Indian Council of Medical Research

Generic Concordance Study Protocol

A generic protocol to determine the true agreement between two versions of an approved technology for the molecular detection of tuberculosis and drug-resistant tuberculosis

Background and rationale

Accurately diagnosing adult pulmonary Tuberculosis (PTB) and Drug-resistant TB remains a major challenge in endemic countries, hindering the achievement of the TB elimination goal. The priority in high-burden countries has been to develop reliable, rapid, and affordable TB tests. Conventional culture-based identification and drug susceptibility tests (DST) require highest biosafety measures in TB laboratories, often limiting their availability to national or higher-level regional laboratories.

Nucleic acid amplification tests (NAATs) for *Mycobacterium tuberculosis* (MTB), specifically amplifying distinct nucleic acid regions, have exhibited promise due to their high sensitivity and specificity. Currently, the National Tuberculosis Elimination Programme (NTEP) has approved and introduced the Xpert® MTB/RIF assay and Truenat® MTB-RIF Dx, both capable of detecting rifampicin resistance[1]. However, these tests are limited to rifampicin resistance detection only.

Recent advancements include development of nucleic acid amplification techniques based closed systems with simultaneous detection of Mycobacterium tuberculosis complex, resistance to rifampicin (RIF) and isoniazid (INH). Recently, Pathodetect™ MTB/RIF-INH assay using Compact XL/Q closed system was found to be on par with the existing closed molecular systems under a multi-centric study conducted by ICMR[2]. Currently the manufacturers have developed a smaller and improved version of the previously approved technology. TB diagnostics is expanding exponentially where different and improved versions of an approved technology have been developed by the manufacturers to accommodate different settings[3].

In order to utilize these modified versions or platforms of the same approved technology, a concordance study is required to demonstrate whether the newer version for molecular detection of tuberculosis and drug resistant tuberculosis can achieve equivalent performance to the existing version or platform. This protocol is for concordance estimation of two exactly same closed systems of NAATs, where one of the systems has been extensively examined by ICMR in field and the new version of the same technology has a different sample testing capacity in a single run. The protocol outlines estimation of true agreement between the already validated closed system with the new version.

Objectives of the study:

1. To determine the true agreement between a previously validated platform and newer version of a molecular test in detecting MTB in confirmed positives and confirmed negatives for *M. Tuberculosis*.
2. To determine the true agreement between a previously validated platform and newer version of a molecular test in detecting drug resistance in confirmed positives and confirmed negatives for drug resistant tuberculosis (rifampicin & isoniazid).
3. To compare the sensitivity and specificity of two versions with respect to the reference molecular test [Xpert MTB or Line Probe Assay (LPA)].

Study Design

This will be a cross-sectional study to determine the level of true agreement† in both confirmed positives and confirmed negatives for mycobacterium tuberculosis (MTB) and multidrug resistant tuberculosis (MDR-TB), between an already validated platform and new version.

Note: †*True agreement refers to the agreement between two versions in detecting the true status of samples i.e true positive and true negative by reference test.*

Study methodology

Sites:

The proposed agreement study will be done only at the designated laboratories approved by the National Tuberculosis Elimination Program (NTEP) using stored sputum samples. If the study is performed for approval or license of the product from the Drug Controller General of India (DCGI), the evaluation may be performed in any of the centres listed by DCGI using this protocol. However, if the product is evaluated for introduction in the national program, ICMR will review the evaluation report (if available). If evaluation has not been done previously, ICMR will assign the concordance testing center.

Samples:

- Sputum samples that are confirmed MTB positive (category 1) and MTB negative (category 2) by NTEP endorsed NAAT (Xpert MTB/RIF) as a molecular reference test (irrespective of LJ/MGIT culture status) will be used for the objective 1.
- Similarly for the objective 2, MTB positive sputum samples with confirmed positive and negative for resistance to rifampicin (RIF) (category 3&4) & isoniazid (INH) (category 5&6) by LPA as a reference will be used.
- Sputum samples stored at -20^{0}C for less than 3 months only should be used for the study[4].
- The quality of the stored samples must be ensured by viability assay performed on previously culture positive samples. Viability with culture positivity should be more than 95 % to proceed with stored samples.
- In case of non-availability or shortfall of stored samples, prospective samples may be considered with approval from institutional ethical committee (if applicable)[5].

