ICMR-CDSCO/IVD/TB/PROTOCOLS/2025

Indian Council of Medical Research and Central Drugs Standard Control Organization
Department of Health Research and Drugs Controller General of India
Ministry of Health and Family Welfare
Government of India
Document No.: ICMR-CDSCO/IVD/TB/PROTOCOLS/2025

Subject: Inviting comments on standard IVD evaluation protocols drafted by ICMR and CDSCO

Licensure of In-Vitro Diagnostics (IVDs) under Medical Devices Rules 2017 requires a detailed evaluation protocol for the performance evaluation of IVDs to evaluate their quality and performance. To facilitate this process, the Indian Council of Medical Research (ICMR) and CDSCO have come together to draft standard evaluation protocols for use by IVD manufacturers testing labs in India. Currently, the following protocols for Tuberculosis have been developed by ICMR and CDSCO:

1. Analytical Performance Evaluation of In-Vitro Diagnostics for Pulmonary Tuberculosis
2. Clinical Performance Evaluation of In-Vitro Diagnostics for Pulmonary Tuberculosis
3. Clinical Performance Evaluation of In-Vitro Diagnostics for Pulmonary Drug Resistant Tuberculosis

These protocols are now being placed in the public domain for comments from relevant stakeholders. This window of opportunity will close on 7th **September 2025**, and, once finalized, there will be minimal scope for change in these documents. Therefore, all interested stakeholders are requested to provide their comments before 7th **September 2025**, at ivdevaluation@gmail.com as per the enclosed format. Once the public consultation period concludes, all comments will be reviewed and considered in finalizing the draft protocols before final clearance by ICMR and CDSCO.

Dated: 27th August 2025
Place: New Delhi

STANDARD IVD PERFORMANCE EVALUATION PROTOCOL

STAKEHOLDER FEEDBACK FORM

S.N.	Name of the Protocol	Document No.	Page No.	Line No.	Current Text	Proposed Text	Explanation/Reference

Name: _____

Designation and Affiliation: _____

ICMR-CDSCO STANDARD PERFORMANCE EVALUATION PROTOCOLS

ANALYTICAL PERFORMANCE EVALUATION OF IN-VITRO DIAGNOSTICS FOR PULMONARY TUBERCULOSIS

ICMR-CDSCO/IVD/TB/PROTOCOLS/1/2025

DIVISION OF COMMUNICABLE DISEASES, ICMR
IN VITRO DIAGNOSTICS DIVISION, CDSCO
AUGUST, 2025
India

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Analytical 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 analytical performance characteristics of the in-vitro diagnostic test in detecting pulmonary tuberculosis and drug-resistant tuberculosis.

II. Purpose

To evaluate the performance characteristics of nucleic acid amplification tests (NAAT) forthe diagnosis of pulmonary Mycobacterium tuberculosis (MTB)using irreversibly de-identified leftover archivedor spiked sputum samples.

III. Study Design

Analytical validation of IVD using irreversibly de-identified leftover clinical/spiked samples.

IV. Ethical Considerations

1. Leftover sputum specimens collected for routine diagnostic evaluation from patients who are suspected of having TB shall be used. No additional specimens should be requested.
2. The probability of harm or discomfort anticipated in the research is nil or not expected.
3. 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.
4. Investigators are required to submit a self-declaration form, as outlined in the ICMR guidelines, to the institutional authorities and ethics committee for information.
5. The protection of privacy of participants should be ensured by using de-identified samples and encrypting the patient identifiers.
6. Respect for the dignity of participants shall be prioritized.

V. Blinding of Laboratory Staff

To ensure the 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 member 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 maintain the database of results. Staff performing the reference test and the test under evaluation, 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. The data should be analyzed only by the PI of the evaluating lab. Refer to Fig. 1.

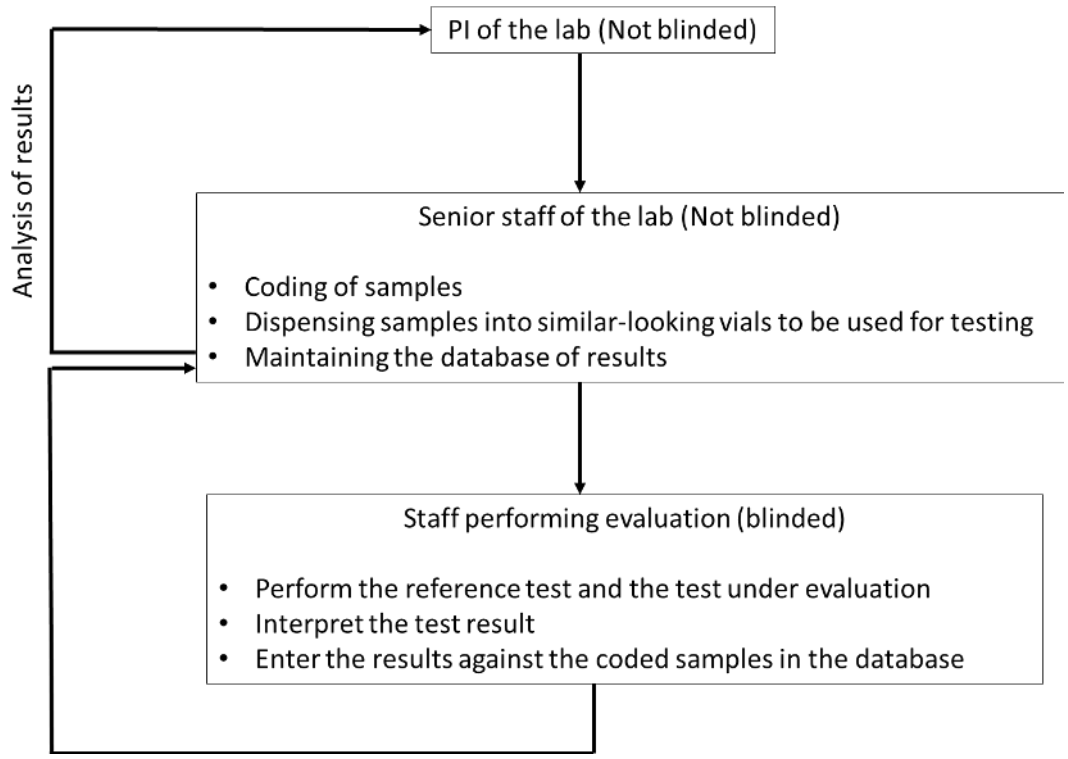


Figure 1 Blinding in evaluation exercise

VI. Procedure

1. Preparation of Evaluation sites/laboratories

A. The laboratory must be approved by the National TB Elimination Program (NTEP).

B. 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].

2. Exclusion

- Extra-pulmonary samples
- Specimens with > 1 freeze-thaw cycle (or according to IFU, if specified)
- Any exclusion criteria stated in the product IFU

