.5)	100 (96.5–100)
PPV %	99.7 (98.5–99.9)	87.5 (63.9–96.5)	100 (82.4–100)
NPV %	98.3 (97.0–99.0)	98.4 (94.4–99.5)	98.1 (93.5–99.5)

PPV-Positive predictive value, NPV- Negative predictive value. Values in parantheses are 95% confidence intervals

Sample type	Induced sputum [n = 71]	Bronchial wash [n = 4]
Sensitivity %	84.2 (62.4–94.4)	100 (34.2–100)
Specificity %	98.0 (89.0–99.6)	100 (34.2–100)
PPV %	94.1 (73.0–98.9)	100 (34.2–100)
NPV %	94.4 (84.8–98.0)	100 (34.2–100)

S K Sharma et al. *Evaluating the Diagnostic Accuracy of Xpert MTB/RIF Assay in Pulmonary Tuberculosis.* PLoS One. 2015; 10(10): e0141011.

Rifampin susceptibility testing by Xpert MTB/RIF and phenotypic DST.

	Rif resistant by DST	RIF sensitive by DST	Total
RIF resistant by Xpert	104	7	111
RIF sensitive by Xpert	6	305	311
Total	110	312	422

Sensitivity- 94.5% (88.6–97.4)

Specificity- 97.7% (95.4–98.9)

Positive Predictive Value- 93.6% (87.5–96.9)

Negative Predictive Value- 98.0% (95.8–99.1)

Data are presented as whole numbers. RIF- Rifampin, DST- Drug susceptibility testing

S K Sharma et al. *Evaluating the Diagnostic Accuracy of Xpert MTB/RIF Assay in Pulmonary Tuberculosis.* PLoS One. 2015; 10(10): e0141011.

EPTB – Tubercular Pleural effusion (TPE)

Diagnostic test	Sensitivity
Thoracoscopic pleural biopsy	93 - 100%
PF ADA	47-100%
PF microscopy	10%
PF culture	20%

ADA	Management
<40	Thoracoscopic pleural biopsy
40-70	ATT given if Pretest probability high*
>70	Most patients receive ATT

*Age of <45 years, nonsmoker, straw-colored effusion, and high tuberculosis prevalence area

Inderpaul Singh Sehgal, Sahajal Dhooria, Ashutosh Nath Aggarwal, Digambar Behera and Ritesh Agarwal.*Diagnostic Performance of Xpert MTB/RIF in Tuberculous Pleural Effusion: Systematic Review and Meta-analysis.* J. Clin. Microbiol. April 2016 vol. 54 no. 4 1133-1136

Gene Xpert in TPE

	Inderpaul et al. (2016)	Denkinger et al. (2014)
Sensitivity	22.7 to 51.4 %	46.4%
Specificity	98.6 to 99.8 %	99.1%

- Low sensitivity: Cannot be used alone for the diagnosis of TPE
- High specificity: Obviate the need for an invasive procedure such as pleural biopsy in patients with high Pretest probability

Inderpaul Singh Sehgal, Sahajal Dhooria, Ashutosh Nath Aggarwal, Digambar Behera and Ritesh Agarwal.*Diagnostic Performance of Xpert MTB/RIF in Tuberculous Pleural Effusion: Systematic Review and Meta-analysis.* J. Clin. Microbiol. April 2016 vol. 54 no. 4 1133-1136

Denkinger CM, **Schumacher SG**,**Boehme CC**,**Dendukuri N**, **Pai M**,**Steingart KR** 2014. Xpert MTB/RIF assay for the diagnosis of extrapulmonary tuberculosis: a systematic review and meta-analysis. Eur Respir J **44**:435–446.10.1183/09031936.00007814

CBNAAT in Non respiratory specimen

Specimen Type	Sensitivity (%)	Specificity (%)
Lymph Node Biopsy	93 (70-99)	98 (all combined)
Lymph Node FNAC	96 (72-79)	
Tissues all types	88 (76-94)	
CSF	85 (75-100)	
Pleural fluid	34 (24-44)	
Other fluid samples (Pericardial, Ascitic, Synovial)	67 (0-100)	
Gastric aspirates	78% (68-85)	99%

