Target Product Profile (TPP) for an Open Real time Polymerase Chain Reaction (RT-PCR) Test for Tuberculosis

Introduction & rationale

Target product profiles (TPPs) are strategic reference documents that are intended to facilitate and accelerate the development of medical products and devices that address the greatest and most urgent public health needs in India. Therefore, the overall objective of this TPP is to align the performance and operational characteristics of open RT-PCR kits to decrease the diagnostic gaps in the TB care cascade in India. ICMR will review the minimum criteria as the lowest acceptable output for any open PCR kit to initiate systematic assessment of these kits.

The target audience comprises test developers and manufacturers interested in entering the TB diagnostic market, regulatory agencies, academia, research institutions, product development partnerships, funders, nongovernmental organizations (NGOs), civil society organizations and donors.

What is open RT-PCR?

For the purpose of this TPP, open RT-PCR is defined as a TB and/or TB-DR (drug-resistance) detection in-vitro diagnostic which is design locked, standardized, commercially procurable with manufacturing license from CDSCO designed for use on multiple validated real-time PCR thermocyclers rather than being limited to a single proprietary or locked PCR platform. Compatibility on multiple thermocyclers should be demonstrated by the manufacturer through appropriate validation data and compliance with the national performance requirements. Open PCR platforms leverages on the existing capacities which are scaled up throughout the country during COVID pandemic.

Note: This document does not define or prescribe software requirements for open RT-PCR assays, recognizing that not all manufacturers will provide dedicated software alongside their test kits. However, where manufacturers choose to develop software for result interpretation, it is important that the user interface is interoperable with the range of real-time thermocycler platforms commonly available and recommended within the country. Such software should support automated result analysis and linkage to Nikshay, provide tools for recording, reviewing, and documenting Ct values, amplification curve, semi-quantitative bacilli load and other relevant assay parameters, and include data analytics capabilities to facilitate quality monitoring. In addition, it should incorporate quality control features, such as early alerts or triggers for potential reagent degradation, control failures, assay drift, contamination events or other indicators of compromised test performance, to support reliable and consistent interpretation of results.

Characteristic	Minimal	Preferred	Notes/comments
Scope			
Document Goal	To provide characteristics for open PCR–based diagnostic kits for rapid detection of Mycobacterium tuberculosis complex (MTBC) in presumptive TB (TB) .patients in India.	To provide characteristics for open, PCR based diagnostic platform and kits for rapid detection of TB and differentiate it from non-tuberculous mycobacteria (NTMs) along with drug resistance testing in India to support Universal Drug Susceptibility Testing (UDST) and early treatment initiation.	This document presents the Target Product Profile (TPP) for diagnostic kits using open PCR technology. For additional classes of molecular technologies, refer to the WHO TPP for tuberculosis diagnosis and drug resistance detection (2024).
Intended use	Detection of MTBC	Detection of MTBC, and differentiate it from NTM and resistance to prioritized anti-TB drugs as mentioned below in the order of decreasing priority: a. Rifampicin (RIF) b. RIF + isoniazid (INH) c. RIF + INH+ Fluoroquinolones (FQ) +Bedaquiline (BDQ) d. Pyrazinamide (PZA) + Linezolid (LZD) +Pretominid/ Delaminid (Pa/DLM) +Clofazamine (CFZ) e. Amikacin (AMK) +Cycloserine f. Any additional drug listed in the Indian treatment guidelines	In the Indian TB testing algorithm, we have MTB and RIF detection upfront. It would be ideal that at the beginning we have a manufacturer with MTBC and NTM detection with a possibility of RIF detection in the same run or as a reflex test The ideal test would be to have a single test with MTBC detection along with drug resistance targets; however, we do acknowledge it might increase complexity and the cost per test. This trade-off will be assessed on number of targets, added benefit and the cost of the test.
Target population	Adults and adolescents presumed to have pulmonary TB, irrespective of HIV status	Adults, adolescents, and children of all ages presumed to have PTB or EPTB disease, irrespective of HIV status	Diagnosis of tuberculosis in children and individuals with extrapulmonary TB is crucial because these cases are typically paucibacillary and represent a vulnerable high-risk group population.

