Newer Advances

Molecular Assays as Initial Tests for the Diagnosis of Tuberculosis

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Tuberculosis (TB) is caused by Mycobacterium Tuberculosis which chiefly affects lungs (PTB) but it can involve other parts of the body (EPTB). TB spreads through droplet infections and is highly contagious. One sputum positive TB patient can infect 10-15 normal contacts during a period of one year. TB still remains one of the world's deadliest communicable diseases because of delay in initial diagnosis and poor control. With timely diagnosis and effective treatment by category 1 first line drugs onward journey of TB transmission can be stopped. According to global tuberculosis report 2019[1], 10 million people developed tuberculosis and 1.5 million died from this disease. One million children contracted this disease and more than 2 Lakhs succumbed to TB. Recent data show that more than 5 Lakhs new cases of multi drugs resistant (MDR) patients are reported annually.

India alone accounts for ¼th of all TB cases. Each day more than 1,000 peoples are estimated to die of TB. Most of these TB deaths could be prevented with early diagnosis and appropriate treatment. Every effort should be made to achieve the END TB GOAL by 2025.

Paradigm Shift in Diagnosis[2] :

In order to curtail the global TB epidemic there should be paradigm shift from the conventional approach to diagnose tuberculosis to more accurate and early diagnosis of all forms of TB pulmonary as well as extra-pulmonary including rapid detection of drug resistance.

The most widely used test to diagnose TB is sputum microscopy for Acid-Fast-Bacilli (AFB). It lacks sensitivity and specificity. Culture is the gold standard in diagnosis of tuberculosis but it is time consuming (4-8 weeks). There has been rapid evolution of molecular techniques in diagnosis of TB. Molecular assays involve polymerase chain reaction (PCR) or real time PCR which are much more sensitive than sputum microscopy and culture. Several test systems based on mycobacterial nucleic acid amplification are available during recent years[3]. These Nuclei Acid Amplification tests (NAATs) will usher a new era of easy, speedy and accurate diagnosis of tuberculosis. Three tests using molecular techniques have been approved by WHO for tuberculosis control program.

(1) Xpert MTB/RIF (Cepheid Sunnyvale, USA) in 2010

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Editor's Comment :

- **Xpert MTB/RIF and Trunat test utilise molecular techniques based on amplification of mycobacterial nucleic acid in test samples.**
- **They are most useful in the rapid diagnosis of pulmonary as well as extra pulmonary tuberculosis patients of all ages.**
- **They are also very useful in TB HIV coinfection.**
- **The use of these sensitive and specific tests is the right step as initial tests for early diagnosis of TB and rifampicin resistance.**

(2) Xpert MTB/RIF-Ultra (Cepheid Sunnyvale, USA)

(3) Truenat MTB, MTB Plus (Molbio diagnostic Goa)

Other tests which are also used are LPA (Line Probe Assay), Urine lipoarabinomannan lateral flow assay and Molecular loop-mediated isothermal amplification assay (LAMP).

(1) Xpert MTB/RIF[4-6] — Gene Xpert TB assay is an automated PCR test and it detects MTB and Rifampicin Resistance (RR) within two hours. Several studies approved by WHO reported that xpert MTB/RIF is a sensitive method for rapid diagnosis of TB as compared to conservative one.

(2) Xpert MTB/RIF-Ultra[7] — Assay was designed by adding two amplification targets. In 2015, alland et al found that xpert MTB/RIF-Ultra is much more sensitive than xpert MTB/RIF. It is likely to be as sensitive as TB culture.

(3) Truenat-TB Test — Truenat TB test is a new molecular TB test which detects TB bacteria and RR TB by using PCR techniques. In other words it is a real time micro PCR system which enables near patient diagnosis of MTB and it issimple, robust and user friendly. It is a chip based PCR and it involves extraction of DNA, amplification and reading the presence of specific genomic sequence (by PCR Analyzer).

The Truenat has been developed by the Indian Firm Molbio diagnostic Private Limited, Goa.

It takes about 25 minutes to do the DNA extraction, another 35 minutes to diagnose TB and additional 1 hour for testing Rifampicin Resistance.

Truenat Machine is a point of care (POC) tool which is not fully automated. It is designed for situations where there is no electricity and where the need is for one test to be done at a time. In contrast the Gene Xpert is designed for larger volumes and needs reliable electrical supply. Truenat MTB is fully fit to be a POC test for diagnosis of TB and Rifampicin Resistance in primary health

centers(PHC) of India and other middle and low income group countries.