Maynard et al. BMJ Inf Dis 2014:14:709

- **A positive test provides useful confirmation so that ATT can be started promptly**
- **A negative test does not always rule out TB**

Specimen type	Comparison (No. of studies, No. of samples)	Median (%) pooled sensitivity (pooled 95% CrI)	Median (%) pooled specificity (pooled 95% CrI)
Lymph node tissue and aspirate	Xpert MTB/RIF compared against culture (14 studies, 849 samples)	84.9 (72–92)	92.5 (80–97)
	Xpert MTB/RIF compared against a composite reference standard (5 studies, 1 unpublished)	83.7 (74–90)	99.2 (88–100)
Cerebrospinal fluid	Xpert MTB/RIF compared against culture (16 studies, 709 samples)	79.5 (62–90)	98.6 (96–100)
	Xpert MTB/RIF compared against a composite reference standard (6 studies, 512 samples)	55.5 (51–81)	98.8 (95–100)
Pleural fluid	Xpert MTB/RIF compared against culture (17 studies, 1385 samples)	43.7 (25–65)	98.1 (95–99)
	Xpert MTB/RIF compared against a composite reference standard (7 studies, 698 samples)	17 (8–34)	99.9 (94–100)
Gastric lavage and aspirate	Xpert MTB/RIF compared against culture (12 studies, 1258 samples)	83.8 (66–93)	98.1 (92–100)
Other tissue samples	Xpert MTB/RIF compared against culture (12 studies, 699 samples)	81.2 (68–90)	98.1 (87–100)

CrI, credible interval; the CrI is the Bayesian equivalent of the confidence interval.

World Health Organization *Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children:Policy update* WHO/HTM/TB/2013.16

GeneXpert in EPTB: INDEX TB guidelines 2017

Recommendations: Diagnosis of EPTB using the Xpert MTB/RIF test
Lymph node TB Xpert MTB/RIF should be used as an additional test to conventional smear microscopy, culture and cytology in fine-needle aspiration cytology (FNAC) specimens. Strong recommendation, low quality evidence for sensitivity estimate, high quality evidence for specificity estimate.
TB meningitis Xpert MTB/RIF may be used as an adjunctive test for tuberculous meningitis (TBM). A negative Xpert MTB/RIF result on a cerebrospinal fluid (CSF) specimen does not rule out TBM. The decision to give anti-tuberculosis treatment (ATT) should be based on clinical features and CSF profile. Conditional recommendation, low quality evidence for sensitivity estimate, high quality evidence for specificity estimate.
Pleural TB Xpert MTB/RIF should not be routinely used to diagnose pleural TB. Strong recommendation, low quality evidence for sensitivity estimate, high quality evidence for specificity estimate.

Central TB Division Ministry of Health and Family Welfare, Government of India, *INDEX-TB guidelines on extra-pulmonary tuberculosis in India*, 2017

WHO recommendation 2013

- Xpert MTB/RIF **should be** used rather than conventional microscopy, culture and DST as the initial diagnostic test in adults and children suspected of having **MDR-TB or HIV-associated TB** *(strong recommendation, high-quality evidence).*
- Xpert MTB/RIF **may be used** rather than conventional microscopy and culture as the initial diagnostic test in **all adults and children suspected of having TB** *(conditional recommendation acknowledging resource implications, high-quality evidence).*

World Health Organization *Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children:Policy update* WHO/HTM/TB/2013.16

WHO recommendation 2013

- Xpert MTB/RIF **may be used as a follow-on test to microscopy** in adults suspected of having TB but not at risk of MDR-TB or HIV-associated TB, especially when further testing of smear negative specimens is necessary
  (conditional recommendation acknowledging resource implications, high-quality evidence).

