NEWER DIAGNOSTIC TESTS IN TUBERCULOSIS

DM SEMINAR
14/9/18

DR VIKRAM D

Outline

- Newer TB diagnostic tests
- Xpert MTB/RIF Ultra
- GeneXpert Omni
- Xpert XDR
- TrueNat MTB
- LPA
- FlouroType MTB, MDRTB
- TB-LAMP
- LAM

Progress In The Development Of TB Diagnostics

Technologies Endorsed By WHO

Molecular detection of TB and Drug resistance
Xpert MTB/RIF Ultra – Cepheid, USA
FL-LPA – Hain Lifescience, Germany
SL-LPA – Hain Lifesxcience, Germany
TB LAMP – Eiken, Japan

Culture – Based Technologies
Commercial Liquid culture systems
Culture based phenotypic DST

Non Molecular technologies
TB-LAM, Alere, USA
IGRA – Qiagen, USA

Microscopy
LED Microscopy

WHO: Global TB report 2017

Scheduled For Evaluation In 2018/19

Molecular detection of TB and drug resistance
Molecular technologies for genotypic Drug resistance - Sequencing
FluoroType MTBDR, Hain Lifescience, Germany
GeneXpert Omni, Cepheid, USA
BD Max MDR-TB, Becton Dickinson, USA
M2000 RealTime MTB system, Abbott, USA

Radiology
CXR
Computer Aided detection

Technologies in development
Gendrive MTB/RIF ID, Epistem, UK
Xpert XDR-TB cartridge, Cepheid, USA
FluoroType XDR-TB assay, Hain lifescience

On the market (evidence for use not submitted to WHO for evaluation)
Truenat MTB, Molbio/Bigtec diagnostics, India
EasyNAT TB diagnostic Kit, Ustar Biotechnologies, China
Genechip, TB drug resistance array, Capital Bio, China

What is the need for Xpert Ultra ?

Xpert MTB/RIF – MTB detection

- Pooled sensitivity and specificity of 89% and 98% respectively.

However it's pooled sensitivity is

- 67% in smear negative PTB,
- 43% in HIV patients with smear negative TB
- As low as 28% for smear negative TB patients from high resource and low TB incidence

Chakravorty S, Simmons AM, Rowneki M, Parmar H, Cao Y, Ryan J et al The new Xpert MTB/RIF Ultra: improving detection of *Mycobacterium tuberculosis* and resistance to rifampin in an assay suitable for point-of-care testing. mBio 8:ea00812-17.

Xpert MTB/RIF – for rifampicin resistance –

- Pooled sensitivity and specificity of 94% and 98% respectively

However, Xpert MTB/RIF has-

- Decreased capacity to detect rpoB C533G mutations responsible for some cases of RIF-R

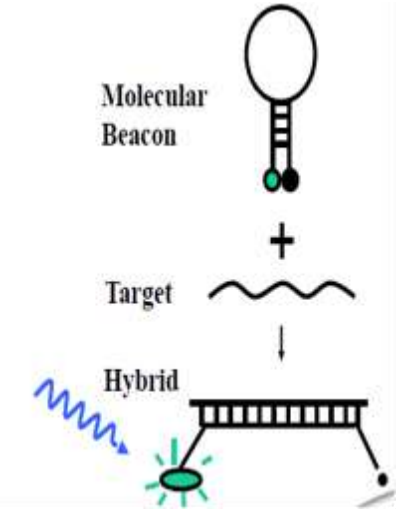
- False recognition of a nonfunctional rpoB F514F silent mutation as conferring RIF-R.

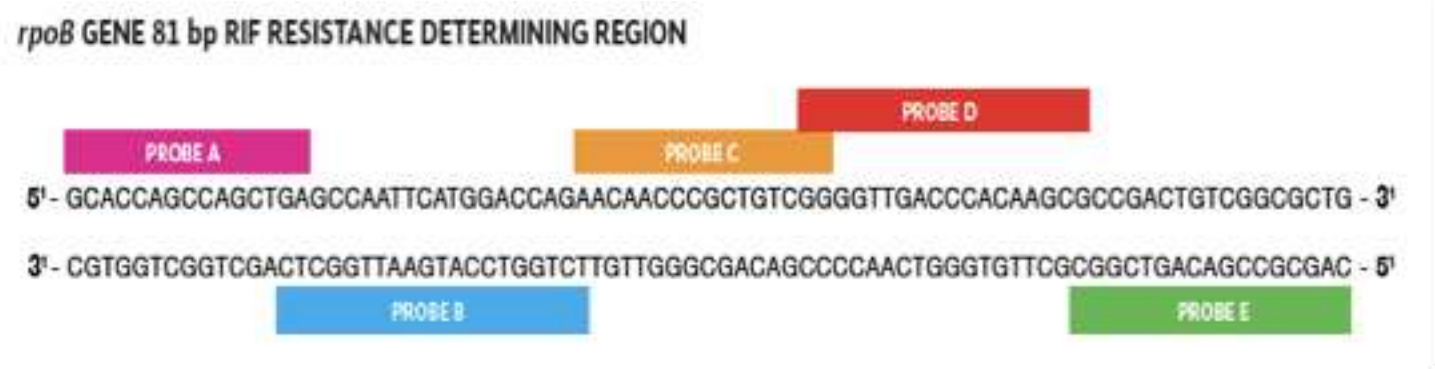
Xpert MTB/RIF Ultra

- Developed by Cepheid, Sunnyvale, USA
- Uses the same GeneXpert platform as Xpert MTB/RIF
- Developed to overcome the limitations of Xpert MTB/RIF
  - imperfect sensitivity – particularly in smear negative and HIV-associated TB
  - determination of rifampicin resistance

	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

Xpert MTB/RIF : Detection



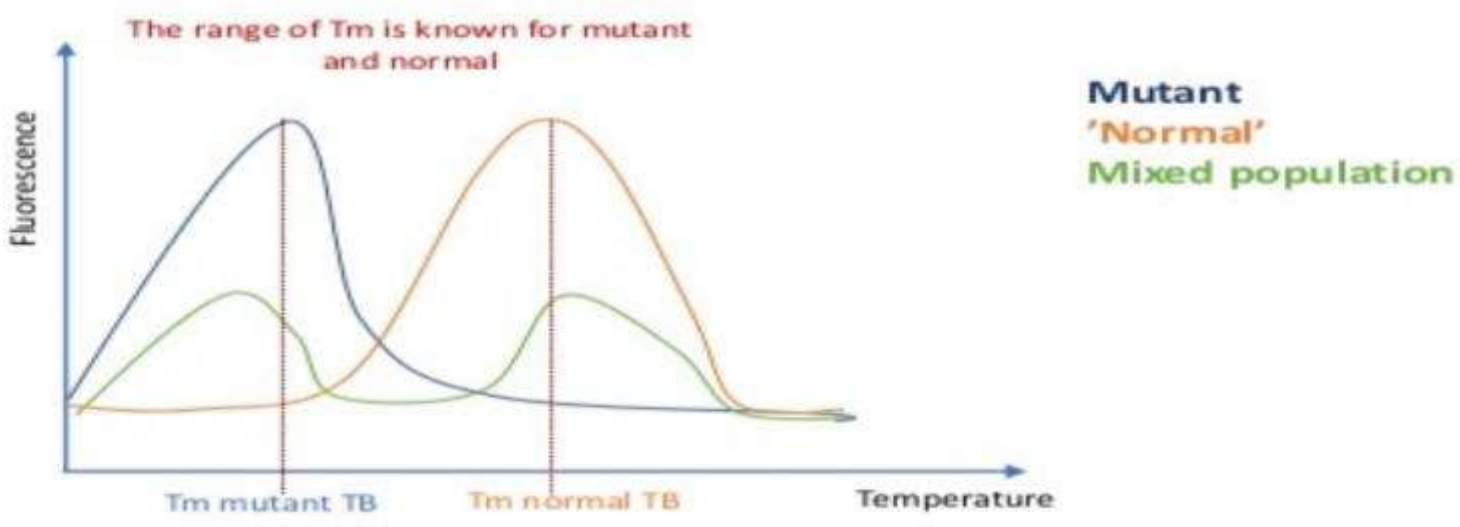


Improved sensitivity for MTB detection

- Uses 2 different multi-copy amplification targets (IS6110 and IS1081)
- Has Larger DNA reaction chamber than Xpert MTB/RIF
- 50 microlitre PCR reaction in Ultra versus 25 microlitre in Xpert MTB/RIF
- Fully nested NAAT, more rapid thermal cycling, improved fluidics and enzymes
- Resulting in LOD of 16 bacterial cfu/ml (vs 131 cfu/ml of Xpert MTB/RIF)