3. Reference tests

- ***For detection of MTB:*** Mycobacterium Growth Indicator Tubes (MGIT) liquid culture.
- ***For MDR-TB:*** MGIT drug sensitivity testing (DST)

4. Preparation of samples

- ***For LOD studies - MTBC-negative sputum:*** smear-negative and NAAT-negative sputum should be used for the spiking analytic studies
- ***For analytical sensitivity and specificity:*** Well characterized archived samples (sputum or processed sputum); MTB positives, MTB negatives and Non-Mycobacterium tuberculosis (NTM) samples confirmed by liquid MGIT culture
- ***For drug sensitivity:***MTB and NTM clinical isolatesthoroughly characterized through MGIT DST and sequencingshould be used.
- For inclusivity/exclusivity, resistance detection, and cross-contamination, mycobacterial strains should be diluted into 7H9 medium at the required concentrations.
- The concentrations (cfu/mL) should be estimated by adjusting the bacterial suspension density to the McFarland standards.

5. Reference Strains

The National Institute for Biological Standards and Control (NIBSC) internal reference standard for Mycobacterium tuberculosis (H37Rv) DNA for Nucleic Acid Amplification Test (NAAT) based assays (NIBSC code: 20/152) will be used for the LOD assay.It was established as the 1st WHO International Standard for Mycobacterium tuberculosis (H37Rv) DNA for NAAT-based assays in 2021. The intended uses of this material are for calibration of secondary or in-house reference materials used in the assays for the molecular detection of M. tuberculosis DNA.It may also be used for assay validation and monitoring the limit of detection of rapid diagnostic tests. This preparation contains an arbitrary unitage of 6.3 log10 (or 2 million) IU per vial.

6. Sample size and sample panel composition

With an anticipated sensitivity of 90% and relative precision of 7%, a minimum of 87 confirmed MTB positive samples by MGIT culture will be required for testing analytical sensitivity. With an anticipated specificity of 95% with 5% relative precision, the minimum sample size required for analytical specificity is 81 confirmed MTB negative samples by MGIT culture. To rule out NTM detection, with an assumed sensitivity of 90% and relative precision of 10%, around 50 confirmed NTM samples may be included to evaluate the index test kit. Hence, approximately 100 confirmed MTB positives, 100 confirmed MTB negatives and 50 NTM samples will be used for pre-validation studies.

The proposed evaluation study will be done using Sputum/MTB isolates stored at the biobank facility of the National TB reference laboratories (NRLs) or the pre-validation labs. The stored sputum/MTB isolate/processed sample/DNA samples will be of the following categories and sub-categories.

Category 1: Positive for MTB by MGIT culture (N = 100)

Category 2: Negative for MTB by MGIT culture (N = 150)

Within the MTB negative group, we propose the following two sub-categories:

i. Negative for all Mycobacteria (N = 100)
ii. Positive for Non-Tuberculous Mycobacterium (N = 50)

Category 3: If resistance detection has to be carried out, within the MTB positive group, we propose to use the following sub-categories:

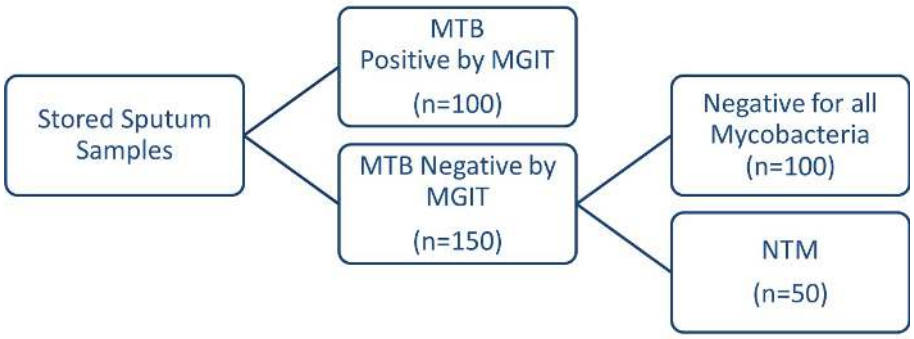
i. Sensitive to Rifampicin and Isoniazid, individually and combined (N = 100) confirmed by Drug susceptibility testing on MGIT liquid culture.
ii. Resistance to both Rifampicin and Isoniazid (N = 100) as detected by Drug susceptibility testing on MGIT liquid culture.
iii. Isoniazid mono-resistance (N = 45) as detected by DST on MGIT liquid culture.
iv. Fluroquinolone resistance (N=45) (If applicable for the index test) as confirmed by DST on MGIT liquid culture.

Table 1: Sample size calculation with 95% confidence level

Anticipated Sensitivity	Relative Precision	Sample size
90%	5%	171
90%	10%	43
90%	7%	87
95%	5%	81
95%	10%	20
95%	7%	41

Analytical sensitivity and specificity:

a)



b)