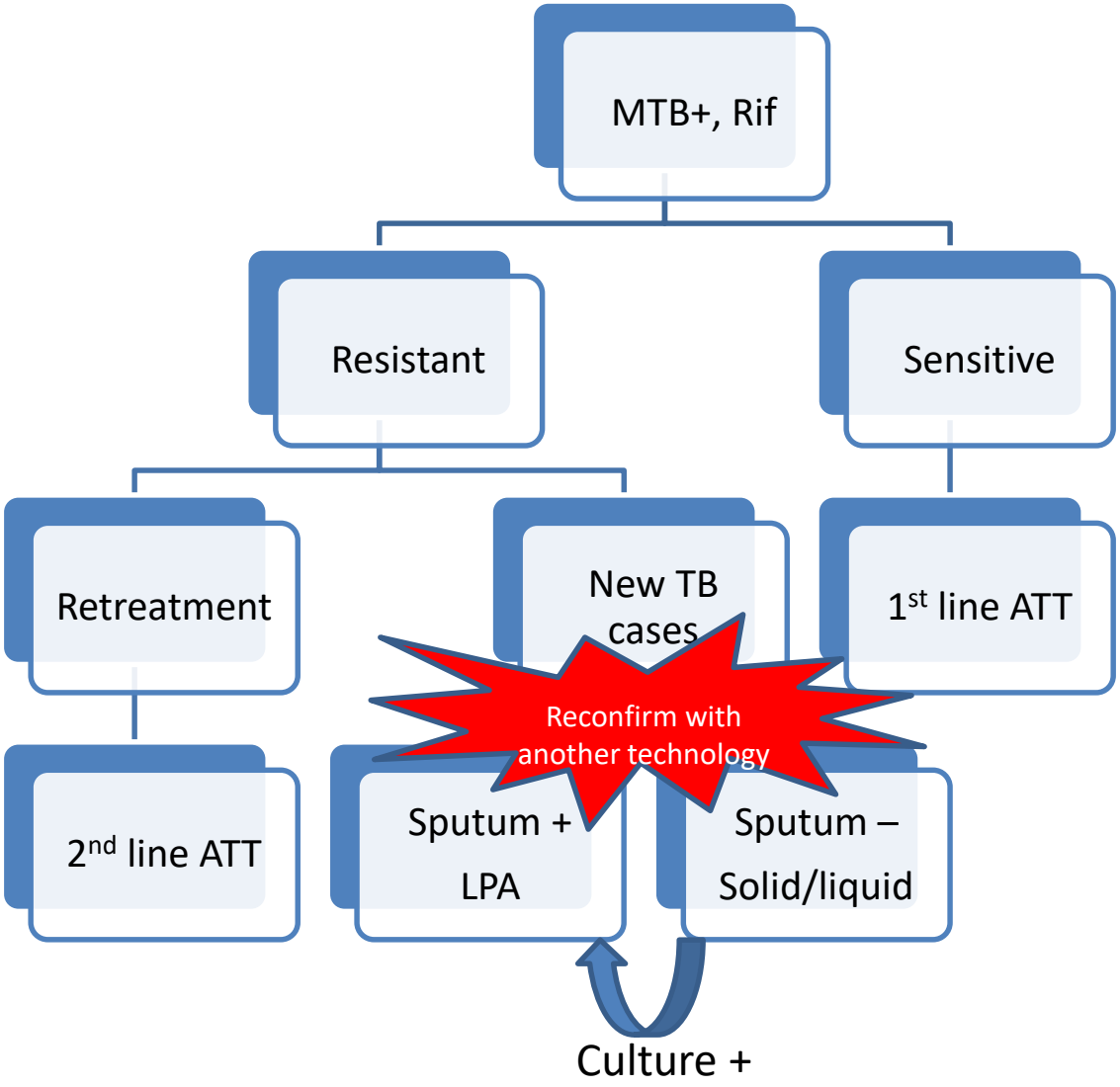
World Health Organization *Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children:Policy update* WHO/HTM/TB/2013.16

RNTCP recommendations 2013

- For MTB +, Rif sensitive results, treat the patient with first line drug regimen
- For MTB +, Rif resistance results-
    - In re-treatment TB cases: 2nd line DST (Rx as MDR-TB)
    - In new TB cases, treat with regimen for MDR-TB after reconfirming Rif resistance with another technology.
        - In smear positive cases, reconfirm with LPA
        - In smear negative cases, offer liquid or solid culture and if culture is positive, the culture isolates must be subjected to LPA as per RNTCP PMDT guidelines

Guidance document for use of CB-NAAT under RNTCP, Sept 2013

RNTCP recommendations 2013



Guidance document for use of CB-NAAT under RNTCP, Sept 2013

Advantages

1. Better sensitivity and specificity than smear microscopy
2. Good accuracy for tuberculosis diagnosis
3. Diagnosis and rifampicin resistance
4. Rapid - 2 hours
5. Simple to use
6. Operators do not need formal laboratory training
7. Does not need advanced biosafety equipment
8. Closed system with low risk of cross-contamination
9. Could potentially be used to test a broad range of samples from extrapulmonary sites