Improved accuracy of Rif resistance detection

- 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



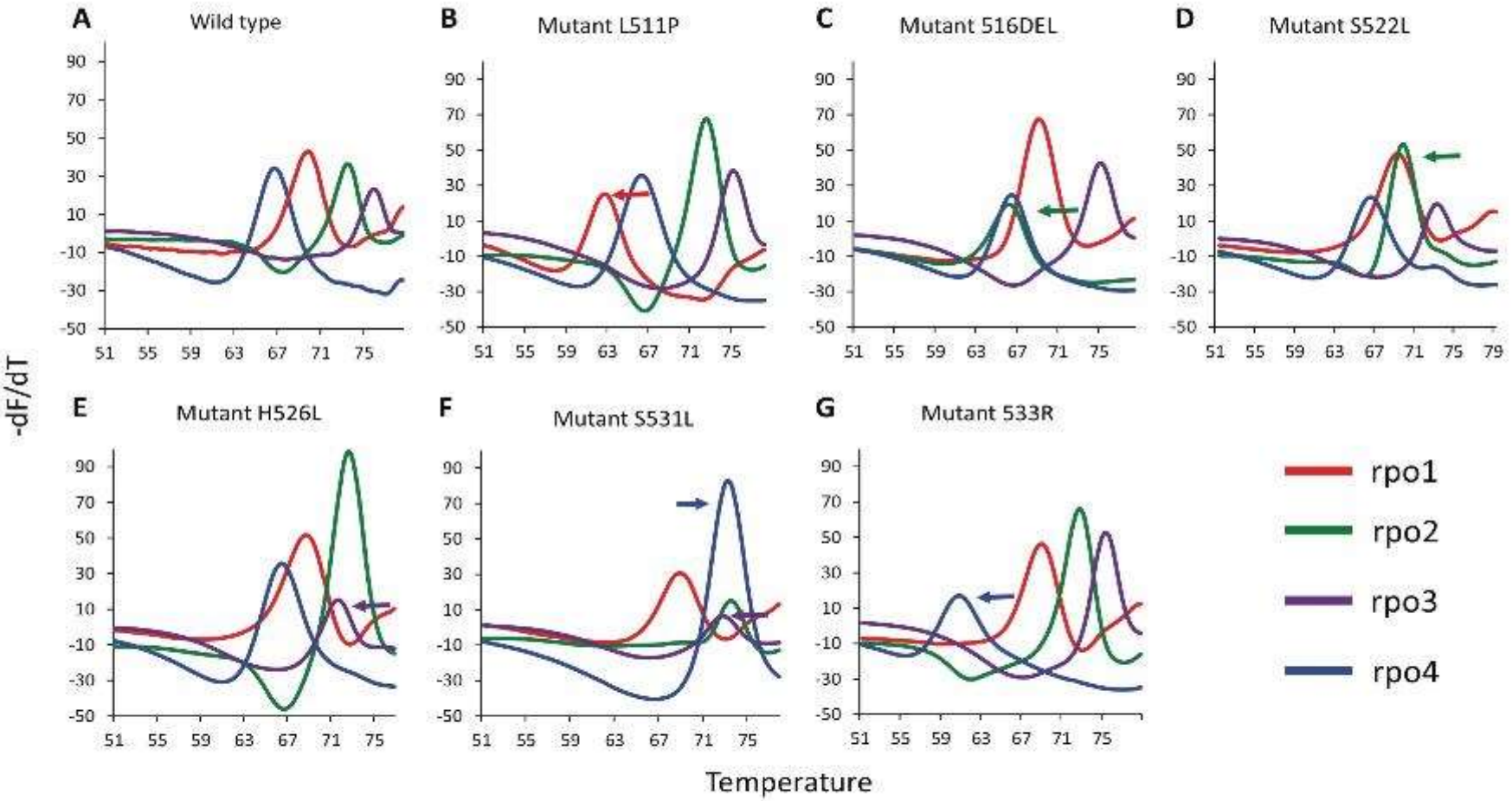


FIG 2 Detection of *M. tuberculosis rpoB* gene mutations associated with rifampin resistance. Derivative-transformed curves of four *rpoB*-specific sloppy molecular beacons (rpo1, rpo2, rpo3, and rpo4) first hybridized and then melted off of their *M. tuberculosis rpoB* gene amplicon target are shown. Each colored peak indicates the melting temperature of the probe corresponding to the colored line. The peaks for wild-type *M. tuberculosis* (A) identify a test sample as rifampin susceptible. The shift in one or more of the peaks away from the wild type's melting temperature (B to G), identify a sample as an *rpoB* mutant and rifampin resistant. The shift in the melt peak is indicated by arrows.

MTB detection with Ultra

Defined as :

- **One or both** of the probes detecting the **multi-copy** targets with cycle thresholds (Cts) **< 37 cycles**

  and

- **At least 2 rpoB** probes with Cts **< 40 cycles**

Quantification into :

- High, medium, low, very low and
- New “trace” category – corresponds to lowest bacillary burden for MTB detection

“MTB detected Trace” – “Trace call” :

- Added to improve sensitivity for MTB detection

For a trace call :

- **One or both** of the probes for the **multi-copy targets** are positive with Cts **< 37** cycles

  and

- **No more than one rpoB** probes have a Ct less than 40 cycles

MTB NOT detected :

- If neither of the multi-copy target probes are positive

  and

- Sample processing control is positive with a Ct < 35 cycles

Rifampicin Resistance detection

Absent :

- if MTB is detected **and**
- All 4 rpoB probes have **identifiable** melting temperature (Tm) peaks in the wild type profile

Present :

- If MTB is detected (not trace) **and**
- All 4 rpoB probes have **identifiable** Tms **and**
- **At least one** of the rpoB probe has a Tm **mutant** profile

Indeterminate :

- If MTB detected with a trace call – then no interpretation can be made regarding rifampicin resistance and results are reported as **MTB detected, trace, RIF indeterminate**

WHO Meeting Report of a Technical Expert Consultation: Non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF

Method:

- Multi-Centre, non inferiority diagnostic accuracy study
- Conducted by FIND, CDRC
- Xpert MTB/RIF and Ultra were performed from the same specimen
- Accuracy was determined with four cultures as the reference standard for TB detection (two MGIT tubes + two LJ slants, performed on two specimens obtained on separate days).
- Phenotypic drug-susceptibility testing as well as sequencing were performed for rifampicin resistance detection

Findings :

- 1,520 persons with signs and symptoms of TB were enrolled.

- Overall, sensitivity of the Ultra was **5% higher** than that of Xpert MTB/RIF (95%CI +2.7, +7.8), but Specificity was **3.2% lower** (95%CI -2.1, -4.7).

- Sensitivity increases were highest among **smear-negative culture-positive patients** and among **HIV infected patients**

sensitivity	Smear -, culture +	HIV
Xpert MTB/RIF	44.5%	75.5%
Ultra	61.3%	87.8%

- For EPTB and pediatric TB, increased sensitivity (primarily due to the 'trace call')

Sensitivity	CSF	Respiratory samples (children)
Ultra	95%	71%
Xpert MTB/RIF	45%	47%

Test	Specificity
Xpert MTB/RIF	98%
Ultra	94.8%

- Specificity decreases were higher in patients with a history of TB (-5.4%, 95%CI -9.1, -3.1) than in patients with no history of TB (-2.4%, 95% CI -4.0, -1.3)
- Likely because Ultra detects non viable bacilli.
- In low TB burden settings the specificity of Ultra is very high (99.3%)

In detection of rifampicin resistance –

- Ultra performed similarly well as Xpert MTB/RIF and
- The specificity of both assays was close to 100% when sequencing data was used to resolve discordant results with phenotypic DST.
- Melting temperature-based analysis - better differentiates silent mutations (such as Q513Q or F514F) from resistance conferring mutations.

Depending on the patient population,

- 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.

Conclusions of the Technical Expert Consultation

- Ultra is **non-inferior** to the Xpert MTB/RIF assay for the detection of MTB and for the detection of rifampicin resistance.

- Ultra has a **higher sensitivity** than Xpert MTB/RIF particularly in **smear-negative culture-positive specimens and in specimens from HIV-infected** patients with at least as good accuracy for rifampicin resistance detection.

- However, as a result of the increased sensitivity, Ultra also detects non-replicating or non-viable bacilli present particularly in patients with recent history of TB, **reducing the overall specificity of Ultra in high-burden TB settings.**

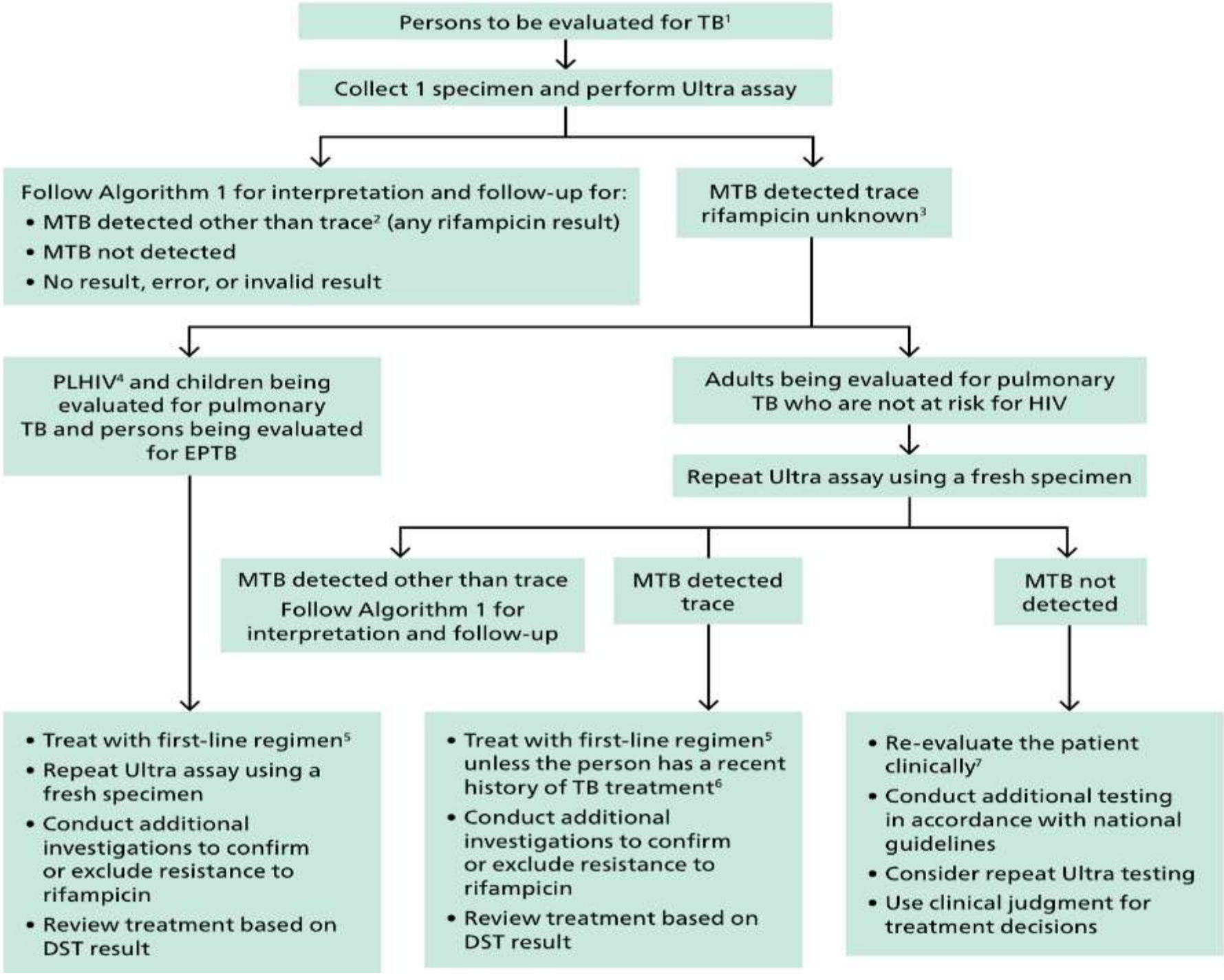
Interpret “trace calls” as follows:

