



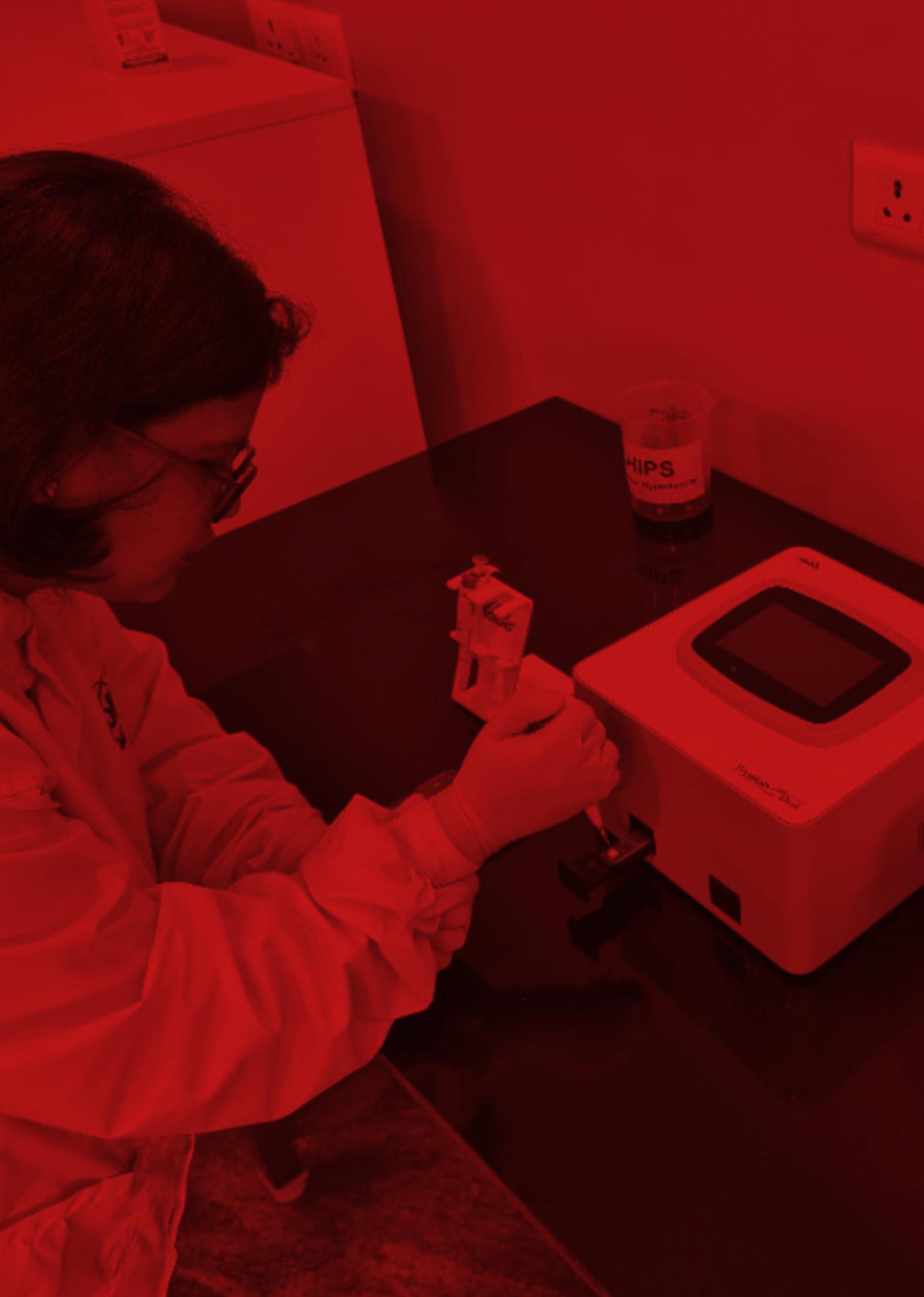
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Practical Guide to Implementation of **Truenat™ Tests** for the Detection of TB and Rifampicin Resistance





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Stop TB Partnership

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Cover photo images: Molbio Diagnostics, Goa, India

Practical Guide to Implementation of Truenat Tests for the Detection of TB and Rifampicin Resistance

About this guide

This guide provides practical guidance to plan for the adoption and implementation of the WHO-recommended Truenat™ tests for the rapid detection of TB and rifampicin resistance. It includes advice on how to translate findings from the WHO policy guidance into an actionable implementation plan, combined with operational considerations on use of the technology and initial field experience from early implementers. The guide also compiles as annexes a number of resources that can be easily adopted by programmes and sites, including a sample SOP for running the assays, checklists for planning and sites assessments, and job aids. The guide will be periodically updated to reflect additional experiences gained by implementers as well as to add as annexes useful tools they have developed that may be easily adopted by other implementers. Please contact any of the authoring organizations to suggest any contributions to this guide.

Target audience

This guide is intended to inform Ministry of Health officials, programme managers, testing site managers, quality assurance unit personnel, supervisory laboratory staff and Truenat users at national, state/provincial and testing site level, as well as technical partners and donors.

Abbreviations

| | |
|--------|--|
| CFU | colony forming units |
| DST | drug-susceptibility testing |
| DR-TB | drug-resistant tuberculosis |
| EQA | external quality assessment |
| FIND | Foundation for Innovative New Diagnostics |
| FN | false-negative result |
| FP | false-positive result |
| GLI | Global Laboratory Initiative |
| HIV | Human Immunodeficiency Virus |
| HR | human resources |
| Hr-TB | isoniazid-resistant, rifampicin-susceptible TB |
| INH | isoniazid |
| LAMP | loop-mediated isothermal amplification |
| MDR-TB | multidrug-resistant tuberculosis |
| MOH | Ministry of Health |
| MTBC | <i>Mycobacterium tuberculosis</i> complex bacteria |
| NTP | National TB Programme |
| NTRL | National TB Reference Laboratory |
| PCR | polymerase chain reaction |
| PLHIV | people living with HIV/AIDS |
| PT | proficiency testing |
| QA | quality assurance |
| QC | quality control |
| RIF | rifampicin |
| RR-TB | rifampicin-resistant TB |
| SOP | standard operating procedure |
| SS- | sputum smear-negative |
| SS+ | sputum smear-positive |
| TN | true-negative result |
| TP | true-positive result |
| TB | tuberculosis |
| TWG | Technical working group |
| WHO | World Health Organization |

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PART

A

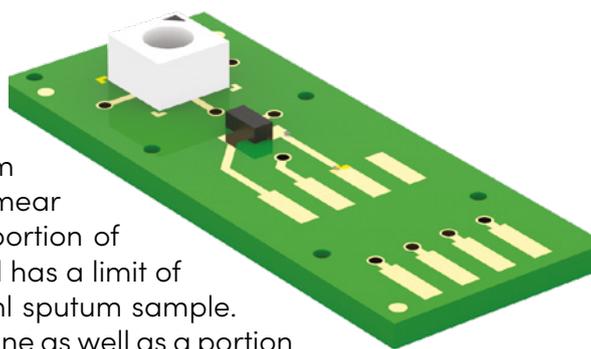


Background

The **Truenat™** (Molbio Diagnostics, Goa, India) testing system uses portable, battery-operated devices to rapidly detect *Mycobacterium tuberculosis* complex bacteria (MTBC) and rifampicin resistance. The system involves two main devices: the **Trueprep® AUTO v2 Universal Cartridge based Sample Prep Device** for the automated extraction and purification of DNA, and the **Truelab® Real Time micro PCR Analyzer** for performing real-time polymerase chain reaction (PCR), resulting in the semi-quantitative detection of MTBC. The system uses room temperature stable reagents (**Trueprep™ AUTO Sample Pre-treatment and Prep kits**) and **Truenat™ micro PCR chips**. The system is designed to be operated in peripheral laboratories with minimal infrastructure and is therefore considered to be the first molecular point-of-care test for TB recommended by the World Health Organization (WHO).