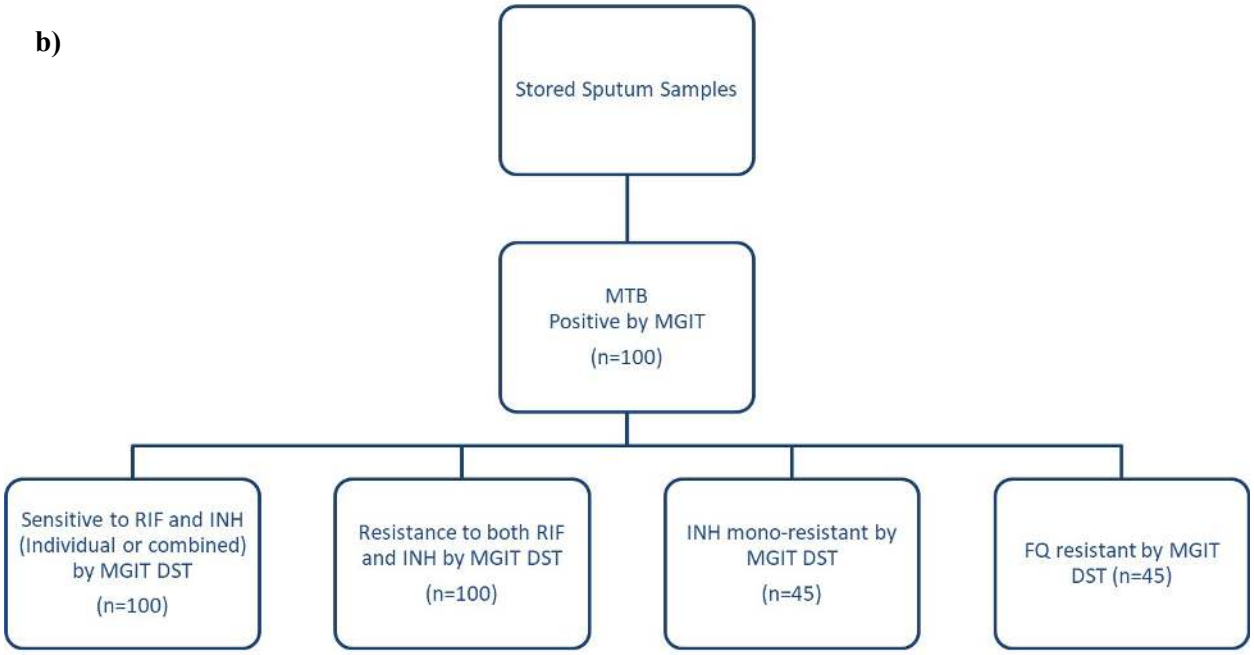


Figure 2. Flowchart for Analytical Validation for detection of; a) MTB detection, b) MDR-TB

7. Limit of Detection (LOD) Assay

The 95% LOD is defined as the minimum concentration of bacterium, expressed as CFU/ml or genomic DNA copy numbers/mL, in a sample volume that can be detected in 95% of tests. Finalize the LOD at least one concentration with a hit rate above 95% and two concentrations with hit rates between 10% and 90%.LOD should be always done with NIBSC H37Rv (20/152) standard and only reported in IU/ml or CFU/ml

Preparation of samples for LOD

1. The volume of sputum required for LOD is based on the IFU (Instruction for use) from the index test manufacturer, which generally varies between 1-2 ml of sputum.
2. A minimum of 200ml of NAAT negative sputum is required for the full LOD studies for a single index test.
3. Sputum samples which are negative by Smear and GeneXpert will be stored at -20C and once the required amount is obtained the samples will be pooled and tested for MTB using molecular and phenotypic test to prove no growth of MTB in the pooled samples.
4. To perform the assay it may take two weeks to one month based on the multiplicities of test suggested in the IFU after the required volume of sputum is collected.

Spiking of sputum samples

1. The spiked sputum will be used *to determine th*e LOD of the test kit. About 1.8 ml of negative sputum *specimen will* be spiked with 200 ul of the respective diluted suspension series of *M. tuberculosis* H37Rv.
2. These dilutions will be added to the sputum to get the final concentration (10000, 1000, 100, and 10 IU/ml). Before spiking, the culture for CFU wil*l be set up for* the different dilutions.
3. NIBSC reference standard will be reconstituted as directed by NIBSC using 1 mL nuclease free molecular biology grade purified water (MBGPW). From this stock 100 µL will be diluted ½ to get 10,00,000 IU/ml and serially diluted to give 100000, 10000, 1000 and 100 IU/ml with MBGW.
4. Each dilution of the WHO International Standard, will be tested 24 times. The 24 replicates will be performed over at least three days by at least two users and, for low-throughput instruments, on at least three different instruments, or sets of instruments if applicable (e.g.,

DNA preparation and amplification instruments). For low through-put instruments, the number of testing days may be increased.

5. Each lot shall comprise different production (or manufacturing, purification, etc.) runs of critical reagents. Inter-lot variation must be evaluated by appropriate statistical means.
6. Lowest dilution at which the test detects M.t*b will be deter*mined a LOD, the corresponding CFU will also be counted and reported in terms of CFU/per ml. The LOD will be presented as IU/mL for each dilution.
7. Analytical sensitivity shall be estimated by determining the 95% LOD with 95% confidence intervals (e.g., by probit analysis).
8. If there are more than four invalid results with the same specimen (i.e. dilution) overall, then the specimen will be retested to get at least 20 valid results for each dilution. For tests that include a claim for drug resistance testing, at least 20 valid results (i.e., sensitive or resistant) for each of the claimed drugs should be obtained for each dilution.
9. To arrive at the LOD a probit analysis should be performed, Probit analysis is defined as a specialized form of regression analysis applied to binomial response variables, transforming a concentration-response curve into a straight line for analysis through methods like least squares or maximum likelihood regression. It is primarily used in molecular biology measurement procedures, such as PCR, to determine the detection probability of analytes at various concentrations.

LOD for detection of drug resistance

1. To test the drug resistant MTB strains, well-characterized MTBC strains of known concentration (expressed as CFU/mL) shall be spiked into each claimed MTBC negative specimen type. DR strains shall be characterized by sequencing.
2. Relevant DR strains (as mentioned in table below) shall be spiked into each claimed MTBC-negative specimen type (e.g., raw and/or processed sputum, and each claimed extra-pulmonary specimen).
3. If the assay detects resistance to more than 1 target drug, the LOD for each target drug in addition to a composite resistance LOD, defined as the highest LOD among the tested target, shall be reported.

4. Analytical sensitivity for resistance detection shall be estimated as the lowest number of colony-forming units (CFU) per specimen that can be reproducibly distinguished from negative specimens with 95% confidence.
5. The determination shall comprise 24 replicate tests (8 replicate tests on each of 3 days) of a minimum 8 8-member 0.5log10 dilution panel. The replicate testing shall be conducted on three different days using 2 lots, and at least 2 dilution series shall be tested.

Table 2: Anti-mycobacterial drugs and common mutations

S.No	Drugs	Resistance mutation of strains to be tested
1	Isoniazid	katG_S315T and fabG1_c-15t
2	Rifampicin	rpoB_S450L; rpoB_D435V; rpoB_H445Y; rpoB_H445D; rpoB_D435Y; rpoB_S450W; rpoB_L452P; rpoB_H445L; rpoB_S450F; rpoB_L430P; rpoB_H445R; one rpoC mutation
3	Levofloxacin (CC) LFX2,3	gyrA_A90V, gyrA_D94G, gyrA_D94H, gyrA_D94N, gyrA_D94Y, gyrA_S91P
4	Moxifloxacin (CC and CB)	gyrA_A90V, gyrA_D94G, gyrA_D94H, gyrA_D94N, gyrA_D94Y, gyrA_S91P
5	Bedaquiline	Rv0678_LoF, pepQ_LoF, atpE_p.Ala63Pro
6	Linezolid	rplC_p.Cys154Arg, rrl_n.2814G>T
7	Ethambutol	embB_M306L, embB_M306V, embB_Q497R
8	Delamanid	ddn_LoF, ddn_p.Leu49Pro, fbiC_LoF
9	Pyrazinamide	pncA_ V139A, pncA_ V139G
10	Amikacin	rrs_ A1401G, rrs_ A1401G, rrs_G1484T, eis /promoter_ C-12T, eis /promoter_C-14T
11	Kanamycin	rrs_ A1401G, rrs_ A1401G, rrs_G1484T, eis /promoter_ C-12T, eis /promoter_C-14T
12	Capreomycin	rrs_ A1401G, rrs_ A1401G, rrs_G1484T, eis /promoter_ C-12T, eis /promoter_C-14T
13	Ethionamide	fabG1_c-15t, inhA_S94A, fabG1_ T-8C
14	Pretomanid[#]	ddn_LoF, ddn_p.Leu49Pro, fbiC_LoF
15	Cycloserine	Alr_C-8T, alr_M319T, alr_Y364D, ald_T-32C, ddlA T365A
16	PAS	thyA_T22A, folC I43T, folC R49W