- Among persons with **HIV, children and extra pulmonary** specimens “trace calls” should be considered to be **true positive** results for use in clinical decisions and patient follow-up

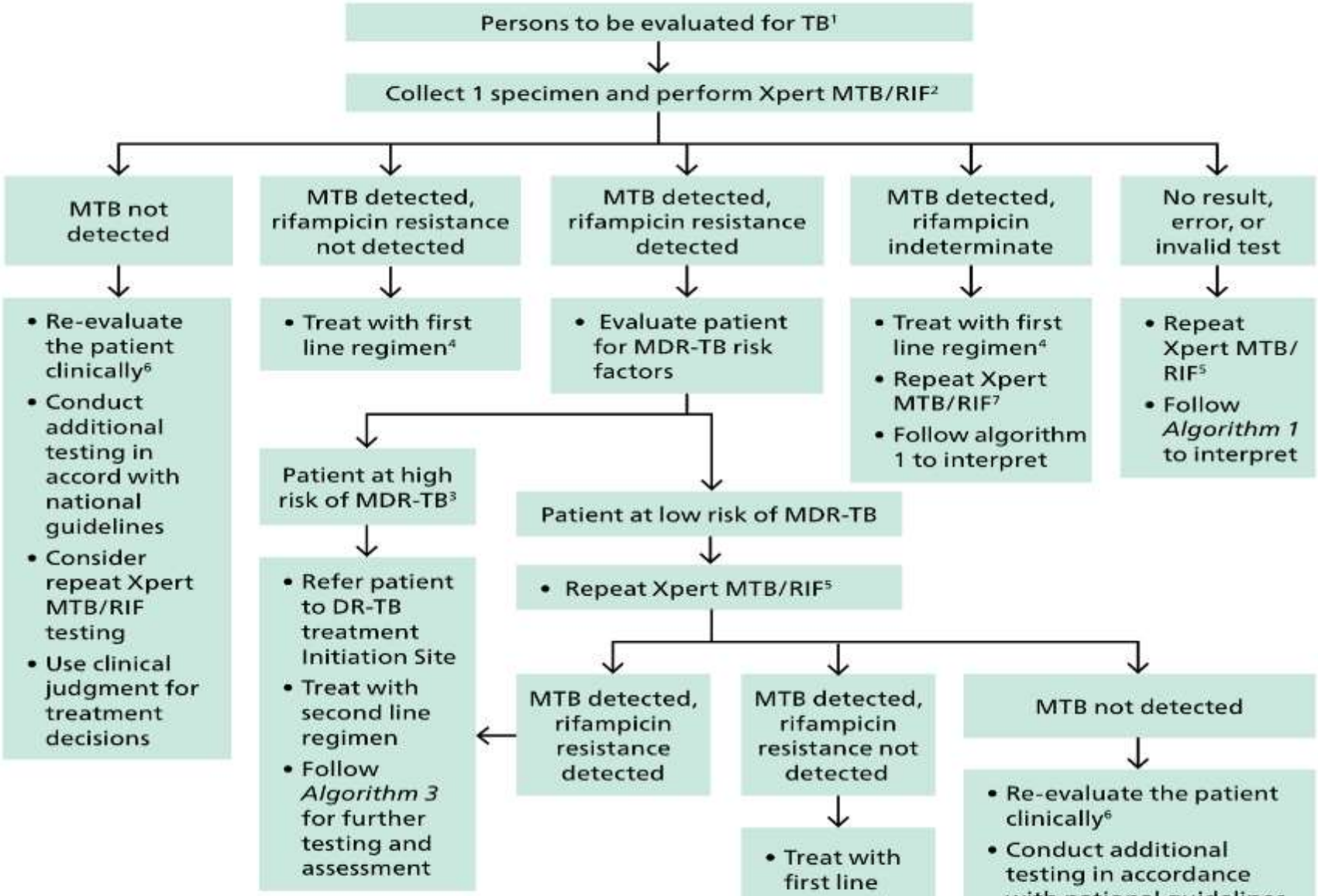
- Among persons **not at risk** for HIV, with an initial “trace call” positive result, a fresh specimen from the patient should **undergo repeat testing** and the result of the second Ultra test be used for clinical decisions and patient follow-up

- A **second “trace call” positive is sufficient to make a diagnosis of pulmonary TB** unless there is a recent history of TB

- Among all persons that test “trace call” positive, additional investigations are needed to confirm or exclude resistance to rifampicin.


Persons to be evaluated for TB[1]
Collect 1 specimen and perform Ultra assay
Follow Algorithm 1 for interpretation and follow-up for:
• MTB detected other than trace[2] (any rifampicin result)
• MTB not detected
• No result, error, or invalid result
MTB detected trace rifampicin unknown[3]
PLHIV[4] and children being evaluated for pulmonary TB and persons being evaluated for EPTB
Adults being evaluated for pulmonary TB who are not at risk for HIV
Repeat Ultra assay using a fresh specimen
MTB detected other than trace
Follow Algorithm 1 for interpretation and follow-up
MTB detected trace
MTB not detected
• Treat with first-line regimen[5]
• Repeat Ultra assay using a fresh specimen
• Conduct additional investigations to confirm or exclude resistance to rifampicin
• Review treatment based on DST result
• Treat with first-line regimen[5] unless the person has a recent history of TB treatment[6]
• Conduct additional investigations to confirm or exclude resistance to rifampicin
• Review treatment based on DST result
• Re-evaluate the patient clinically[7]
• Conduct additional testing in accordance with national guidelines
• Consider repeat Ultra testing
• Use clinical judgment for treatment decisions

(Algorithm 1 in the 2017 *GLI Model TB Diagnostic Algorithms*)



[1] Persons to be evaluated for TB include adults and children with signs or symptoms suggestive of TB or with a chest X-ray with abnormalities suggestive of TB. This algorithm may also be followed for the detection of MTB using CSF, lymph node and other tissue specimen from persons being evaluated for extrapulmonary TB. For persons being evaluated for TB who are HIV positive and have CD4 counts ≤100 cells/µl or are seriously ill, see Algorithm 4.

[2] Programmes may consider collecting two specimens upfront. The first specimen should be promptly

GeneXpert Omni

- Small, portable, automatic secured cloud based connectivity
- Integrated battery, low power consumption
- Can use both Xpert MTB/RIF and Ultra cartridges

- Expands diagnostic testing to disseminated locations.
- Dramatically reduce time to diagnosis and detect rifampicin resistance.
- Processes one sample at a time – not suitable for high attendance clinics.

Xpert XDR Assay

- Works on GeneXpert platform
- For INH, FQ and SLI resistance detection
- Due to funding gap – will not be available until 2019 at the earliest
- Till XDR cartridge is available, second-line LPA is the only relatively quick way to determine second line drug resistance.

Drug resistance detection	Sensitivity	Specificity
INH	98.1%	100%
FQ	95.8%	100%
Kanamycin	92.7%	100%
Amikacin	96.8%	100%

Boheme C. New diagnostic Technologies for MDR TB. Oral abstact presented at annual CROI.

TrueNat MTB

- Developed by the Indian firm MolBio Diagnostics Pvt Ltd Goa.
- Funded by Bigtec Labs, India.
- battery operated and portable

- 25 minutes for DNA extraction.
- 35 minutes to diagnose TB.
- additional one hour for testing for rifampicin resistance

- Will be an important competitor to Xpert Omni, however lacks automation.

Battery operated devices are used for –

- Extraction of DNA (Trueprep Auto device)
- Amplification (TrueNat MTB chip) and
- Reading the presence of specific genomic sequences (TrueLab PCR analyser).
- Any resistance to rifampicin (RR) is detected by doing a second RTPCR.

Notes: Trueprep™ AUTO, an automated and cartridge-based sample preparation extraction tool (image A). Truelab™ Uno and Truelab™ Quattro, single and 4x scaled test reactors for amplification and analysis of test data (image B) from the Truenat™ reaction chip (image C).

Source: Images reproduced with permission of Molbio Diagnostics.

- The test is now entering the final stage of performance validation and operational feasibility testing by the ICMR.

- ICMR plans to take the test to Public Health Centers which currently use smear samples to test for TB.

Truenat	Xpert MTB/RIF
Not fully automated	Automated
Designed for situations where there may not be electricity and	Needs A reliable electricity supply
Where the need is for one test to be done at a time.	Designed for larger volumes
0.5ml sample required	1ml sample required

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

Abstract

Background: Control of the global Tuberculosis (TB) burden is hindered by the lack of a simple and effective diagnostic test that can be utilized in resource-limited settings.

Methods: We evaluated the performance of Truenat MTB™, a chip-based nucleic acid amplification test in the detection of *Mycobacterium tuberculosis* (MTB) in clinical sputum specimens from 226 patients with suspected pulmonary tuberculosis (TB). The test involved sputum processing using Trueprep-MAG™ (nanoparticle-based protocol run on a battery-operated device) and real-time PCR performed on the Truelab Uno™ analyzer (handheld, battery-operated thermal cycler). Specimens were also examined for presence of MTB using smear microscopy, liquid culture and an in-house nested PCR protocol. Results were assessed in comparison to a composite reference standard (CRS) consisting of smear and culture results, clinical treatment and follow-up, and radiology findings.