Available Truenat chips for the detection of MTBC with the Truelab micro PCR Analyzer include the Truenat™ MTB chip and a more sensitive Truenat™ MTB Plus chip. WHO recommends use of Truenat MTB or MTB Plus on sputum specimens as the initial diagnostic test for TB rather than smear microscopy or culture. The Truenat MTB chip amplifies a portion of the ribonucleoside-diphosphate reductase gene, *nrdB*, and has a limit of detection (LOD) of about 100 colony forming units (CFU)/ml sputum sample. The Truenat MTB Plus chip amplifies a portion of the *nrdZ* gene as well as a portion of the multicopy IS6110 element and has a LOD of about 30 CFU/ml. Extraction of DNA and detection of MTBC takes approximately one hour.

If the Truenat MTB or MTB Plus test (hereafter 'Truenat TB' test refers to either the Truenat MTB test or the Truenat MTB Plus test) result is positive, an aliquot of the already extracted DNA may be loaded onto a Truenat™ MTB-RIF Dx chip and analyzed in the Truelab micro PCR Analyzer. Mutations associated with rifampicin (RIF) resistance are detected by a probe melt analysis of the real-time PCR products. In addition to the hour required to extract DNA and detect MTBC, the detection of rifampicin resistance in an MTBC-positive sample requires approximately an additional hour.



Performance of the Truenat TB tests

Evidence reviewed by WHO on the use of the Truenat TB tests for the detection of MTBC and RIF resistance was generated through a multicenter prospective clinical evaluation study in 19 clinical sites (each with a microscopy center attached) and 7 reference laboratories in 4 countries, conducted by the Foundation for Innovative New Diagnostics (FIND). The study assessed the diagnostic accuracy of the Truenat TB assays when performed in the intended settings of use (i.e., microscopy centers), relative to microbiological confirmation (culture) as the reference standard. Compared to a microbiologic reference standard, key performance characteristics of these tests among persons with signs and symptoms of pulmonary TB being evaluated at health care facilities are reflected in Table 1.

Table 1. Diagnostic accuracy of Truenat MTB, MTB Plus and MTB-RIF Dx tests relative to culture, in microscopy center settings, FIND evaluation study

| Test | Sensitivity (all patients) | Sensitivity (SS+ patients) | Sensitivity (SS- patients) | Specificity (all patients) |
|--------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| Truenat MTB | 0.73 | 0.91 | 0.37 | 0.98 |
| Truenat MTB Plus | 0.80 | 0.96 | 0.46 | 0.97 |
| Truenat MTB-RIF Dx | 0.84 | 0.88 | 0.67 | 0.95 |

The performance characteristics of the Truenat TB tests were also compared to those of the Cepheid Xpert MTB/RIF test on the same specimens in reference laboratories as part of this assessment. The performance characteristics of the Truenat MTB, MTB Plus and MTB-RIF Dx tests were generally comparable to those of the performance of the Xpert MTB/RIF test; see Table 2. Specificities of both the Truenat TB tests and the Xpert MTB/RIF test were reduced at comparable levels in individuals who presented with a prior history of TB disease; see Table 3.¹

Table 2. Diagnostic accuracy of Truenat MTB, MTB Plus and MTB-RIF Dx tests and Xpert MTB/RIF tests among individuals being evaluated for TB, reference laboratory settings

| Test | Sensitivity (all patients) | Sensitivity (SS+ patients) | Sensitivity (SS- patients) | Specificity (all patients) |
|--------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| Truenat MTB | 0.84 | 0.98 | 0.45 | 0.97 |
| Truenat MTB Plus | 0.87 | 0.99 | 0.55 | 0.95 |
| Xpert MTB/RIF | 0.85 | 0.99 | 0.48 | 0.97 |
| Truenat MTB-RIF Dx | 0.82 | 0.86 | 0.33 | 0.98 |
| Xpert MTB/RIF | 0.84 | 0.89 | 0.33 | 0.98 |

Table 3. Effect of prior treatment on specificity of Truenat MTB and MTB Plus tests and Xpert MTB/RIF test

| Test | Specificity - No History of TB treatment | Specificity - History of TB treatment |
|------------------|--|---------------------------------------|
| Truenat MTB | 0.977 (0.964-0.986) | 0.922 (0.830-0.966) |
| Xpert MTB/RIF | 0.976 (0.962-0.985) | 0.906 (0.810-0.956) |
| Truenat MTB Plus | 0.959 (0.942-0.972) | 0.885 (0.782-0.943) |
| Xpert MTB/RIF | 0.975 (0.961-0.984) | 0.902 (0.802-0.954) |

1 WHO consolidated guidelines on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection. Web Annex 4. Evidence synthesis and analysis. Geneva: World Health Organization; 2020. <https://apps.who.int/iris/bitstream/handle/10665/334150/9789240010260-eng.pdf>

WHO Recommendations

Based on a review of these data, WHO recommendations for the use of the Truenat MTB, MTB Plus and MTB-RIF Dx tests are:²

- **In adults and children with signs and symptoms of pulmonary TB, the Truenat MTB or MTB Plus may be used as an initial diagnostic test for TB rather than smear microscopy or culture**
 - This recommendation applies to the use of the test with sputum specimens. There were no data available to assess the accuracy of the test in different respiratory specimens or with extrapulmonary specimens.
 - This recommendation applies to the use of the test with sputum specimens from HIV-positive persons based on extrapolation of the data on test performance with smear-negative sputum specimens.
 - This recommendation applies to the use of the test with sputum specimens from children based on extrapolation of the data from adults, although the test is expected to be less sensitive in children. In the case of children, there were no data available to assess the accuracy of the test in different specimens, and not enough indirect evidence to extrapolate for specimens other than sputum.
- **In adults and children with signs and symptoms of pulmonary TB and a Truenat MTB or MTB Plus positive result, Truenat MTB-RIF Dx may be used as the initial test for detection of rifampicin resistance**
 - There was a very low certainty of evidence for test accuracy and there is a need for additional evidence because of the small number of RIF-resistant samples tested and the limited spectrum of rpoB mutations represented in the tested samples.

How to perform the assay: equipment, reagents and procedures

The assay involves **three** main steps using **two** main components of the Truelab[®] Real Time micro PCR system and **three** reagent packs. All reagents and consumables required for the test procedures are provided by the manufacturer, with the exception of personal protective equipment (same level of protection as required for microscopy or Xpert MTB/RIF), a timer, and hypochlorite-based disinfectant.

