Report on Truenat Pilot in Cambodia, Including Best Practices for Successful Truenat Implementation

INFECTIOUS DISEASE DETECTION AND SURVEILLANCE (IDDS) PROJECT

MAY 2024

Acknowledgements

This report was prepared by USAID's Infectious Disease Detection and Surveillance (IDDS) project. The operational research study was conducted by Cambodia's National TB Program (CENAT) in collaboration with USAID's IDDS project and Community Mobilization Initiatives to End Tuberculosis (COMMIT) project. Supply of Truenat commodities was provided by Stop TB Partnership's Global Drug Facility with funding from USAID under the *introducing New Tools Project* (iNTP). We thank the CENAT leadership and staff and the IDDS local team for successfully conducting this research.

Contents

Figures and Tables

Abbreviations

CENAT	National Centre for Tuberculosis and Leprosy Control
CI	Confidence Interval
COMMIT	Community Mobilization Initiatives to End Tuberculosis
CXR	Chest X-ray
DOT	Directly Observed Treatment
DNA	deoxyribonucleic acid
GDF	Global Drug Facility
GF	The Global Fund to Fight AIDS, Tuberculosis and Malaria
GX	GeneXpert
HC	Health Center
IDDS	Infectious Disease Detection and Surveillance
KHANA	Khmer HIV/AIDS NGO Alliance
MDR TB	Multi-drug Resistant TB
MDR/RR TB	Multi-drug Resistant and Rifampicin Resistant TB
MOH	Ministry of Health
MTB	Mycobacterium tuberculosis
NGO	Non-governmental Organization
NTP	National Tuberculosis Program
NTRL	National TB Reference Laboratory
NSP	National Strategic Plan
OD	Operational District
OR	Operations Research
PCR	Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
RR TB	Rifampicin Resistant Tuberculosis
SOC	Standard of Care
TB	Tuberculosis
USAID	United States Agency for International Development
WHO	World Health Organization

Executive Summary

Tuberculosis (TB) remains a public health problem in Cambodia (estimated population of 16.4 million), although the National TB Program's TB case notification and mortality numbers have been showing a declining trend over the last two decades, indicating the successes of TB prevention and control interventions.

In 2021, the estimated total TB incidence burden was 288 (197–396, 95 percent CI) cases and estimated mortality was 21 (14–28, 95 percent CI) per 100,000, according to the World Health Organization (WHO). There were about 1,000 cases of multi-drug resistant (MDR)/rifampicin-resistant (RR) TB in the same year. In absolute numbers, there are an estimated 48,000 (33,000–66,000, 95 percent CI) TB cases, of which the National TB Program notified a total of 21,661 patients in the year 2021. Detecting and treating "missing" people with TB remains a priority for the TB control program to potentially curtail the transmission of TB, especially at the hard-to-access peripheral point-of-care health centers (HCs) and communities. Molecular diagnostic technologies offer more sensitive, timely, and accurate TB and RR detection and, consequently, higher TB detection yields than sputum microscopy. Truenat TM TB tests, approved by the WHO in 2020, are more suitable for peripheral level point-of-care sites (USAID, GLI, Stop TB Partnership, 2021).

Truenat MTB and MTB Plus assays are chip-based Real-Time PCR tests for the semiquantitative detection of Mycobacterium tuberculosis (MTB) directly from a sputum specimen (Molbio Diagnostics Private Limited, 2020). The assay uses automated, battery-operated devices to extract, amplify, and detect TB deoxyribonucleic acid (DNA) (Molbio Diagnostics Private Limited, 2020) and enables wireless and real-time data acquisition and monitoring of test results. Therefore, the Truenat TB test is proposed as ideal for peripheral point-of-care or near point-of-care rapid molecular tests in resource-limited settings (MacLean et al., 2020).

Cambodia's National TB Program (CENAT) in collaboration with USAID's Infectious Disease Detection and Surveillance (IDDS) project and Community Mobilization Initiatives to End Tuberculosis (COMMIT) project conducted an operational research (OR) study to determine whether performing Truenat TB test using sputum samples is feasible in peripheral HCs for pulmonary TB and RR-TB detection among adults and children.

The OR study data showed that the Truenat MTB/MTB Plus along with the RIF reflex test is feasible to operate at peripheral HCs. The study included a feedback survey administered to end users and care providers, which found the test is of moderate complexity and suitable at the HC level to reduce the result turnaround time from one week to one day and improve the accessibility of WHO-recommended molecular diagnostics (mWRDs). A high proportion (99.7 percent) of valid Truenat MTB results further strengthens the case for feasibility of Truenat testing at the lowest level of health system in Cambodia.

1. Objectives

The OR examined pulmonary TB diagnosis among individuals evaluated for TB using the Truenat TB test, as a new mWRD, covering the population seeking TB services across 14 peripheral primary HCs in Cambodia.

1.1. Primary Objective

The primary objective of the OR was to assess the technical feasibility of introducing the Truenat TB test for rapid molecular detection of TB in people presenting with signs and symptoms of pulmonary TB at peripheral primary HCs. This included assessing Truenat testing performance indicators, including test positivity and invalid results for TB and rifampicin resistance detection, as well as assessing the impact of Truenat on access to mWRD testing and TB case finding compared to historical data.

1.2. Secondary Objective

The secondary objective of the OR was to assess the ease-of-use and acceptability of the Truenat TB devices and assay procedures among test users and care providers at peripheral primary HCs. This included assessing operational feasibility issues, including commodity availability, equipment failure and service interruption events, as well as appraisals from users and care providers around complexity and satisfaction.

2. Methodology

Prior to participant enrollment, the study protocol was approved by the Cambodia National Ethical Committee for Health Research (no. 313 dated December 27, 2021) and FHI 360's Protection of Human Subjects Committee (IRBNet project no. 1791495-1, dated November 26, 2021).

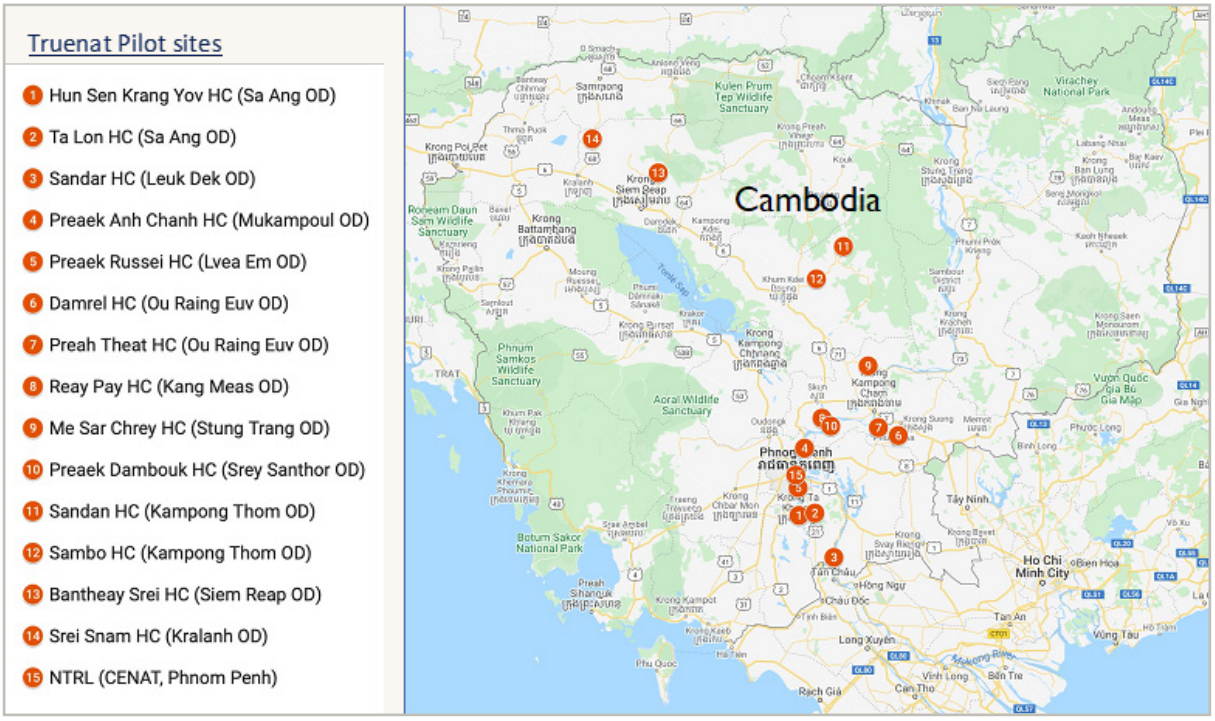
A representative sample of a minimum of 1,068 presumptive TB individuals were needed across all 14 target sites to have a confidence level of 95 percent with a margin of error of 3 percent for the feasibility assessment. We targeted a conservative total sample size of 1,200 presumptive TB individuals (Dean et al., 2013).