Results: Based on the CRS, 191 patients had "Clinical-TB" (Definite and Probable-TB). Of which 154 patients are already on treatment, and 37 were treatment naïve cases. Remaining 35 were confirmed "Non-TB" cases which are treatment naïve cases. The Truenat MTB test was found to have sensitivity and specificity of 91.1% (CI: 86.1–94.7) and 100% (CI: 90.0–100) respectively, in comparison to 90.58% (CI: 85.5–94.3) and 91.43% (CI: 76.9–98.2) respectively for the in-house nested PCR protocol.

Conclusion: This preliminary study shows that the Truenat MTB test allows detection of TB in approximately one hour and can be utilized in near-care settings to provide quick and accurate diagnosis.

Line probe assay

- DNA is **extracted** from *M. tuberculosis* isolates (indirect testing) or directly from clinical specimens (direct testing).

- PCR **amplification** of the resistance-determining region of the gene under question is performed using biotinylated primers.

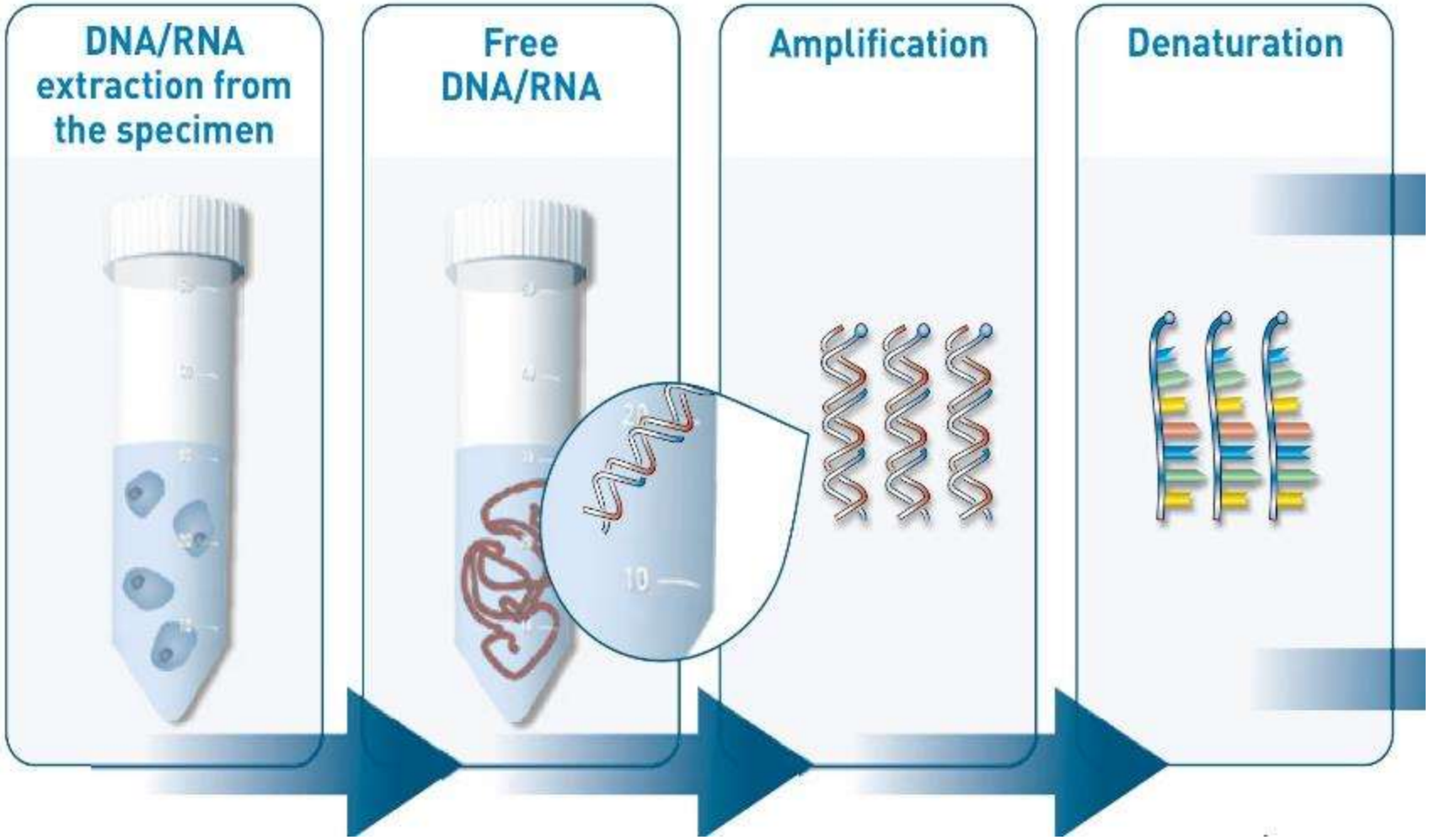
- Labeled PCR products are **hybridized** with specific oligonucleotide probes immobilized on a strip.

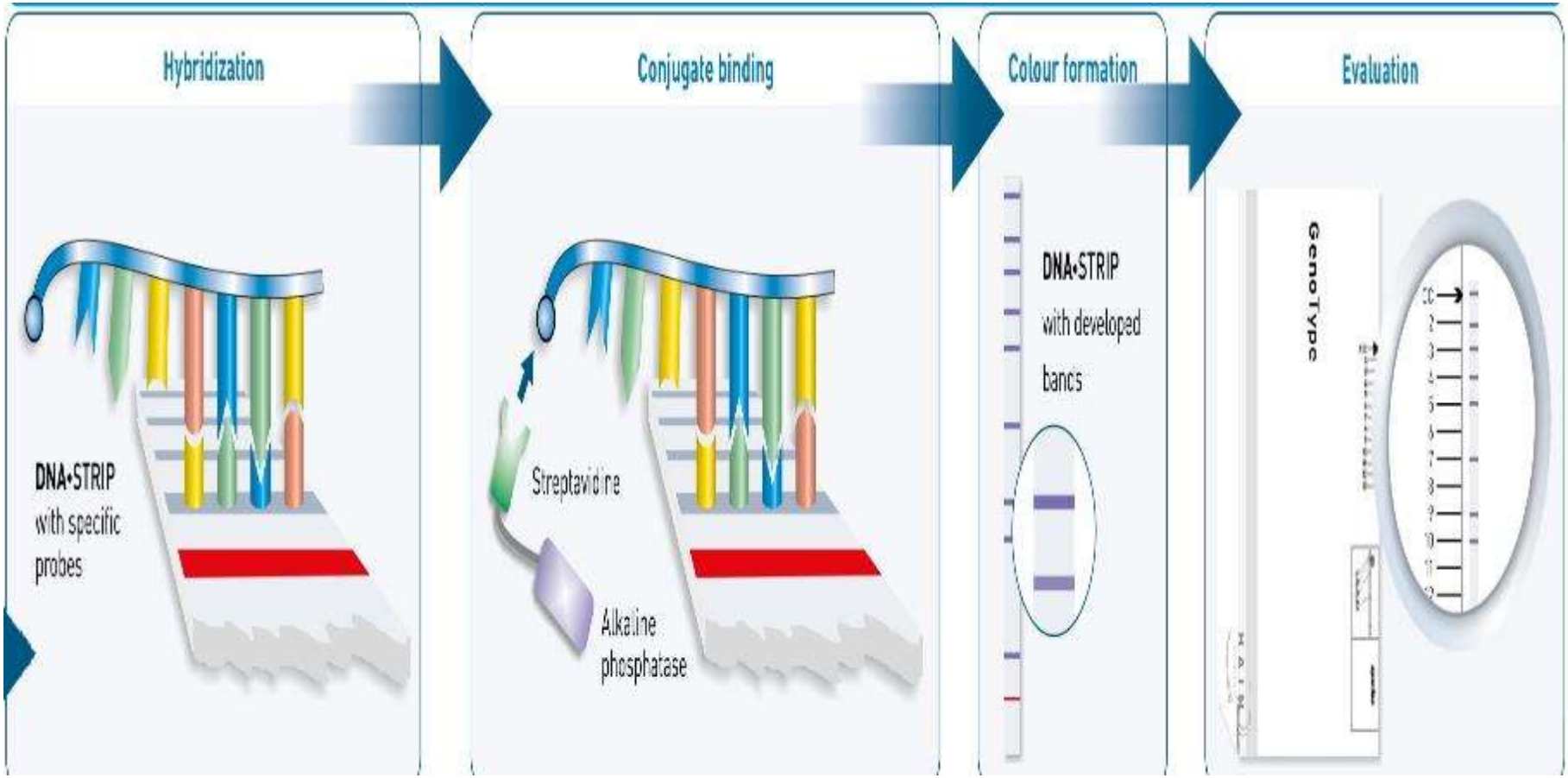
- Captured labeled hybrids are detected by **colorimetric development**, enabling detection of the presence of *M. tuberculosis* complex, as well as the presence of wild-type and mutation probes for resistance.

- If a mutation is present in one of the target regions, the amplicon will not hybridize with the relevant probe.

- Mutations are detected by **lack of binding to wild-type probes,** as well as by **binding to specific probes** for the most commonly occurring mutations.

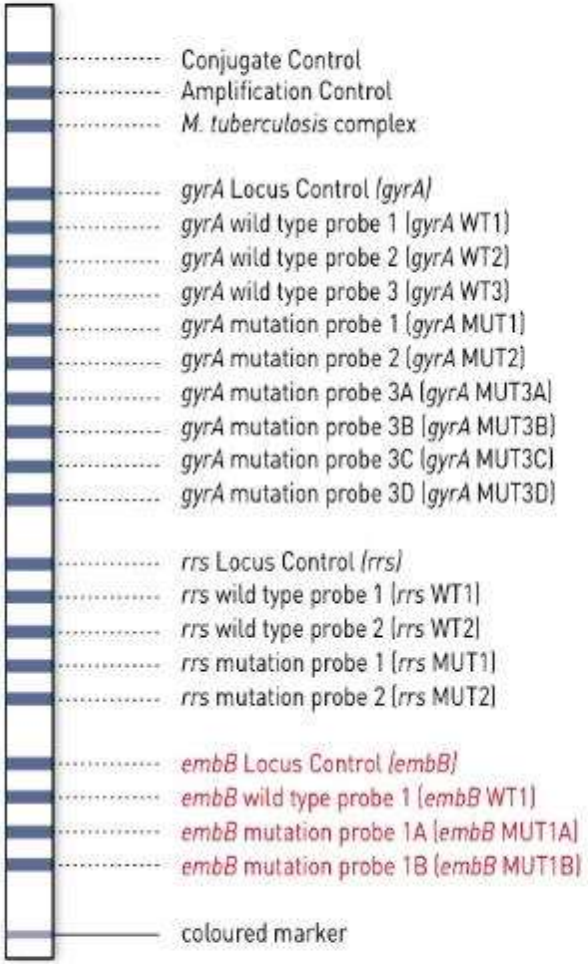
- The post hybridization reaction leads to the development of colored bands on the strip at the site of probe binding.


