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Mini Review

Application of New Molecular Methods for TB Eradication: Gene Xpert Revisited

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Abstract

While use of Gene Xpert (CBNAAT) has led to early detection of tuberculosis, using rifampicin (Rif) susceptibility as a surrogate marker for Multidrug resistance (MDR) TB may have been an over reach, with evidence emerging that INH monoresistance (IMR) occurs and if standard treatment is instituted based in Rif susceptibility, during continuation phase for four months onlyone effective drug would be administered, leading to compromised clinical response and promoting development of MDR TB.

Keywords: Multidrug Resistance; Computer Aided Diagnosis (CAD); Rifampicin

It is essential that both INH and Rif susceptibility should be probed and reported simultaneously so that first line drugs could be prescribed with confidence and with out delay. Recently WHO has approved four new molecular methods which not only detect MTBC but also simultaneously report on INH and Rif susceptibility. These moderately complex methods offer high throughput testing of large number of samples in a central laboratory. Point of care tests performing the same on single to upto 8 samples are also available and are being evaluated by regulatory authorities. This should revolutionize the management of drug susceptible TB (DS-TB) which accounts for 86% in India (2022), if it is combined with symptom screen and Computer aided diagnosis (CAD) using artificial intelligence to screen for hot spots in the lung followed by molecular testing and immediate initiation of appropriate treatment, not only would patient benefit by early detection and treatment, but society too would benefit from reduced transmissionof infection and together would help in eradication of this ancient disease.

Tuberculosis is one of the top ten global causes of mortalities and poses a severe threat to public health and poverty elimination efforts in developing countries like India. Conventional TB diagnostic tests – including sputum smear microscopy for ZN or AO staining, chest X Ray and culture on solid LJ media have not been able to contribute significantly to TB control efforts and are unlikely to perform any better now that the focus has shifted from mere control to eradication of TB as a public health problem. Conventional methods suffers from either low sensitivity (smear) or low specificity (X Ray) or taking upto 8 weeks for culture results and even longer for species identification. In the past, Global TB control efforts, therefore, had been severely hampered by the lack of diagnostic tests that were accurate, simple to use and could be applied to point of care. This had been further compounded by a widespread inability to test for drug resistance [1].

The development of Xpert MTB/RIF assay, aka Gene Xpert as well as CBNAAT, a molecular assay that could be used close to the point

of care and a closed system requiring minimal technical expertise as well as use of single use disposable cartridge (preventing cross contamination) with complete information becoming available in under two hours, thus appeared to be the answer to all the diagnostic needs of the community [2,3].

Following endorsement by the World Health Organization in 2010, Gene Xpert was widely implemented for rapid molecular diagnosis in India and has been available free of charge in government set up and at fixed price in the private laboratory set up.

More than 40 million cartridges have been used worldwide for diagnosis of tuberculosis. It soon became evident that Gene Xpert was not only the best diagnostic method for smear and culture positive sputum samples but also for smear negative and culture positive samples and but also for extra pulmonary samples [4,5].

Subsequently it emerged that while Gene Xpert was excellent for respiratory samples, it performance was suboptimal in paucibacillary tubercular infections such as meningitis, childhood tuberculosis, genital TB and household contacts of TB patients. The limit of detection (LOD) ofGene Xpert aka CBNAAT was 131 cfu/ml. Consequently, Gene Xpert Ultra with LOD of 16 cfu/ml was developed and this increase in sensitivity made Gene Xpert Ultra better than Gene Xpert and MGIT Culture in the field. Even Gene Xpert XDR has been developed, but recently the definition of XDR has been changed and following success of all oral BPal regimen, WHO has discouraged the use of injectable anti TB drugs, making Gene Xpert XDR only partially suitable for this purpose [6-10].

Differences between Gene Xpert and Gene Xpert Ultra

	Gene Xpert	Gene Xpert Ultra
Diagnosis	MTBC	MTBC
Resistance	Detects Rifampicin resistance as asurrogate for MDR TB	Detects Rifampicin resistance as asurrogate for MDR TB
Amplification	Single target: rpoB region	Multiple copy target: rpoB regionInsertion Sequences IS 6110 IS 1081
Resistance detection	RT PCR 5 probes in TB rpoB region	Melting curve 4 probes bind to rpoB region
Sample size	2 ml	2 ml
PCR reactionvolume	25 ul	50 ul
PCR reaction time	112 minutes	65 to 87 minutes
Limit of detection	131 cfu/ml	16 cfu/ml
Cost	US$ 9.98	US$ 9.98
Use	Initial diagnostic tests from adultsand children	Initial diagnostic tests from adultsand children
Special advantage		Paucibacillary samples: from pediatric patients, EPTB like CSF, Lymph Nodes, Tissues, Fluids