Objective 1: The stored sputum samples tested by Xpert MTB/RIF should be used as reference test in each of the following categories for objective 1:

Category 1: MTB positive sputum samples (n=325) by reference test
Category 2: MTB Negative sputum samples (n=325) by reference test

[Note: MTB positive sputum samples under category 1 should include all types of grades like 3+, 2+, 1+ and scanty and high, medium and low by Xpert]

Objective 2: The stored MTB confirmed positive sputum samples tested by LPA as reference test in each of the following categories will be used for objective 2:

Category 3: MTB positive sputum samples, resistance to rifampicin (n=325)
Category 4: MTB positive sputum samples, sensitive to rifampicin (n=325)
Category 5: MTB positive sputum samples, resistance to isoniazid (n=325)
Category 6: MTB positive sputum samples, sensitive to isoniazid (n=325)

[Note: All prevalent *rif* mutations should be included for category 3 and samples for both *kat G* and *inh A* mutations should be included for category 5]

Sample size determination

Any new test aimed at ruling-in MTB (i.e treatment will be initiated based on the MTB positive test result) should have specificity of more than 95%. Sample size determination for determining the true agreement between an already validated platform and newer version with different configuration with same approved technology;

$$N = \frac{[Z_{(1-\alpha/2)}]^2 * (A) * (100-A)]}{d^2}$$

where,
N: sample size for true agreement
A: assumed % true agreement between the two versions
Z $(_{1-\alpha/2})$: 1.96 for confidence level of 95%
d: half width of confidence interval (±d)

Table 1: Sample size required to determine percent true agreement between two versions at a given 95% confidence interval.

		Width of Confidence Interval						
		5%	**7.5%**	**10%**	**12.5%**	**15%**	**17.5%**	**20%**
% True Agreement between two versions in positive or negative samples	**99%**	61	28	16	10	7	5	4
	97.5%	150	67	38	24	17	13	10
	95%	292	130	73	47	33	24	19
	92.5%	427	190	107	69	48	35	27
	90%	554	246	139	89	62	46	35
	87.5%	673	299	169	108	75	55	43
	85%	784	349	196	126	88	64	49
	82.5%	888	395	222	142	99	73	56
	80%	984	438	246	158	110	81	62
	77.5%	1072	477	268	172	120	88	67
	75%	1153	513	289	185	129	95	73

The accepted true agreement should be equal to or more than 95% for new system with already approved system/technology. Given a confidence level of 95%, a sample size of 292 would be required to yield 95% true agreement between two versions with 5% confidence interval. Testing around 325 samples in each category would ensure that these parameters are met even with 10% invalid samples.

Sample processing and testing:

All sample processing procedures, preparation of reagents and testing methods (Culture, Gene Xpert, LPA) should be followed as per the NTEP guidelines[6] and the manual of ICMR-National Institute of Research in Tuberculosis (NIRT)[7]. The evaluation must be done in a double-blind coded manner.

Sputum samples in each category will be divided in two equal aliquots. One aliquot will be tested in previously validated platform and the other aliquot will be tested in parallel in the new version under similar environmental conditions as per manufacture's instructions. Reference test used for molecular detection of MTB i.e. Xpert MTB/RIF and MDR-TB (DST or LPA) should be specified when study is performed (Figure 1).