DNA/RNA extraction from the specimen
Free DNA/RNA
Amplification
Denaturation

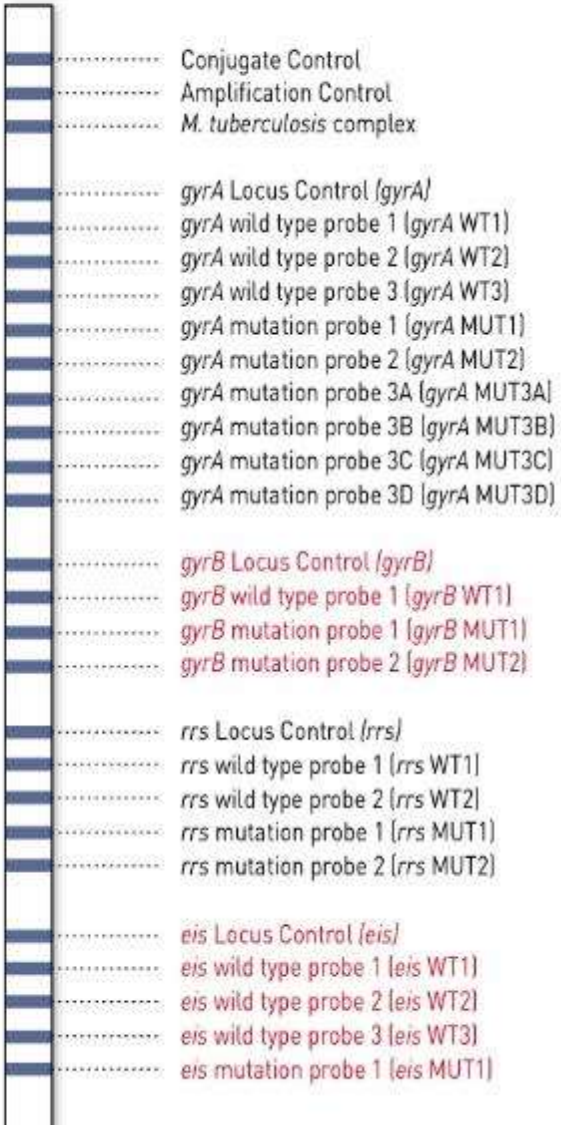

Hybridization
Conjugate binding
Colour formation
Evaluation
DNA-STRIP
with specific
probes
Streptavidine
Alkaline
phosphatase
DNA-STRIP
with developed
bands
GenoType

	Mutations detected	Drug
INNO-LiPA Rif.TB	rpoB	Rif
Genotype MTBDR	rpoB, KatG	Rif INH
Genotype MTBDRplus	rpoB, KatG inhA	Rif INH INH
Genotype MTBDRsl v1	rrs gyrA EMB	SLID FQ Ethambutol
Genotype MTBDRsl v2	rrs, eis gyrA, gyrB	SLID FQ

GenoType **MTBDRsl** VER 1.0



GenoType **MTBDRsl** VER 2.0



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

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)	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.

The use of molecular LPAs for the detection of resistance to second line ATT drugs: WHO policy guidance 2016

MTBDRsl version 2.0

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

Indirect testing	sensitivity	specificity
FQ resistance	84-100%	99-100%
SLID resistance	72-89%	90-99%
XDR-TB	75-80%	91-100%

The use of molecular LPAs for the detection of resistance to second line ATT drugs: WHO policy guidance 2016

WHO Policy recommendations

- For patients with confirmed rifampicin-resistant TB or MDR-TB, SL-LPA **may be used as the initial test**, instead of phenotypic culture-based DST, to detect resistance to **fluoroquinolones**

(**Conditional recommendation**; moderate certainty in the evidence for test accuracy for direct testing of sputum specimens; low certainty in the evidence for test accuracy for indirect testing of *Mycobacterium tuberculosis* cultures).

- For patients with confirmed rifampicin-resistant TB or MDR-TB, SL-LPA **may be used as the initial test**, instead of phenotypic culture-based DST, to detect resistance to the **second-line injectable drugs**

(**Conditional recommendation**; low certainty in the evidence for test accuracy for direct testing of sputum specimens; very low certainty in the evidence for test accuracy for indirect testing of *Mycobacterium tuberculosis* cultures).

- Resistance conferring mutations detected by SL-LPA are highly correlated with phenotypic resistance to **ofloxacin** and **levofloxacin**.

- However, the correlation of these mutations with phenotypic resistance to **moxifloxacin and gatifloxacin is unclear** and the inclusion of moxifloxacin or gatifloxacin in a MDR-TB regimen is best guided by **phenotypic DST** results

- Mutations in some regions (e.g., the *eis* promoter region) may be responsible for causing resistance to one drug in a class (group) more than other drugs within that class (group).

- Given the test's sensitivity and specificity when SL-LPA are done directly on sputum, **SL-LPA can be used for the testing of all sputum specimens** from patients with confirmed rifampicin-resistant TB or MDR-TB, irrespective of whether the microscopy result is positive or negative.

- These recommendations apply to the diagnosis of XDR-TB while acknowledging that the accuracy for detecting resistance to the fluoroquinolones and to the SLIDs differs and hence the accuracy of a diagnosis of XDR-TB overall is reduced

Diagnostic accuracy of GenoType® MTBDR*sl* VER 2.0 in detecting second-line drug resistance to *M. tuberculosis*

R. Yadav,* A. Saini,* P. Kaur,* D. Behera,† S. Sethi*

Departments of *Medical Microbiology and †Pulmonary Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh, India

SUMMARY

SETTING: A tertiary care hospital in North India.

OBJECTIVE: To evaluate the GenoType® MTBDR*sl* VER 2.0 assay for rapid diagnosis of second-line drug resistance to *Mycobacterium tuberculosis*.

DESIGN: The MTBDR*sl* VER 2.0 assay was performed on 431 multidrug-resistant *M. tuberculosis* clinical isolates and specimens. The results were compared with phenotypic drug susceptibility testing (DST) and DNA sequencing. Molecular characterisation of drug resistance using DNA sequencing was performed for *gyr*A, *gyr*B, *rrs* and *eis*.

RESULTS: Of the 415 isolates, respectively 176 (42.4%) and 40 (9.6%) were resistant to levofloxacin (LVX) and kanamycin (KM). The sensitivity and specificity of MTBDR*sl* VER 2.0 compared with phenotypic DST in detecting LVX resistance were respectively 97.2% (95%CI 93.5–99.1) and 99.1% (95%CI 97–99.9), and for KM resistance they were respectively 92.5% (95%CI 79.6–98.4) and 99.5% (95%CI 98.1–99.9).

CONCLUSION: The MTBDR*sl* VER 2.0 assay showed very high sensitivity and specificity for the detection of second-line drug resistance, suggesting it has potential for the rapid, early detection of such cases.

KEY WORDS: tuberculosis; resistance; diagnosis; line-probe assay; GenoType MTBDR*sl* VER 2.0

HAIN lifescience Mycobacteria Product series

	Test	
TB **screening**	FluoroType MTB ver 1.0	Detects MTBC from specimen
Differentiation	GenoType MTBC ver 1.x	Differentiation of MTBC from cultures
	GenoType Mycobaterium CM ver 2.0	Detects MTBC and 27 clinically relevant **NTM** from cultures
Culture identification	TB check MPT64 ver 1.0	Rapid detection of MTBC from **liquid** cultures

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 as a **melting curve**.