Equipment

The main components of the system are the **Trueprep AUTO v2 Universal Cartridge Based Sample Prep Device** and the **Truelab Real Time micro PCR Analyzer**. A printer is also available.



Trueprep AUTO Universal Cartridge Based Sample Prep Device



Truelab micro PCR Analyzer Available with 1, 2 or 4 chip ports



Truelab micro PCR printer

² WHO consolidated guidelines on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection. Geneva: World Health Organization; 2020. <https://www.who.int/publications/i/item/who-consolidated-guidelines-on-tuberculosis-module-3-diagnosis---rapid-diagnostics-for-tuberculosis-detection>

The number and models of Trueprep Sample Prep Devices and Truelab micro PCR Analyzers to be procured should be adjusted to match anticipated site-level demand. The Truelab Analyzer is available with 1 (Uno) chip port as well as with 2 (Duo) or 4 (Quattro) chip ports which allow for independent testing of multiple samples at once. Table 4 shows the anticipated throughput for the instruments based on the manufacturer's calculation and an optimized workflow.² Early implementers have reported however that the 'real-world' throughput is significantly less (about 75% of the manufacturer's calculation), similar to what was seen by early implementers of the Xpert MTB/RIF test.

Table 4. Device combinations and throughput

| Devices | Throughput per 8-hour shift with optimized work flow ³ | Estimated throughput with "real-world" conditions |
|---|---|---|
| 1 Trueprep Device + 1 Truelab Analyzer Uno | 10-12 specimens | 7-9 specimens |
| 1 Trueprep Device + 1 Truelab Analyzer Duo | 20-24 specimens | 15-18 specimens |
| 2 Trueprep Devices + 1 Truelab Analyzer Quattro | 40-48 specimens | 30-36 specimens |

Procedures

For details on procedures, see a sample SOP in [Annex 1](#). For a sample job aid depicting the steps visually, see [Annex 11](#). A video demonstrating the sample preparation and DNA extraction steps. PCR amplification and MTB detection steps can be found online [here](#).

 **8 - 10 minutes**

Step 1.

Sample preparation using the liquefaction and lysis buffers (**Trueprep AUTO MTB Sample Pre-treatment Pack**). Summary procedures:

→ **Collect 2-5 ml of sputum sample**

- Add 2 drops of liquefaction buffer to sputum container containing sample
- Close the cap of the container and swirl gently to allow buffer to mix with sample
- Incubate for 10 minutes at room temperature. If sample is not pipettable after 10 minutes, incubate for another 5 minutes with swirling at 2 minutes intervals
- Transfer 0.5 ml of liquefied sample to a lysis buffer bottle using a 1 ml graduated transfer pipette
- Add 2 drops of liquefaction buffer to the lysis buffer bottle
- Swirl gently to mix
- Wait at least for 3-5 minutes, until sample is completely liquefied and lysed



Trueprep AUTO MTB Sample Pre-treatment Pack
(for 5, 20 or 50 tests)

Contents:

- Graduated transfer pipettes (1 ml)
- Lysis buffer bottles containing 2.5 ml of buffer
- Liquefaction buffer bottle

⁴ The manufacturer's calculation for an optimized workflow assumes that while DNA amplification is being run for a sample in the Truelab Analyzer, the next sample is being processed and undergoing DNA extraction in the Trueprep device at the same time.

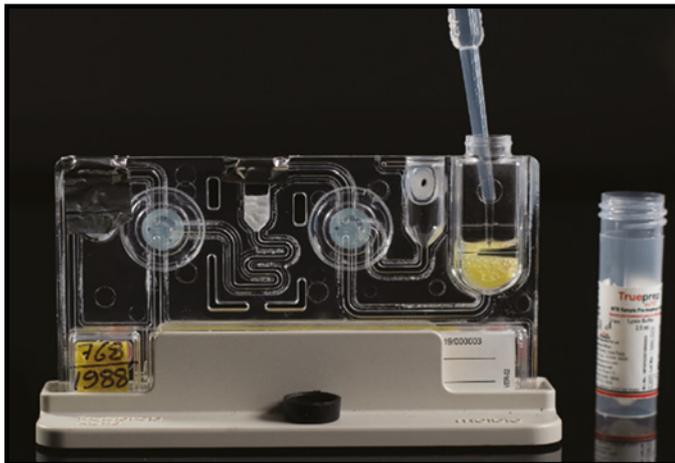
 22 minutes

Step 2.

Extraction and purification of DNA using the **Trueprep AUTO v2 Universal Cartridge Based Sample Prep Kit** and the **Trueprep AUTO v2 Universal Cartridge Based Sample Prep Device**.

Summary procedures:

- Transfer entire contents of lysis buffer bottle to sample chamber of cartridge using 3ml transfer pipette
- Load cartridge into Trueprep AUTO v2 Sample Prep Device
 - Automated extraction and purification take **20 minutes**
- After cartridge is automatically ejected from the Trueprep AUTO v2 Device at the end of the run, use the pipette from the cartridge pouch to transfer the entire volume of DNA eluate from the cartridge into an Elute Collection Tube (ECT)⁴



Pre-treated, lysed sample being loaded into a cartridge



Loading a cartridge into the Trueprep AUTO v2 device



Trueprep AUTO v2 Universal Cartridge Based Sample Prep Kit (for 5, 25 or 50 tests)

Contents:

- Reagent pack
- Transfer pipettes (3 ml)
- Cartridge pouches, each containing:
 - Cartridge
 - Elute collection tube (ECT)
 - Transfer pipette

⁴ In a study comparing six nucleic acid extraction technologies, the Trueprep AUTO Device was found to be highly suitable for use in resource limited settings, based on diagnostic accuracy, sample input and output volumes, total processing time, user-required manual steps and cost estimates: Beall SG, Cantera J, Diaz MH, Winchell JM, Lillis L, White H, et al. (2019) Performance and workflow assessment of six nucleic acid extraction technologies for use in resource limited settings. PLoS ONE 14(4): e0215753. <https://doi.org/10.1371/journal.pone.0215753>. Research is still needed however to confirm the ability to use the eluate with other testing methods, including line probe assays and Next-Generation Sequencing.

Step 3.

PCR amplification and fluorescent probe-based detection of MTB using **Truenat chips**, a **Truepet® 6µl Precision Micropipette** and the **Truelab micro PCR Analyzer**. Summary procedures:

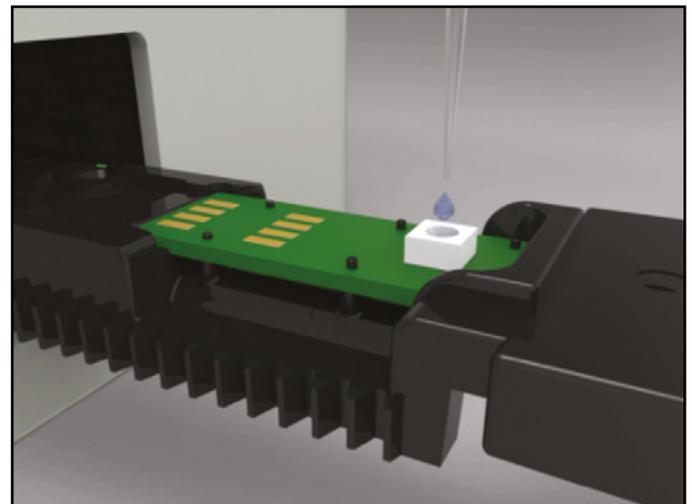
- Prepare the Truelab micro PCR Analyzer for a TB test by using the flatscreen digital interface: select “MTB” (or “MTB Plus”, if using an MTB Plus chip), enter patient details and select “Sputum” as the sample type
- Use the Truepet 6µl Precision Micropipette and a filter barrier tip from the chip pouch to pipette out 6µl of purified DNA eluate from the Elute Collection Tube (ECT) into the microtube containing freeze-dried PCR reagents. Do not mix.
- Wait 30–60 seconds
- Use the same tip to transfer 6µl of the solution from the microtube to a Truenat chip.
Note: this transfer of solution into the white reaction well of a chip requires sufficient training to ensure proficiency
- Load Truenat chip into the Truelab micro PCR Analyzer
 - Automated PCR amplification and fluorescent probe-based detection takes **35 minutes**. An optional “Plot” view on the digital interface allows for monitoring test progress in real time.
- At the end of the run, the “Result” screen for the Truenat MTB test indicates whether MTB has been “DETECTED” or “NOT DETECTED”. For the Truenat MTB test, if MTB is detected, the estimated number of bacteria in terms of colony forming units per ml (CFU/ml) in the original sample is also reported. For the Truenat MTB Plus test, the MTB DETECTED results are described as high, medium, low or very low.
- Optional: Press “Print” to send the result via Bluetooth to the Truelab micro PCR printer.



Truenat Chip Pack: MTB, MTB Plus or MTB-RIF Dx (for 5, 20 or 50 tests)

Contents:

- Individual chip pouches, each containing:
 - Truenat™ chip
 - Microtube containing freeze-dried PCR reagents
 - Filter barrier pipette tip



Transferring the solution to a Truenat chip



Truepet 6µl Precision Micropipette
Included in instrument kits, with a free replacement every 6 months to ensure calibration

| Truenat™ MTB | | | | | Truenat™ MTB Plus | | | | | | |
|------------------------|--------------------------------------|-------------|-----------------------|-------------|-------------------|------------------------|-------------------|-------------|-----------------------|-------------|--------|
| Center | Molbio QC | Operator | Service | Bay | 1 | Center | molbio QC | Operator | Service | Bay | 1 |
| Profile | MTB | Date | Tue 18 Jun 2019 22:42 | | | Profile | MTB Plus | Date | Wed 29 May 2019 10:21 | | |
| Lot | TB100 | Expiry Date | 05-21 | Sample | Sputum | Lot | MTP11 | Expiry Date | 06-20 | Sample | Sputum |
| Patient Details | | | | | | Patient Details | | | | | |
| Name | XX | ID | XX | | | Name | XX | ID | XX | | |
| Age | XX | Gender | Male/Female | Referred By | XX | Age | XX | Gender | Male/Female | Referred By | XX |
| Result | | | | | | Result | | | | | |
| Control C _i | 27.33 | MTB | 29.0 | | | Control C _i | 29.9 | MTB Plus | 32.0 | | |
| Run Status | Valid | | | | | Run Status | Valid | | | | |
| MTB | DETECTED 6.6x10 ⁰³ CFU/ml | | | | | MTB Plus | DETECTED Very Low | | | | |

Results screens for a Truenat MTB test and a Truenat MTB Plus test. Note the red circle indicating that MTB has been detected.

 60 minutes

Step 4.

PCR amplification and fluorescent probe-based detection of rifampicin resistance using **Truenat MTB-RIF Dx chips**, a **6µl Truepet Precision Micropipette** and the **Truelab micro PCR Analyzer**.

- If MTB is detected in a sample, a portion of the same DNA eluate can be used to test for rifampicin resistance using a Truenat MTB-RIF Dx chip. Repeat the procedures in Step 3 above, selecting “MTB RIF” as the test type in the Truelab micro PCR Analyzer. Automated PCR amplification and fluorescent probe-based detection of rifampicin resistance takes an additional **60 minutes**, resulting in a total runtime of ~2 hours to detect MTB and rifampicin resistance.



Truelab® Real Time PCR Workstation Field Case

The Truenat TB system is also available as part of a portable system, which may make it particularly useful for active case finding activities or providing testing in hard-to-reach areas or populations. The Truelab Real Time PCR Workstation Field Case contains all the instruments (Trueprep Device, Truelab Uno Dx micro PCR Analyzer, micro PCR printer and Truepet SPA fixed volume (6µl) Precision micropipette) and reagent packs (Sample Pre-treatment Pack, Sample Prep Kit, Truenat TB Chip Pack) needed to conduct the Truenat TB tests. As security measures, the Truelab PCR analyzer includes GPS tracking (triangulation based on connection to cell towers when the device is switched on) and password protection. The field case can also be stored in a lockable location when not in use.

PART

TB



Image: IRL New Delhi Tuberculosis Centre, India NTEP

Implementing the Truenat TB tests

Placement of the Truenat TB test in the tiered structure of a laboratory network

Considerations that guide the placement of any diagnostic test within the existing laboratory network structure include established targets for expanding patient access to rapid testing, current and planned testing algorithms, projected testing volumes, infrastructure requirements, biosafety requirements, trained human resources (HR) capacity, links to other laboratories for further testing, specimen referral and result reporting systems and possibility of integration with testing for other diseases.

The Truenat TB assays use automated, battery-operated devices and are designed to be operated in sites with minimal infrastructure, including peripheral health centers or mobile vans. Batteries can be recharged using the main electrical system or solar power. The devices can also be powered directly from the electrical system using the provided AC/DC adapter and a functioning, well-grounded electrical socket.

In many countries, the intended setting for use will be the peripheral microscopy laboratory. While countries should consider the advantages and disadvantages of using Truenat TB tests versus other WHO-approved rapid molecular tests for detection of MTBC, e.g., the Xpert MTB/RIF test or TB-LAMP test, a country does not necessarily need to select only one test to meet its needs for rapid testing. Positioning the Truenat TB test or TB-LAMP test at lower levels within the healthcare network than the Xpert MTB/RIF test (e.g., at peripheral microscopy centers or clinical laboratories, clinic settings) can increase patient access to rapid molecular testing for TB, decentralize testing for rifampicin resistance, reduce the need for patient travel especially in hard-to-reach areas, and help countries reach targets to replace sputum smear microscopy as an initial diagnostic test. Note that adoption of any molecular test including Truenat TB tests does not eliminate the need for microscopy, as microscopy is still required for monitoring treatment of TB patients; needed microscopy capacity may however be greatly reduced.

Multiplexing opportunities

The Truenat testing technology allows for multiplexing opportunities using the same pieces of equipment for nucleic acid extraction and PCR amplification and detection. For some testing, the same eluate of extracted nucleic acid may also be used with various testing chips to detect multiple diseases. As of the date of publication of this guide, the Truenat tests for Hepatitis C virus (Truenat™ HCV) and Human Papillomavirus High Risk Types 16, 31 and 18, 45 (Truenat™ HPV-HR) were undergoing review by the WHO Prequalification Department. Many other Truenat tests are also [available](#) from the manufacturer, including a test for SARS-CoV-2.