8. Reproducibility

Within-run (same operator, same measuring system, same operating conditions, and same location), Between-run, -lot, -day, -site, -operator.

1. Three specimens will be used; MTB sensitive (H37Rv), MTB resistant and MTB negative.
2. The effect of operator-to-operator variation on IVD performance will be included as part of the precision studies. Each lot will comprise different production (or manufacturing, purification, etc.) runs of critical reagents.
3. The nucleic extraction/purification component will also be considered for estimating precision.
4. Contrived specimens will be used (i.e., MTBC strains with specific/most common mutations in the target genes spiked into a clinical matrix claimed in the IFU) for repeatability and reproducibility studies.
5. DR specimens at the concentrations specified for each DRTB (i.e. RR-TB, Hr-TB, MDR-TB, TB resistant to fluoroquinolones) as described in the table on resistance detection.
6. If there are two or more invalid results for the same specimen in the same run, then the run should be repeated for this specimen. Invalid results should be reported.
7. Results will be statistically analyzed by ANOVA or other methods to identify and isolate the sources and extent of any variance.
8. Furthermore, the percentage of correctly identified, incorrectly identified, and invalid results will be compiled for each specimen and separately categorized by site, lot, and other factors.

9. Inclusivity and exclusivity

1. Inclusivity MTBC stains: *M. bovis*
2. Exclusivity NTM strains: *M. avium, M. kansasii, M. intracellulare*
3. Representative MTBC and non-tuberculosis mycobacteria (NTM) strains will be tested in triplicate forinclusivity and exclusivity verification.
4. Resistance detection: For assays with a claim for detection of drug resistance, the applicable specimens from the resistancedetection panelwill be tested in triplicate.
5. The concentration of MTBC isolates used in inclusivity studies will be at levels at or near the specific LOD and will be confirmed by plating/ counting bacterial CFUs (estimated

using Truenat).

6. The selection of specific MTBC strains with relevant genetic variations linked to DR will be made to support the claims in the IFU.
7. This will involve testing strains that carry the most common mutations, including associated or interim resistance mutations, covering at least 80% of the resistance mechanisms observed globally for each of the assay target drugs (as shown in table 2).

10. Cross-contamination/carry-over

1. The experiment will allow the determination of the well-to-well or vial-to-vial cross-contamination rate ofhigh-throughput platforms or potential carryover in low-throughput instruments.
2. This will be assessed byalternating one high-positivespecimen with one negative specimen and repeating thissequence twenty times.
3. For high-throughput assays, this will be achieved by alternating high-positive andhigh-negative specimens in the same plate/run.
4. For low-throughput assays, each sequence of highly positivespecimens followed by negative specimens should be done on the same instrument.
5. If more than one instrument isused, each run (i.e same instrument and same day) should include a minimum of 2 sets of alternating high-positive and negative specimens.
6. Contrived specimens prepared by spiking MTBC strains into MTBC negative clinical sputum will be used for these studies.

> **Note:** The strains used for assessment of reproducibility, inclusivity/exclusivity, resistance detection, and carry-over may be commercially acquired or locally prepared, well-characterized strains (by phenotypic DST and sequencing).

11. Resolution of discrepancy:

- The results of MGIT culture should be used to resolve any discrepancy in detection of MTB
- Results of phenotypic DST and sequencing should be used to resolve discrepancy in detection of MDR-TB.

VII. Statistical Analysis Plan

1. The index molecular test should be evaluated for its analytical sensitivity and analytical specificity.
2. 95% Confidence interval should be calculated for each of the parameters.

$$\% \text{ Sensitivity} = \frac{\text{Positives by index test}}{\text{Confirmed positives by MGIT culture}} \times 100 = [a/a+c] * 100$$

$$\% \text{ Specificity} = \frac{\text{Negatives by index test}}{\text{Confirmed negatives by MGIT culture}} \times 100 = [d/b+d] * 100$$

VIII. Acceptance Criteria

Acceptance criteria for Diagnostic tests:

Expected sensitivity: ≥90%

Expected specificity: ≥95%

Sample Size: ~ 100 confirmed MTB positives (by MGIT culture), ~ 100 confirmed MTB negatives (by MGIT culture) and ~ 50 NTM samples (confirmed by culture and identification)

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

IX. Publication Rights

The PI(s) of the evaluating labs shall retain publication rights of the evaluation as lead author(s).

IMPORTANT NOTE

Once a kit is determined to be "Not of Standard Quality", following the procedure outlined in this document, no further requests for repeat testing of that kit will be accepted.

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%.

References

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PERFORMANCE EVALUATION REPORT FORMAT

Performance Evaluation Report For MTB/MDR-TB 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:

		Reference assay ……..…… (MGIT/MGIT DST for RIF/INH/FQ/others)		
		Positive	Negative	Total
Name of MTB or MDR-TB 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**********

ICMR-CDSCO STANDARD PERFORMANCE EVALUATION PROTOCOLS

FIELD PERFORMANCE EVALUATION OF IN-VITRO DIAGNOSTICS FOR PULMONARY DRUG RESISTANT TUBERCULOSIS

ICMR-CDSCO/IVD/TB/PROTOCOLS/3/2025

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IN VITRO DIAGNOSTICS DIVISION, CDSCO
AUGUST, 2025
India

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Field Performance Evaluation of IVD for Pulmonary DR-TB

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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 the in-vitro diagnostic test in detecting pulmonary drug resistant tuberculosis (DR-TB).

II. Purpose

To evaluate the clinical performance characteristics of nucleic acid amplification tests (NAAT) for diagnosis of pulmonary drug resistant tuberculosis (DR-TB) using prospectively collected sputum samples in clinical settings.

