Final Report on Operational feasibility and performance of TrueNat MTB RiF assays in field settings under the Revised National Tuberculosis Control Program

Background :

- Study commissioned by Ministry of Health and Family Welfare – 28th October, 2016 – to be carried out in 50 select districts
- Joint decision to conduct the study in two designated Microscopy Centres of each district totalling to 100 DMCs taken by DG-ICMR and Central TB Division
- Protocol approved by DG-ICMR, India TB Research Consortium-ICMR, Scientific Advisory Committee of National Institute for Research in Tuberculosis, Chennai, and National Operational Research Committee
- Protocol was written by – NIRT, Chennai
- Site selection was done by Central TB Division – Dr. Kiran Rade (WHO), Dr. Malik Parmar, Dr. S. Anand, Dr. Puneet Devan
- Protocol inputs and site selection approved by an expert -Dr. Ranjani Ramachandran

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State TB Officer – Tamil Nadu
State TB Officer – Tripura
State TB Officer – Uttar Pradesh

Site In Charge
District TB Officers – 50 districts (Annexure)

Summary and analysis

A. Feasibility Study of TrueNat (M.TB and Rif.) was conducted at 100 Designated Microscopy Centres of India by NIRT, Chennai with support from CTD, MOH&FW and WHO. The study was co-ordinated by ICMR Hqrs., New Delhi.

The data analysis was undertaken by NIRT Chennai and WHO India after cleaning the data for pooled TB diagnosis in the study for sensitivity purpose and comparison of bacterial load (Colony Forming Units/ml of Sputum) between discordant results. It is based on the recommendations of the International Scientific Advisory Group (ISAG) and the National Committee constituted by ICMR for evaluation that when a new test outperforming the existing validated test, then comparison with pooled / composite TB is preferable especially when issues of differences in technologies prevail which includes: in–vitro vs in-vivo, clinical disease vs bacteriologically proven, dead vs live bacilli demonstration in test. For discrepant samples, DNA samples from the sites were received several months later and hence could not be used for discrepancy resolution. In the given constraints, further analysis was undertaken.

Results:

MTB Detection

The analysis included 10878 samples for which all three results – Sputum AFB Smear, TrueNAT and Xpert - were available. Positivity rates of each test along with pooled positivity in each of the 10 States and the overall total is given in Table 1. Smear, Truenat and Xpert yielded overall positivity rates of 13.29%, 18.80% and 18.11% respectively. Sensitivity of Smear, Truenat and Xpert were found to be 59.4%, 84.1% and 81.0% respectively when compared to the Pooled TB results. The difference in the sensitivity for detection of MTB between Truenat and Xpert was statistically significant. (p Value - <0.001)

Table:1 State-wise and Overall Distribution of Positivity rates of Smear, Truenat and Xpert

	Assam	Gujarat	Haryana	Jharkhand	Karnataka	MP	Maharashtra	Tamil Nadu	Tripura	Uttar Pradesh	Overall
Detected TB											
Truenat	172	220	165	332	258	128	247	198	85	240	2045
Xpert	176	196	168	344	234	137	205	163	97	250	1970
Smear	129	149	106	248	169	85	186	113	77	184	1446
Pooled TB	193	273	194	390	305	158	306	225	104	285	2433
No TB	429	1148	494	895	1170	399	967	1576	532	835	8445
Total	**622**	**1421**	**688**	**1285**	**1475**	**557**	**1273**	**1801**	**636**	**1120**	**10878**
Sensitivity of Test to Diagnose TB by any Method											
Truenat	**89.1%**	**80.6%**	**85.1%**	**85.1%**	**84.6%**	**81.0%**	**80.7%**	**88.0%**	**81.7%**	**84.2%**	**84.1%**
Xpert	**91.2%**	**71.8%**	**86.6%**	**88.2%**	**76.7%**	**86.7%**	**67.0%**	**72.4%**	**93.3%**	**87.7%**	**81.0%**
Smear	**66.8%**	**54.6%**	**54.6%**	**63.6%**	**55.4%**	**53.8%**	**60.8%**	**50.2%**	**74.0%**	**64.6%**	**59.4%**