Test accuracy considerations for selecting which Truenat TB test to use

The predictive values of any test vary depending on the prevalence of TB in the population of patients being tested. Table 5 provides population-level projections of the results of testing with the Truenat MTB and MTB Plus tests based on sensitivity and specificity estimates that were extracted from the WHO policy statement⁵ for the tests. The impact of false-negative results may be missed opportunities to treat TB. The impact of false-positive results may be over-treatment of patients without TB.

In deciding whether to select Truenat MTB or MTB Plus, countries will need to consider the possible trade-offs between higher or lower sensitivity and higher or lower specificity based on the prevalence of TB, DR-TB and TB/HIV in their country. For example, in a population with a high prevalence of HIV, a more sensitive test (i.e., Truenat MTB Plus) may be the more appropriate test because of its increased sensitivity for the detection of MTBC in smear-negative samples.

Table 5. Predictive values of the Truenat TB tests in populations with different prevalences of TB

| | | | Population-level projection of true and false results | | |
|--------------------|--------------|--------------------------------------|---|----------------|-----------------|
| Patient population | Truenat Test | Sensitivity (Se) Specificity (Sp) | 2.5% prevalence | 10% prevalence | 30% prevalence |
| All patients | MTB | Se: 0.73 | TP: 18/FN: 7 | TP: 73/FN: 27 | TP: 220/FN: 80 |
| | | Sp: 0.98 | TN: 957/FP: 18 | TN: 884/FP: 16 | TN: 687/FP: 13 |
| SS+ patients | MTB | Se: 0.91 | TP: 23/FN: 2 | TP: 91/FN: 9 | TP: 273/FN: 27 |
| | | Sp: N/A | | | |
| SS- patients | MTB | Se: 0.37 | TP: 10/FN: 15 | TP: 37/FN: 63 | TP: 111/FN: 189 |
| | | Sp: 0.98 | TN: 955/FP: 20 | TN: 881/FP: 19 | TN: 685/FP: 15 |
| All patients | MTB Plus | Se: 0.80 | TP: 20/FN: 5 | TP: 80/FN: 20 | TP: 239/FN: 61 |
| | | Sp: 0.97 | TN: 941/FP: 34 | TN: 868/FP: 32 | TN: 675/FP: 25 |
| SS+ patients | MTB Plus | Se: 0.96 | TP: 24/FN: 1 | TP: 96/FN: 4 | TP: 288/FN: 12 |
| | | Sp: N/A | | | |
| SS- patients | MTB Plus | Se: 0.47 | TP: 12/FN: 13 | TP: 47/FN: 53 | TP: 142/FN: 158 |
| | | Sp: 0.97 | TN: 941/FP: 34 | TN: 868/FP: 32 | TN: 675/FP: 25 |

SS+: sputum smear positive; SS-: sputum smear negative; TP: true positive; FN: false negative, TN: true negative, FP: false positive. N/A: not applicable

5 WHO consolidated guidelines on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection. Geneva: World Health Organization; 2020. <https://apps.who.int/iris/bitstream/handle/10665/334150/9789240010260-eng.pdf>

Furthermore the use of chest X-ray as a screening tool to triage who should be tested with a Truenat TB test may improve the pre-test probability for TB and, thus, the predictive value of the Truenat TB test; such a strategy also reduces the number of individuals requiring a Truenat TB test and the associated costs.

Adoption and roll-out of Truenat: Experience of the National TB Elimination Programme of India

Following a successful validation study supported by the Ministry of Health and Family Welfare (MoHFW) and conducted under programmatic settings to evaluate operational feasibility and performance, Truenat has become recommended in India for the detection of MTB and rifampicin resistance in sputum samples from people with signs and symptoms of TB. The study was conducted under the aegis of the Indian Council of Medical Research (ICMR) at 100 microscopy centers under the National TB Elimination Programme (NTEP) in 50 districts of 10 states, identified in consultation with the Central TB Division (CTD), MoHFW.

Based on results from 10,878 samples, the study found overall MTB positivity rates of 13.3% for smear microscopy, 18.1% for Xpert MTB/RIF and 18.8% for Truenat. Sensitivity of Truenat was found to be higher than Xpert MTB/RIF (84.1% vs 81.0%, respectively); 79% of discrepant cases were resolved by PCR in favor of Truenat. Regarding detection of rifampicin resistance, Truenat performed similarly to Xpert MTB/RIF, without a statistically significant difference.

The study found the following operational advantages of Truenat:

- Cost-effectiveness: low equipment and test costs;
- Patient access: use of Truenat at primary healthcare level eliminates the need for sample transport for detection of rifampicin resistance during the first patient visit;
- Time taken for the assay: MTB detection is completed in 35 minutes and the rifampicin assay is done only as a reflex test;
- Availability of DNA: With Truenat, DNA is available for repeat testing and any further investigation and quality control purposes.

In order to expand access to molecular testing and detect additional cases in line with the National Strategic Plan (2017-2025), the India MoHFW decided to deploy Truenat machines at NTEP sites in a phased manner. In the first phase, 1,512 high workload microscopy centers have been identified for deployment of 2-chip Truelab Duo equipment; these sites exclude facilities where 1,238 GeneXpert machines have already been deployed. The selected Truenat sites are expected to test approximately 4,000,000 people with signs and symptoms of TB annually across the country. The NTEP is currently considering whether to eliminate smear microscopy as a diagnostic test with further saturation of Truenat. The Truenat platform is also currently being used for COVID-19 testing.

From experience gained so far in use of Truenat in the field, the NTEP has found advantages in the Truenat equipment being portable and battery-operated, having direct connectivity with a mobile interface for data sharing, and not requiring air conditioning. However, performing a Truenat test does require a dedicated and skilled technician, and periodic refresher trainings are needed to maintain adequate technical proficiency.

Steps and processes for implementing the Truenat TB tests

As an initial step in implementing a new diagnostic test, countries should review WHO policies, guidance and reports as well as any available implementation guides from WHO⁶, GLI⁷, and implementing partners. Particular attention should be paid to WHO policies and recommendations of the use of the test, the limitations of the test and how to interpret test results. External technical assistance may be needed; please contact any of the author organizations for more information on possible support.

Steps to implementing a new diagnostic test such as the Truenat TB tests are described in detail in the *WHO operational handbook on tuberculosis. Module 3*.⁸ The section below briefly describes aspects of implementation with an emphasis on aspects specific to the Truenat TB tests. [Annex 2](#) contains an example of a high-level checklist for implementation of the Truenat TB tests. **Note that the implementation steps described below are not listed in sequential order.** Many of the steps may be pursued at the same time (see Gantt chart in [Annex 5](#)). Adopting a new technology requires a conscious effort at change management to ensure that staff understand and are supported in making changes. Change management is systematic, structured approach to ensure that changes are smoothly and efficiently implemented and that the changes lead to desired benefits.

1. Policies and planning

Establish a technical working group (TWG) to lead the planning for the implementation, perform a situational analysis and develop a costed operational plan with timelines and milestones. The TWG should lead a review of existing national diagnostic algorithms in light of the intended use and placement of the Truenat TB tests, country epidemiology, existing testing algorithms, sample referral systems, opportunities for multiplexing by testing for other diseases on the same devices and other operational considerations, and make recommendations to the Ministry of Health (MOH) or NTP on proposed revisions to algorithms, specimen transport systems and linkages to referring and referral laboratories. A model algorithm for the use of the Truenat TB tests is described in detail in Section C.