DNA extraction

Amplification and detection

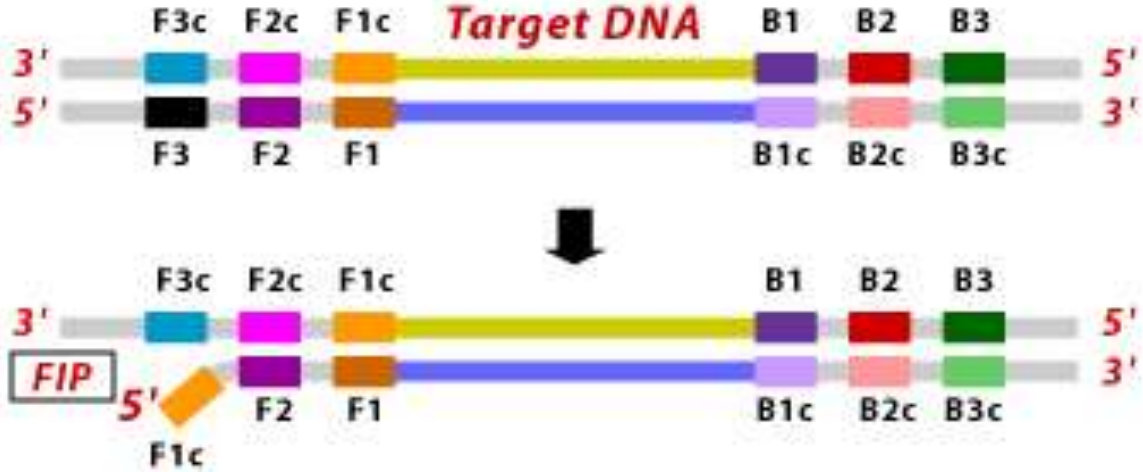
Results in approx. 3 hours

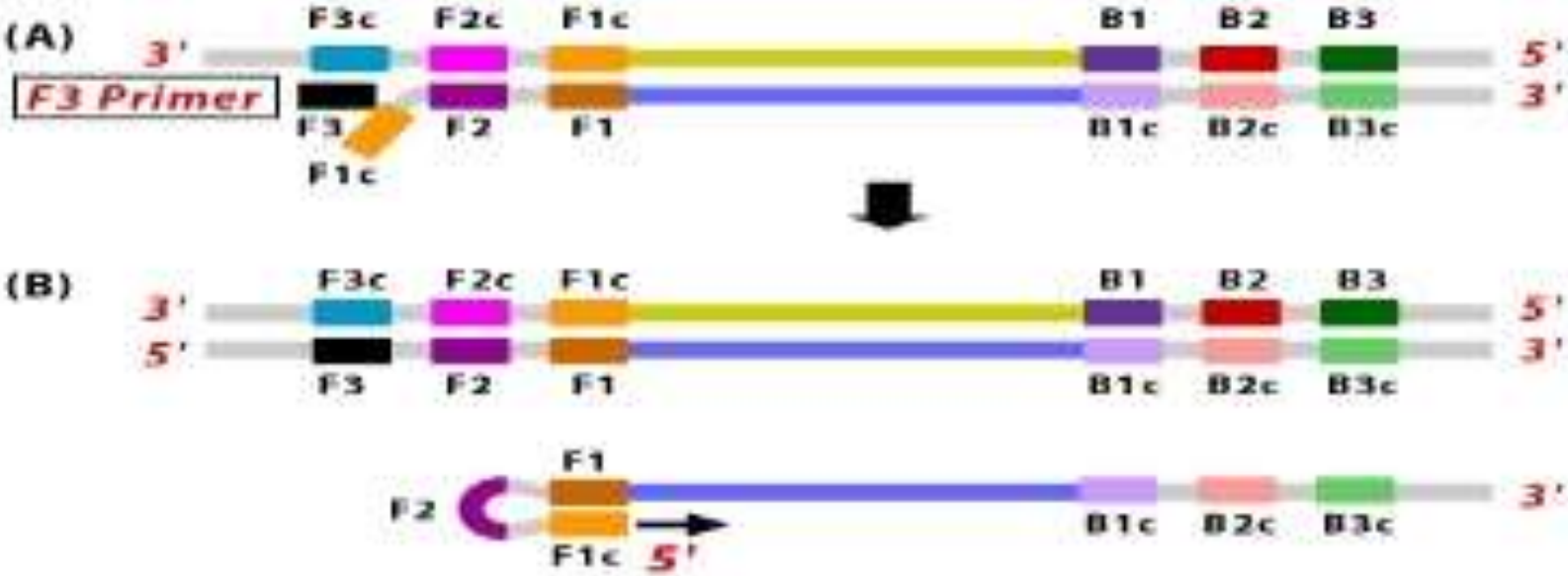
Fluorotype MDR TB

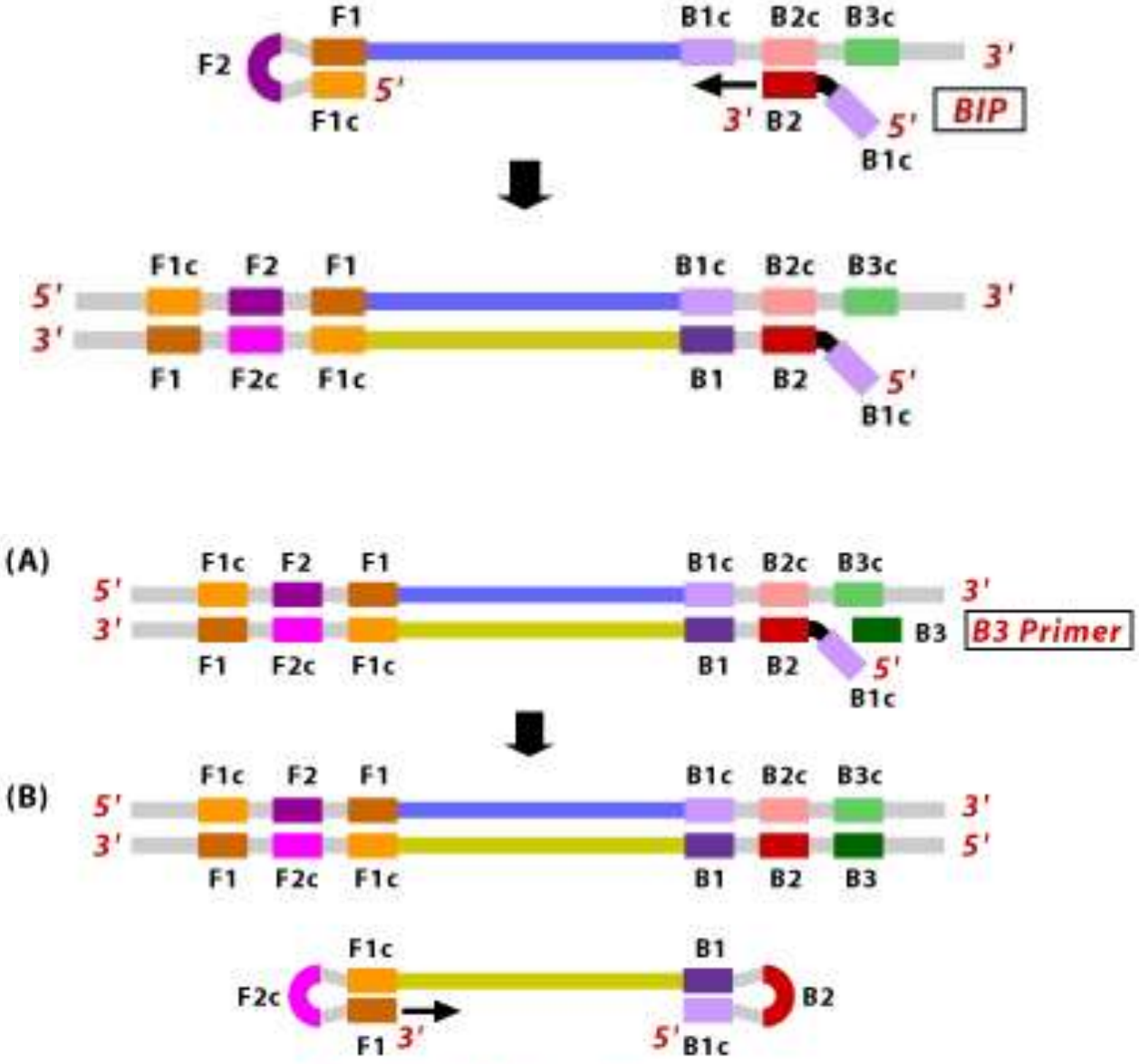
- Launched in April 2017, Hain Lifescience, Germany
- Based on **FluoroType** technology
- Detects **Rifampicin and INH** resistance within **3** hours
- To be reviewed by WHO in 2018

TB-LAMP
Loop mediated isothermal amplification

- Unique, **temperature-independent** NAAT
- Developed by Eiken Chemical Company (Tokyo, Japan)
- **Faster** (40 minutes) and **less expensive** than Xpert.
- **Limited infrastructure** requirement and **relative ease of use** - being explored as an alternative to smear microscopy in resource limited settings.
- LAMP methods have been used to detect **malaria and several neglected tropical diseases.**

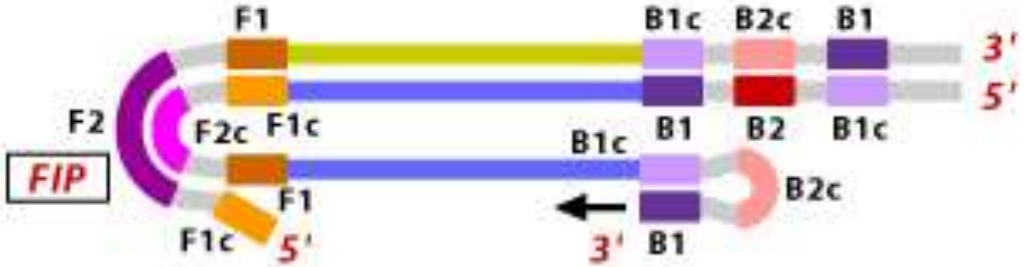

F3c
F2c
F1c
Target DNA
B1
B2
B3
3'
5'
F3
F2
F1
B1c
B2c
B3c
FIP
F1c

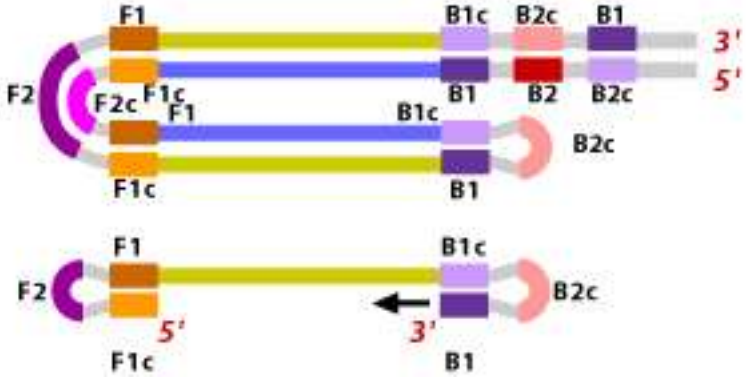

(A)
F3 Primer
(B)
F1
F2
F1c
5'
3'

