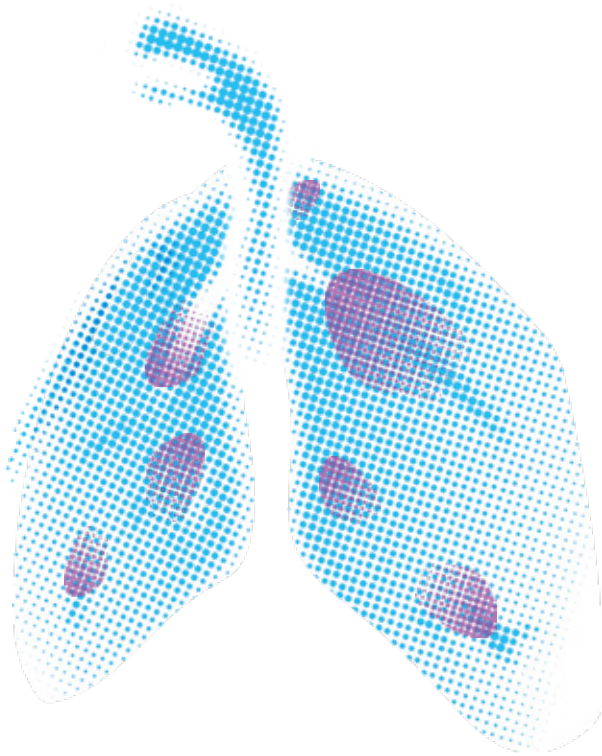


A Compendious **TB Diagnostics Solution**

(Dr Iravatham - Medgenome
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TB Prevalence

Worldwide, Tuberculosis (TB) is one of the top 10 causes of death, and the leading cause from a single infectious agent (above HIV/AIDS).

According to a WHO report,

India saw

2.7 TB cases
Million (incidence + relapse)
in 2017

India accounted for

27% of global
TB deaths

Globally,

3.4% of new
TB cases and

&

18% of previously
treated cases

Had multi- drug resistant/rifampicin
resistant TB (MDR/RR-TB)

India is one of the top 3 countries
with the largest number of MDR/RR-TB
cases that constitute 43% of global
MDR/RR-TB cases.

TB Diagnostics- Need of the hour

**Whole
Genome
Sequencing for
Diagnosis and Drug
Resistance Detection of Mtb**

Atypical identification test

Sensitivity for Atypical Mycobacterium

Xpert MTB RIF Assay

Line Probe Assay 1st Line Drugs

Line Probe Assay 2nd Line Drugs

Molecular Tests

AFB Direct Smear/ Conc Method

AFB Culture (Conventional Method)

AFB Culture Bactec (MGIT Method)

AFB Drug Sensitivity for 1st and 2nd line drugs

Conventional Tests



Conventional Tests

- AFB Direct Smear/ Conc Method

Direct Smear

Microscopic examination of smears prepared directly from patient's specimens for **Acid Fast Bacilli (AFB)**

Conc. Smear

Microscopic examination of smears prepared from processed specimens after centrifugation to concentrate the material.



Applications

- Initial diagnosis of Tuberculosis
- Treatment monitoring



Accuracy

- This test has a **sensitivity of 65%** and **specificity of 98%** among pulmonary tuberculosis^[1]



Advantages

- Proven, widely used **simple diagnostic test** for all types of specimens.
- **Fluorescent stain method** as well as ZN stain method available



Limitations smear microscopy

- Requires a **High Bacterial load**
- Cannot differentiate between the **viable and dead bacilli**
- Does not provide **species identification**

Hence for confirmation and varied applications, such as species identification and drug resistance detection, more sophisticated techniques such as culture and molecular tests needs to be applied.

- AFB Culture and Drug sensitivity

Culture technique is considered as the gold standard for diagnosis as well as drug resistance detection



Applications

- Confirmatory diagnosis of Tuberculosis
- Species Identification
- Phenotypic Drug resistance detection

The automated liquid MGIT culture technique has a comparatively higher sensitivity than conventional solid culture, however, being a gold standard, quantification of accuracy levels is impractical.