The TWG should consider priorities and gaps in their National Laboratory or TB Laboratory Strategic Plans to maximize synergies and collaborations with other disease programmes and improve engagement with the private sector. The TWG should also lead a review of guidelines for the use of the Truenat TB test results in patient care decisions. Clinical guidelines should provide clear guidance to clinicians, nurses and health care professionals on the intended use of the new tests, target patient populations and how to order the tests and interpret and use the results. The TWG must also consider the revisions required to test requisition forms, registers and other recording and reporting forms, and how to integrate the reported data into existing central surveillance systems.

Activities at a glance

- ❑ Establish a Technical Working Group (TWG)
- ❑ Review WHO policies and available technical and implementation guides
- ❑ Define immediate and future purposes of the test
- ❑ Update national diagnostic algorithm and guidelines
- ❑ Perform a situational analysis
- ❑ Develop a costed operational plan for implementation

6 https://www.who.int/tb/areas-of-work/laboratory/policy_statements

7 <http://stoptb.org/wg/gli>

8 WHO operational handbook on tuberculosis. Module 3: diagnosis - rapid diagnostics for tuberculosis detection. Geneva: World Health Organization; 2020. <https://www.who.int/publications/i/item/who-operational-handbook-on-tuberculosis-module-3-diagnosis---rapid-diagnostics-for-tuberculosis-detection>

A situational analysis of the existing laboratory network and capacities should be conducted to inform the plans for implementing the new diagnostic test. For most tests, key elements to be assessed include: regulatory requirements; laboratory and network infrastructure; existing sample transportation system; staff skills, expertise, and experience; IT capabilities; diagnostics connectivity; availability and adequacy of SOPs; supply chain; financial resources; recording and reporting forms, M&E tools and quality assurance systems. The high-level checklist for implementation of the Truenat TB tests in [Annex 2](#) may be useful to guide the situational analysis.

The TWG may also be involved in the selection of sites to conduct Truenat TB testing, although sites are usually selected by the NTP or National TB Reference Laboratory (NTRL). Site selection should be based on factors such as TB epidemiology, geographic considerations, testing workload, availability of qualified staff, efficiency of referral networks and patient access to services. For the prospective testing site, assessments will be needed of the suitability of the testing sites with respect to physical facilities, staffing and infrastructure (see [Annex 6](#) for a testing site assessment checklist).

Introducing Truenat TB testing at sites that previously had to refer specimens to other sites for rapid TB testing and detection of rifampicin resistance will have implications on the design of specimen referral networks. For resources on specimen referral planning, implementation and monitoring, see the GLI Specimen Referral Toolkit (<http://stoptb.org/wg/gli/srt.asp>).

A detailed, costed, prioritized action plan for phased implementation with targets and timeline should be developed. Budget considerations are summarized in [Annex 3](#).

2. Regulatory

Countries should work closely with the manufacturer and its authorized distributors to determine importation and registration requirements and to enable initiation of country verifications, if required. Most countries will need to only conduct small-scale verification studies (as opposed to large-scale validation studies) to demonstrate that 1) the laboratories can achieve the same performance characteristics as indicated by the manufacturer and 2) the method is suitable for its intended use in the population of patients being tested. For cost saving and efficiency, a more extensive verification study may be done at the NTRL using 50–60 samples (e.g., leftover or frozen sputum samples with known results) that will give a mix of results (e.g., positive and negative results, different semi-quantitative results). Verification at each testing site with a limited number of samples (e.g., 4–10 samples from proficiency testing panels) could serve multiple purposes including verification, demonstration of fitness-for-purpose of the instruments and competency assessment of users after on-site training.

Activities at a glance

- Determine importation requirements
- Conduct country verification study, if required
- Complete national regulatory processes

3. Equipment and site preparation

An essential step of the implementation process is the selection of instruments to fit the needs of the testing algorithm, anticipated workload and intended setting of use the Truenat TB tests. Laboratories should select the appropriate number and models of Truelab micro PCR Analyzers to match anticipated demand (see Table 4 for estimation of throughput). Note that the ability of a testing site to utilize all of the capacity of a workstation may not be an important factor for selecting Truenat TB testing sites,

especially considering that the Truenat TB test is designed to be placed at peripheral microscopy centers.

All instruments must be documented to be “fit for purpose” through testing with known positive and/or negative material prior to commencing testing of clinical specimens.

Planning for service and maintenance

Truelab and Trueprep instruments require minimal preventive maintenance as per the manufacturer. For an example of a preventive maintenance log that shows routine daily and monthly activities as well as ad hoc activities to be conducted as necessary, see [Annex 4](#).

The manufacturer offers warranty packages for equipment service and maintenance including through its growing network of in-country service providers. Annual warranties that provide comprehensive service and maintenance including on-site visits are available through the Stop TB Partnership's Global Drug Facility (GDF); see the [GDF Diagnostics Catalog](#) for more details. Countries planning to implement Truenat should inform the manufacturer in advance to allow the manufacturer to plan as needed for expansion of its network of service providers.

Site preparation and assessment of readiness

As part of the situational analysis, each of the selected testing sites will have been evaluated for suitability for conducting Truenat TB testing using a standardized checklist (see [Annex 6](#)). Key infrastructure and operational considerations for site preparation include:

- **Power:** In-built batteries in both devices allow for testing without power for up to 8 hours; devices will not allow for a cycle to start if battery power is too low. Batteries are expected to last five years. Note that power is still required to charge batteries: recharging time is approximately 4 hours for the micro PCR analyzers, 9 to 10 hours for the Trueprep device and 4 to 6 hours for the micro printer. Use of instruments while charging is possible, when the electrical socket is well-grounded. The devices are able to operate within the 100–240 voltage range; no additional voltage stabilization is required in settings with instabilities or low voltage. Solar panels may be a solution for settings without any access to power (see Box on specifications). Power may also be required to possibly cool the storage room for test chips; as described below, chips should be stored at $\leq 30^{\circ}\text{C}$.

Activities at a glance

- Select, procure, install and set-up equipment
- Verify the instruments are fit for purpose
- Arrange for preventive maintenance and servicing
- Prepare site infrastructure for Truenat instruments and testing
- Ensure a safe and functional testing site



Suggested specifications of a solar panel as per the manufacturer:

- Panel: 150 Watts. Dimensions (LxWxH): 1490 x 665 x 35 mm
- Battery: 12V 18Ah Lead Acid
- Solar Charge Controller + DC to DC boost converter (12V to 170V, 100 Watts)

Controller and converter available from Molbio; panel, battery and installation to be locally sourced.