Lancet Infect Dis March 2013

Disadvantages

1. Expensive
2. Yearly calibration and maintenance
3. Continuous electrical power supply
4. Relatively short shelf life of cartridges (18 months)
5. Cannot be used to monitor treatment success or failure, or relapse
6. Use with extrapulmonary samples is not yet fully defined

Lancet Infect Dis March 2013

XPERT MTB/RIF ULTRA

Xpert MTB/RIF Ultra assay (Ultra)

- Xpert MTB/RIF sensitivity is imperfect, particularly in **smear negative and HIV-associated TB**

WHO meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTF/RIF Ultra compared to Xpert MTB/RIF. Geneva: World Health Organization; 2017 (WHO/HTM/TB/2017.04). Licence: CC BY-NCSA 3.0 IGO

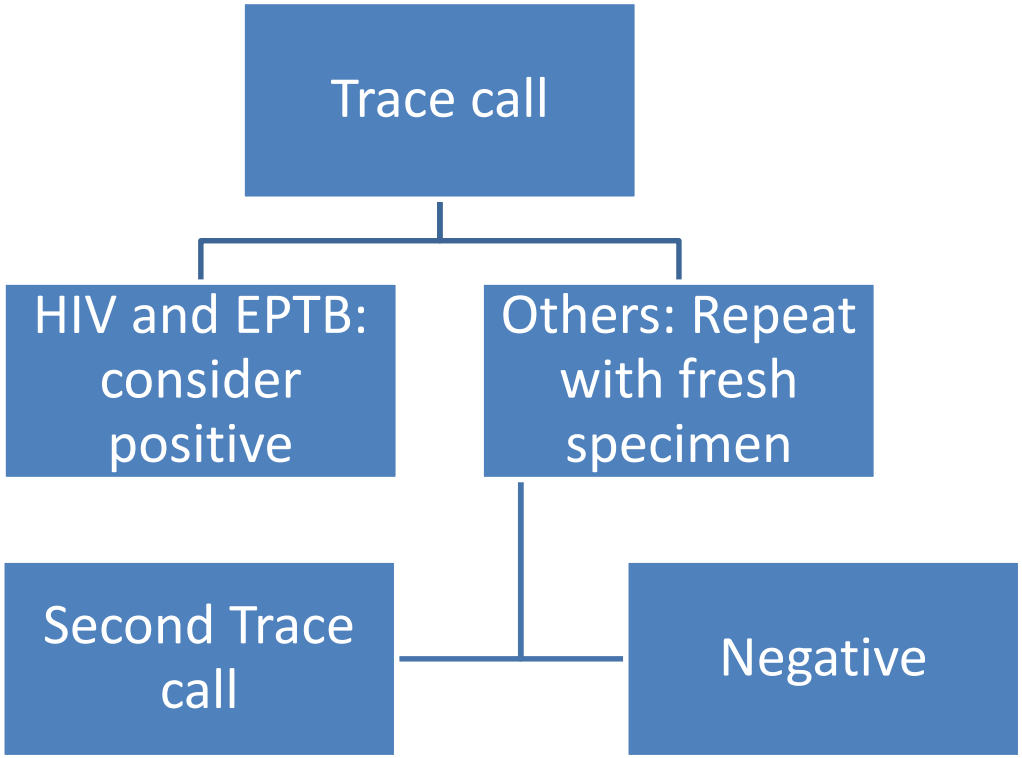
XpertUltra vs Xpert

	Xpert MTB/RIF Ultra	Xpert MTB/RIF
Mechanism of rifampicin resistance	Melting temperature based PCR	Real time PCR
Amplification targets	**IS6110 and IS1081,** 4rpoB	rpoB
DNA chamber	50µl PCR reaction	25µl PCR reaction
Amplification	Fully nested	Hemi nested
Thermal cycling	More rapid	
Fluidics and enzymes	Improved	
Limit of detection (LOD)	16 cfu/ml	114 cfu/ml