Rapid Communication of WHO, January 2020 :

There is a rapid communication from WHO which aims to provide latest evidences on the use of molecular assays as initial diagnostic test for PTB, EPTB and RR-TB in adults and children.

Key Findings of Latest Evidences by Using Molecular Assays in Initial Diagnosis of Tuberculosis and Resistant TB:

(1) Results of expert MTB/RIF as initial tests to diagnose pulmonary TB have diagnostic accuracy and improved patient outcomes replacing sputum smear microscopy.

(2) Xpert-Ultra has got high diagnostic accuracy replacing smear microscopy. It shows additional advantage of simultaneous detection of Rifampicin Resistance.

(3) Xpert MTB/RIF and xpert-Ultra both are better tools to diagnose TB and Rifampicin Resistance in respiratory and non-respiratory specimens in children.

(4) Xpert MTB/RIF and Xpert Ultra offer better results in diagnosis of TB and detection of Rifampicin Resistance.

(5) Truenat MTB and MTB plus also show high diagnostic accuracy and specificity in diagnosis of tuberculosis replacing sputum smear microscopy and sequentially detect Rifampicin Resistance comparable to Xpert MTB/RIF and Xpert MTB/RIF-Ultra.

Studies and Trials :

Xpert MTB/RIF — 70 studies involving 30 Thousand patients from 37 Countries show that xpert MTB/RIF offer high diagnostic accuracy of pulmonary TB in adults with 85% sensitivity (smear positive and smear negative both) and 98% specificity. In diagnosis of TB with HIV co-infection sensitivity is 81% and specificity 98%. Data suggest that xpert MTB/RIF may be replaced for sputum smear microscopy as initial test.

48 studies involving 8 Thousand patients from 33 Countries confirm simultaneous detection of Rifampicin Resistance with overall sensitivity (96%) and specificity 98% when compared to phenotypic drug sensitivity testing.

Xpert MTB/RIF-Ultra — 6 studies involving 2 Thousand patients from 14 Countries exhibit high diagnostic accuracy of xpert MTB/RIF-ultra with 90% sensitivity with 98% specificity. In HIV co-infection results are similar to xpert MTB/RIF.

5 studies involving 1 Thousand patients from 12 Countries showed similar results of xpert ultra for simultaneous detection of Rifampicin Resistance as compared to xpert MTB/RIF.

Xpert MTB/RIF and Xpert Ultra in Children — Results of 43 studies involving 6 Thousand patients from 21 Countries showed variable results in sensitivity in different specimens (Nasopharyngeal-46%, Stool-61%, Sputum-65% and Gastric Specimen-73%) but specificity was 98-100%. Similar results were obtained in xpert ultra. Results of Rifampicin Resistance showed 90% sensitivity and 98% specificity.

Xpert MTB/Rif and Xpert Ultra in EPTB — EPTB always poses diagnostic difficulties in obtaining extra pulmonary specimens and confirmation of diagnosis. Molecular assays have proved a pillar in diagnosis of extra pulmonary tuberculosis.

Results from 59 studies from 26 Countries showed variable sensitivity from 50% from pleural fluid to 97% from synovial fluid. The specificity also varies from 79% for Lymph node Biopsy to 99% for pleural fluid. Both xpert molecular assays showed overall high performance for simultaneous detection in Rifampicin Resistance.

Truenat — FIND (The Foundation for Innovative New Diagnostics) coordinated multi-central field evaluation study from 4 countries involving 744 participants showed overall sensitivity of the truenat MTB assay (83%) and MTB Plus assay (89%) and specificity of both assays was 98-99%. Truenat MTB/RIF Dx showed comparable diagnostic accuracy for sequential detection of Rifampicin Resistance.

Data Collected from National Tuberculosis Elimination Program (NTEP) Jawaharlal Nehru Medical College, Bhagalpur (Bihar).

Using the conventional approach of LED sputum microscopy in more than 6,000 patients only 10.13% positivity could be detected during 2018 & 2019 (Table 1).

Using Xpert MTB/RIF (CBNAAT) involving more than 6,000 patients more than 22% patients detected to be MTB Positive while there was simultaneous detection MTB Resistance in more than 3% cases. Data proves that use of CBNAAT gives clear advantage for early initial diagnosis of tuberculosis over conventional sputum microscopy. Limitation of this data is non-confirmation of this data with reference to culture and DST (Table 2).

Table 3 shows data involving more than 900 patients when subjected to CBNAAT testing showed more than 19% positivity in smear negative cases.