Primary Objectives

1. To determine the diagnostic accuracy of new multi-drug resistant (MDR) NAAT test against culture based drug sensitivity testing (DST) in detecting first line drug resistance [Rifampicin (RIF), Isoniazid (INH)] among the microbiologically confirmed TB patients (positive by smear or NAAT test).
2. To determine the diagnostic accuracy of new NAAT test against culture-based drug sensitivity testing (DST) in detecting fluroquinolone (FQ) drug resistance among MDR-TB/ RR-TB pulmonary tuberculosis patients

III. Study Design

Cross-sectional prospective multi-centric diagnostic accuracy study of IVD for detection of pulmonary drug resistant TB, using Mycobacterium Growth Indicator Tube culture and drug sensitivity testing (MGIT-DST) 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 (GeneXpert MTB/RIF, MGIT DST, LPA 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 evaluation study data should be analyzed only by the PI of the evaluating lab (Fig. 1).

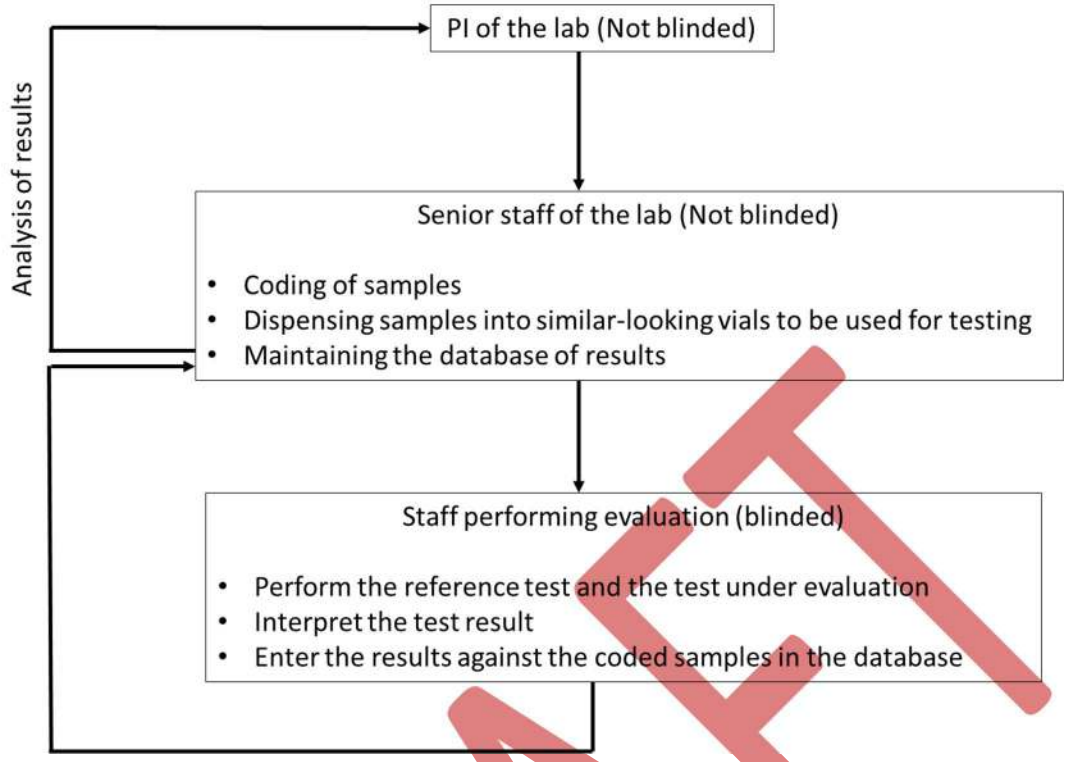


Figure 1 Blinding in evaluation exercise

VI. Procedure

1. Preparation of Evaluation sites/laboratories

- Laboratory must be approved by the 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

i. ***For First Line Drug Resistance:*** People with microbiologically confirmed pulmonary TB by smear and/or NTEP approved NAAT test attending hospital OPDs/Chest clinics/district microscopy centers (DMCs) and Directly Observed Therapy Short Course (DOTS) centers.

ii. ***For Second Line Drug Resistance:*** All patients with microbiologically confirmed MDR-TB/RR-TB (RIF resistant TB by NAAT test) attending the hospital OPDs/Chest clinics/DMCs/DOTS centers

All such consecutive cases (not currently receiving ATT) and willing to provide consent should be enrolled in the study.

3. Eligibility of Participants

Inclusion criteria for testing First Line Drugs

i. Individuals positive for TB by smear or any approved NAAT test (Xpert® MTB/RIF) and not receiving ATT

ii. Individuals willing to give consent

iii. Individuals who are able and willing to give two good quality mucopurulent sputum samples of ≥ 3 ml

Inclusion criteria for testing Second Line Drugs

i. All patients with microbiologically confirmed MDR-TB/RR-TB (RIF positive by NAAT test)
ii. Individuals who are able and willing to give two sputum samples of ≥ 3 ml

Exclusion criteria

i. Individuals on TB treatment for >10 days
ii. Individuals not consenting for the study
iii. Individuals unable to produce two sputum samples of ≥ 3 ml

4. Reference and Index tests

	Index test	**Reference Test**	**Comparator**
First Line Drug Resistance	New NAAT test for RIF/INH	MGIT Culture DST for RIF and INH	FL-LPA: GenoType MTBDRplus
Second Line Drug Resistance	New NAAT test for FQ	MGIT Culture DST for Moxifloxacin (0.25, 1 mg) and Levofloxacin (1 mg)	SL-LPA: GenoType MTBDRsl

5. Sample size

Sample size for RIF and INH resistance among TB patients

The expected sensitivity of the index test is about 90% with 5 % precision and the expected specificity is 95% with 5% precision. With a confidence interval of 95 % and assuming 10 % loss due to indeterminate results, the sample size required is estimated to be approximately **200** patient's positive each for INH and RIF resistance either alone or in combination. The average prevalence of Isoniazid and Rifampicin are ~18 % and 7.3 % respectively, among the new and previously treated TB patients combined together (Report of drug resistance survey, 2014-16). The number needed to screen to obtain 200 drug resistant cases will be approximately 1111 for INH resistance and 2857 for RIF resistance. The participants will be enrolled till the required sample size is achieved for INH and RIF resistance.