WHO meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTF/RIF Ultra compared to Xpert MTB/RIF. Geneva: World Health Organization; 2017 (WHO/HTM/TB/2017.04). Licence: CC BY-NCSA 3.0 IGO

Interpretation of results

- New semiquantitative category “trace call”- lowest bacillary burden for MTB detection



WHO meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTF/RIF Ultra compared to Xpert MTB/RIF. Geneva: World Health Organization; 2017 (WHO/HTM/TB/2017.04). Licence: CC BY-NCSA 3.0 IGO

Ultra Performance

N = 1520	Sensitivity of Ultra vs GeneXpert	Specificity of Ultra vs GeneXpert
Overall	5% higher (95%CI +2.7, +7.8)	3.2% lower (95%CI -2.1, -4.7)
Smear – Culture +	**+17%** (95%CI +10, +25)	
HIV infected	**+12%** (95%CI +4.9, +21)	
Pediatric EPTB: CSF	95% vs 45%	
Pediatric PTB:	71% vs 47%	

WHO meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTF/RIF Ultra compared to Xpert MTB/RIF. Geneva: World Health Organization; 2017 (WHO/HTM/TB/2017.04). Licence: CC BY-NCSA 3.0 IGO

Rifampicin resistance by Ultra

- Ultra performed similarly well as Xpert MTB/RIF
- ‘Trace call’: Indeterminate rifampicin result
- If low risk for rifampicin resistance (e.g. new TB cases not at risk for MDR-TB) a positive rifampicin resistance result should be repeated

WHO meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTF/RIF Ultra compared to Xpert MTB/RIF. Geneva: World Health Organization; 2017 (WHO/HTM/TB/2017.04).

Advantages

- Better sensitivity in **paucibacillary specimens** (Smear negative culture+, HIV+, pediatric, EPTB esp CNS TB)
- **Better differentiate silent mutations** (such as Q513Q or F514F) from resistance conferring mutations

WHO meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTF/RIF Ultra compared to Xpert MTB/RIF. Geneva: World Health Organization; 2017 (WHO/HTM/TB/2017.04).

Limitations

- **False positives:** If past h/o TB +
  - Picks non replicating and non viable bacilli also

WHO Recommendation

- Use as initial diagnostic test for all adults and children with signs and symptoms of TB.
- Use in the testing of selected extrapulmonary specimens (CSF, lymph nodes and tissue specimens)

WHO meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTF/RIF Ultra compared to Xpert MTB/RIF. Geneva: World Health Organization; 2017 (WHO/HTM/TB/2017.04).

XPERT OMNI

GeneXpert Omni

- 9 inches(23 cm) tall 2.2 pounds (1kg)
- Proven Cartridge Technology similar to GeneXpert
- Battery operated, wireless, web enabled
- Able to transmit instrument and time information in real time

Loop Mediated Isothermal Amplification (LAMP)

- Developed by Eiken chemical, Tokyo, Japan
- Manual NAAT, DNA can be amplified $10^{1º}$ times in 15-60 min
- Targets gyrB gene (M, tuberculosis, M. avium, and M. intracellulare)
- Sensitivity (smear positive)- 97%

  (smear negative)-62
- Specificity-96.3%
- Advantages: 1.High speed (35 mins for solid media, 60 mins for liquid media and sputum)

  2.No use of thermal cycler (isothermic - 63° C)

  3.Can be used in peripheral level

Loop-mediated isothermal amplification for direct detection of Mycobacterium tuberculosis complex, M. avium, and M. intracellulare in sputum samples. Iwamoto T J Clin Microbiol 2003; 41 : 2616 - 2622