Table 1

One aspect which has troubled introspective scientists is that Gene Xpert detects only Rifampicinsusceptibility and this is used as a surrogate for MDR TB as it is presumed that INH susceptibility mirrors that of Rifampicin susceptibility, which means if the isolate is Rifampicin susceptible, the isolate was termed as DS TB without determining susceptibility of INH and if isolate is Rifampicin resistant, then the isolate would be considered to be MDR TB, which has been shown to be factually incorrect. However when INH susceptibility was determined, usually by Line Probe Assay 1 (LPA 1), it was found that INH mono-resistant (IMR) was not infrequent in India. This would mean that if the isolate is Rifampicin susceptible but IMR, then during the continuation phase, the patient would receive only one effective drug (Rifampicin) for four months and could lead to emergence of MDR TB, on the other hand if Rifampicin was resistant but INH susceptible, and we treat this patient as MDR TB, we would deny the benefit of INH which has excellent anti- bactericidal activity and make the patient take MDR TB treatment, which runs the risk of default and non-compliance.

When Gene Xpert was being evaluated and endorsed by WHO, it was suggested after a computer modelingexperiment that while detection of Rifampicin has far reaching influence on successful treatment,additional detection of INH resistance added minimally to its effectiveness. Subsequent reports from different parts of the world indicated that IMR had a significant effect on treatment and may lead to more deaths than if IMR did not exist [11-16].

To overcome this drawback in Gene Xpert Ultra, what is required is that the method should not only detect MTBC but also simultaneously report on both INH and Rifampicin susceptibility. A recent report indicated that in India 86% of isolates were both INH and Rifampin susceptible andin these cases only one test would be enough to put the patient of standard four drugs treatment with confidence [17].

Recently WHO has validated, approved and recommended for use four moderately complex automated molecular tests for detection of MTBC but also simultaneous reporting on INH and Rifampicinsusceptibility. Application of any one of these tests would enable initiation of appropriate treatment quickly and decrease the chance of further spread of infection in the community.

Abbott real time MTB and MTB RIF/INH assays

The automated Real Time MTB (Abbott, Chicago, IL, USA) assay can diagnose MTBC in high- throughput mode (96 samples including two assay controls) in a two step process, with positive specimens reflexed to the MTB RIF/INH assay (24 samples including two assay controls) for full MDR-TB diagnosis within 10.5 hours. DNA extraction and PCR preparation is first performed by the Abbott m2000sp instrument, after which the PCR plate is sealed and transferred to the m2000rt instrument for real-time PCR. For the diagnosis of MTBC, the assay targets the insertion elementIS6110 as well as the pab genes. As a reflex test the detection of resistance to rifampicin and isoniazid the assay targets the rpoB gene, and the katG gene and inhA promoter region, respectively. The assay can discriminate and report high (katG) and low (inhA) isoniazid resistance. DNA extraction relies on the capturing of bacterial DNA to magnetic micro particles subsequent to cell lysis [18].

Becton Dickinson MAX MDR-TB assay

The Becton Dickinson MAX MDR-TB assay is a realtime PCR assay that can be run on the BDMAX System to detect MTBC through targeting IS6110 and IS1081. Detection of resistance to rifampicin and isoniazid is done through targeting the rpoB gene, and the katG gene and inhA promoter region. The assay can discriminate and report high (katG) and low (inhA) isoniazid resistance. The assay can include up to 24 sputum samples per run and reports results within 4 hours. Both DNA extraction and the BD MAX MDR-TB assay procedures are done by the BD MAX System. Bacterial cell lysis is done chemically and by heat, and the released nucleic acids are then captured by magnetic affinity beads. This methodology thereby may also include the capturing of extracellular DNA and has increased sensitivity being able to detect 0.5 cfu/ml and report resistance on a bacterial load of 6 cfu/ml. (18)

Hain lifescience fluorotype MTbdR assay

The Hain Lifescience (Hain) FluoroType MTBDR assay (Hain Lifescience, Nehren, Germany) uses LATE-PCR amplification and lightson/lights-off chemistry to detect MTBC by targeting the rpoB gene. Detection of resistance to rifampicin and isoniazid is done through targeting the rpoBgene, and the katG gene and inhA promoter region. The high throughput platform can include upto 96 samples (including assay controls) per run and reports results

within 4 hours. Not only can the assay differentiate between high- and low-level isoniazid resistance, the run report also includes the specific mutations identified for the three gene targets. DNA extraction and PCR preparation are done by the GenoXtract 96 (GXT96) instrument, after which the PCR plate is transferred to the FluoroCycler XT instrument for the FluoroType MTBDR assay. For the DNAextraction by the GXT96, the methodology includes the capturing of intact cells to magnetic beads, from where the cells are washed and then lysed. The binding of extracellular DNA to the magnetic beads are very low and not competitive to the bacteria binding but dependent on the salt concentration and pH which may vary between raw specimens, decontaminated specimens and cultured isolates [18].

The Roche cobas MTB assay (Roche, Basel, Switzerland) uses real-time PCR to detect MTBC by targeting 16S rRNA and 5 esx genes. It can generate results for 96 tests (including assay controls) in one 3.5 hour run. MTBC positive specimens are reflexed to the RIF/INH assay (96 tests including assay controls per run) for MDR-TB diagnosis in an additional 3.5 hours.