- **Biosafety:** Truenat TB tests require the same biosafety precautions as microscopy, Xpert MTB/RIF or TB-LAMP. A well-ventilated room minimizes the risk of infection from aerosols during the initial specimen processing steps. None of the steps in the Truenat TB assay procedures requires use of a biosafety cabinet. Consult the *WHO Tuberculosis Laboratory Biosafety Manual*⁹ for more information on minimum biosafety precautions including appropriate use of bench space and personal protective equipment (PPE) when conducting procedures that are considered to carry a low risk of TB infection. For countries considering use of Truenat for COVID-19 testing, consult the most recent WHO guidance on laboratory biosafety.¹⁰
- **Waste management:** The assay results in a significant amount of plastic waste, which should be properly disposed of and incinerated as per national regulations. The manufacturer recommends submerging the used Truenat chips, microtube, microtube cap, transfer pipette, pipette tips etc. in freshly prepared 0.5% sodium hypochlorite solution for 30 minutes before disposal as per the standard medical waste disposal guidelines.
- **Room layout:** The Truelab instruments should be installed on a flat, stable surface (preferably non-metallic) away from instruments that may cause vibrations or electromagnetic interference and out of the path of direct sunlight or close to any radiating or heating apparatus. A surface with dimensions of 1.2 m by 0.6 m (4-foot by 2-foot) should be sufficient for the equipment and procedures. Exact dimensions of equipment are: Truelab Analyzer Uno, 248 mm x 185 mm x 112 mm; Truelab Analyzer Duo, 240 mm x 242 mm x 159 mm; Truelab Analyzer Quattro, 400 mm x 242 mm x 159 mm; Truelab micro PCR printer, 120.5 mm x 84 mm x 50.5 mm. Three well-grounded electrical outlets are recommended for operating or charging the instruments at once.
- **Security:** All laboratory equipment should be kept in a secure, lockable facility. The portable Truelab Real Time PCR Workstation Field Case and equipment should be stored in a secure lockable location when not in use.
- **Ambient temperature:** Trueprep AUTO v2 Devices and Truelab Analyzers are designed to be used at ambient temperatures (between 15°C to 40°C). For reference, the maximum room temperatures for use of GeneXpert (Xpert MTB/RIF) is 30°C, and for HumaLoop (TB-LAMP) is 40°C.
- **Humidity:** Trueprep AUTO v2 Devices and Truelab Analyzers can be used in humid settings (relative humidity: 10–80%)
- **Dust:** The Truelab Analyzer does not require air intake to allow for the PCR process, so Truenat use may not be compromised in dusty settings. Nevertheless, the manufacturer recommends installing the instruments in a dust-free environment when possible.

Preparing a safe and functional testing site is an important step for many of the other key implementation steps that follow including training, standardized procedures, quality monitoring etc. (Sections 4-10), Following completion of all implementation steps and prior to beginning testing of clinical specimens for patient care decisions, the site and staff should be evaluated for readiness using a standardized checklist (see [Annex Z](#) for an example).

Clinical site preparation

Clear clinical protocols and guidance will be needed for the selection of patients to be tested, ordering tests, interpreting test results, and making patient care decisions.

⁹ https://www.who.int/tb/publications/2012/tb_biosafety/en/

¹⁰ <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance-publications>

Clinical staff involved in the diagnosis and management of patients must be sensitized on updated testing algorithms that incorporate Truenat TB testing and new protocols and procedures prior to beginning the use of the Truenat TB test with clinical samples (see Section 9). A checklist for assessing the readiness of a clinical site is in [Annex 8](#).

4. Supply chain

Uninterrupted availability of reagents and disposables at the testing site is essential to ensure building of technical capacity in the early stages of implementation (avoiding delays between training and availability of reagents and disposables) and to ensure consistent service during routine use. The shelf life of reagents and their required storage conditions must be taken into consideration in the design of procurement, distribution and storage systems. The recommended storage conditions for the Truenat TB chips is 2°C–30°C and for the Sample Pre-treatment Pack and Prep Kit is 2°C–40°C. The shelf-life of all reagents is 2 years under recommended storage conditions. Truenat TB chips can be stored for up to 6 months at a temperature under 40°C, if conditions do not allow for storage under 30°C.

New lot testing, also known as lot-to-lot verification ensures the quality of the testing materials and prevents the use of test kits that generate test failures. The manufacturer recommends running positive and negative controls whenever a new shipment of Truenat TB test kits is received and for each new test kit lot. Controls can be purchased as part of the Truenat™ Positive Control Kit-Panel I.

For countries planning an initial order, the numbers of instruments and reagents needed per site will depend on the expected number of tests to be performed per day. Table 6 reflects the number of reagents to order for 1 year to match the anticipated number of tests to be performed and the required instrumentation.

Activities at a glance

- Review forecasting, ordering and distribution procedures
- Develop procedures to monitor reagent quality and shelf-life

For pricing of equipment, reagents and service packages through Stop TB Partnership's Global Drug Facility (GDF), see the [GDF Diagnostics Catalog](#).



Truelab® micro PCR Analyzer Uno Dx, Duo, Quattro

Table 6. Number of reagents to order for 1 year of testing based on planned average number of tests per day (260 working days)

| Average number of tests per day | Needed instruments | | |
|---------------------------------|--|---|---|
| | 1 Truelab Analyzer Uno Dx + 1 Trueprep AUTO v2 Device | 1 Truelab Analyzer Duo + 1 Trueprep AUTO v2 Device | 1 Truelab Analyzer Quattro + 2 Trueprep AUTO v2 Devices |
| 2 | 11 MTB/MTB Plus kits (Pre-treatment, Prep, Chip kits, 50 tests each) 3 MTB-RIF Dx kits (50 tests each) | Consider procuring a lower throughput model, unless testing is expected to increase over time | |
| 4 | 22 MTB/MTB Plus kits 5 MTB-RIF Dx kits | | |
| 6 | 33 MTB/MTB Plus kits 7 MTB-RIF Dx kits | | |
| 8 | 44 MTB/MTB Plus kits 9 MTB-RIF Dx kits | 44 MTB/MTB Plus kits 9 MTB-RIF Dx kits | |
| 10 | | 55 MTB/MTB Plus kits 11 MTB-RIF Dx kits | |
| 16 | | 88 MTB/MTB Plus kits 18 MTB-RIF Dx kits | 88 MTB/MTB Plus kits 18 MTB-RIF Dx kits |
| 24 | Procure a higher throughput model to meet testing needs | | 132 MTB/MTB Plus kits 27 MTB-RIF Dx kits |
| 32 | | | 175 MTB/MTB Plus kits 35 MTB-RIF Dx kits |

Note: The number of MTB-RIF Dx kits to order should depend on the anticipated proportion of people tested that will be MTB positive, and therefore in need of a test for rifampicin resistance. The anticipated number of tests needed should include the number of repeat rifampicin resistance tests that will be required given the need to confirm rifampicin-resistant results among patients in whom the result is unexpected and for tests that give errors or indeterminate results (see recommended algorithm in Section C). In the table above, an estimate of 20% is used. Tests are increased by 5% to account for potential wastage, and the resulting number of tests is rounded up to the nearest kit (50 tests per kit). Note that other kit sizes are available (kits with 5 or 20 tests).

5. Procedures

A well-defined, comprehensive set of standard-operating procedures (SOPs) that addresses all aspects of the Truenat testing processes from sample collection to results reporting is essential. Some SOPs will rely on the manufacturer’s protocols included with the commercial kits. Other SOPs may need to be developed or modified for Truenat TB testing. An example of an SOP for Truenat TB testing is in [Annex 1](#).