Sample size for FQ resistance among MDR/RR TB patients

The expected sensitivity of the index test for detecting FQ resistance is 90 % with 5 % precision and the expected specificity is 95 % with 5 % precision. Assuming 10 % loss, the sample size required is 200 FQ resistant cases. The prevalence of FQ resistance among MDR/RR TB patients is 20 % (Report of drug resistance survey, 2014-16). Hence, the number needed to screen will be approximately 890. The participants will be enrolled till the required sample size is achieved for FQ resistance. Table 1 shows sample sizes required for RIF, INH and FQ drug resistance.

Table 1. Sample sizes for RIF, INH and FQ Drug Resistance

	Assumptions for Sensitivity	Assumptions for Specificity
Sensitivity/Specificity of the new test (%)	90	95
Relative precision (d) (%)	5	5
Desired confidence level (1- alpha) %	95	95
Number of drug resistance (INH and RIF) cases required	178	84
Number of drug resistant cases required with 10 % loss due to indeterminate results	~200	~93
Number needed to be screened assuming a combined weighted average prevalence of ~18 % for INH resistance among the new and previously treated TB patients	1111	517
Number needed to be screened assuming a combined weighted average prevalence of ~7 % for RIF resistance among the new and previously treated TB patients	2857	1329
Number needed to be screened considering a prevalence of 20 % for FQ resistance among MDR/RR TB	890	465

Other disease controls (to check cross-reactivity in real patients)

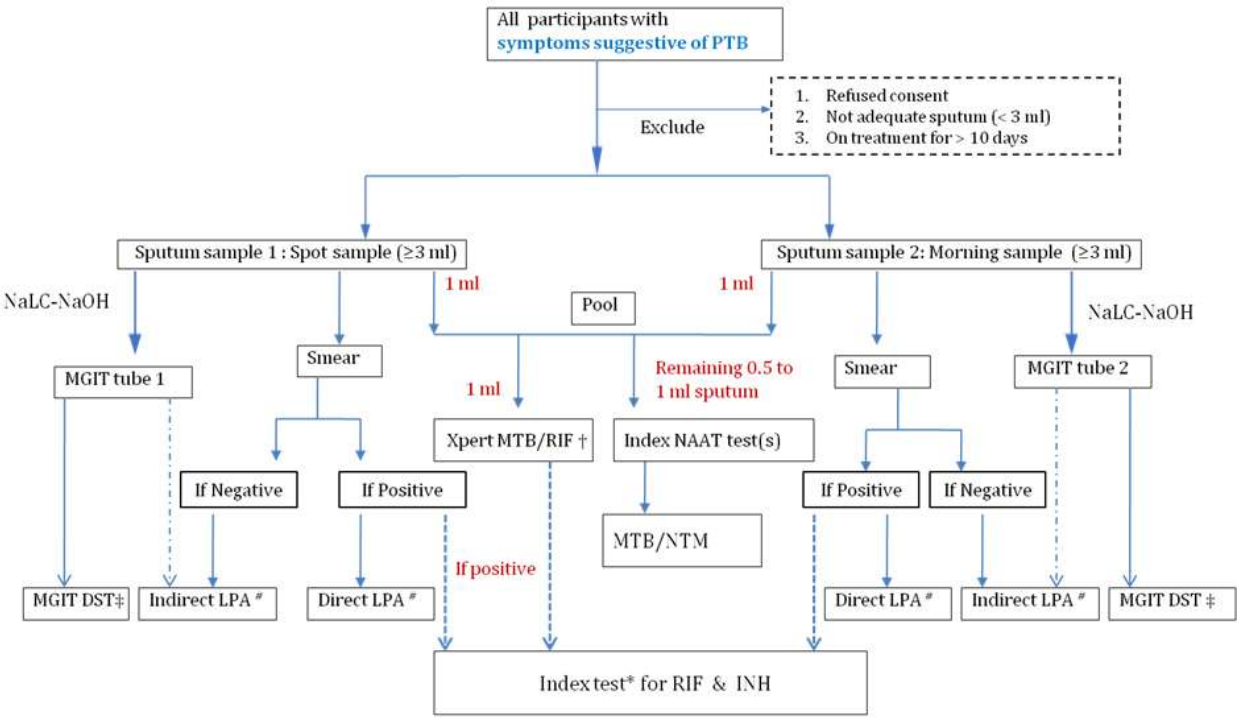
Include people with common alternative diagnoses to mirror programmatic reality and probe false positives. This subset helps characterize clinical exclusivity beyond simple “TB-negative” status:

i. Non-Tuberculous Mycobacteria (Culture or PCR confirmed): ~30

ii. Other respiratory diseases [e.g., bacterial pneumonia, chronic obstructive pulmonary disease (COPD), lung cancer, chronic fungal (like Histoplasmosis or Aspergillosis)]: ~30 patients combined.

6. Implementation Plan

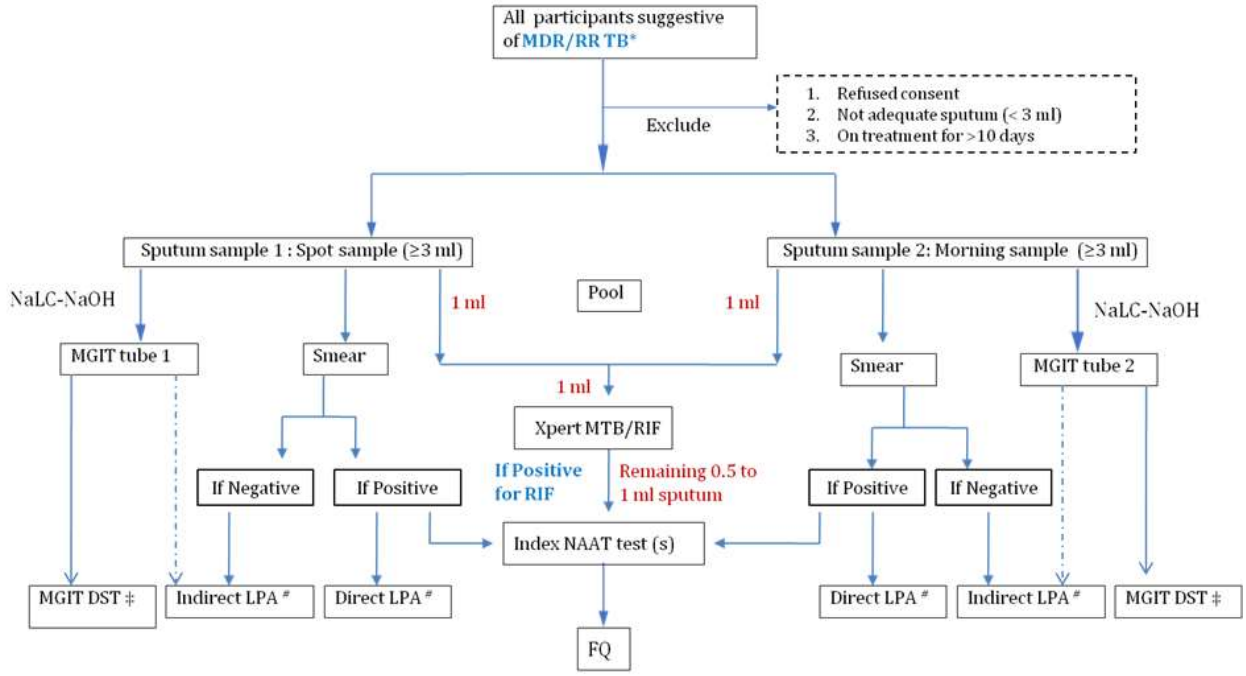
The samples will be collected and tested as per the routine practice for smear, Xpert MTB/RIF®, LPA, MGIT culture and DST. The samples with positive result for MTB either in smear or NAAT test should be tested for first line drug resistance (RIF and INH). The samples that are positive for MDR/RR (positive for rifampicin resistance by NAAT test) should be used for testing drug resistance for second line drugs.



