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

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 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).

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 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 drug resistance (FQ) among the microbiologically confirmed TB patients (positive by smear or NAAT test).

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°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).

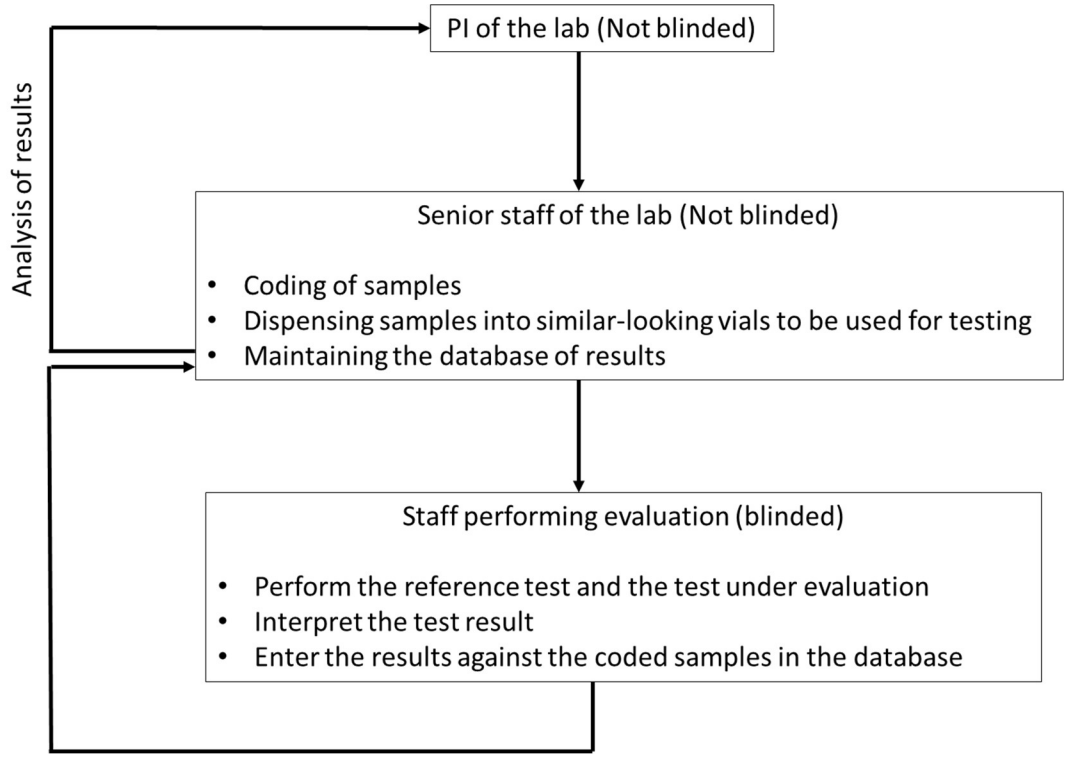


Figure 1 Blinding in evaluation exercise

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

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. 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

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.

The expected sensitivity of the index test for detecting FQ resistance is 85 % with 7 % precision and the expected specificity is 95 % with 5 % precision. Assuming 10 % loss, the sample size required is 111 FQ resistant cases. The prevalence of FQ resistance among TB patients is ~3 % (Report of drug resistance survey, 2014-16). Hence the number needed to screen will be approximately 3333. 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

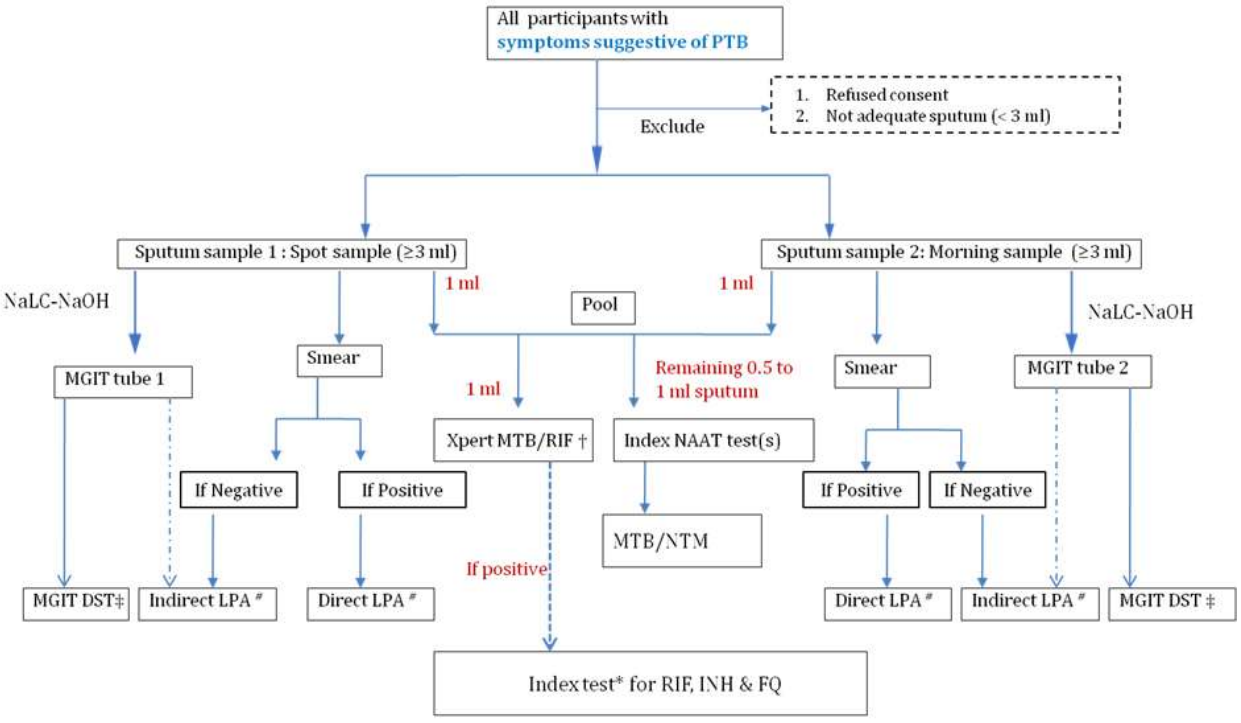
Other disease controls (to check cross-reactivity in real patients)

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 and second line drug resistance (RIF, INH and FQ).



** **Index test RIF and INH:** Samples tested positive by either smear or Xpert will be tested by Index test for drug resistance*
*$ **Index Test**: New NAAT Test under evaluation*
*# **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, INH and FQ among pulmonary TB (PTB) 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 -20°C for later use. Decontaminated sediments and one positive culture per patient should be stored at -80°C, 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 -20°C 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. Xpert MTB/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%.

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

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**********