ICMR-CDSCO STANDARD PERFORMANCE EVALUATION PROTOCOLS

FIELDPERFORMANCE EVALUATION OF IN-VITRO DIAGNOSTICS FOR PULMONARY TUBERCULOSIS

ICMR-CDSCO/IVD/TB/PROTOCOLS/2/2025

DIVISION OF COMMUNICABLE DISEASES, ICMR
IN VITRO DIAGNOSTICS DIVISION, CDSCO
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India

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Field Performance Evaluation of IVD for Pulmonary Tuberculosis

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I. Background

CDSCO and ICMR, New Delhi, have aimed at facilitating the availability of Quality-Assured diagnostic kits appropriate for use in India. This protocol gives the methods to be used for evaluating the clinical performance characteristics of nucleic acid amplification based in-vitro diagnostic test in detecting pulmonary tuberculosis.

Note: According to CDSCO guidelines, "performance evaluation" refers to "analytical validation" required for obtaining "test license", while “field evaluation” refers to "clinical validation” performed in clinical samples in real world setting.

II. Purpose

To evaluate the clinical performance characteristics of nucleic acid amplification tests (NAAT) for diagnosis of pulmonary Mycobacterium Tuberculosis (MTB) using prospectively collected sputum samples in clinical setting.

III. Study Design

Cross-sectional prospective multi-centric diagnostic accuracy study of IVD for detection of pulmonary TB using Mycobacterium Growth Indicator Tube (MGIT) liquid culture as the microbiological reference standard.

IV. Ethical Considerations

1. The study should be compliant to the ICMR’s Guidance on Ethical Requirements for Laboratory Validation Testing, 2024. Performance evaluation activities using irreversibly de-identified leftover clinical samples are exempt from ethics approval as per ICMR’s Guidance on Ethical Requirements for Laboratory Validation Testing, 2024. Investigators are required to submit a self-declaration form, as outlined in the ICMR guidelines, to the institutional authorities and ethics committee for information.
2. Sputum specimens should be collected, as required for routine diagnostic evaluation, from patients who are suspected of having pulmonary TB as per algorithm. Probability of harm or discomfort anticipated in the research is nil or not expected.
3. Enrolment of subjects should be continued till the sample size is met or till the project duration is completed.

4. If additional sputum sample is obtained, written consent must be obtained as per the ICMR National Ethical Guidelines for Biomedical and Health Research Involving Human Participants. The institutional ethics committee of each participating site should be intimated about the study for necessary approval prior to initiating the study. Assent form should be collected in addition to Informed Consent in case of adolescents (13 to 16 yrs). For children between 7 and 12 years old, oral assent should be obtained in presence of parent or legal guardian. For children under 7 years old, written informed consent should be obtained from parent or legal guardian.
5. The protection of privacy of research participants will be ensured by encrypting the patient identifiers.
6. Patients shall receive the best possible diagnostic work-up as per the routine practice and the National Tuberculosis Elimination Program (NTEP) guidelines. There should not be delay in sending report due to the study.
7. TB treatment decisions should not be made based on the result of the index test under evaluation, but on the basis of the routine clinical and laboratory methods (smear, solid / liquid culture, standard NAAT results, and clinical work-up).
8. Respect for the dignity of research participants should be prioritized.
9. No compensation shall be provided to the participants since there is no additional cost or travel involved in sample collection for the study. Patients should be compensated for travel and time only if they are asked to pay additional visits exclusively for the sake of the study and not during regular treatment visits.
10. Follow-up visits may be required for a very limited number of discrepant patients to exclude TB.
11. Leftover sputum samples and deposits should be stored for resolving discrepancies. One positive culture and two DNA samples per patient should be stored at -80^{o}C for use later.
12. All the sites should follow up with all study participants till the final diagnosis is made and the patient should be initiated on appropriate treatment as per NTEP norms. Those found to be *M. tuberculosis* complex (MTB)positive by standard NAAT test should be started on anti-tuberculosis treatment (ATT) by medical officer of the study site as per NTEP guidelines.
13. The findings of the study should be made accessible through reports.