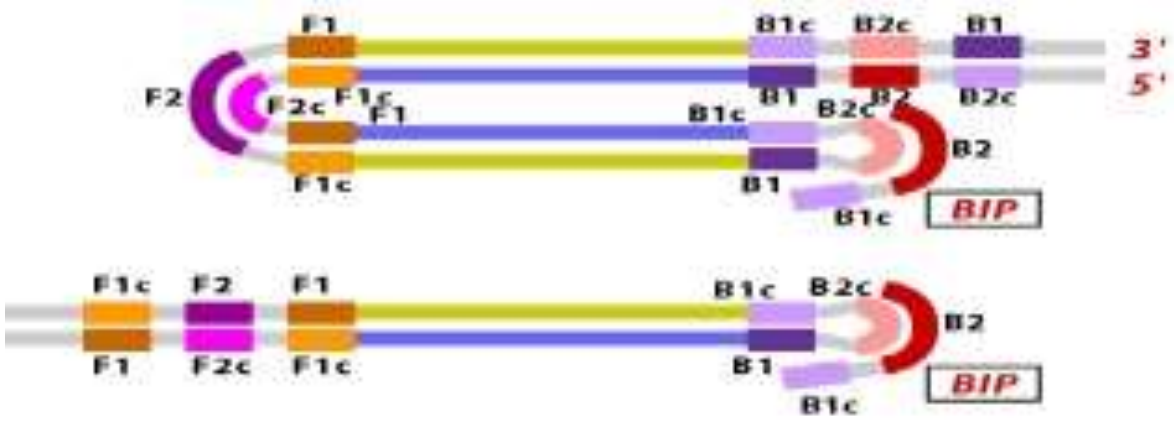


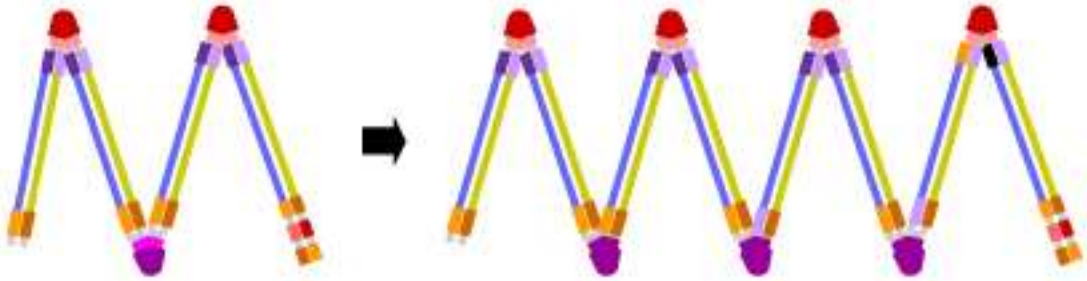
Dumbbell Shape Structure

Stemloop Structure









Amplification Proceeds

- Sample preparation (10–20 minutes)
- Amplification (40 min) – at 67C
- Visual detection of fluorescent light from the reaction tube using ultraviolet light (0.5–1 minute)

Figure 3. Schematic description of the workflow for TB-LAMP

- More sensitive (42.2%) than smear microscopy in smear negative samples
- However doesn’t detect drug resistance and requires several manual steps.
- May be an improvement over smear, in settings with low rates of HIV

The use of LAMP for diagnosis of PTB : WHO policy guidance 2016

WHO policy recommendations

- 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 smear negative specimens is necessary** (conditional recommendation, very low-quality evidence).

- Its use with other samples (e.g., urine, serum, plasma, cerebrospinal fluid or other body fluids) has not been adequately evaluated.

- Due to the limited evidence, it is unclear whether TB-LAMP has additional diagnostic value over sputum-smear microscopy for testing persons living with HIV who have signs and symptoms consistent with TB.

- TB-LAMP **should not replace the use of rapid molecular tests** that detect TB and resistance to rifampicin, especially among populations at risk of multidrug-resistant TB.

- Adoption of TB-LAMP **does not eliminate the need for smear microscopy**, which should be used for monitoring the treatment of patients with drug-susceptible TB.

- However, the demand for conventional sputum microscopy may decrease in settings where TB-LAMP fully or partially replaces conventional sputum microscopy.

- TB-LAMP should not replace the Xpert MTB/RIF assay because the Xpert MTB/RIF assay simultaneously detects M. tuberculosis and rifampicin resistance; it is automated; and the procedure is relatively simple.

- In settings where the Xpert MTB/RIF assay cannot be implemented (e.g., owing to an inadequate electric supply, or excessive temperatures, humidity, or dust), TB-LAMP may be a plausible alternative.

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

SUMMARY

SETTING: A tertiary care hospital in North India.

OBJECTIVE: To evaluate a commercial kit-based loop-mediated isothermal amplification (TB-LAMP) assay for the diagnosis of pulmonary tuberculosis (PTB).

DESIGN: A total of 530 patients presenting with PTB symptoms were enrolled and one sputum sample was collected from each patient. The TB-LAMP assay (Loopamp™ MTBC Detection kit) was performed on the raw sputum sample. The remaining sample was used for smear microscopy and mycobacterial culture. A cartridge-based nucleic acid amplification test (NAAT, Xpert® MTB/RIF assay) was also performed on the processed pellet.

RESULTS: The sensitivity and specificity of the TB-LAMP assay in culture-positive samples obtained from 453 patients presenting with PTB symptoms (77 specimens were excluded) were respectively 100% (95%CI 94.7–100) and 99.2% (95%CI 97.8–99.8). The sensitivity and specificity of Xpert in culture-positive samples were respectively 82.6% (95%CI 71.5–90.6) and 94.9% (95%CI 92.2–96.8). A concordance of 0.75 was obtained between the two NAATs (TB-LAMP assay and Xpert) using the κ statistic.

CONCLUSION: 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.

KEYWORDS: nucleic acid amplification test; diagnosis; tuberculosis; Xpert® MTB/RIF; TB-LAMP

TB-LAM

- LAM (lipoarabinomannan) antigen is a lipopolysaccharide present in mycobacterial cell walls
- Released from metabolically active or degenerating bacterial cells
- Appears to be present only in people with active TB disease.
- Urine-based testing - easy to collect and store, and lacks the infection control risks associated with sputum collection.
- Alere Determine TM TB LAM Ag, Alere Inc, Waltham, MA, USA

HIV-positive patients with TB disease may be missed for the following reasons:

- Sputum bacillary load is typically low in these patients
- They may not be able to provide sufficient and high quality sputum specimens
- Substantial proportion of these patients have extra pulmonary TB without pulmonary TB.

- Improved sensitivity of urinary LAM in the presence of HIV-TB co-infection, which further increases with lower CD4 counts.

- This finding is in contrast to traditional diagnostic methods for TB in people with HIV.

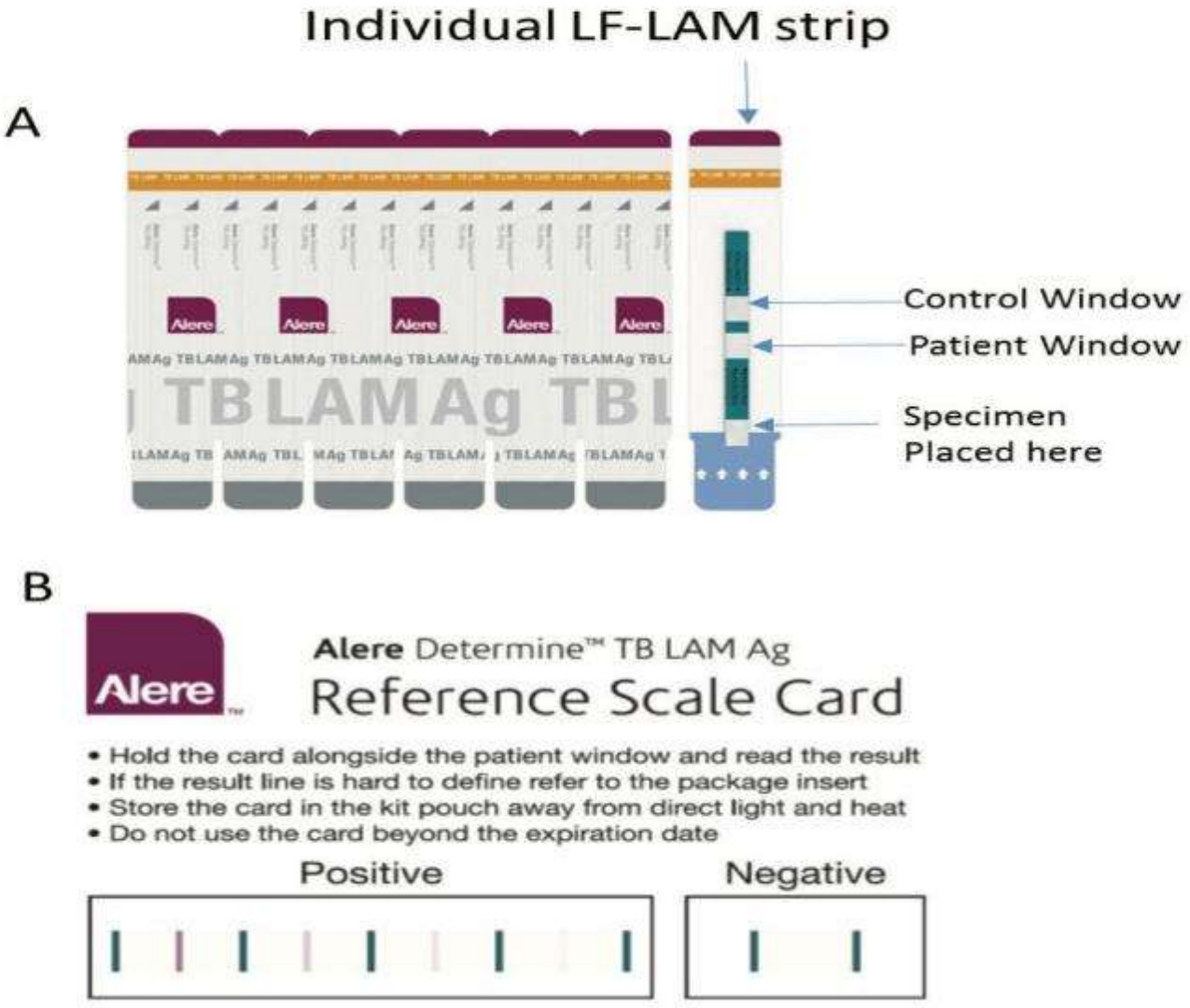
Can be due to –

- Higher bacillary burden and antigen load,
- Greater likelihood of TB in the genitourinary tract,
- Greater glomerular permeability to allow increased antigen levels in urine.

- Immunochromatography based test.
- Performed manually by applying 60µL of urine to the test strip.
- Incubation at room temperature for 25 minutes.
- The intensity of any visible band on the test strip is graded by comparing it with the intensities of the bands on a manufacturer-supplied reference card.