The OR was a cross-sectional feasibility study of 1,200 presumptive TB individuals that presented to 14 peripheral HCs *(Hun Sen Krang Yov, Ta Lon, Sandar, Preaek Anh Chanh, Preaek Russei, Damrel, Preah Theat, Reay Pay, Me Sar Chrey, Preaek Dambouk, Sandan, Sambo, Bantheay Srei, Srei Snam)* in 11 operational districts (ODs) *(Leuk Dek, Saang, Muk Kampoul, Lvea Em, Srey Santhor, Kang Mease, Stung Trang, Ou Raing Euv, Kampong Thom, Siem Reap, and Kralanh)* in five provinces *(Kandal, Kampong Cham, Tbong Khmum, Kampong Thom, and Siem Reap)* (Table 1 and Figure 1). Most of the target sites did not have an on-site TB diagnostic tool such as sputum microscopy or rapid molecular tools. They were at the lowest level of health care structure in Cambodia and used to refer the sputum to the nearest district-level GeneXpert facilities. The catchment population for "commune" ranged from an estimated 5,730 (Sandar HC) to 21,369 (Sandan HC), according to Cambodia's general population census in 2022. Nine sites were in COMMIT-supported ODs and five were in ODs supported by the Global Fund to Fight AIDS, Tuberculosis and Malaria (GF). The study intake period was six months, from May to November 2022.

Table 1: *List of Peripheral Health Facilities, ODs and Provinces in the OR Study*

Province	Operational District (OD)	Peripheral Health Facility
Kampong Cham	Stung Trang	Me Sar Chrey HC
	Srey Santhor	Preaek Dambouk HC
	Kang Meas	Reay Pay HC
Kampong Thom	Kampong Thom	Sambo HC
	Kampong Thom	Sandan HC
Kandal	Saang	Hun Sen Krang Yov HC
	Muk Kampoul	Preaek Anh Chanh HC
	Lvea Em	Preaek Russei HC
	Leuk Dek	Sandar HC
	Saang	Ta Lon HC
Siem Reap	Siem Reap	Bantheay Srei HC
	Kralanh	Srei Snam HC
Tbong Khmum	ORaing Ov	Damrel HC
	ORaing Ov	Preah Theat HC

Figure 1: *Map of Truenat Pilot Sites*



The OR was conducted in two parts, as per the primary and secondary objectives. Participants for the primary objective were presumptive TB individuals attending targeted health facilities for TB diagnosis and the participants for the secondary objective were the health care workers engaged as (1) Truenat TB testing staff as users and (2) care providers.

All the health care providers (clinicians, nurses, OR coordinators), laboratory technicians (end users), and supervisors were oriented prior to the OR study enrollments and specific trainings were provided to the health care staff on OR study enrollment, informed consent/assent taking, the diagnostic algorithm, and patient flow. The end users were trained on specimen processing, operations, and data management using IDDS standard training materials.

2.1. Inclusion Criteria

(Individuals evaluated for TB): To participate in the OR, the individual met the following minimum criteria:

- All individuals who met the criteria for presumptive pulmonary TB as per the HC's standard of care protocols and CENAT guidelines (see CENAT's TB diagnosis algorithm in Appendix 1). These include individuals without history of previous TB treatment and individuals who were previously treated for TB (relapse, treatment after failure, and treatment after loss to follow-up patients) who return to the HCs with clinical symptoms of active pulmonary TB after having received anti-TB drugs for at least 30 days or more, as per CENAT guidelines.

  - Presumptive TB, based on clinical symptoms, was defined as having one or more of the four common symptoms:
    - Cough for more than or equal to two weeks
    - Fever (38°C or above) for more than or equal to two weeks after exclusion of common causes of fever (i.e., malaria, pneumonia)
    - Night sweats for more than two weeks
    - Weight loss or failure to thrive
  - All individuals who agreed to participate in the evaluation through written informed consent or written consent by parent/guardian (in case of children) and assent of the child (10 years of age and older).

(TB health workers): Individuals participated in the OR if they were:

- All TB care providers from OR facilities who participated in the OR for TB screening, enrollment, care, and management (for the provider acceptability survey only)
- All TB testing staff from OR facilities that provided Truenat TB testing services and operationalized the testing devices (for the ease-of-use survey only)
- At least 18 years of age
- Must agree to participate in the survey through written informed consent

2.2. Exclusion Criteria

(Individuals evaluated for TB): Individuals did not participate in the OR if they were:

- TB patients currently on drug-susceptible (DS) or drug-resistant (DR) TB treatment
- TB patients presumptive or confirmed for extrapulmonary TB.
- TB patients who did not meet the criteria for presumptive TB, per the HC's standard of care protocols and CENAT guidelines
- Client or guardian/parent did not provide appropriate consent to participate in the OR
- Children aged 10 years and older did not provide assent to participate in the OR
- People who were unable to provide good quality sputum specimens for testing for TB
- People whose treatment was initiated before the sputum specimens were provided
- Children younger than five years

A CENAT-approved testing algorithm (Appendix I) was followed and Truenat TB assays were conducted per the manufacturer's instructions.

(TB health workers): Individuals did not participate in the OR if they were not involved in the Truenat TB testing protocols or patient care management by the OR team.

The OR was conducted as per the details of the approved OR study protocol.

Study-specific data collection forms were used to supplement the CENAT's standard TB screening and testing forms. TB health workers were trained for the OR at each facility to administer the forms. The trained OR focal person handled the informed patient consent, TB screening, and enrollment. The Truenat TB testing person handled the Truenat TB test setup, results, and related test operational conditions. Monthly monitoring visits were conducted by the OR facilitators for data completeness, correctness, and consistency at each HC. Norms for patient data confidentiality were maintained at all levels. Data was entered into the MS Access database, validated, and analyzed as per the details provided in the OR study protocol.

2.3. Quality Control

All health care staff (focal person, laboratory technician and coordinators) involved in the OR were properly trained prior to enrollment. Participating laboratories conducted the test ensuring high quality of data and documentation and they were cross-checked by the study coordinators on monthly supervisory visits. An EQA program was introduced at all the participating HCs to ensure the quality of Truenat testing.

2.4. Confidentiality

All individuals being evaluated for presumptive TB who provided informed consent were enrolled by OR focal point using an enrollment form and asked to see the Truenat TB testing staff within the HC for submission of a sputum specimen. Signed consent forms were securely filed at the HC under the custody of the TB health worker (OR focal point). Each individual was assigned a unique OR identification number, which was recorded on the consent form. Similarly, the health care providers appraisal survey data was securely kept at the HC.

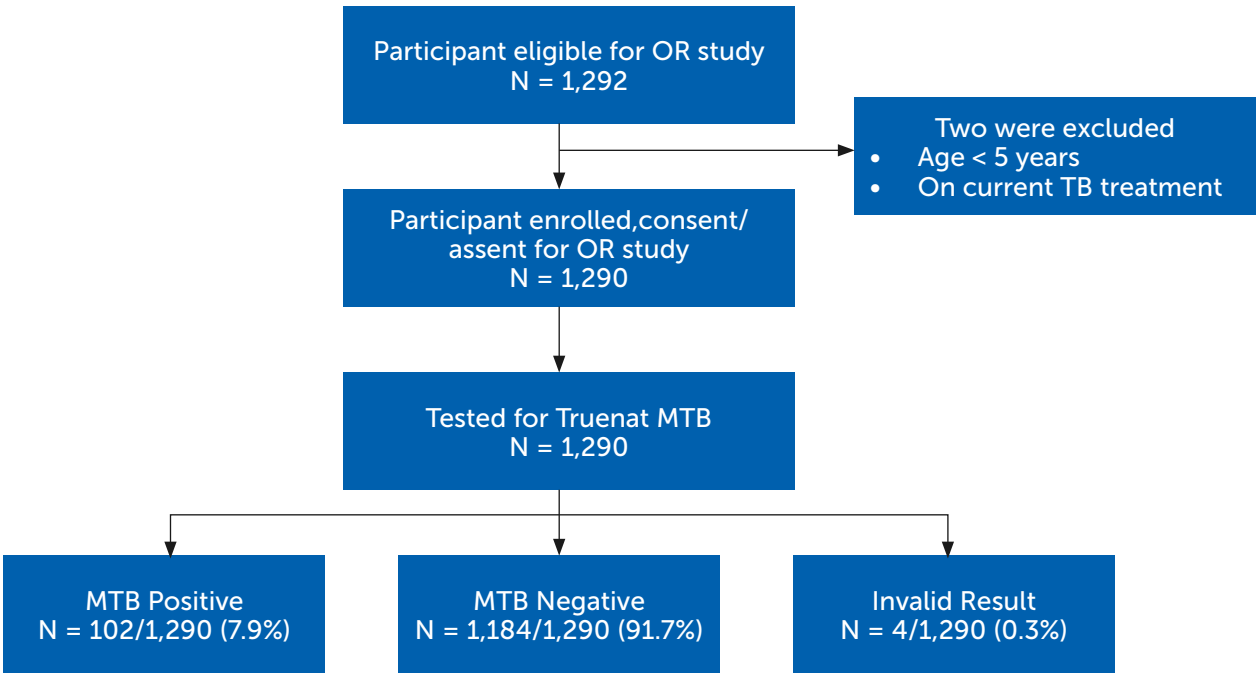
3. Results

Prior to participant enrollment, the study protocol was approved by the Cambodia National Ethical Committee for Health Research (no. 313 dated December 27, 2021) and FHI 360's Protection of Human Subjects Committee (IRBNet project no. 1791495-1, dated November 26, 2021).