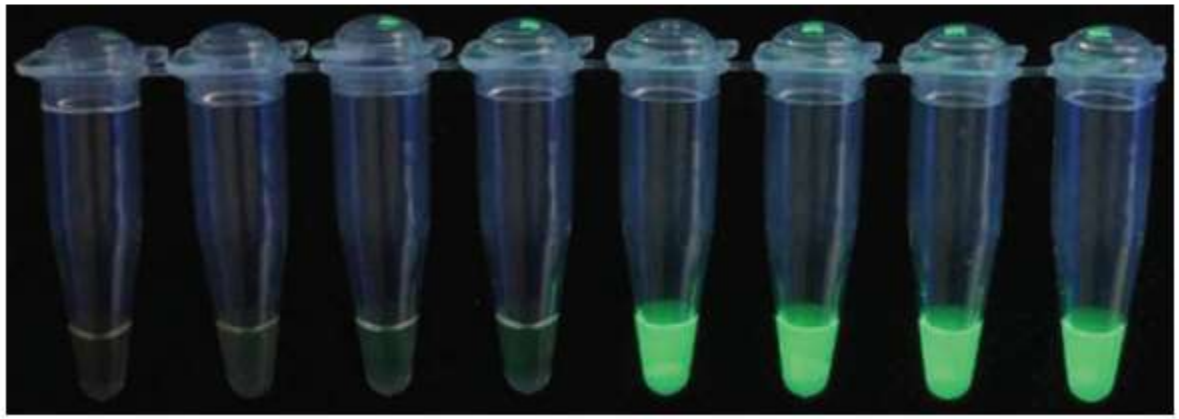
Steps

World Health Organization 2016 *The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis* WHO/HTM/TB/2016.11

Detection

- Double-stranded DNA binding dyes, such as SYBR green detect turbidity caused by precipitating magnesium pyrophosphate

Figure 2. Visual display of TB-LAMP results under ultraviolet light



World Health Organization 2016 *The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis* WHO/HTM/TB/2016.11

Molecular principles

- Loop primers contain sequences complementary to the ss loop region on the 5′-end of the hairpin structure, speeds the reaction by providing a greater number of starting points for DNA synthesis
- Using loop primers, amplification by 109 to 1010 times can be achieved within 15–30 minutes.

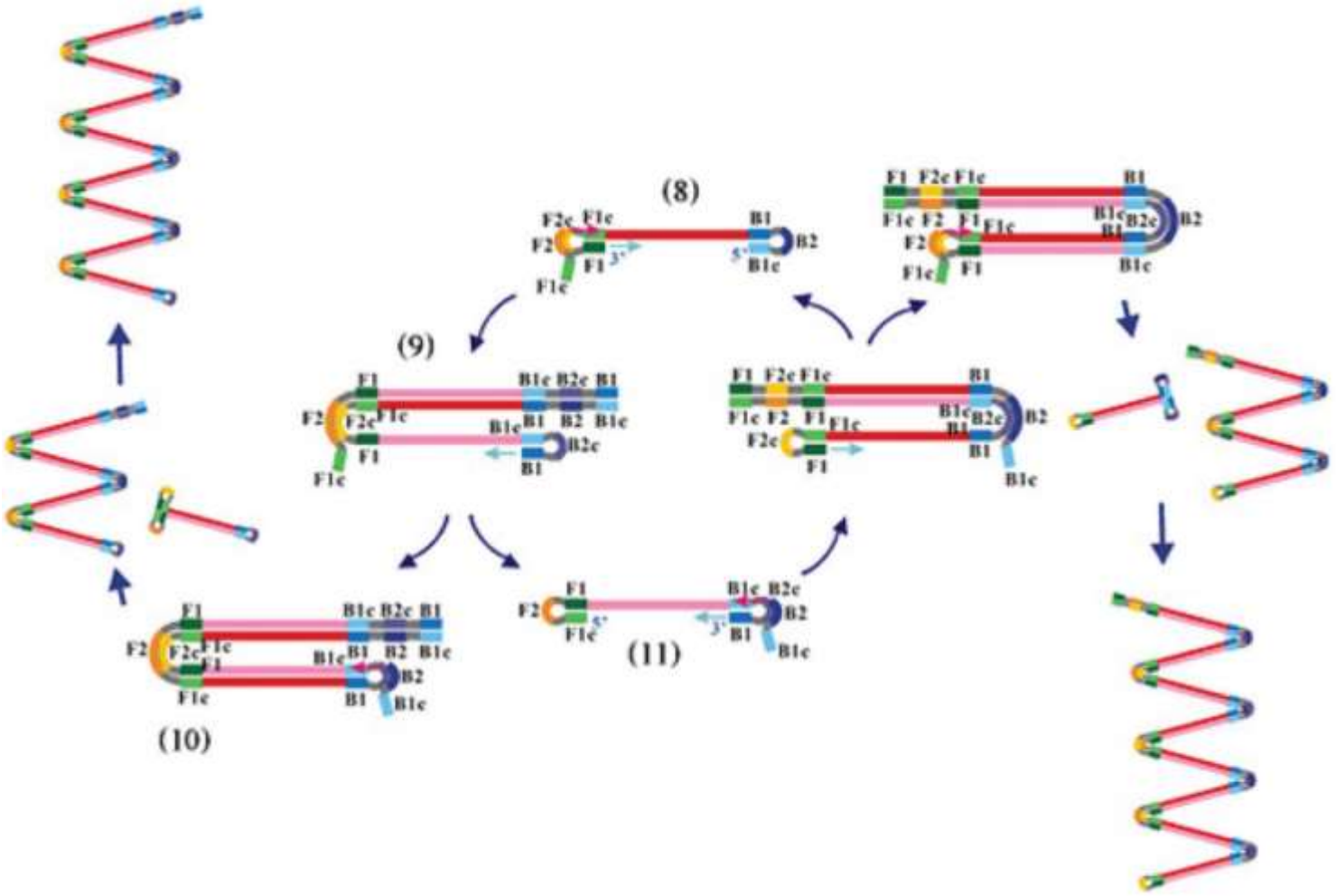
World Health Organization 2016 *The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis* WHO/HTM/TB/2016.11