Target user of test	Trained laboratory personnel or healthcare workers with moderate molecular diagnostic skills, including nucleic acid extraction, precision pipetting, and PCR operation.	Minimally trained laboratory personnel or healthcare workers using simplified open molecular workflows with automated extraction, assay setup, and result interpretation.	Open PCR testing for TB will be introduced in laboratories that previously conducted viral testing during the COVID pandemic. Therefore, procedures should be simple to ensure easy adoption in these facilities.
Target interpreter of the test	Microbiologist (MD/PhD)	Microbiologist (MSc/ MD/PhD)	Interpretation and understanding of Ct values, thresholds etc. require trained people to assess and analyze the results of the PCR
Setting (level of the Healthcare system)	Medical college laboratories, Intermediate Reference Laboratories (IRLs), culture and drug-susceptibility testing (CDST) labs	Mobile diagnostic units, and designated microscopy centers where there is a designated workspace to create ecosystem for this RT-PCR testing District Hospitals, Integrated Public Health Laboratories (IPHL), Block Public Health Laboratory (BPHL) with designated workspace to create ecosystem for RT- PCR testing	Do not use it in settings that do not have working space for PCR testing or do not have trained personnel as it could cause cross-contamination or invalid results.
Pricing and Market Access			
Cost per test **Notes- to have a list of commonly available equipment like dry bath, vortex etc.**	≤ 250 INR‡ for MTB detection and ≤ 450 INR for drugs *If there are ancillary equipment like a sonicator or lysis device which is kit specific then the cost per test could be ≤ 350 INR for depending on number of drugs included in assay*	≤ 200 INR for MTB detection only while <400 INR for MTB plus DR detection depending on number of drugs included in assay	The costs for drug resistance would depend on the number of drugs included in the test and the targets per drug included. The cost mentioned here refers to the cost per test, including the costs of reagents, consumables, any ancillary equipment needed for running the tests but excluding the price of shipping and import, and any ceiling price (i.e. a

			price control imposed by a government). It is important to note that these costs are indicative and not rigid estimates.
Regulatory requirements	Manufacturing and quality systems should comply with ISO 13485 and applicable IVD regulatory standards. Assays and platforms should be licensed by Central Drugs Standard Control Organization (CDSCO) under applicable Medical Device Rules and preferably validated under the programmatic conditions in India.	The product should obtain WHO prequalification, clearance, or certification from a stringent regulatory authority (SRA) or founding member regulatory authority of the International Medical Device Regulators Forum (IMDRF). The platform should be licensed by Central Drugs Standard Control Organization (CDSCO), demonstrate interoperability, quality assurance compatibility, and suitability for implementation under programmatic conditions in India.	
Performance			
Diagnostic sensitivity	Sputum: ≥85%[1] Non Sputum: 80%	≥ 95%[1]	*Reference standard here is MGIT culture.* *The sensitivity of the test should be similar (±2%) or better than the currently recommended molecular test by the WHO when evaluated with culture as a reference standard.* *For example, if in the same clinical evaluation, Xpert MTB/RIF has a sensitivity of 87%, the acceptable*

			sensitivity of the open PCR being evaluated will be 85%-89%. Non sputum sensitivity might differ based on fluid specimen being tested. It is important to note that for NTEP integrated Diagnostic and Treatment algorithm, it is still using Xpert MTB/RIF and has not transitioned to Ultra which also impacts these estimates.
Sensitivity for drug resistance detection	≥85 %[‡] for rifampicin resistance and isoniazid resistance compared with reference standard.	≥ 95%[‡] for rifampicin resistance, ≥ 90% for isoniazid resistance, fluoroquinolone resistance.	Drug specific performance should be evaluated separately. Reference standard here is phenotypic DST. The sensitivity of the test should be similar (+/- 2%) or better than the currently recommended molecular testby the WHO when evaluated with culture as a reference standard.
Diagnostic Specificity	≥ 95%[‡]	≥ 98%[†]	Specificity of the test when compared with MGIT culture: The specificity of the index test should be similar (+/- 2%) or better than the currently recommended molecular tes tby the WHO when evaluated alongside culture as a reference standard. *For example, if in the same clinical evaluation, currently recommended NAAT has a specificity of 94%, the acceptable specificity of the open PCR being evaluated will be 92%-96%.*

Specificity for drug resistance detection	≥98†		Drug specific performance should be evaluated separately. Reference standard here is phenotypic DST. The specificity of the index test should be similar (+/- 2%) or better than the currently recommended molecular test by the WHO when evaluated alongside culture DST as a reference standard.
Non-actionable (Invalid/error results)	≤5%†	<1-2%	Invalid, error and rifampicin resistance-indeterminate results should be characterized and reported separately by the manufacturer including the underlying causes where identifiable. Invalid/errors non-actionable results in open TB PCR refer to tests where amplification fails or results cannot be reliably interpreted. Such results do not confirm or exclude TB and require repeat testing or alternative diagnostic methods.
Rifampicin (indeterminate results)	≤5%†	≤3%	Evaluate indeterminate samples among MTB-positive specimens in which rifampicin resistance is assessed where assay fails to provide a definitive rifampicin-susceptible or rifampicin-resistant result on the initial test. Rifampicin indeterminate rate is taken as a separate criterion because of its clinical importance as it may delay