* ***Index test RIF and INH:*** *Samples tested positive by either smear or Xpert will be tested by Index test for drug resistance (DR cartridge) - RIF & INH*
*# **LPA**: Any one positive sample will be used for LPA- Direct LPA if smear positive and indirect LPA if smear negative and culture positive.*
‡ ***MGIT DST***: Any one positive culture (tube 1 or 2) will be used for DST

Storage: *Leftover sputum samples and DNA elutes to be stored at -20°C, One positive culture and two decontaminated sediments per patient will be stored at -80°C for later use*

Figure 2. Flowchart for evaluating IVDs for testing drug resistance to RIF and INH among pulmonary TB (PTB) patients



*Enrolment in the study: MDR/RR TB- Positive for Rif by NAAT test
LPA: Any one positive sample will be used for LPA- Direct LPA if smear positive and indirect LPA if smear negative and culture positive.
‡ MGIT DST: Any one positive culture (tube 1 or 2) will be used for DST

Storage: Leftover sputum samples and DNA elutes to be stored at -20°C, One positive culture and two decontaminated sediments per patient will be stored at -80°C for later use

Figure 3.Flowchart for evaluating IVDs for testing drug resistance to FQ among MDR/RR TB patients

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. All smear positive or NAAT positive samples will be tested by Line Probe Assay (LPA).
6. The resultant deposit should be used for inoculation into two MGIT960 tubes.
7. 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).
8. The positive cultures should be tested for drug sensitivity.
9. All sputum samples should be stored at -20ºC for later use. Decontaminated sediments and one positive culture per patient should be stored at -80ºC, if necessary for later use.
10. Two DNA samples (one DNA sample extracted for index test and one for LPA) per patient should be stored at -20ºC till the end of the study for resolution of discrepant results.
11. 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. MGIT drug sensitivity testing (DST) for Rif, INH: Drug sensitivity testing will be carried out from any one positive MGIT culture.
iv. MGIT drug sensitivity testing for moxifloxicin (0.25 mg and 1 mg) and levofloxacin (1 mg). Drug sensitivity testing should be carried out in from any one positive MGIT culture.
v. Speciation of culture: Rapid immunochromatographic test (ICT) of MGIT culture
vi. LPA: LPA shall be carried out as per routine practice and as per NTEP guidelines. Direct LPA should be carried out from any one smear positive sample. If the sample is smear negative and culture positive, indirect LPA should be carried out from culture. First line LPA (FL-LPA) will be carried out (Rif and INH resistance)
vii. XpertMTB/RIF (one test per patient)

9. Index test

i. Index test will be performed as per manufacturer's instructions following blinded study protocols.
ii. At least 2 different lots of reagents should be tested across the study population to demonstrate consistency of test performance and minimize lot-related bias.
iii. The results of the index test will not be disclosed to study participants or clinicians and will not be used to guide treatment decisions.

10 . 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. All Invalids/Indeterminates/errors should be recorded and reported.
ii. Results for new patients and previously treated patients should be entered separately. Result analysis will be carried out for these two populations separately as well as combined.
iii. A subgroup analysis may be carried out for pediatric population.

11. Quality Control (QC) measures

All sites should ensure high quality 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.

Drug sensitivity testing (DST): Standard ATCC strains should be used for each drug as reference control. QC should be performed whenever a new batch of drugs is prepared, after servicing of the instrument and after long gap of setting up DST.

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.

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 will be evaluated for its performance with reference to MGIT DST (for RIF/INH/FQ).

iii. Similarly, the performance of NTEP approved molecular test (Xpert MTB/RIF and LPA) should be estimated with reference to MGIT DST.

iv. The agreement between the index test and molecular test for drug resistance (LPA) should be calculated using kappa statistic.

VIII. Acceptance Criteria

Expected minimal sensitivity for MTB and Drug Resistant TB: ≥85 ± 2%

Expected minimal specificity for MTB and Drug Resistant TB: ≥95 ± 2%

Sample size: ~200 positives for each drug resistance (RIF or INH or FQ etc) (either alone or in combination) and ~ 100 negatives for each drug resistance (RIF or INH or FQ etc).

IMPORTANT NOTE

Once a kit is determined to be "Not of Standard Quality", following the procedure outlined in this document, no further requests for repeat testing of that kit will be accepted.

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%.

References

1) Report of the first national anti-tuberculosis drug resistance survey India, 2014-2016.
2) Technical and operational guidelines for tuberculosis control in India 2016. Central TB Division.
3) RNTCP Standard Operating Procedures for Tuberculosis lab for culture and DST, 2009.
4) Standard Operating Procedures (SOP) for Mycobacteriology laboratory, ICMR-NIRT, 2010.
5) Practical manual on tuberculosis laboratory strengthening, 2022 update. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO.
6) Mycobacteriology laboratory manual, Global laboratory initiative, First edition, April 2014, Stop TB Partnership.

PERFORMANCE EVALUATION REPORT FORMAT

Performance Evaluation Report For MDR-TB 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				
	Number of samples tested	**Sensitive**	**Resistant**	
FL LPA – RIF				
FL LPA - INH				

SL LPA- FQ				
MGIT-DST- RIF				
MGIT-DST-INH				
MGIT-DST-FQ				
New IVD- RIF				
New IVD-INH				
New IVD-FQ				

		Reference assay ……..…… (MGITDST – RIF/INH/FQ)*		
		Positive	Negative	Total
Name of MDR-TB kit	Positive			
	Negative			
	Total			

	Estimate (%)	95% CI
Sensitivity		
Specificity		

***Report RIF/INH/FQ as separate tables**

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 Nos.……), 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**********

ICMR-CDSCO STANDARD PERFORMANCE EVALUATION PROTOCOLS

FIELD PERFORMANCE 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
AUGUST, 2025
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.