V. Blinding of Laboratory Staff

To ensure rigor of the evaluation process, laboratory staff performing the evaluation should be blinded to the status of the clinical samples. The PI of the evaluation exercise should remain unblinded, i.e., privy to the status of the samples. Another senior laboratory staff selected by the PI may remain unblinded and carry out coding of samples and dispensing them into similar-looking vials to be used for testing, and maintaining the database of results.

Staff performing the reference test and the test under evaluation (index test), interpretation of the test result, and entering the results against the coded samples in the database, should remain blinded to the status of samples till the completion of evaluation.

Operators conducting routine laboratory tests (smear, Xpert MTB/RIF, MGIT culture etc) will not participate in the index test evaluation. Instead, dedicated operators, who are not involved in routine testing and are blinded to the routine test results, will perform the index test. The results will be recorded independently for each test without any patient identifiers. The result sheets will be shared with the investigator for result analysis. The data should be analyzed only by the PI of the evaluating lab (Fig. 1).

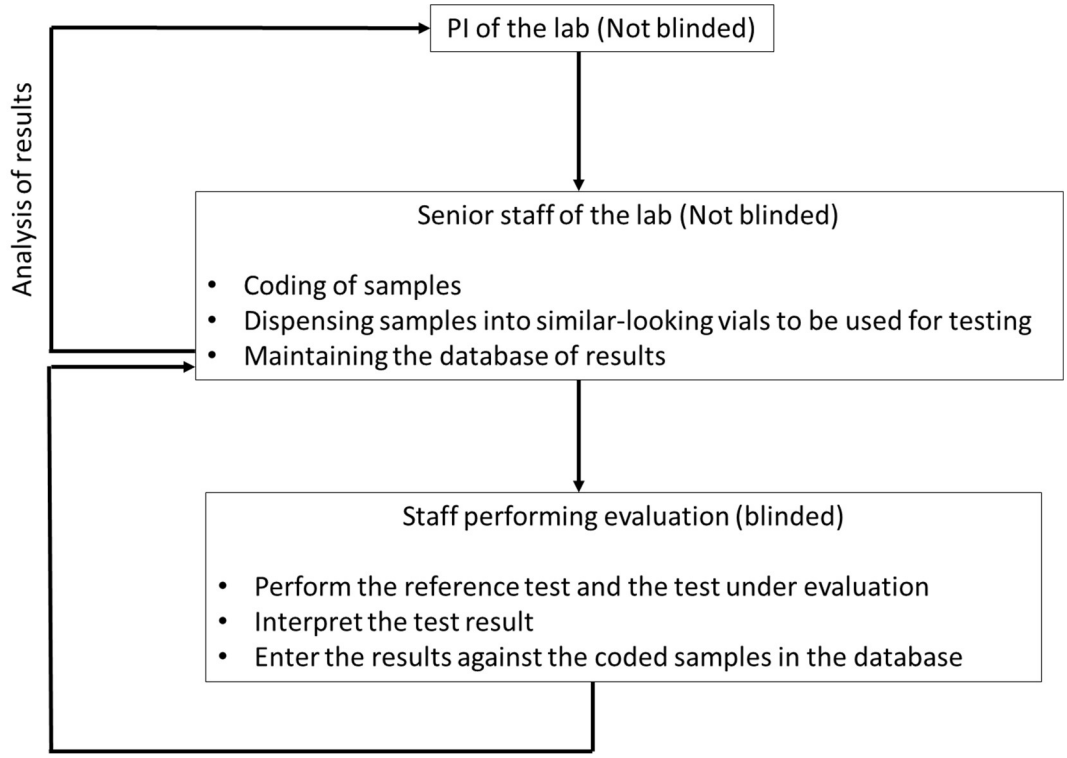


Figure 1 Blinding in evaluation exercise

VI. Procedure

1. Preparation of Evaluation sites/laboratories

- Laboratory must be approved by the National TB Elimination Program (NTEP).
- Accreditation for at least one Quality management system [accreditation for Testing Lab / Calibration Lab (ISO/IES 17025), Medical Lab (ISO 15189), PT provider ISO/IEC 17043 or CDSCO approved Reference laboratory].
- Three or more sites from different geographical regions should perform clinical validation for representation of population in real world setting.