A representative sample of a minimum of 1,068 presumptive TB individuals were needed across all 14 target sites to have a confidence level of 95 percent with a margin of error of 3 percent for the feasibility assessment. We targeted a conservative total sample size of 1,200 presumptive TB individuals (Dean et al., 2013).

Of the 1,292 individuals who were originally eligible for the OR study, 2 were excluded, resulting in 1,290 enrolled participants who provided consent (Figure 2). The 1,290 participants were tested using Truenat, resulting in 102 (7.9%) with positive results, 1,184 (91.7%) with negative results, and 4 (0.3%) with invalid results.

Figure 2: *Truenat Test Flow and Results*



Among 1,290 eligible individuals, cough of more than 2 weeks (with or without other TB symptoms) remained the primary TB symptom leading to a Truenat test request (Table 2) for 1,263 (97.9 percent) of the total tested. Fever for more than two weeks was recorded in 763/1,290 (59.1 percent), night sweats in 799/1,290 (61.9 percent), and 827/1,290 (64.1 percent) presented with weight loss.

Table 2: *Distribution of TB Symptoms Among Individuals in the OR Study*

TB Symptoms	Number of Participants (n=1,290)
Cough > 2 weeks	1,263 (97.9%)
Fever > 2 weeks	763 (59.1%)
Night Sweat	799 (61.9%)
Weight Loss	827 (64.1%)

Prior history of contact with confirmed TB cases was found in 402 (31.2 percent) individuals including 8 out of the 17 children enrolled. The majority of the individuals evaluated for TB were between 15 and 64 years old (n=866, 67.1 percent) with 9 percent MTB positivity. The MTB positivity among individuals aged above 65 years was 5.9 percent. None of the 17 (1.3 percent) individuals younger than 14 years had TB detected (Figure 3).

Figure 3: *Truenat Positivity and Age Group*

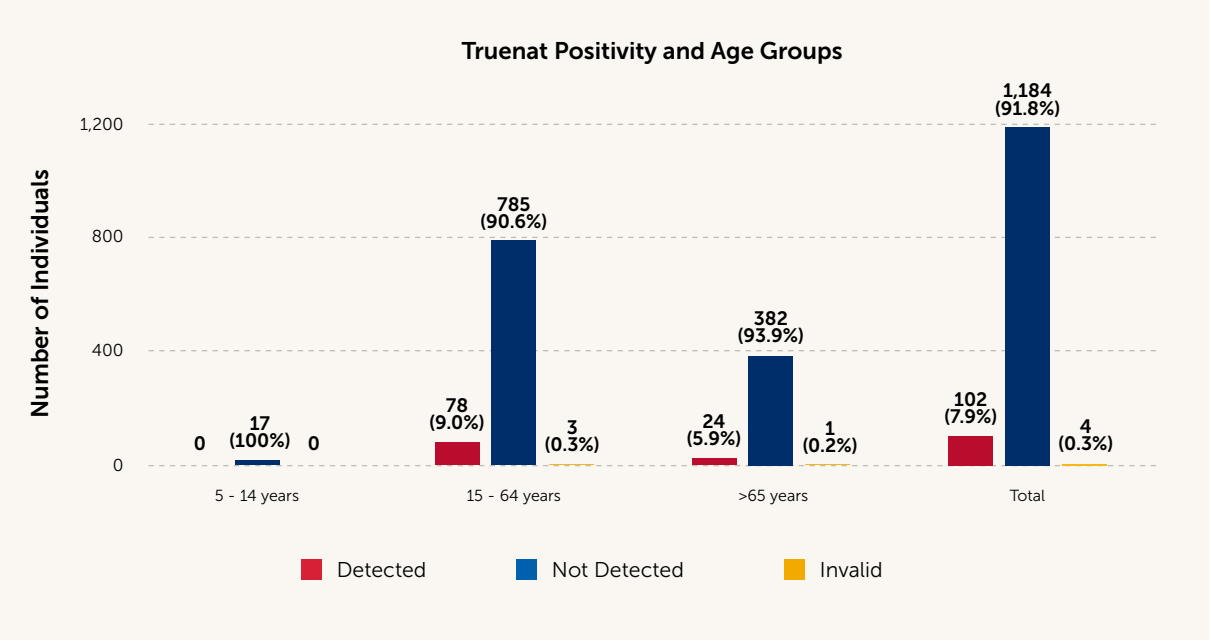
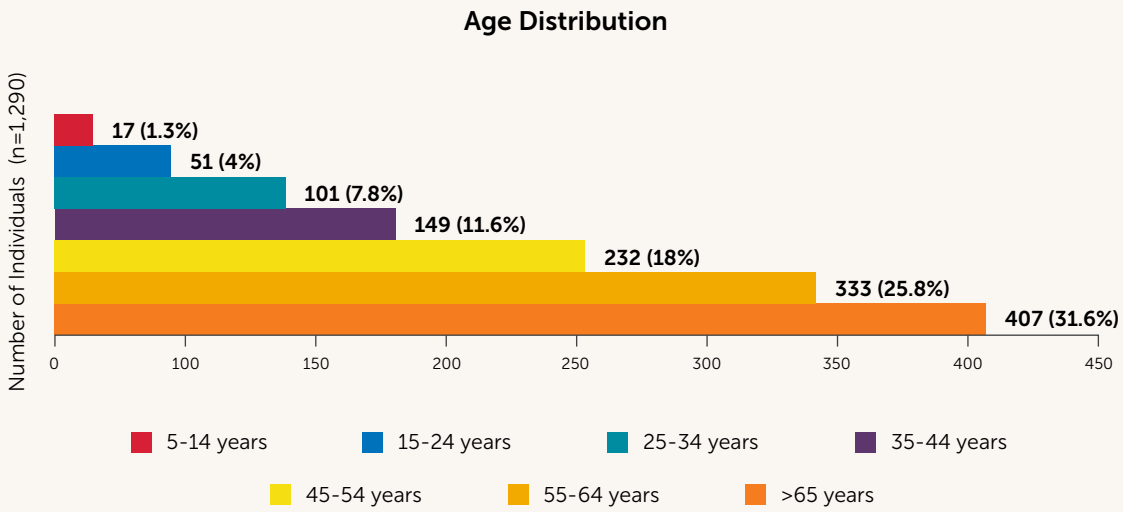
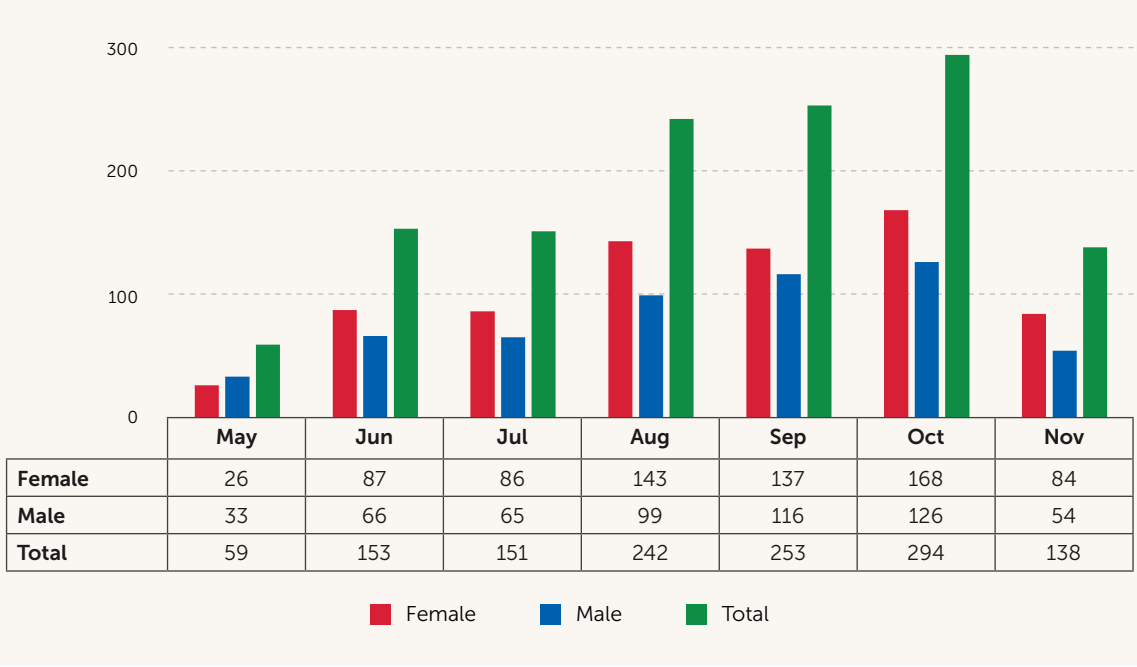