In more than 700 extra-pulmonary specimens 11% of these showed Rifampicin Sensitivity and about 2% showed Rifampicin Resistance (Table 4).

In cases of TB and HIV co-infection MTB detection was found to be around 10% and Rifampicin Resistance was detected in more than 2% (Table 5).

Table 1 — *Details of specimen tested by smear microscopy*

Year	Total specimen tested by smear microscopy (LED)	Total AFB positive detected	No of M TB detected	Rifampicin resistance detected
2018	3066	282 (9.19 %)	NA	NA
2019	3695	403 (10.90 %)	NA	NA
Total	6761	685 (10.13%)		

LED - Light Emitting Diode

Table 2 — *Details of specimen tested by CBNAAT (Xpert MTB/RIF)*

Year	Total specimen tested by CBNAAT	No of M TB detected	Rifampicin resistance detected
2018	3169	604 (19.05 %)	113 (3.56 %)
2019	2926	745 (25.46 %)	88 (3.00 %)
Total	6095	1349 (22.13%)	201 (3.29%)

Table 3 — *Details of smear negative cases further tested by CBNAAT (Xpert MTB/RIF)*

Year	Number of smear negative cases further tested by CBNAAT	No of M TB detected	Rifampicin resistance detected
2018	246	39 (15.85 %)	3 (1.21 %)
2019	667	136(20.38 %)	15 (2.24 %)
Total	913	175 (19.16%)	18 (1.97%)

M TB - Mycobacterium Tuberculosis,
CBNAAT - Cartridge Based Nucleic Acid Amplification Test

Table 4 — *Details of extra-pulmonary specimen tested by CBNAAT (Xpert M TB/RIF)*

Year	Number of extra-pulmonary specimen tested by CBNAAT	No of M TB detected	Rifampicin resistance detected
2018	312	38 (12.17 %)	8 (2.56 %)
2019	443	50 (11.28 %)	7 (1.58 %)
Total	755	88 (11.65%)	15 (1.98%)

In pediatrics cases MTB detection was more than 10% and Rifampicin Resistance was detection in more than 1%.

Data from NTEP Jawaharlal Nehru Medical College, Bhagalpur also shows that if Xpert MTB/RIF (CBNAAT) is used as initial test for diagnosis of tuberculosis in adults with pulmonary and extra pulmonary TB, HIV co-infection and pediatric tuberculosis it will lead to early diagnosis and hence early initiation of treatment of tuberculosis.

Conclusions :

(1) All the latest evidences reviewed by WHO recommend the continued use of Xpert MTB/RIF and Xpert MTB/RIF-Ultra as initial diagnostic test of PTB of all ages.

(2) These data support the use of both these molecular assays in the diagnosis work-up of EPTB and Childhood tuberculosis.

(3) Both assays also exhibit high diagnostic accuracy in diagnosing Rifampicin Resistance simultaneously.

(4) Performance of Truenat MTB, MTB Plus and MTB/RIF Dx assays show comparable accuracy with xpert MTB/RIF and xpert Ultra for TB detection and for sequential Rifampicin Resistance (Truenat MTB/RIF Dx)

(5) Truenat MTB and MTB Plus assays are comparable to TB-LAMP assay as replacement test for sputum smear microscopy.

(6) Truenat MTB/RIF Dx is comparable to Line Probe Assays in diagnostic accuracy.

Table 5 — *Details of specimen of PL-HIV cases tested by CBNAAT (Xpert MTB/RIF)*

Year	Specimen of PL-HIV cases tested by CBNAAT	No of M TB detected	Rifampicin resistance detected
2018	379	28 (7.38 %)	7 (1.84 %)
2019	200	28 (14.00 %)	7 (3.50 %)
Total	579	56 (9.67%)	14 (2.41%)

Table 6 — *Details of specimen of pediatric cases tested by CBNAAT (Xpert MTB/RIF)*

Year	Specimen of pediatric cases tested by CBNAAT	No of M TB detected	Rifampicin resistance detected
2018	159	18 (11.32 %)	2 (1.25 %)
2019	208	21 (10.09 %)	2 (0.96 %)
Total	367	39 (10.62%)	4 (1.08%)

The updated Guidelines on Molecular Assays as initial tests for the diagnosis of TB are to be released in April 2020. Meantime "National TB Programmers and other stakeholders are encouraged to conduct high-quality implementation/operational research to collect more evidence on the accuracy,effectiveness, feasibility,acceptability,cost,and impact of WHO-recommended diagnostic tools for TB".

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