Figure 1. Alere Determine™ TB LAM Ag test

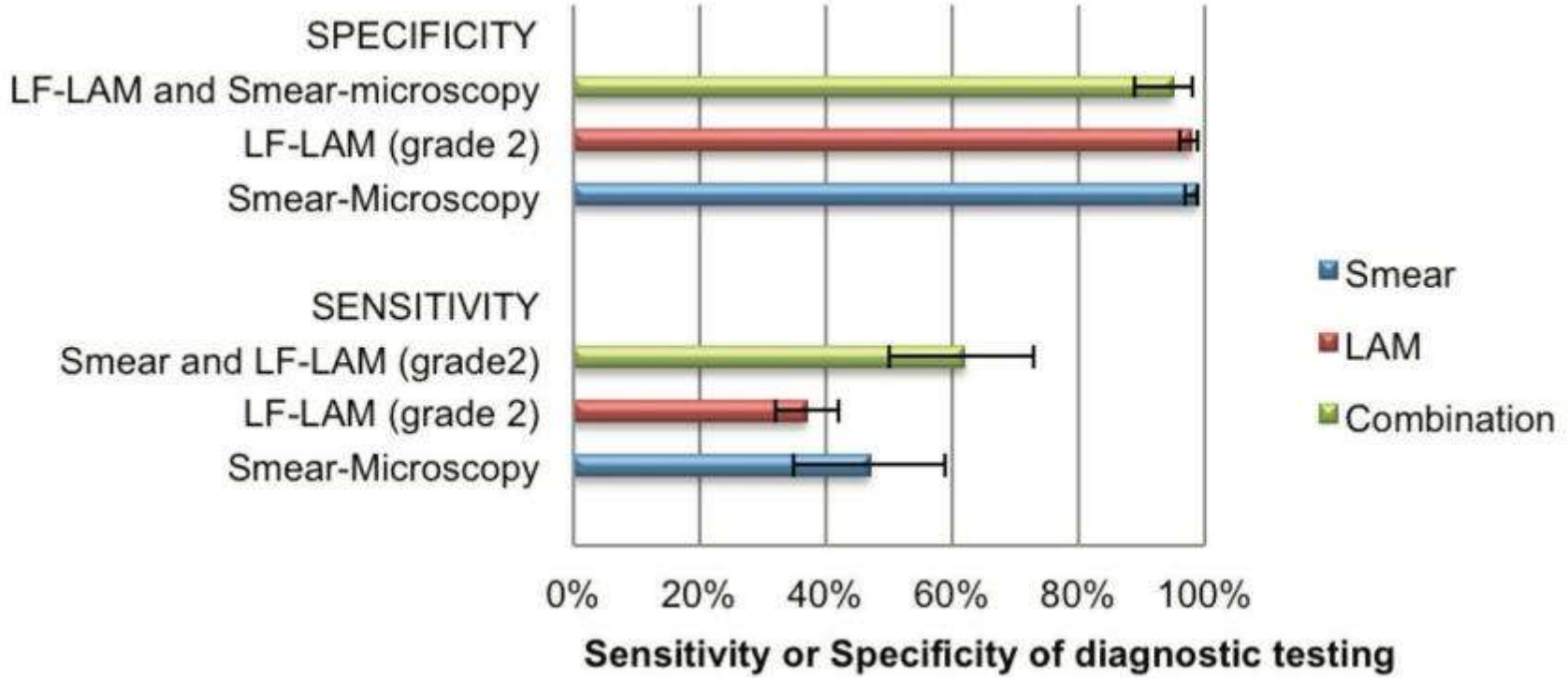
(A) Alere Determine™ TB LAM Ag tests. To the sample pad (white pad marked by the arrow symbols) 60 µL of urine is applied and visualized bands are read 25 minutes later. (B) Reference card accompanying test strips to 'grade' the test result and determine positivity (33). Copyright© [2014] [Alere Inc]: reproduced with permission.



	Pooled sensitivity	Pooled specificity
Sputum M/S	47%	98%
LF-LAM	37%	95%
LF-LAM + sputum M/S	62%	91%
Xpert MTB/RIF	77%	96%
LF-LAM + Xpert MTB/RIF	83%	91%

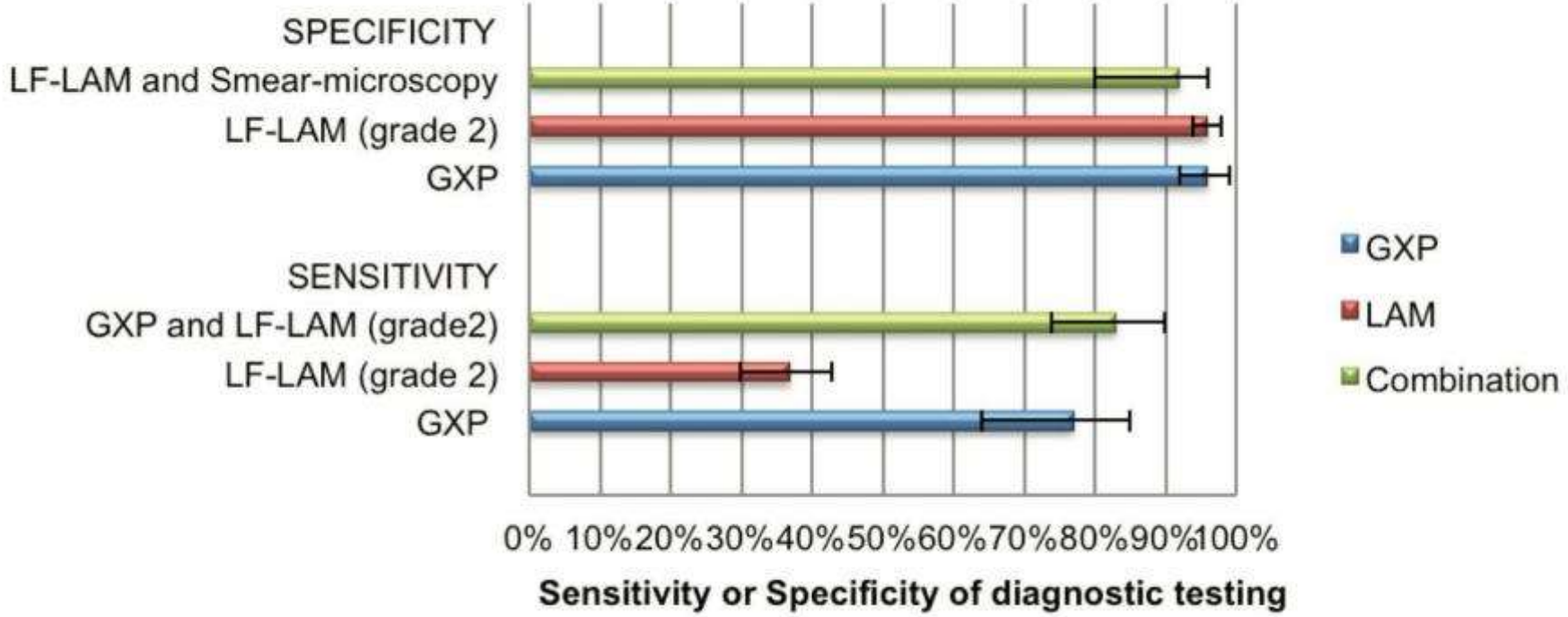
The use of LF-LAM for the diagnosis and screening of active TB in people living with HIV: WHO policy guidance 2015

Figure 13. Sensitivity and specificity of LF-LAM in combination with and in comparison to sputum microscopy for TB diagnosis in adults living with HIV



Bars represent point estimates of pooled sensitivity or specificity of tests used individually or in combination. Error bars represent 95% credible intervals.

Figure 14. Sensitivity and specificity of LF-LAM in combination with and in comparison to Xpert MTB/RIF for TB diagnosis in adults living with HIV



Legend: GXP, sputum Xpert MTB/RIF. Bars represent point estimates of pooled sensitivity or specificity of tests used individually or in combination. Error bars represent 95% credible intervals.

WHO policy recommendations

- **Except** for persons with **HIV infection with low CD4 counts or who are seriously ill**, LF-LAM **should not be used** for the diagnosis of TB (strong recommendation, low quality of evidence).

- LF-LAM may be used to **assist in the diagnosis of TB** in HIV positive adult in-patients with signs and symptoms of TB (pulmonary and/or extra pulmonary) who have a **CD4 cell count less than or equal to 100 cells/µL, or HIV positive patients who are seriously ill** regardless of CD4 count or with unknown CD4 count (conditional recommendation; low quality of evidence).

- LF-LAM should **not be used as a screening test for TB.** (strong recommendation; low quality of evidence)

- LF-LAM does not differentiate between the various species of mycobacterium and cannot be used to distinguish M. tuberculosis from other species.

- However, in areas endemic for tuberculosis the LAM antigen detected in a clinical sample is likely to be attributed to M. tuberculosis.

- Implementation of LF-LAM in the targeted patient groups does not eliminate the need for other diagnostic tests - Xpert MTB/RIF, culture or sputum-smear microscopy - as these tests exceed LF-LAM in diagnostic accuracy.

- Whenever possible, a **positive LF-LAM should be followed up with a confirmation test** such as Xpert MTB/RIF, line probe assay or bacteriological culture and drug-susceptibility testing.

- LF-LAM is designed to detect mycobacterial LAM antigen in human urine. Other samples (e.g. sputum, serum, plasma, CSF or other body fluids) or pooled urine specimens should not be used.

Conclusion

- Ultra is **non-inferior** to the Xpert MTB/RIF assay.
- Till Xpert XDR cartridge is available, second-line LPA is the only relatively quick way to determine second line drug resistance.
- TrueNat MTB will be an important competitor to Xpert Omni, however lacks automation and evidence as of now.
- TB-LAMP **may be used as a replacement test** for SSM to diagnose PTB in adults with signs and symptoms consistent with TB
- **Except** for persons with **HIV infection with low CD4 counts or who are seriously ill**, LF-LAM **should not be used** for the diagnosis of TB