2. Study Participants

Individuals with symptoms of presumptive pulmonary TB attending hospital OPDs/Chest clinics/district microscopy centers (DMCs) and Directly Observed Therapy Short Course (DOTS) centers. All such consecutive cases willing to provide consent will be enrolled in the study.

Definition of Presumptive PTB:
Patients with any of the following symptoms regardless of duration will be considered to have 'presumptive TB': cough for two weeks or more, fever for two weeks or more, night sweats, unintentional weight loss, hemoptysis, chest pain or loss of appetite, with any abnormality in chest radiograph (one or more of the following findings by standardized interpretative criteria: cavitary lesion(s), apical infiltrates, hilar lymphadenopathy, new infiltrates and other suggestive radiological findings).

3. Eligibility of Participants

Inclusion Criteria

1. Individuals positive for TB by smear or any approved NAAT test (Xpert® MTB/RIF)
2. Individuals willing to give consent
3. Individuals who are able and willing to give two good quality mucopurulent sputum samples of ≥ 3 ml

Exclusion criteria

1. Individuals on TB treatment for >96 hrs
2. Individuals not consenting for the study
3. Individuals unable to produce two sputum samples of ≥ 3 ml

4. Reference and Index tests

Reference test: Mycobacterium Growth Indicator Tubes (MGIT) liquid culture

Comparator: NTEP approved NAAT test (Xpert® MTB/RIF)

5. Sample size

The anticipated sensitivity of an index test is 90 % and with absolute 5 % precision, while the anticipated specificity is 99 per cent with 1 % precision. A higher precision for specificity would be required to minimize false positivity. The minimum sample size requirement has been calculated as ~150 positives and ~470 negatives for MTB by the gold standard culture. With a prevalence of 24 % culture positives among presumptive cases in hospital setting (Penn-Nicholson et al., 2021) and a 5 % loss due to indeterminate results, approximately 610 consecutive cases meeting the inclusion and exclusion criteria would be required to be enrolled for the detection of MTB (Jayaprakasam et al., 2024). Enrolment would be continued till the required number of participants is covered.

The formula for calculating sample size for determining sensitivity/specificity of the index test:

$$N_{Se} = \frac{[Z\ (1-\alpha/2)]^2 * (Se) * (1-Se)]}{d^2}$$

or

$$N_{Sp} = \frac{[Z\ (1-\alpha/2)]^2 * (Sp) * (1-Sp)]}{d^2}$$

N_{Se}: Sample size for estimating sensitivity,

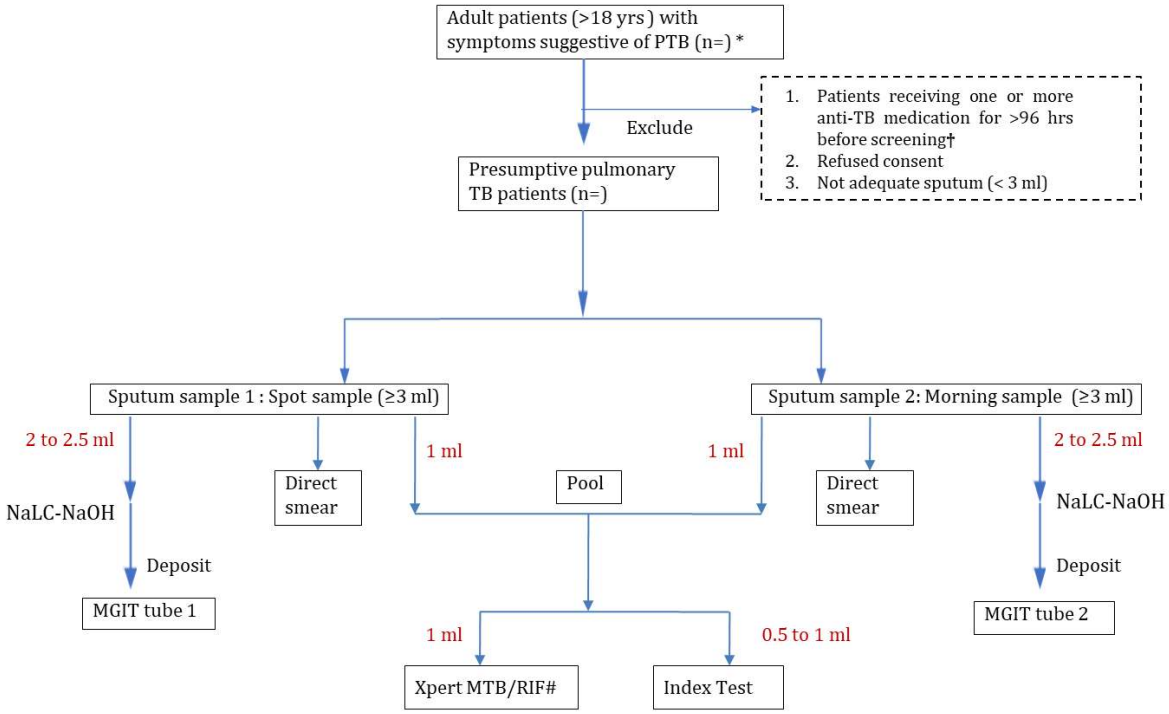
Se: Anticipated sensitivity with reference to culture DST

Sp: Anticipated specificity with reference to culture DST

Z (1-α/2):1.96 for confidence level of 95%

d: Absolute precision

6. Implementation Plan



* ***Screening***: *Medical history & clinical examination as per NTEP guidelines*
† To ensure that dead bacilli are not detected and no treatment failure cases are enrolled
*# **Comparator**: Xpert MTB/RIF*
Storage: *One positive culture and 2 decontaminated samples per patient stored at -80°C for later use. Two DNA samples stored at -20°C for resolution of discrepant results.*

Figure 2 Flowchart for evaluating NAAT test for detection of Mycobacterium Tuberculosis (MTB) among individuals with presumptive pulmonary TB (PTB)

7. Sample collection, processing and storage

1. Two sputum samples each of minimum 3 ml should be collected (one spot and one morning specimen) and sent to laboratory.
2. Approximately 1 ml of sample should be taken from each sample and pooled under sterile conditions (total of 2 ml).
3. Around 1 ml of pooled sample should be tested by the standard NAAT (Xpert MTB/RIF®) and remaining sample used for index test(s).
4. The remaining portion of each sputum sample should be subjected to direct smear and decontamination by NaLC-NaOH method individually.
5. The resultant deposit should be used for inoculation into two MGIT960 tubes.
6. All positive cultures should be identified using rapid Immuno-chromatography test (ICT). (Ideally, positive MGIT tubes are tested within 5 days of instrument positivity. Interpretation of the result should be done within 15 minutes).
7. All sputum samples should be stored at -20°C for later use. Decontaminated sediments and one positive culture per patient should be stored at -80°C, if necessary for later use.
8. Two DNA samples per patient should be stored at -20°C till the end of the study for resolution of discrepant results.
9. The index tests should be carried out as per the algorithm (figure 2) and as per the manufacturers' instructions in the instructions for use (IFU).

All conventional test procedures for smear, culture (solid and liquid) and Xpert MTB will be performed as per NTEP national laboratory guidelines (CTD, 2016; RNTCP 2009) and laboratory manual of ICMR-NIRT (NIRT, 2010). Standard operating procedures for index test(s) will be provided by the manufacturer(s) including use of positive and negative controls. All procedures for preparation of media, reagents, washing, decontamination, disposal and storage will be performed according to the standard operating procedures (SOP) of ICMR-NIRT (NIRT, 2010) and WHO, (WHO, 2022).