Similar to the Abbott RealTime platform, the assay targets the rpoB gene, and the katG gene and inhA promoter region for detection of resistance to rifampicin and isoniazid. DNA extraction, PCR preparation, and the cobas MTB and MTB-RIF/INH assays are done in cobas 6800/8800 systems. The sample preparation procedure requires sonication and centrifugation, for which additional instrumentation is needed. Bacterial cell lysis is done chemically (lysis reagent), enzymatically (proteinase), and physically (sonication). Subsequently, the released bacterial DNA is captured by magnetic glass particles. This methodology implies that extracellular DNA may also be captured [18].

The comparative analytical evaluation showed that the platforms from BD, Abbott, and Roche had a similar or lower Limit of Detection (LoD) for MTBC compared to Xpert MTB/RIF. The platform from Hain Lifescience showed an increased LoD compared to Xpert MTB/RIF, but the clinical significance of this difference is uncertain. All assays were comparable (or slightly moresensitive) to Hain Lifescience GenoType MTBDRplus for the detection of rifampicin and isoniazid resistance.

Similarly, the systematic review and meta-analyses showed overall similar performance of the centralized platforms to the WHO-endorsed assays, although the amount and quality of availableclinical data were very limited with available head-to-head studies. Available direct head-to-headstudies showed comparable performance of Abbott Realtime MTB, BD MAX MDR-TB, and Roche cobas MTB for MTBC detection to Xpert MTB/RIF [18].

The assays from Roche, Abbott, and BD showed lower or similar LoD for MTBC compared to Xpert MTB/RIF, while the assay from Hain Lifescience showed 5-6 fold increase in the limit to detect MTBC compared to Xpert MTB/RIF. The higher analytical sensitivity of Abbott RealtimeMTB, BD MAX MDR-TB, and Roche cobas MTB compared to Xpert MTB/RIF is most likely explained by these assays targeting multiple genetic regions for MTBC DNA amplification.

These three assays also have the capability of capturing extracellular DNA, which may increase the sensitivity of these assays in analytical studies. All centralized assays assessed with the resistant strain panel showed similar or increased accuracy for the detection of rifampicin and isoniazid resistance compared to the comparator test Genotype MTBDRplus. Sensitivity for detection of tested mutations conferring rifampicin and isoniazid resistance was ≥95% of testedstrains for all assays when accounting for the global frequency of tested mutations [18].

While all these four tests are high throughput and best suitable for a centralized model of laboratory diagnosis, recently SD Biosensor based in South Korea but with a branch in Manesar. I has developed a single-use cartridge-based POCT, which is able to do the same, and its small size makes it suitable for decentralized use. It is under evaluation of ICMR and WHO.

In a meta-analysis of newer molecular methods, Kohli., *et al.* [19] included 21 studies, which contributed a total of 26 datasets. Data limitations meant that they could only meta-analyze data for three out of the five identified assays. For TB detection, the included assays had a sensitivity of 91% or higher and a specificity ranging from 97% to 100%. All included assays for rifampicin resistance detection had a sensitivity of over 92% and a specificity of 99-100%. Sensitivity for isoniazid resistance detection varied

from 70% to 91%, but specificity remained high at 99-100% across all index tests. Studies that compared these assays with Xpert MTB/RIF for detection of TB and rifampicin resistance indicated comparable diagnostic accuracy. Centralized molecular assays showed similar diagnostic accuracy to the WHO-recommended Xpert MTB/RIF assay fordetecting TB, rifampicin resistance, and isoniazid resistance in people with symptoms of pulmonary TB.

Comparison of moderately complex NAAT approved by WHO

Attribute	Abbott	BD Max	Bruker Hains	Roche
detect	a. MTBC b. INH+RIF	MTB+INH+RIF	a. MTBC b. INH+RIF	a. MTBC b. INH+RIF
Target/s	IS 6110, pab geneRRDR of rpoB + INH promotor + kat G	IS 6110, IS 1081, both multi-copy+ devR + RRDR of rpoB + INH promo-tor+ katG 315	IS 6110 RRDR of rpoB +inh A+ kat G	16SrRNA+ esx RRDR of rpoB+INH promotor+katG
LOD	17 cfu/ml 60 cfu/ml for DST	0.5 cfu/ml 6 cfu/ml for DST	15 cfu/ml 20 cfu/ml for DST	7.6 cfu/ml 8.8 cfu/ml for DST
Sample prepa-ration	Automated, 4.5 hrs	Manual, 1.5 min/ sample	automated	Automated
PCR	Automated RealTime PCR	Automated multiplex PCR	Automated RealTime PCR	Automated RealTime PCR
IQC	+ & - controls	In every cartridge	+ & - controls	+ & - controls
Tests perrun	96	24	96	96, 384

Table 2

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