Figure 4: *Age Distribution of the Individuals in the OR Study*



The proportion of female participants enrolled in the study, by months, were higher than male participants, with the range from 54 to 61 percent, except the first month of the study (44 percent). Out of the enrolled participants 731 (56.7 percent) were females and 559 (43.3 percent) were males (Figure 5). The Truenat positivity was higher (11.9 percent) in males compared to females (4.7 percent).

Figure 5: *Sex Distribution of the Individuals in the OR Study*



	May	Jun	Jul	Aug	Sep	Oct	Nov
Female	26	87	86	143	137	168	84
Male	33	66	65	99	116	126	54
Total	59	153	151	242	253	294	138

3.1. Invalid Test Results

Out of 1,290 tests performed in the 14 Truenat study sites, 4 tests showed invalid test results, in Bantheay Srei HC, Preah Theat HC, and Sambo HC. One invalid test result occurred in May, one in September, and two in October. They resulted from either device or technical errors.

3.2. Specimen Quality and Truenat Test

A total of 1,007 (78.1 percent) specimens were mucopurulent, 274 (21.2 percent) were salivary, and 9 (0.7 percent) contained blood stains. The quality of the specimens tested is shown in Figure 6. Two sites reported a maximum of salivary samples: Ta Lon, and Krang Yov HCs. The quantity of sputum was mostly adequate, about 2.5–4 ml for 983 (76.2 percent). Ninety-six participants (7.4 percent) could collect the sputum in the small amount of 0.5–2 ml, and 211 (16.4 percent) collected less than 2 ml. Most sputum samples (91.2 percent) were delivered, tested, and had results reported on the same day.

A total of 1,290 patients were tested during the study period. The distribution per site is given in Table 3. According to the total Truenat tests per site, Preaek Dambouk HC performed the most tests, with 210 (16.3) of the total OR intake, followed by Preah Theat HC with 170 (13.2 percent) and Bantheay Srei HC with 147 (11.4 percent). The lowest sample intake was noticed at Reay Pay HC, which tested 25 (1.94 percent) of the total. Me Sar Chrey HC practically remained non-functional, with only two tests performed. This was mainly due to the reluctance of HC management to test for TB with Truenat.

Figure 6: *Quality of Sputum of TB Individuals in the Study*

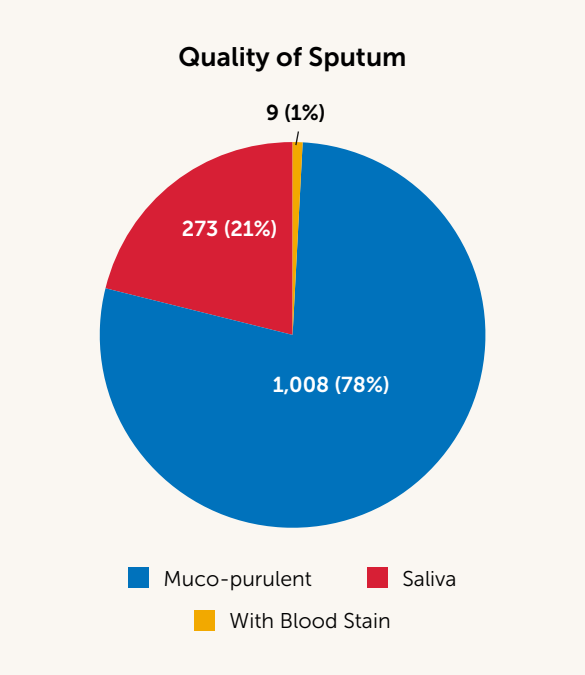


Table 3: *Number of Truenat TB Tests Performed at the OR sites*

Peripheral Health Facility	Level	Population Covered by HC in 2022*	Total Specimens with Valid Test Results
Bantheay Srei HC	District/Commune	10,055	148
Damrel HC	Commune	10,060	86
Hun Sen Krang Yov HC	Commune	18,593	101
Me Sar Chrey HC	Commune	13,851	2
Preaek Anh Chanh HC	District/Commune	14,166	107
Preaek Dambouk HC	Commune	13,555	210
Preaek Russei HC	Commune	10,028	64
Preah Theat HC	Commune	12,307	170
Reay Pay HC	Commune	14,601	25
Sambo HC	District/Commune	15,297	66
Sandan HC	District/Commune	21,369	87
Sandar HC	Commune	5,730	60
Srei Snam HC	District/Commune	8,454	89
Ta Lon HC	Commune	21,134	76
Total		**189,200**	**1,286**

* Data from HCs

Test positivity remained above 10 percent in 6 out of 14 sites. Sandan HC showed the highest positivity rate at 20.7 percent, while the positivity rates at the other 5 sites (Bantheay Srei HC, Preaek Anh Chanh HC, Reay Pay HC, Sambo HC, Ta Lon HC) were around 11 to 17 percent. For the other 6 sites (Damrel HC, Hun Sen Krang Yov HC, Preah Theat HC, Preaek Dambouk HC, Sandar HC, Srei Snam HC) there was a range of positivity rates around 2 to 8 percent (on average, 3 percent). Me Sar Chrey HC and Praek Russei showed no positivity of MTB detection. However, only two TB individuals were recruited at the Me Sar Chrey HC.

Table 4: *Site-wise Positivity Rate of MTB by Truenat*

Peripheral Health Facilities	Total Number of Individuals Evaluated for TB	MTB Detected, n (%)
Bantheay Srei HC*	146	15 (10.3)
Damrel HC	86	3 (3.5)
Hun Sen Krang Yov HC	101	4 (4)
Me Sar Chrey HC	2	0
Preaek Anh Chanh HC	107	13 (12.1)
Preaek Dambouk HC	210	13 (6.2)
Preaek Russei HC	64	0
Preah Theat HC*	169	3 (1.8)
Reay Pay HC	25	3 (12)
Sambo HC*	64	11 (17.2)
Sandan HC	87	18 (20.7)
Sandar HC	60	1 (1.7)
Srei Snam HC	89	7 (7.9)
Ta Lon HC	76	11 (14.5)
	1,286	**102**

*Facility showed invalid results

Of 102 MTB positive tests, only 2 (2 percent) showed RR-TB by Truenat detection, as shown in Figure 7. Twenty-two (21.6 percent) showed indeterminate results even though all repeated the test, and 17 (77.3 percent) of them had very low levels of MTB detection. Finally, two (2 percent) had medium and high levels of MTB.