However due to a comparatively longer turn-around time higher possibility of contamination and subsequent repeat testing with advanced infrastructure and highly trained personnel, the paradigm has shifted to advanced molecular tests still requiring the culture for the confirmation of TB.





Molecular Tests

- Xpert MTB RIF Assay

Rapid and more sensitive detection of Mtb (Mycobacterium Tuberculosis) and RIF (rifampicin) resistance for MDR (multi-drug resistant) strains in both pulmonary as well as extra pulmonary TB with results available in 2 hours. Over and above, appropriate treatment initiation, rapid results aid in accelerating the implementation of MDR-TB control measures, and ultimately reducing TB case incidence.



Accuracy

- Xpert MTB/RIF pooled sensitivity was **98% in smear-positive** and **67% in smear-negative** pulmonary TB cases ^[2,3]
- The sensitivity, specificity of Xpert MTB/RIF assay for diagnosis of Extra Pulmonary (EPTB) cases were **84.91% and 86.72%**. ^[2,3]

However, due to limitation of drug resistance detection of only Rifampicin, more sophisticated technique such as (LPA) Line Probe Assay has been introduced

- Line Probe Assay

A DNA-strip based test to determine the drug resistance pattern of TB strains by the binding of DNA amplicons to the probe that targets resistance associated mutations to both first line as well as second line drugs.



Accuracy

- LPA can be performed on DNA extracted from Culture or Direct Sputum.^[4]
- First Line LPA (FL-LPA) assay (GenoType MTBDRplus V2,) showed a sensitivity and specificity for the detection of Rif resistance of 96.7% and 98.8%, respectively, and for the detection of H resistance, a sensitivity and specificity of 90.2% and 99.2%, respectively.^[4]
- Secone Line LPA (SL-LPA) assay (GenoType MTBDRsl V1 & 2) showed a pooled sensitivity and specificity for the detection of fluoroquinolone resistance by direct testing of 86.2% and 98.6%, respectively, and a pooled sensitivity and specificity for the detection of second-line injectables drugs resistance of 87.0% and 99.5%, respectively.^[4]
- Limitations of the test as it depends on detection of specific genes for drugs and NOT ALL gene loci will be detected.^[4]



Whole Genome Sequencing of Mtb

Whole genome sequencing (WGS) offers the opportunity to screen not only the loci included in rapid molecular tests, but also other known resistance-associated loci thereby enabling identification of new drug resistance-associated mutations that are not explained by currently available diagnostics.



Advantages

Single test for diagnosis and drug resistance prediction



Sample Requirement

The use of sputum eliminates the requirement of growing a culture and hence reduces the time delay in diagnosis.



Multiple applications

Due to its voluminous data availability, this test can be used for strain typing, epidemiology studies and disease surveillance.



Comprehensive Drug Panel

The existing molecular techniques have a limitation of covering only a few drugs. The whole genome sequencing of Mtb covers all drug resistance markers – reported as well as novel.



Accuracy

This Methodology has **100% sensitivity to resistance variants profiled by line probe assay (LPA)** and **98% accuracy with the phenotypic drug susceptibility tests for 6 anti-tuberculosis drugs**^[5]

References

1. Approaches to improve sputum smear microscopy for tuberculosis diagnosis, expert group meeting report geneva: 31 october 2009. (https://www.Who.Int/tb/laboratory/egmreport_microscopymethods_nov09.Pdf)
2. Xpert MTB/RIF and Xpert MTB/RIF Ultra for pulmonary tuberculosis and rifampicin resistance in adults (Review). Horne DJ et.al., Cochrane Library, Cochrane Database of Systematic Reviews.
3. Diagnostic Accuracy of Xpert MTB Compared to Smear Microscopy in Pulmonary vs Extrapulmonary Tuberculosis. Niveditha S1, Jagmohan S V2, Abhishek K Verma3, Minni Meka4. IJCMR., 2019.
4. Line probe assays for drug resistant tuberculosis detection. Interpretation and reporting guide for laboratory staff and clinicians. (www.stoptb.org/wg/gli)
5. Soundararajan L et al. Whole genome enrichment approach for rapid detection of Mycobacterium tuberculosis and drug resistance-associated mutations from direct sputum sequencing. Tuberculosis 121 (2020) 101915.

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