Using the quantification factor – colony forming units - yielded by Truenat whenever MTB was detected, the mean and median values were calculated for GenXpert positive and GenXpert negative samples (Table 2)

Table 2: Colony Forming Units / ml of sample detected by Truenat among Gene Xpert positive and negative samples

CFU Among Gene Xpert Positives											
Mean	544125	555118	511948	404315	8.02E+21	599390	243624	295362	353147	487734	1.04E+21
Median	**19000**	**28000**	**120000**	**43000**	**25000**	**31000**	**21000**	**47500**	**49000**	**60500**	**42000**
CFU Among Gene Xpert Negatives											
Mean	732071	558792	37675	16780	37687	89432	22200000	1061	140429	133000000	12800000
Median	**460**	**920**	**490**	**410**	**500**	**1005**	**270**	**210**	**280**	**500**	**390**

Interpretation: Sensitivity of Truenat for detection of MTB is higher as compared to Gene Xpert. Average CFU/ml among Truenat positive results indicates that GenXpert detects MTB in samples with higher CFU but not in samples with low CFU/ml. Hence, Truenat is a sensitive test for detection of MTB in sputum samples. Considering the discordance between any two tests for diagnosis of TB, the potential impact on TB needs to be considered.

Impact on Programme: Assuming 9 million tests are being done currently by the Programme annually, if tests with different potentials are deployed in different scenarios of implementation, the number of microbiologically confirmed patients by each technology will be different which will impact the program. Number of positive patients identified by each technology if implemented at different frequencies based on their respective positivity rates is presented in Table 3.

Table 3: Number of positive patients identified by Smear, Truenat and Xpert under different frequencies of implementation

Test	Positivity Rate	Implementation			
		100%	75%	50%	25%
Truenat	**18.80%**	**1691947**	**1268960**	**845974**	**422987**
Xpert	**18.11%**	**1629895**	**1222421**	**814948**	**407474**
Smear	**13.29%**	**1196360**	**897270**	**598180**	**299090**

Additional yield in identifying positive patients if smear is replaced 100% by Truenat and Xpert will be 4,95,587 (41.42%) and 4,33,535 (36.23%) respectively. However, the smear can be replaced completely by TrueNat only at DMC level because it does not require an airconditioned laboratory, whereas the Xpert cannot be placed at PHC level without an AC lab.

Distribution of microbiologically confirmed TB patients who can be diagnosed if smear is replaced by Truenat or Gene Xpert at different frequencies is given in Table 4.

Table 4: Distribution of microbiologically confirmed TB patients diagnosed with the implementation of molecular tests at different frequencies

Test	Positivity Rate	Implementation			
		100%	75% ^	50% ^^	25% ^^^
Truenat	**18.80%**	**1691947**	**1568050**	**1444153**	**1320256**
Xpert	**18.11%**	**1629895**	**1521511**	**1413127**	**1304744**

^ - includes the number of positives with 75% coverage by Truenat and 25% coverage by smear
^^ - includes the number of positives with 50% coverage by Truenat and 50% coverage by smear
^^^ - includes the number of positives with 25% coverage by Truenat and 75% coverage by smear

As 70% patients are diagnosed at the Block and District level health facilities and if these 6000 DMCs are replaced by the molecular tests additional 2 to 2.5 lakhs of patients will be diagnosed (2.48 lakhs by Truenat and 2.16 lakhs by Xpert). (As per Table 3, with 100% implementation in 13000 DMCs all over the country, the number of patients diagnosed additionally by Truenat will be 4.95 lakhs and 4.33 lakhs by Xpert). However as per the feasibility and testing requirements, the replacement at PHC is possible only by TrueNat and not by Xpert. T Also the detection of TB as well as MDR-TB (Rif Resistance) at DMC level would eliminate need for Xpert.

Resolving Discrepant results:

The discrepant results were resolved by doing PCR on the discrepant sample.