Molecular principles

- Requirement for homogeneous sequences at multiple binding sites preserves the specificity of the assay even in the absence of a probe
- LAMP method is relatively insensitive to the accumulation of DNA and DNA by-products (pyrophosphate salts), so the reaction proceeds until large amounts of amplicon are generated

World Health Organization 2016 *The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis* WHO/HTM/TB/2016.11

Molecular principles



Performance

Table 4. TB-LAMP as a replacement test for smear microscopy: estimates of pooled sensitivity and specificity

Reference standard[a]	Pooled sensitivity[b]	Pooled specificity[b]
Standard 1	77.7 (71.2-83.0)	98.1 (95.7-99.2)
Standard 2	76.0 (69.9-81.2)	98.0 (96.0-99.0)
Standard 3	80.3 (70.3-87.5)	97.7 (96.1-98.7)

[a] All reference standards classify patients as having TB if ≥ 1 positive culture was confirmed as *M. tuberculosis* by speciation testing. To be classified as not having TB, patients had to have no positive and at least (i) two negative cultures on two different sputum specimens (Standard 1), (ii) two negative cultures on the same or different sputum specimens (Standard 2), or (iii) at least one negative culture (Standard 3).
[b] Values are percentages (95% confidence intervals).

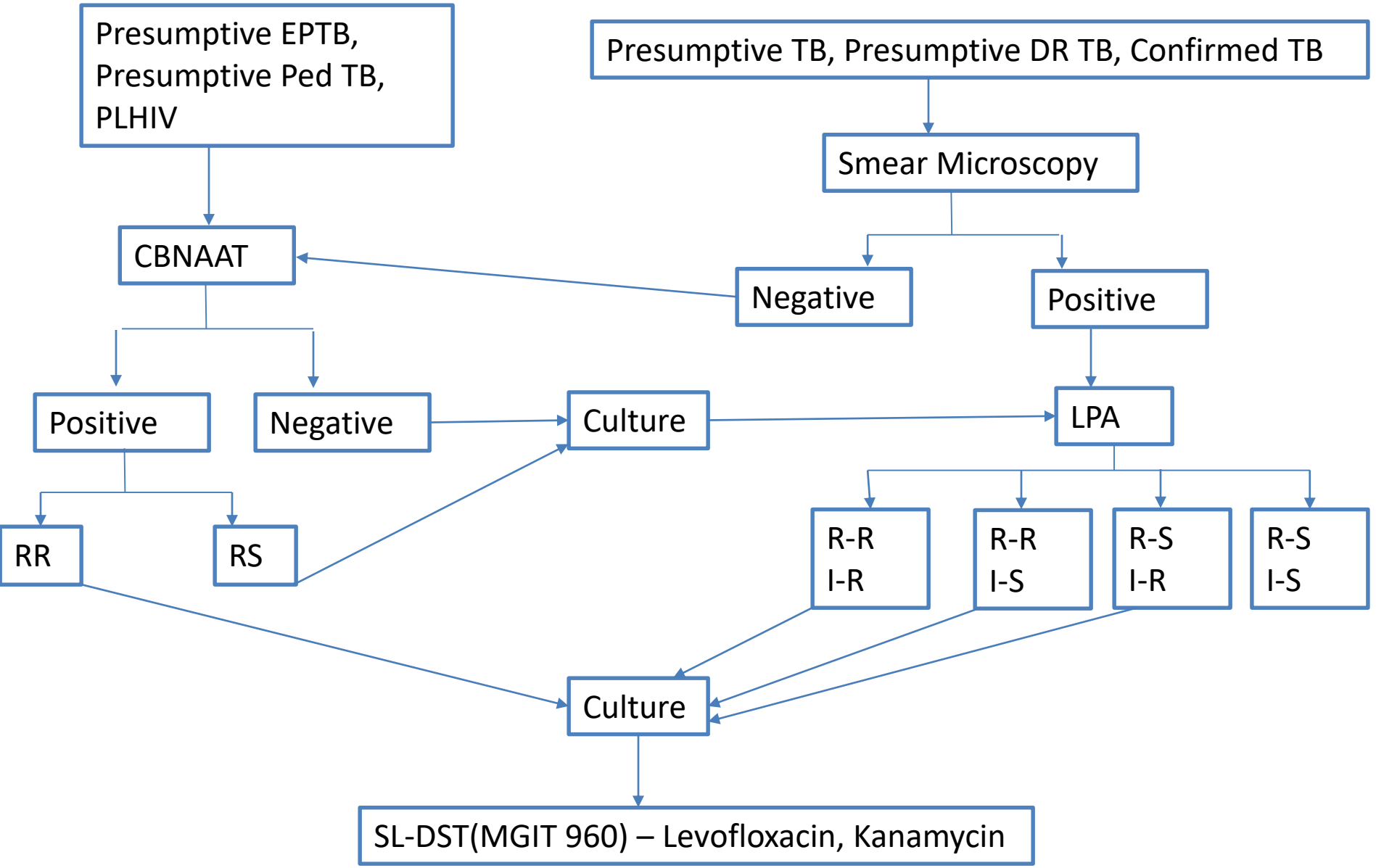
World Health Organization 2016 *The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis* WHO/HTM/TB/2016.11

WHO recommendations 2016

- TB-LAMP **may be used** as a replacement test for sputum-smear microscopy to diagnose pulmonary TB in adults with signs and symptoms consistent with TB *(conditional recommendation, very low-quality evidence).*