Figure 7: *Rifampicin Resistance Detection by Truenat*

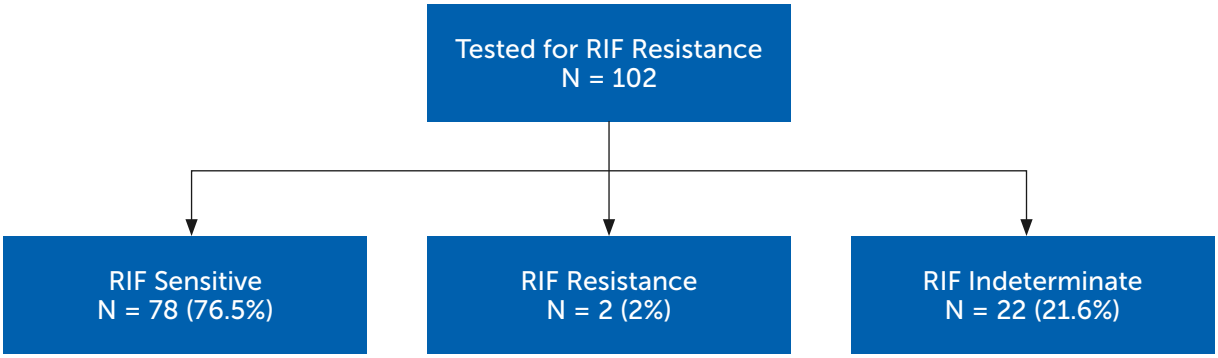


Table 5: *Rifampicin Resistance Detection by Truenat*

Quantity	Truenat Results: Rifampin Resistance Detection			
	Not detected	Detected	Indeterminate	Total
Very low	5	0	17	**22 (21.6%)**
Low	35	0	3	**38 (37.3%)**
Medium	36	1	2	**39 (38.2%)**
High	2	1	0	**3 (2.9%)**
Total	**78 (76.5%)**	**2 (2%)**	**22 (21.6%)**	**102**

To ensure the quality of testing, all sites participated in the commercial Truenat MTB Plus & MTB-RIF Dx external quality assessment (EQA) program conducted by SmartSpot Quality, an accredited manufacturer of EQA materials based in South Africa. Three rounds of EQA were conducted during the study period.

The scoring system calculated a panel score, panel statistics (Table 6), and frame statistics. Out of the 15 sites (14 HCs and NTRL), 11 showed the panel outcome as "Pass" in Cycle 1; 10 in Cycle 2 and 11 in Cycle 3. Two sites showed "acceptable" results in Cycle 1, four in Cycle 2, and two in Cycle 3. Only two sites showed "concern" results in Cycle 1, one in Cycle 2, and two in Cycle 3. None of the participating sites showed "unacceptable" scoring (Figure 8). The most common incorrect result occurred showed MTB Detected, RIF Resistance Detected control, but the sites reported as MTB Detected, RIF Resistance Indeterminate. The possible cause could be insufficient DNA elution, incorrect detection of resistance, or cross-contamination. The feedback was shared with the sites and followed up by super-users (who are trained on Truenat troubleshooting) on monthly visits.

Figure 8: *EQA results by Cycle in 15 sites (14 HCs and NTRL)*

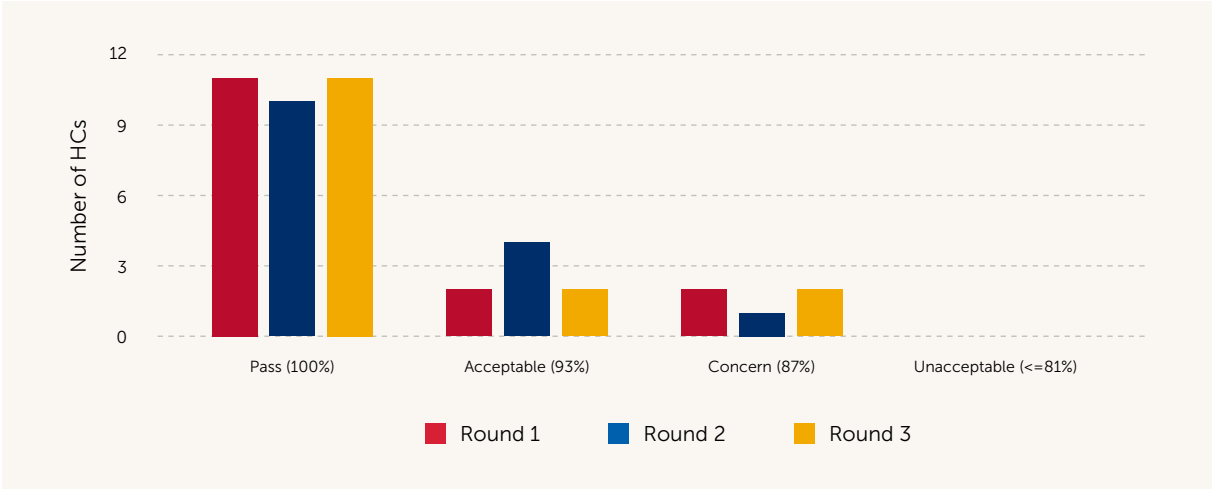


Table 6: *Panel Score Statistic Calculation*

Panel Score	Panel Score (%)	Panel Outcome
16/16	100%	**Pass**
15/16	93%	**Acceptable**
14/16	87%	**Concern**
≤ 13/16	81≤%	**Unacceptable**

Correct result = 2 ; Error (any) = 1 ; Invalid = 1 ; Rif indeterminate = 1 ; Incorrect result = 0

The frame score is generated from the three panels test scoring (Table 7).

Table 7: *Frame Score Statistic Calculation*

Frame Score (%) (average last three (3) Panel Scores)	Frame Outcome
90% - 100%	**Pass**
85% - 90%	**Acceptable**
≤85%	**Concern**

A consolidated OR sitewise EQA performance summary is shown in Table 8.

Table 8: *Rifampicin Resistance Detection by Truenat*

Site Name	Cycle Score (%)			Frame Score (%)		
	2022 - 1	2022 - 2	2022 - 3	Previous	Current	Trend
Banteay Srey HC (OD Siem Reap) Siem Reap	**100.0**	**100.0**	**100.0**	N/A	**100.0**	N/A
Damrel HC (OD Oraing Ov) Tbong Khmum	93.8	**100.0**	**100.0**	N/A	97.9	N/A
Hun Sen Krang Yov HC (OD SarAng) Kandal	**100.0**	**100.0**	**100.0**	N/A	**100.0**	N/A
Me Sar Chrey HC (OD Stoeung Trang) Kampong	**100.0**	92.9	**100.0**	N/A	97.6	N/A
National TB Reference Laboratory (NTRL) CENAT	**100.0**	**100.0**	85.7	N/A	95.2	N/A
Preaek Anh Chanh HC (OD Muk Kampoul) Kandal	**100.0**	**100.0**	92.9	N/A	97.6	N/A
Preaek Dambouk HC (OD Srey Santhor) Kampong	87.5	**100.0**	**100.0**	N/A	95.8	N/A
Preaek Russei HC (OD Lvea Em) Kandal	**100.0**	**100.0**	**100.0**	N/A	**100.0**	N/A
Preah Theat HC (OD Oraing Ov) Tbong Khmum	93.8	92.9	85.7	N/A	90.8	N/A
Reay Pay HC (OD Kang Meas) Kampong Cham	87.5	**100.0**	**100.0**	N/A	95.8	N/A
Sambo HC (OD Kampong Thom) Kg. Thom	**100.0**	**100.0**	**100.0**	N/A	**100.0**	N/A
Sandan HC (OD Kampong Thom) Kg. Thom	**100.0**	92.9	**100.0**	N/A	97.6	N/A
Sandar HC (OD Leuk Dek) Kandal	**100.0**	**100.0**	92.9	N/A	97.6	N/A
Srey Snom HC (OD Kralanh) Siem Reap	**100.0**	85.7	**100.0**	N/A	95.2	N/A
Ta Lon HC (OD SarAng) Kandal	**100.0**	92.9	**100.0**	N/A	97.6	N/A

4. Operational Feasibility Issues

During the study, the information about the operation of Truenat such as availability of the commodities, equipment failure due to high temperature or humidity, and service interruption events was collected from all the participated health centers and it was found that:

- No site reported stockout of Truenat testing reagents/kits. Consequently, no service interruptions related to stock-outs were reported.
- Test utilization remained low compared to test kit stocks obtained based on estimates.
- No issue related to the equipment/device batteries or backup during the testing duration was reported.
- Room temperatures of testing sites ranged from 24–36°C during the OR period. This parameter was not recorded and researchers used their best guess according to the weather forecast records.

However, a few services interruptions were reported:

- The TruePrep instrument used for extracting DNA had a few instances of interrupting services for 3–7 days during the study period. The most frequent problem encountered was with resetting the buffer for testing due to leakage of reagent containers at five sites (Sandan, Preaek Dambouk, Damrel, Preaek Anchanh, and Talon).
- Printer connectivity and functionality issues were reported at two sites: Talon and Sambo HCs.
- One site, Banteay Srey HC, reported an issue with the TrueLab touch screen, which became non-responsive. The optional physical keyboard was used to continue testing.

Any technical issue reported with the instrument functionality was recorded by the OR study site staff and communicated to IDDS staff for remote support. If the issue wasn't resolved, then it was escalated to the Molbio service support agency, Tekmax Vietnam, within 24 hours.