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.
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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.
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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.

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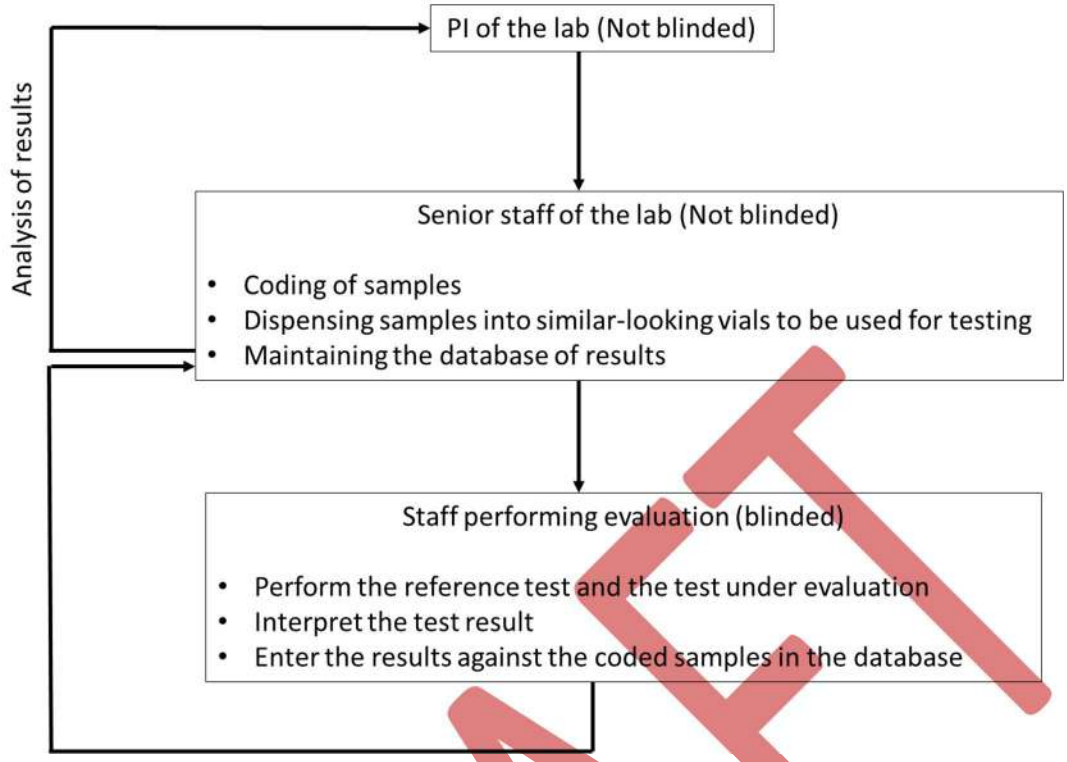

Figure 1 Blinding in evaluation exercise

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

198 ## 6. Implementation Plan

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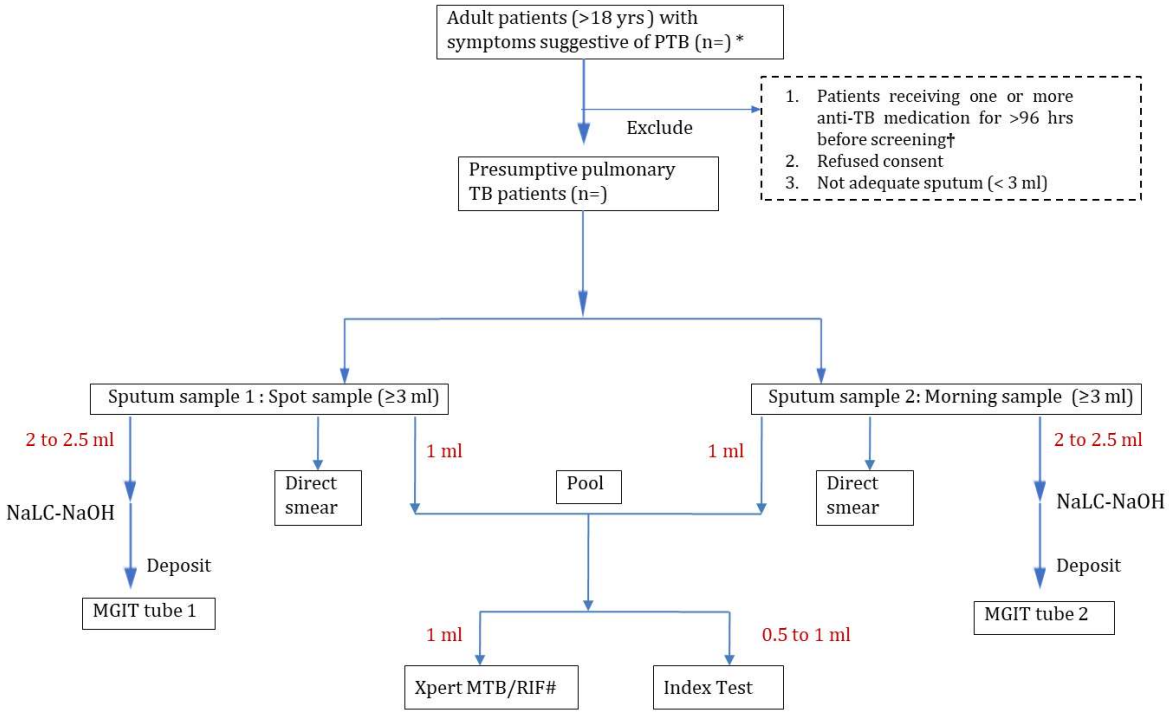

* ***Screening***: *Medical history & clinical examination as per NTEP guidelines*
† To ensure that dead bacilli are not detected and no treatment failure cases are enrolled
*# **Comparator**: Xpert MTB/RIF*
Storage: *One positive culture and 2 decontaminated samples per patient stored at -80°C for later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

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

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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 -20ºC for later use. Decontaminated sediments and one positive culture per patient should be stored at -80ºC, if necessary for later use.
8. Two DNA samples per patient should be stored at -20ºC 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 immunochromatographic 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

Expected sensitivity: ≥85 ± 2%

Expected specificity: ≥95 ± 2%

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

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%.

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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4. RNTCP Standard Operating Procedures for Tuberculosis lab for culture and DST, 2009.
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**********