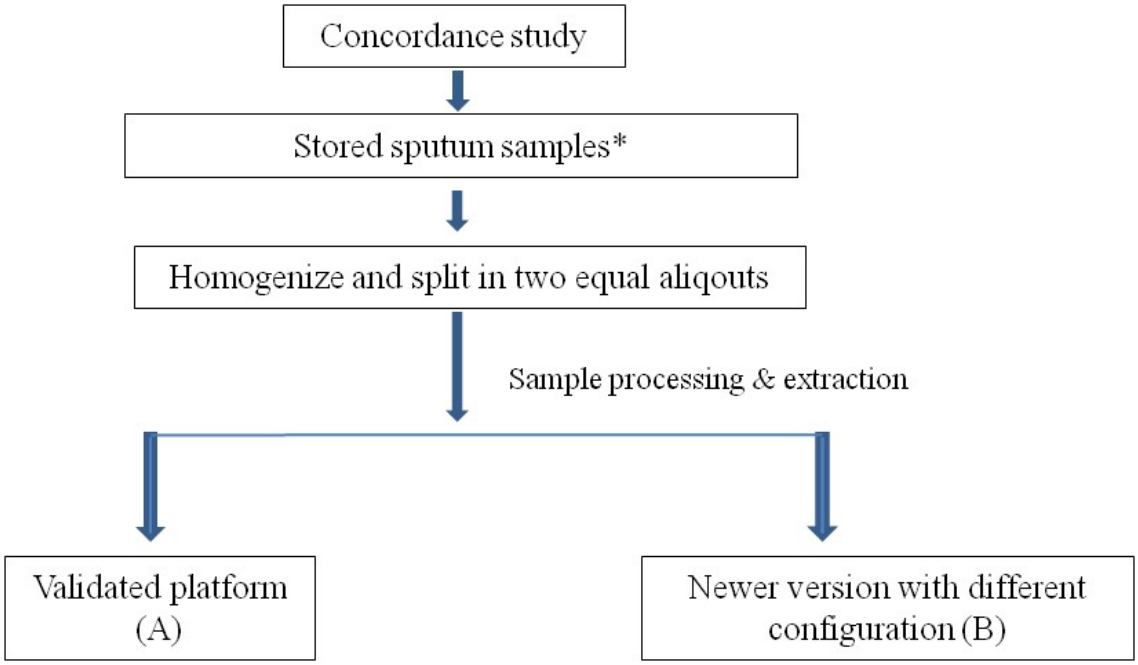

Figure 1: Flowchart for evaluating the true agreement between two version of an approved technology for the molecular detection of tuberculosis and drug resistant tuberculosis.
* Samples from each category (1 to 6) should be processed and tested separately.

Result Analysis

Evaluating test results:

- Standard laboratory protocols as per the ICMR-NIRT/NTEP manual should be followed. Any deviation/modifications should be noted down.
- The results obtained with validated platform (A) and newer version (B) must be read as per the manufacturer's instructions/ software. Test result with inadequate sample or error in either of the platform will not be included in final analysis.
- True agreement will be calculated in each of the six categories (confirmed positive & negative for TB and MDR-TB), between the validated platform (A) and new version (B) for molecular detection of TB and MDR-TB using stored sputum samples (Table 2).

$$\text{True agreement in positive samples} = \frac{[\text{Number of positives by both versions (A\&B)}]}{\text{Total number of positive samples by reference test (Xpert)}}$$

$$\text{True agreement in negative samples} = \frac{[\text{Number of negatives by both versions (A\&B)}]}{\text{Total number of negative samples by reference test (Xpert)}}$$

Table 2: 2 x 2 table for samples tested by validated platform (A) & newer version (B) for determining true agreement

		Validated platform (A)		Total
		Positive	Negative	
New version (B)	Positive	a	b	a+b
	Negative	c	d	c+d
Total		a+c	b+d	N (a+b+c+d)

Note: Table 2 should be prepared separately for each of the six categories

True agreement in TB positive samples (category 1) = (a / N) x 100

In addition, Cohen's Kappa (k) will be calculated to account for chance agreement between version A & version B for the detection of TB and MDR-TB as;

$$\text{Cohen's Kappa (k):} \quad \frac{(P_0 - P_e)}{(1 - P_e)}$$

Where, proportionate agreement (P_0): [(a+d) / N] and,
Probability of random agreement (P_e) = sum of probability of being positive (P+ve) and probability of being negative (P-ve)

i.e., P+ve = [(a+b) / N] x [(a+c) / N]
P-ve = [(c+d) / N] x [(b+d) / N]

Note: The 2x2 table, percentage true agreement, corresponding 95 % CI and Cohen's Kappa should be calculated for each of the six categories separately.