4.1. Ease of Use: User Appraisal

To understand the feedback on Truenat implementation and ease of use and acceptability, a survey questionnaire was administered to six TB health workers (46 percent), five TB assistants (35 percent), and three technicians (21 percent) who performed Truenat testing and participated in the OR implementation. The questions mainly assessed satisfaction of respondents with Truenat system operations (Appendix 2).

The usability test survey indicated that the space needed for Truenat device installation was adequately available at all sites. The majority (92 percent) of them were satisfied with the performance of the assays concerning ease of use and TB detection. All participants agreed that Truenat MTB and MTB-RIF DX assays are a practical means for testing for TB and meet the requirements of peripheral (point-of-care) level HCs. Except for one, all participants indicated that the test was "less complex" or "as complex as microscopy" for diagnosis of TB. The workload at the sites, in the opinion of the users, varied from 1–2 tests (35 percent), 3–4 tests (42 percent), to more than 10 tests (21 percent) per day. It took about 1–2 hours of hands-on time to perform the test for most (85 percent) of the staff. Ease of use for installing Truenat devices (TrueLab, TruePrep, TrueLab printer) was of "moderate difficulty" for 64 percent of the total users. Preparation of sputum sample for DNA extraction was "easy" for 92 percent, and the addition of eluted DNA to the Truenat chip was "easy" for 100 percent of the users. Waste management was adequate at all sites as per the requirements indicated during the training. Though information displayed on the Truenat devices was in English, 92 percent of the users found it "easy" to interpret and understand.

Charging batteries for Truenat devices was either "very easy" or "easy" for all the users. It took less than 8 to 12 hours to fully charge the batteries, which did not affect performing the Truenat testing. All users estimated that about 5 to 6 tests could easily be performed on a fully charged battery. No battery charge-outs or battery-related interruptions were reported during the testing in the OR period. Using Bluetooth TrueLab printers was said to be difficult at one of the sites. Five (36 percent) out of 14 users indicated difficulties in service and maintenance. In most cases, service and maintenance issues were resolved within 24 hours. However, four users noted that service and maintenance took about 2 to 7 days, mainly due to the remoteness of these health facilities and unavailability of the service provider in the country. Except for two users who felt the Truenat TB test was "moderately difficult," all thought the test was easy or very easy to perform at the health centers. Though all the technicians were trained prior to performing the Truenat TB Assay, about 35 percent of staff indicated that 1 training session was only partially adequate, indicating the need for follow-up trainings.

4.2. Acceptability: Care Provider Appraisal

From the total of 14 participants, 5 trained TB health workers (35 percent), 3 medical officers (21 percent), 2 midwives (14 percent), and other clinical staff screened the patients for TB symptoms and requested Truenat tests at the 14 HCs during the study. The national program trained all the staff for identification and management of people with TB. About 71 percent of the staff had more than 6 years of work experience in screening for TB symptoms, identifying presumptive TB individuals, and referring them for testing. The workload for the clinicians at the health facilities during the OR was one to five newly diagnosed people with TB per week. Further, introduction of Truenat helped reduce the burden on the patient and on the health system by removing the need for the patient to return another day to receive test results, since Truenat results were delivered on the same day.

All the care providers who participated in the survey mentioned that Truenat TB and RIF resistance results were obtained within the same day of the test request, which was critical in promptly initiating the right treatment. More than half of care providers responded that the test was less difficult than other methods for diagnosing TB at the HC level. Information provided by the Truenat TB test results was sufficient for clinicians (85 percent) to diagnose TB or RIF resistance. Five sites faced difficulties in printing results through the Bluetooth-enabled printer due to connectivity issues with the testing equipment. All the survey participants agreed that Truenat TB assays might replace smear microscopy (64 percent) for TB diagnosis and help in early TB detection (36 percent) at health facilities. However, most (78 percent) care providers also expressed a need for adequate space in the HC for Truenat devices. Overall, all care providers indicated that Truenat TB assays were easy to implement at deployed sites, including those without any prior experience with TB diagnostics.

Overall, the test users and the TB care provider surveys indicated that Truenat TB assays were easy to implement and acceptable for TB diagnosis at the peripheral levels of health care. A survey questionnaire is attached as Appendix 2.

5. Impact on TB Case Finding

TB case notification data from the past two years (2020 and 2021) as well as the study period were collected from the national TB information system (TB-MIS) and compared to see the increase in the case notification. The aggregated data from all 14 sites showed a substantial increase (93 percent) in the bacteriological confirmed cases compared to the similar timeframe of the previous year, while the overall case notification rose to 45 percent. This could be due to the smaller number of people visiting the facilities in 2021, due to fear of catching COVID-19 in that year. Therefore, we compared the study period data with 2020 data (prior to COVID-19 impact on TB detection) and found a reasonable increase of 16 percent and 13 percent in the bacteriological and overall case notification respectively. Though selective referral cannot be ruled out, an increased number of bacteriologically confirmed cases were expected due to the use of a highly sensitive method compared to smear microscopy. Interestingly, the proportion of bacteriologically confirmed cases among all notified newly diagnosed people with TB improved by 8 percent compared to the previous year and remained almost the same compared to the year 2020 (Figure 9, Table 8).

Moreover, we compared the proportion of presumptive TB patients diagnosed with WRD in the same period, before and during the Truenat implementation from May 2021 to March 2022 and from May 2022 to March 2023. The data showed the presumptive TB patients diagnosed with WRD increased from 59.7 to 96.9 percent (Figure 9).

Figure 9: *Percentage of TB tests with WRD (before and during implementation)*

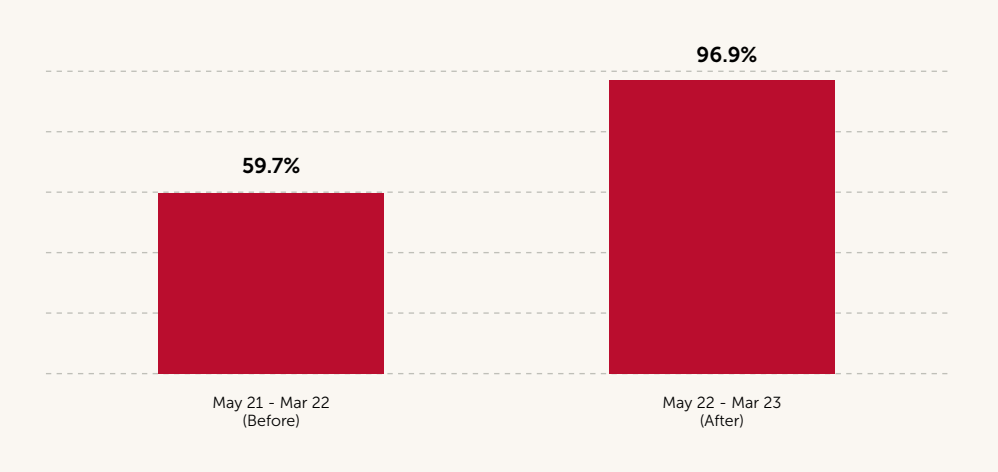


Table 9: *Historical and Study Period TB Case Notifications at Truenat HCs*

No.	OR Site	Total case notified May-Nov 2020			Total case notified May-Nov 2021			Total case notified May-Nov 2022 (study period)		
		Bact +ve	Bact -ve / clinical	All forms	Bact +ve	Bact -ve/ clinical	All forms	Bact +ve	Bact -ve/ clinical	All forms
1	Banteay Srey	14	5	**19**	9	1	**10**	12	3	**15**
2	Damrel	5	7	**12**	2	24	**26**	3	28	**31**
3	Kraing Yov	6	23	**29**	8	58	**66**	12	72	**84**
4	Mesor Chey	4	10	**14**	2	5	**7**	3	7	**10**
5	Preah Theat	1	8	**9**	0	22	**22**	2	19	**21**
6	Prek Anchanh	8	22	**30**	2	0	**2**	15	21	**36**
7	Prek Dambok	1	0	**1**	4	1	**5**	5	3	**8**
8	Prek Russey	6	10	**16**	2	4	**6**	2	3	**5**
9	Reay Pay	2	16	**18**	3	3	**6**	6	2	**8**
10	Sambo	9	20	**29**	10	14	**24**	13	13	**26**
11	Sandan	25	7	**32**	12	1	**13**	21	4	**25**
12	Sandar	4	24	**28**	0	7	**7**	1	0	**1**
13	Srey Snam	1	6	**7**	0	3	**3**	9	1	**10**
14	Talon	7	19	**26**	2	12	**14**	4	22	**26**
Total		**93**	**177**	**270**	**56**	**155**	**211**	**108**	**198**	**306**

6. Truenat TB Test Utilization Challenges

- All Truenat OR sites were at the "commune" level of health structure, which caters to a limited population. Six out of the 14 sites serve a population of less than 10,000 per site. Epidemiologically, all TB incidence estimated was 288 cases/100,000 population in 2021. Therefore, the catchment area population screened for TB was probably less given the passive TB finding strategy employed for testing at the health facility. Consequently, fewer testing volumes were notified.
- NGOs and community TB screening increase the index of TB suspicion and requests for Truenat testing. This activity could have varied among NGOs (COMMIT or GF-supported OD), showing low testing volumes.
- Truenat increased the accessibility of TB molecular testing at peripheral levels for the population, which is a good trade-off to low test utilization rates as the turnaround time of test results report were substantially improved at the OR sites.
- Specimen transport from other neighborhood facilities to the Truenat sites could not be implemented for various operational reasons, including transport costs facilitated by CENAT, which were paid based on the distance. GeneXpert instruments were placed farther from the commune than the Truenat sites.
- Higher intake in some Truenat OR sites could probably be due to GeneXpert system failures or GeneXpert cartridge stockouts at the nearby facilities, especially at Preaek Dambouk. Patients might have been referred to the OR sites for this reason. GeneXpert cartridge stockout was reported at some sites during the OR period.