8. Laboratory Tests

i. Smear microscopy: Two direct sputum smear
ii. MGIT culture (decontaminated with 1-1.5% final NaOH); Two MGIT tubes (one per specimen) for each patient
iii. Speciation of culture: Rapid immune-chromatographic test (ICT) of MGIT culture
iv. Xpert MTB/RIF (one test per patient)

9. Data Analysis and resolution of discrepancy

i. If the index test produces error or indeterminate results, then only one repeat is allowed. The results of first test and repeat test should be recorded separately.
ii. All Invalids/Indeterminates/errors should be recorded and reported.
iii. A subgroup analysis may be carried out for pediatric population.

10. Quality Control (QC) measures

All sites should ensure high quality of laboratory procedures, data recording and documentation. There should be no deviation from the protocol. All the sites should participate in internal quality control (IQC) and external quality assurance (EQA) for all methods as per the standard manuals of Global Laboratory Initiative (GLI, 2014).

Culture: Positive (Reference strain H37Rv or H37Ra) and negative controls for MGIT and LJ cultures would be tested as per NTEP guidelines. MGIT Time to detection QC for MTB reference strain would be performed every month/new lot of reagents/machine service. Sterility and performance testing of culture media would be performed with every new batch or lot.

Smear: Smear QC should be performed as per NTEP guidelines at regular intervals and with new lot of reagents.

ICT Identification of MTB complex: Culture of *M. tuberculosis* reference strain in MGIT broth should be used as positive control. Culture of Mycobacteria other than tuberculosis (e.g., a well characterized strain of *M. avium* complex/*M.kansasii*) in MGIT broth should be used as negative control. QC for ICT should be performed every 3 months.

Molecular diagnostics: For molecular diagnostics internal quality control includes control supplied by the manufacturer and control prepared by the lab from the previous testing. The

internal control should be used whenever batch of test kit changes, machine is serviced, and newly trained person is introduced into the system.

Avoiding Cross-contamination: Unidirectional workflow: The workflow of a molecular lab should be in one direction only. PCR master mix reagents and samples that may contain templates for PCR should be prepared in the pre-PCR room only. Tubes that have undergone amplification in the post-PCR room contain amplicons and will not be opened or introduced in the pre-PCR room. Consumables and PPE (lab coats, gloves, goggles, etc.) that have been used in the post-PCR room should not be placed back in the pre-PCR room without thorough decontamination. Aerosol resistant pipettes will be used for all procedures and standard aseptic cleaning technique should be carried out before and after PCR for work surface, bench top and equipment.

VII. Statistical Analysis Plan

i. The performance of the diagnostic kits should be evaluated by calculating the sensitivity, specificity, positive predictive value, negative predictive value and accuracy with reference to the gold standard. 95% Confidence interval should be calculated for each of the parameters.
ii. The index molecular test should be evaluated for its performance with reference to the MGIT culture.
iii. Similarly, the performance of standard molecular test (Xpert MTB/RIF) should be estimated with reference to MGIT culture.
iv. The sensitivity and specificity of index test vs MGIT culture should be compared with that of Xpert® MTB/RIF Vs MGIT culture.
v. The agreement between the index test and standard NAAT test (Xpert MTB/RIF) should be calculated with kappa statistic.

VIII. Acceptance Criteria for diagnostic tests

Expected sensitivity: ≥85 ± 2%

Expected specificity: ≥95 ± 2%

Sample size: ~150 MTB positives and ~470 MTB negatives by MGIT culture

For screening tests the acceptability criteria will be as per WHO TPP 2025

Acceptance criteria for Screening tests:

Test Type	Minimal Accuracy	Optimal accuracy
High Sensitivity high specificity screening test	90% sensitivity 80% specificity	95% sensitivity 95% specificity
High Sensitivity screening test	90% sensitivity 60% specificity	95% sensitivity 85% specificity
High specificity screening test	60% Sensitivity 98% specificity	70% sensitivity 98% specificity

Source: WHO TPP 2025

IMPORTANT NOTE

After following due procedure as defined in this document, once any kit is found to be Not of Standard Quality, thereafter, no request for repeat testing of the same kit will be acceptable.