Total number of samples tested by TRC4 PCR were 94. The results are given below:

a. Xpert positive but **Truenat negative** cases were 44 – of these, 4 were positive and **40 were negative by PCR**

b. Xpert negative but **Truenat positive** were 50 – of these, **34 were positive** and 16 were negative by PCR

In all 74 out of 94 test results resolved in favour of Truenat (78.7%) indicating that the TrueNat is superior to Xpert in detection of M.TB in Indian settings.

Detection of Rifampicin Resistance

Data was cleaned and data of the total cases detected positive for Rif among all TB positive cases was analysed to determine the rates of detection of rifampicin resistance by Truenat and Gene Xpert. Valid results were available for a total of 1298 samples. Distribution of the rates of rifampicin resistance detection by each test state-wise and overall is given in Table 5.

Table 5: State-wise and overall distribution of rifampicin resistance detection by th molecular tests

Test	Assam	Gujarat	Haryana	Jharkhand	Karnataka	MP	Maharashtra	Tamil Nadu	Tripura	UP	Overall
Truenat	3	8	6	24	9	5	9	6	1	12	83
Xpert	2	5	7	17	6	5	10	8	1	11	72
Total	**111**	**134**	**107**	**254**	**155**	**84**	**100**	**110**	**68**	**175**	**1298**

Percentage detection of rifampicin resistance among the TB positives by either method is given in Table 6, and it was found to be 6.4% and 5.5% for Truenat and Xpert respectively. This difference was not statistically significant.

Table 6: Percentage detection of rifampicin resistance by both tests

	Resistant	Sensitive	Total
Truenat	83 (6.4%)	1215 (93.6%)	1298
Xpert	72 (5.5%)	1226 (94.5%)	1298

Conclusions: Based on the results of the feasibility study of TB and MDR-TB detection at DMC level using TrueNat in comparison to Xpert in State level laboratories, the Replacement of smear microscopy and Gene Xpert by Truenat would be beneficial to achieve NSP targets in view of the following observations:

1. Significantly Incremental detection by TrueNat over microbiologically confirmed TB diagnosis.
2. Higher rate of rifampicin resistance detection by Truenat as compared to Gene Xpert. Truenat is also a sensitive test for detection of MTB in sputum samples as Trunat can detect M.TB in samples with lower CFU while GenXpert detects MTB in samples with higher CFU but not in samples with low CFU/ml. Hence,
3. Similarly, sensitivity of Truenat (Rif.) for detection of Rifampicin resistance in samples positive for TB is higher as compared to Gene Xpert.
4. The PCR results of discrepant cases showed that 74 out of 94 test results resolved in favour of Truenat (78.7%) indicating that the TrueNat is superior to Xpert in detection of M.TB in Indian settings.
5. The feasibility aspects reveal that Truenat can be implemented in the PHCs which is the first point of contact for TB patients and higher. It also has additional benefit of its ease of use.
6. Use of Truenat at Primary Health Care (PHC) level eliminates the need for sample transport as is done in case of GeneXpert thus adding cost benefit besides detecting TB /MDR-TB during the first visit of the patient.
7. Operational requirements of Truenat – Portable, Battery Operated, Direct Connectivity with mobile interface for data sharing, whereas Xpert requires continuous power supply, air-conditioning and connectivity is only through a computer system
8. Time taken for assay – Design of the Truenat assay is such that MTB detection is completed in 35 minutes and Rifampicin assay is done only as an add on test. Hence samples with negative results can be reported much earlier whereas with Xpert, even negative results require 120 minutes as both MTB detection and rifampicin resistance assays are done simultaneously. However, in positive cases, since rifampicin test is done as an add-on assay by Truenat, the possibility of human error is greater and
9. Availability of DNA – With Truenat, DNA is available for repeat or any further investigation and QC, whereas with Xpert, the cartridge is discarded after the completion of the assay and no DNA is available.
10. The cost of equipment and the test is much lower than Gene Xpert and the machine is battery operated which also has option of solar battery. Cost – Details are provided in Annexure III
11. Moreover, this TrueNat platform can be used simultaneously in other diseases for detecting far more infections that require molecular diagnosis. (Annexure III). This will enhance

capacity to detect other infections and monitoring where it is required at all levels of health care including PHCs in future.

Approved document