- Two of the sites had low intake: one site was not operational at all because HC staff are not willing/commit to do testing at their HCs. This could have reduced the consumption of test kits. There are reasons to shift instruments from this site to another immediately.
- Five sites (Bantheay Srei, Sambo, Sandan, Srei Snam and Prek Anchanh) functioned as microscopy sites and established referral mechanisms from the commune level, which resulted in higher testing volumes.
- It was observed that the health staff at some facilities operated mainly in the morning sessions. This could have affected TB screenings and, consequently, testing.
- The motivational level of staff at microscopy centers was higher than that of the HC staff at the commune level.
- The initial annual estimation of Truenat testing kits/reagents was probably based on 4 tests per site per 250 days at 15 sites (totaling 15,000 tests). However, implementation experience indicates that testing at selected sites could hardly reach about half of this capacity (i.e., 2 tests per day during the OR). This could be due to low test referrals or inefficient screening systems at some sites, especially with higher population catchment areas. Another possible reason is the lack of an efficient specimen referral system from the nearby HCs; thus, the Truenat tests were conducted only from the OR sites.

7. Study Limitations

- The study was conducted in a controlled manner ensuring strict adherence to the SOPs which may vary in the real-life situation.
- The HCs selected, though geographically diverse, were within the USAID and GF-funded areas with additional resource availability to support the timely technical and troubleshooting support, which could reflect facility biases.
- The impact of the Truenat implementation on the overall case notification is difficult to ascertain due to involvement of other factors such as community mobilization and TB awareness.
- The OR study was focused primarily on the implementation feasibility of a new technology; the impact on treatment outcomes due to early and accurate diagnosis was not assessed.
- The cost effectiveness of the test was not measured, which is critical for the nationwide rollout of the technology.

8. Conclusions and Key Considerations for Future Expansion

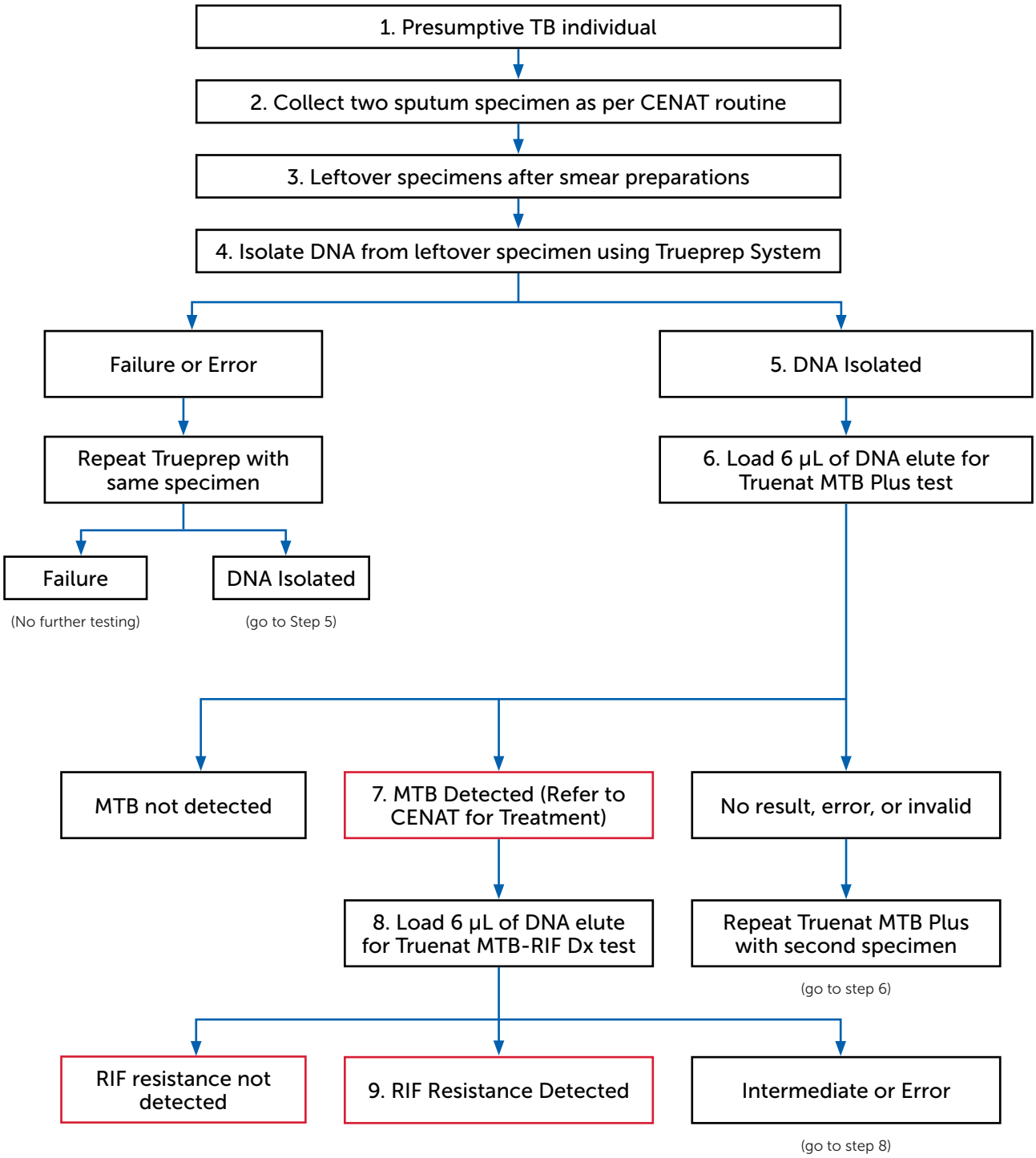
Overall, the OR study demonstrated a positive outcome on technical and operational feasibility including improved access to mWRD testing and TB case finding, reliable commodity availability, good functioning and maintenance of equipment, as well as positive appraisals from users and care providers at the peripheral level. Below are the major results that should be considered while expanding Truenat in Cambodia:

- All sites reported the Truenat test results to the clinicians within a few hours, compared to one week for GeneXpert, which facilitated same-day treatment initiation. This saves additional patient visit and out-of-pocket travel expenses. Further, this is a critical public health measure to break the chain of transmission of TB.