Any request of re-validation from the same manufacturer for the same test type will only be entertained after a minimum of 3 months and only if a high-level technical summary of modifications or functional improvements to the kit design is submitted, without explicit disclosure of proprietary information.

Clinical samples are precious, therefore, repeat evaluation of a kit using the same/ different well-characterized sample panel at a different laboratory may be considered only for kits which claim high performance characteristics (sensitivity and specificity 95% and above), but which fail the performance evaluation by a margin of 5%.

Atleast two different lots or batches should be used for the field validation of any new molecular test.

References

1. Penn-Nicholson, A., Gomathi, S. N., Ugarte-Gil, C., Meaza, A., Lavu, E., Patel, P., Choudhury, B., Rodrigues, C., Chadha, S., Kazi, M., Macé, A., Nabeta, P., Boehme, C., Gangakhedkar, R. R., Sarin, S., Tesfaye, E., Gotuzzo, E., du Cros, P., Tripathy, S., Ruhwald, M., … Members of the Truenat Trial Consortium: (2021). A prospective multicentre diagnostic accuracy study for the Truenat tuberculosis assays. The European respiratory journal, 58(5), 2100526.
2. Jayaprakasam, M., Pandey, R. M., Choudhary, H., Shanmugam, S., Sivaramakrishnan, G. N., & Gupta, N. (2024). Evaluation of molecular diagnostic test for detection of adult pulmonary tuberculosis: A generic protocol. The Indian journal of medical research, 159(2), 246–253.
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5. Standard Operating Procedures (SOP) for Mycobacteriology laboratory, ICMR-NIRT, 2010.
6. Practical manual on tuberculosis laboratory strengthening, 2022 update. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO.
7. Mycobacteriology laboratory manual, Global laboratory initiative, First edition, April 2014, Stop TB Partnership.

PERFORMANCE EVALUATION REPORT FORMAT

Performance Evaluation Report For MTB Kit

Name of the product (Brand/generic)		
Name and address of the legal manufacturer		
Name and address of the actual manufacturing site		
Name and address of the Importer		
Name of supplier: Manufacturer/Importer/Port office of CDSCO/State licensing Authority		
Lot No /Batch No.:		
Product Reference No/Catalogue No		
Type of Assay		
Kit components		
Manufacturing Date		
Expiry Date		
Pack size (Number of tests per kit)		
Intended Use		
Number of Tests Received		
Regulatory Approval: Import license / Manufacturing license/ Test license License Number: Issue date: Valid Upto:		
Application No.		
Sample Panel	Sample type	
	Positive samples (provide details: strong, moderate, weak)	
	Negative samples (provide detail: clinical/spiked, including cross reactivity panel)	

Results:

Test	Number of samples tested	Positive	Negative	Invalids/ Indeterminates/Error/ Contamination (culture)
Smear				
MGIT culture				
Xpert MTB/RIF				
New MTB kit				

		Reference assay …….…… (MGIT culture)		
		Positive	Negative	Total
Name of MTB kit	Positive			
	Negative			
	Total			

	Estimate (%)	95% CI
Sensitivity		
Specificity		

Conclusions:

- Sensitivity, specificity
- Performance: **Satisfactory / Not satisfactory**

(Sensitivity and specificity have been assessed in controlled lab setting using kits provided by the manufacturer from the batch mentioned above using ….. sample. Results should not be extrapolated to other sample types.)

DISCLAIMERS

1. This validation process does not approve / disapprove the kit design
2. This validation process does not certify user friendliness of the kit / assay

Note: This report is exclusively for ……Kit (Lot No……), version ……with the gene targets ……...manufactured by …… (Supplied by …….).

Evaluation Done on ……

Evaluation Done by …….

Signature of Director/ Director-In-charge …… Seal ……

**********End of the Report**********