			appropriate DR-TB management.
Carry over contamination	It is important for manufacturer to use mechanisms to reduce carry over contamination (e.g. use of dUTPs). Manufacturer should also conduct studies to assess any potential carry over contamination.		
Lot to Lot variability	Manufacturers should follow CLSI guidelines and conduct lot to lot variability testing		
Limit of detection	The analytical limit of detection (LoD) should be defined as the lowest concentration of the target analyte that can be reliably detected with a probability of at least 95% (LoD_{95}) in the intended specimen matrix, with a minimum of 24 replicate tests performed at the proposed LoD concentration to verify detection performance The test should have comparable limit of detection (LoD) of the currently recommended molecular tests for both MTB detection and claimed resistance detection.		This refers to difference in LOD between MTB detection and drug-resistance detection due to different target genes. In many cases, resistance prediction requires a higher LOD. Therefore, the minimum acceptable sensitivity for MTB resistance detection should be ≥60% in smear-negative/paucibacillary samples (TPP DST 2021).
Quality controls	<u>External controls</u> Positive and negative (no template controls) should be specified in the IFU and should be provided as an integral component of these kits in sufficient quantities for 1. Pre use verification of each lot 2. Use in each test run	<u>Minimal plus having internal controls</u> to assess for specimen adequacy as well.	Manufacturer should provide enough number of additional positive and negative controls with the test kits to ensure operationalization of the flexible loading of specimens.
Operational characteristics			

Multi-disease platform	Yes		This refers to the PCR platform and any ancillary extraction or sample preparation device should have functionality for other diseases- especially respiratory pathogens with similar symptoms as well.
Specimen type	Sputum	Sputum and Non—sputum specimens, preferably which can also be self-collected	Open PCR kits should support a broad range of specimens, including samples from extrapulmonary TB (EPTB) and vulnerable groups (children, PLHIV), such as stool, urine, cerebrospinal fluid, lymph node aspirates, tissue samples, pulmonary samples like BAL, ET aspirate, GA and minimally invasive options like tongue/oral swabs. These specimen types enable improved case detection where sputum is difficult to obtain or has low bacillary load.
Manual preparation of samples (steps needed after obtaining sample)	≤ 5 manual steps for including both sample preparation, extraction and processing for RT PCR	≤2 manual steps – preferable lyophilized prefilled reagents to reduce steps in RT PCR Rapid and automated extraction platforms or extraction-free protocols	PCR workflow steps, including sample preparation, should be simple and safe, **without** generating aerosols that require BSL-3 facilities.
Time to result	Within 1 day (batch testing)	Within ≤5 hours	The time required from sample collection, processing and getting results from the test for MTBC detection. The time to result will increase based on the drug resistance testing and the targets we have for reflex tests

Daily throughput	Flexible loading with a minimum of 6 samples or more At least 2 runs per day	Flexible loading with 1-96 samples per run At least 2 runs per day	Manufacturer should provide enough number of controls with the test kits to ensure operationalization of the flexible loading of specimens.
Kit compatibility	The open PCR kit should be compatible with at least 3 open PCR platforms available and recommended for use in the country	The open PCR kit should be compatible with all PCR platforms available and recommended for use in the country	
Biosafety	Minimum biosafety requirement with clean area for molecular work with appropriate sample handling and DNA extraction precautions, separate sections for DNA extraction and PCR set up	Minimal biosafety requirement through closed/semi-closed workflow with minimal infectious aerosol risk using lyophilized PCR reagents	It is important to note that the sample is processed with an initial inactivation step (either a lysis buffer or a heat inactivation step) to ensure safety for the healthcare workers.
Waste disposal- solid	Standard biomedical waste disposal procedures as per current national biomedical waste management guidelines.	Minimal waste generation through closed/semi-closed workflows. Reusable, recyclable, or non-plastic alternatives to disposable materials	Central pollution board documents for waste disposal
Waste disposal – infectious	Similar to those for currently used standard of care molecular tests	Similar to or less than smear microscopy (low-risk TB laboratories)	
Reagent kit – transport	Should be able to tolerate temperature stress during transport for at least 72 hours at –20 °C to +50 °C		
Reagent kit – storage and stability	12 months at -20 °C to +30 °C with up to 70% humidity; cold chain may or may not be required during transportation.	2 years at +5 °C to +40 °C with up to 90% humidity; should be able to tolerate stress during transport for at least 72 hours at +50 °C; no cold chain should be required	

Training and Education	3–5 days of training for staff	1-2 days of training for staff	Procedures should be easy for basic laboratory staff to learn with minimal training and should not require advanced molecular biology expertise. 1-2 days would only be done where people have already been trained on PCR testing
Environmental Impact	Tests and any associated instruments should minimize adverse impact on the environment. This includes the potential to produce tests locally, minimizing waste and maximizing reusability and recycling of by-products, multi-use ancillary equipment, recycling of equipment at the end of their life.		

Footnotes:

ǂ Based on the Health Technology Assessment (HTA) Report of Department of Health Research (DHR) on the Open RT-PCR kits

‖ Based on the published evidence available for the sensitivity and specificity reported for the existing NTEP approved NAAT tests (Xpert MTB or Truenat) for detection of MTB and RIF resistance.

† Based on WHO TPP 2024