- TB-LAMP **may be used** as a follow-on test to smear microscopy in adults with signs and symptoms consistent with pulmonary TB, especially when further testing of sputum smearnegative specimens is necessary *(conditional recommendation, very low-quality evidence).*

World Health Organization 2016 *The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis* WHO/HTM/TB/2016.11

Mycobacteriology Lab Workflow – PGIMER



PGIMER- Available lab equipment

- DMC (Designated Microscopy Centre) (New OPD/1/1031)
- Culture & DST Laboratory (Research Block A/ 2 /221)
- Auramine stain is used under LED based microscope since 2011
- BACTEC 460 and MGIT are available
- Gene Xpert and LPA are also available
- Solid culture and DST for First Line DST (RIF + INH + STR + ETM)- 2011
- Line Probe Assay For First Line DST (RIF+ INH) - April 2013
- Liquid culture and DST for First Line DST (RIF + INH + STR + ETM) – Feb 2015
- Liquid culture and DST for Second Line DST (OFLx + AMK + KAN + CAP) – Sept 2015
- LAMP and second line LPA are also available and is currently used for research purposes

Take home points

- Rapid molecular tests donot eliminate the need for conventional Culture and DST
- GeneXpert (R): poor sensitivity in body fluids (PF)
- GeneXpert Ultra (R): better sensitivity in paucibacilly specimens (sputum- and EPTB)
- LPA (H&R): only in sputum + specimens
- Second line LPA: In all MDR-TB, to rule out resistance to FQ/SLID

New cases

80%

drop in new TB cases by 2030

TB deaths

90%

drop in people dying of TB by 2030

Reducing poverty

100%

of TB-affected families protected from catastrophic costs by 2030

Thank You