- None of the users or clinicians reported the increased workload due to the implementation of Truenat at their facility. Instead, some of them stated that the technology had helped improve their professional satisfaction due to same-day result reporting.
- The number of tests conducted per day was low (2 to 5). The access to the WHO-recommended technology closer to the patients is a logical public health measure and probably a good trade for access while compromising on the utility. A multiplexing strategy can be considered to improve the utility, return on investment, and lower the maintenance cost burden on a single public health program.
- No test interruption due to power failure or equipment malfunction due to hot weather was reported, which eliminates the necessity of air-conditioning for instrument operation and reagent storage. Further, this reduces the initial implementation cost in addition to the lesser test price compared to GeneXpert.
- Service interruptions at some sites were reported for 2 to 5 days due to technical problems with the Truenat instrument. A buffer stock of a few instruments can be maintained at the central level to quickly replace any broken instrument.
- None of the 17 children enrolled was found MTB positive, as they were unable to produce good quality of sputum. Stool Xpert testing should be considered for this group of patients.
- Most of the end users and care providers found the test useful for the patients. The involvement of 2 HCs remained very limited, and a fewer number of cases were enrolled at some of the sites, which was a missed opportunity. NTP should ensure the proper advocacy and obtain commitment when selecting a site for Truenat.
- MTB positivity rates varied among OR sites, which could be due to quality of TB screening and referral for Truenat testing. The health care staff training for TB screening and orientation about the diagnostic technology including results interpretation is critical.
- Five sites reported that the reagent containers leaked, which could be due to a particular batch of reagents, as such cases weren't reported elsewhere.
- Most of the users, even those without previous experience on TB diagnostics, found the Truenat TB test "moderately difficult," but about 35 percent of staff indicated that one training session was not sufficient for confidently running the test. Therefore, a refresher training should be considered after a few months of initial training.
- A high number of indeterminate RIF test results was observed which was expected in very low to medium MTB positive cases. A repeat test should be considered to overcome the issue as the manufacturer supplies a sufficient number of RIF tests with MTB or MTP Plus chips.
- The super-users were found to be very useful in providing onsite technical and troubleshooting support. NTP should expand the pool of super-users while expanding the instrument fleet.
- EQA is an integral part of the quality management systems and this OR study provided an opportunity to gain experience in EQA program implementation. This should be budgeted along with the annual operational expenses of Truenat.
- We did not compare Truenat MTB assay with smear microscopy, but the test is highly sensitive and has the potential to replace microscopy in line with WHO recommendations.
- The OR study demonstrated that the Truenat tests performed at peripheral health facilities by trained health care providers had very low error/invalid test rates at 0.3 percent. Of 1,290 participants enrolled for the study and sample tests performed by Truenat, during the study period 1,286 (99.7 percent) yielded valid test results and 4 (0.3 percent) yielded invalid test results.

Appendices

Appendix 1. Testing Algorithm



Appendix 2. Survey Questionnaire: User Appraisal of Truenat TB Tests

Questionnaire 1: User appraisal of Truenat TB tests

Unique OR site ID:

Name of the Health Center:

Operational district:

Date of Survey:

(a) Background of the participant: (please select and tick the correct answers(s))

1. Role of the participant in the HC:
   Technician TB assistant TB health worker
   Any other .....
2. How many months/years of experience do you have in making smear for TB testing? (if applicable)
   None <6 months 6 mon-2 years > 2 years
3. Are you trained for Truenat testing by the OR staff
   Yes No
4. Is the training adequate for performing all the activities for the Truenat assay
   Adequate Partially adequate Inadequate Don't know

(b) Usability (please select and tick the correct answer (s))

5. Is the space provided for all of the Truenat devices sufficient for the room?
   Space Sufficient Space insufficient Don't know
   Any comments:
6. Are you satisfied with the performance of Truenat MTB, MTB-RIF Dx assays
   Yes No Don't know
   Any comments:

7. Do you feel Truenat MTB, MTB-RIF Dx assays are practical means of testing TB in your health center settings?
   Yes No Don't know
   Any comments:
8. Do you feel that the use of Truenat MTB, MTB-RIF Dx assays, meets the peripheral (point-of-care) health center level requirements?
   Yes No Don't know or N/A
   Any comments:
9. How difficult was it to use Truenat assays compared to smear microscopy? (if applicable)
   More difficult Same Less difficult Don't know
   Any comments:
10. How much is the hands-on time required for Truenat assays?
    <1 hour 1 -2 hours 3-4 hours Don't know
    Any comments:
11. How many tests, on average estimate, were performed in a day
    1-2 tests 3-4 tests 5-10 tests >10 tests
    Don't know

(c) Ease of Use:

12. How easy was it to install Truelab, TruePrep, Truelab printers?
    Very easy Easy Moderately Difficult Difficult Don't know or N/A
    Any comments:
13. How easy was it to prepare sputum sample for DNA extraction?
    Very easy Easy Difficult Don't know or N/A

Any comments:

14. How easy was it to add extracted DNA sample to test Chip of Truenat assay?
    Very easy Easy Difficult Don't know or N/A
    Any comment:
15. Are you satisfied with the waste management measures for used Truenat test chips?
    Yes No Don't know
    Any comment:
16. How easy was the information displaced on the Truenat analyzer screen to use, report?
    Very easy Easy Difficult Don't know or N/A
    Any comments:
17. How easy was it to recharge the batteries of Truenat devices?
    Very easy Easy Difficult Don't know or N/A
    Any comments:
18. How long did it take to re-charge the Truenat batteries?
    <8 hours 8-10 hours 11-12 hours >12 hours Don't know
    Any comments:
19. How many TB tests were performed once the battery is fully charged?
    2-4 tests 5-6 tests 7-8 tests 9-12 tests
    Don't know
    Any comments:

20. Were there any battery related interruptions in testing, once the battery is fully charged
    Yes No Once in a while Don't know
    Any comments:
21. How easy was it to use the Bluetooth Truelab printer in result reporting?
    Very easy Easy Difficult Don't know or N/A
    Any comments:
22. How was your experience with any equipment down-times due to Truenat Service and maintenance (if applicable)
    Very good Good Difficult Don't know or N/A
    Any comments:
23. What was the maximum duration for resolving in case of equipment down-times
    Same day 24 hours 2-3 days >3 days
    No Service interruptions or Don't know
    Any comments:
24. Tell us about how you felt performing Truenat TB test compared to other TB diagnosis methods that performed before:
    Very easy Easy Moderate difficulty Difficult
    Don't know or N/A
25. Additional comments, difficulties, remarks: please list out:
    a.
    b.
    c.

Appendix 3. Survey Questionnaire: User Appraisal of Truenat TB Tests

Questionnaire 2: Care Provider appraisal of Truenat TB tests

Unique OR site ID:

Name of health facility :

Operational district:

Date of Survey:

(a) Background of the participant:

1. Role of the survey participant in the HC:

   Medical officer Nurse TB health worker Mid-wife
   Multicomponent staff Others.....

2. Are you trained by national program (CENAT) in TB care and management?

   Yes No Don't know

3. How much experience do you have in screening for TB signs and symptoms?

   <6 months 6 months-2 years > 2 years

4. How many presumptive TB individuals you refer for diagnostic testing?

   <1 per week 1-5 per week 6-10 per week
   >10 per week

(b) Use of Truenat TB assays for clinical decision making:

5. How difficult was it to diagnose TB without Truenat assays compared to other methods in general population?

   More difficult Same Less difficult Don't know

   Any comments:

6. How much time it takes to return the results (result turn-around-time (TAT)) for the Truenat TB assay?

   <= 2 hours Same day 1 -2 days > 2 days Don't know

Any comments:

7. How much time it takes to return the results (result TAT) for Truenat assay for TB compared to smear microscopy or other diagnosis?

   More time Same time Less time Don't know

   Any comments:

8. How much time it takes to return the results (result TAT) for Truenat assay for MDR TB compared to other diagnosis methods?

   More time Same time Less time Don't know

   Any comments:

9. Was the information provided by the test result sufficient for clinical decision-making in difficult cases?

   Yes No Need more information

   Any comments:

10. How difficult was it to use the report generated by the Bluetooth Truelab printer for clinical purposes

    Difficult Easy Don't know Not used

    Any comments:

11. What was the maximum number of clients that are diagnosed per day by the Truenat

    0-1 patient 2-4 patients >4 patients Don't know

    Any comments:

12. Advantage (s) of the Truenat TB assays (more than one answer allowed)

    Early RIF result (for MDR TB)
    May replace smear microscopy
    Early TB detection
    It takes less time than smear microscopy
    Not applicable/None

Appendix 4. Operational Issues with TruePrep, TrueLab, and Printer Devices and Solutions

No.	TruePrep Device	Solution	TrueLab Device	Solution	Printer Device	Solution
1	E1: Cartridge Valve Error	Started a fresh by processing remainder of sample in lysis buffer and load into new cartridge	E5: Probe Check Error	Repeated the run using a fresh chip and reload	Broken	Replaced with the new one
2	E2: Cartridge Error	Started a fresh by processing remainder of sample in lysis buffer and load into new cartridge	E1: Thermal Cycling	Repeated the run using a fresh chip and reload		
3	E3: Cartridge Clogged	Repeated extraction with new cartridge	E2: Test Stopped Manually	Repeated the run using a fresh chip and reload		
4	E8: Used Reset Card	Used new reset card	E3: Incorrect Optical profile	Repeated the run using a fresh chip and reload		
5	E9: Reset Card Read Error	Used new reset card	Touch Screen not responding	Used external keyboard		
6	E14: Cartridge Error	Repeated extraction with new cartridge				
7	Reagent Leak and Wet (Cannot extract DNA)	Changed new reagent				
8	TruePrep cannot reset buffer	Tried to reset buffer				
9	Cannot Reset buffer (optics don't recognize the QR and Lens of Camera is dirty)	Cleaned optic lens				
10	Solenoid valve broken	Pumped with air by using syringe				