Further, sensitivity and specificity of each version (A & B) for detection of MTB or MDR-TB will be evaluated separately using well characterized stored sputum samples. Gene Xpert RIF and LPA test will be used as reference test for MTB and MDR-TB respectively (Table 3 & 4).

Table 3: 2 x 2 table for sensitivity and specificity for MTB or MDR-TB detection using version A

		Reference test	
		+	-
Index test using version A (validated platform)	+	a	b
	-	c	d
Total		a+c	b+d

Sensitivity of index test using platform A = [a / (a+c)]*100

Specificity of index test using platform A = [d / (b+d)]*100

Table 4: 2 x 2 table for sensitivity and specificity for MTB or MDR-TB detection using version B

		Reference test	
		+	-
Index test using version B (New version)	+	a	b
	-	c	d
Total		a+c	b+d

Sensitivity of index test using platform B = [a / (a+c)]*100

Specificity of index test using platform B = [d / (b+d)]*100

Note: Sensitivity and specificity of version A should be compared with the sensitivity and specificity of version B for detection of MTB, RIF and INH separately.

Study outcomes:

Percentage true agreement between the two versions of an approved technology will be estimated with 95% confidence along with Cohen Kappa statistics in confirmed positives and confirmed negatives for tuberculosis (MTB) and drug resistant tuberculosis for RIF and INH resistance (MDR-TB). Additionally, the sensitivity and specificity of each version with respect to the reference molecular test will be known.

Evaluation of Report:

Final report with raw data will be submitted to ICMR for review by the expert committee.

Regulatory and ethical consideration:

No human subject should be enrolled for the agreement study. Anonymized stored sputum samples shall be utilized for this purpose. Administrative or regulatory permission should be obtained prior to start of the study as per ICMR guideline on ethical requirements for laboratory validation testing 2024[7].

Quality Control:

- The quality and adequate volume of stored sputum specimen shall be ensured while utilizing for the concordance study[4]. The laboratory may ensure that samples are not degraded and stored in appropriate condition.
- Study site/sites shall ensure high quality of laboratory procedures, biosafety practices[8], data recording and documentation.

References:

1. Guidelines for programmatic management of tuberculosis and drug resistant tuberculosis in India March 2021. National TB Elimination Programme, Central TB Division, Ministry of Health & Family Welfare, Government of India
2. https://main.icmr.nic.in/sites/default/files/upload_documents/Revised_list_of_TB_kits_validated_as_on_24052023.pdf
3. Georghiou SB et al. Equivalence of the GeneXpert System and GeneXpert Omni System for tuberculosis and rifampicin resistance detection. PLoS One. 2021 Dec 17;16(12):e0261442.
4. Prajwal P, Neary T, Rohrbach K, Bittel P, Göller PC, Buch T, Dümcke S, Keller PM. Optimizing mycobacteria molecular diagnostics: No decontamination! Human DNA depletion? Greener storage at 4 °C! Front Microbiol. 2023 Apr 11;14:1104752. doi: 10.3389/fmicb.2023.1104752.
5. ICMR guidance on ethical requirements for laboratory validation testing, February 2024. https://main.icmr.nic.in/sites/default/files/upload_documents/Guidance_on_Ethical_Requirements_for_Laboratory_Validation_Testing.pdf
6. RNTCP Standard Operating Procedures for Tuberculosis lab for culture and DST, 2009.
7. Standard Operating Procedures (SOP) for Mycobacteriology laboratory, ICMR-NIRT.
8. Singh S. Biosafety precautions in tuberculosis laboratory, Indian J tuberculosis. 2013; 60 (3): 135-137.