In addition to laboratory-related SOPs, clear clinical protocols and guidance will be needed for the selection of patients to be tested, ordering tests, completing test requisition forms, specimen collection, specimen referral, interpreting test results, and making patient care decisions. The availability of clear protocols and sensitization of clinical staff are important to strengthen the clinical-laboratory interface and ensure that clinical staff at all sites that should use the test, actually order the test.

Activities at a glance

- ❑ Develop standard operating procedures
- ❑ Update clinical procedures and strengthen the clinical-laboratory interface

6. Digital data

Truelab micro PCR analyzers offer the opportunity for multiple uses of digital data through in-built connectivity via SIM card, WIFI or Bluetooth. Data connectivity allows for automatic and rapid transmission of test results to clinicians by email or SMS, as well as potential for transmission of patient data and results to central electronic patient registers or LIMS (Laboratory Information Management Systems) via a customized API (application programming interface). Data can also be easily exported in CSV format for analyses. Third-party connectivity softwares (e.g., GxAlert/Aspect, DataToCare) can be configured to allow for integration of Truenat data into those platforms. For a discussion of the benefits and requirements of diagnostics connectivity, see the *GLI Quick Guide to TB Diagnostics Connectivity Solutions*.¹¹

Truelab analyzers can also be configured to send data on device performance to the manufacturer's (default configuration) or local servers to allow the manufacturer and/or the national TB programme to monitor instrument performance on a real-time basis. This allows for the identification and possible prevention of instrument malfunctions or breakdowns, detection of user errors and retraining needs, and monitoring of instrument and test utilization across fleets of instruments. The analyzers' software is configured to prevent patient data from being sent to the manufacturer's servers. For countries interested in utilizing the manufacturer's server and when national data regulations permit, the manufacturer can provide a free password-protected web-based dashboard to monitor utilization of instruments and test results. The analyzers utilize an Android operating system, and updates can be provided via the mobile network or WIFI

Truelab analyzers can store up to 20,000 test results in internal memory. Past test results can be viewed on the device, with search options to find results of specific patients or referring clinicians. As with any electronic data system, there is a risk of loss of testing data. An SOP for regular backing up of data (e.g., to an external drive) is essential as well as an SOP for data retrieval. There also must be policies and procedures to ensure the security of laboratory data and confidentiality of patient data. The manufacturer is working on incorporating a remote data wipe functionality in case the analyzer is stolen.

Activities at a glance

- Digital data and diagnostics connectivity
- Develop procedures for data backup, security and confidentiality

Realtime Truenat Data Dashboard in India

The National TB Elimination Program (NTEP) in India has worked with the manufacturer to develop a customized online dashboard of realtime data on Truenat use. The dashboard currently includes data on numbers of Truenat and Truenat Rif-Dx tests conducted, stratified by site, by time period, by age and gender, as well as numbers of TB cases and rifampicin resistant cases detected. The dashboard also provides data on inventory status to facilitate procurement and supply chain management. The NTEP is working with the manufacturer to enhance the dashboard to include automated analyses to facilitate monitoring, including information on malfunctioning machines and numbers of testing hours lost, trends in machine utilization, reports on errors that can guide user action and troubleshooting, and alerts when specific actions are required.

¹¹ GLI Quick Guide to TB Diagnostics Connectivity Solutions. Geneva, Global Laboratory Initiative, 2016. http://www.stoptb.org/wg/gli/assets/documents/gli_connectivity_guide.pdf

7. Quality assurance, control and assessment

Implementation of a laboratory quality management system including a comprehensive system of quality assurance is needed to ensure the accuracy, reliability and reproducibility of Truenat test results. Essential elements of a quality assurance system include standardized documents, use of Good Molecular Biology practices, competency assessment, internal quality controls, external quality assessment (EQA) including proficiency testing (PT) or blinded rechecking, on-site supervision and continuous quality improvement processes. Documentation of adherence or completion of each of the quality elements is also needed. A comprehensive discussion of the essential elements of a quality assurance system for any rapid TB diagnostic test may be found in the *GLI Practical Guide to Implementing a Quality Assurance System for Xpert MTB/RIF Testing*.¹²

Activities at a glance

- Implement a comprehensive quality assurance program
- Establish and monitor quality controls
- Develop an external quality assessment (EQA) program
- Monitor and analyze quality indicators (performance indicators)

The Truenat TB assays incorporate an internal positive control that undergoes all the processes that the specimen undergoes, from extraction to amplification, thereby assessing the validity of the test run from sample to result.

To ensure that the Truelab micro PCR Analyzer is working accurately, the manufacturer recommends running positive and negative controls (which can be purchased as part of the Truenat™ Positive Control Kit- Panel I) periodically. The positive and negative controls can also be used for lot-to-lot verification and assessment of reagents if the temperature of storage areas falls outside of the recommended ranges.

The Truenat system is a closed amplification system (i.e., the amplified product is sealed in the chip) and an enzyme system is incorporated in the reaction mix to prevent previously amplified material from getting re-amplified. Nonetheless, it is recommended that testing sites perform negative control tests using Trueprep AUTO lysis buffer reagent and sterile PBS monthly or when contamination is suspected (e.g., unusually high proportion of specimens with 'MTB detected'). Swab testing of work surfaces and both the Truelab and Trueprep machines should be conducted monthly.

Similar to what is recommended for AFB- smear microscopy¹³, testing of 10–15 specimens per week is recommended to maintain proficiency of staff conducting the Truenat TB tests. External quality assessment programmes for the Truenat TB tests are not yet available¹⁴, but can be modeled after the proficiency testing programme used for the Xpert MTB/RIF test. Similarly, monitoring of the quality indicators, also known as performance indicators, used for the Xpert MTB/RIF test should also be used for the Truenat TB test. These are described in the *GLI Practical Guide to Implementing a Quality Assurance System for Xpert MTB/RIF Testing*.

Laboratories should routinely collect, analyze and report key quality indicators, also known as performance indicators. An unexpectedly high frequency of errors may indicate that retraining of technicians is required or that the instruments require servicing; the Truelab analyzer manual includes a [table](#) of possible errors and their interpretations. [Annex 9](#) contains a list of recommended performance indicators for the Trueprep DNA isolation; Truenat MTB and MTB Plus tests; and the Truenat MTB-RIF Dx test.

12 Practical Guide to Implementing a Quality Assurance System for Xpert MTB/RIF Testing. Global Laboratory Initiative. 2019. Geneva, Switzerland. <http://www.stoptb.org/wg/gli/pgiqas.asp>

13 Practical Guide to TB Laboratory Strengthening. Global Laboratory Initiative. 2017. Geneva, Switzerland. http://www.stoptb.org/wg/gli/assets/documents/GLI_practical_guide.pdf

14 FIND will be piloting a Truenat EQA program in India in the second half of 2020

8. Recording and reporting

Depending on the current format of the country's requisition (specimen examination request) form, it may be necessary to make revisions to accommodate the Truenat TB tests. Similarly, laboratory and clinical registers may need to be modified to record the results of the Truenat TB tests and Truenat MTB-RIF Dx tests. Because the Truenat TB tests and the Xpert MTB/RIF tests generate the same type of information (e.g., MTB detected or not detected), the forms and registers being used for the Xpert MTB/RIF test may be suitable for use with the Truenat TB tests. As such, generic forms suitable for use with Xpert TB tests, Truenat TB test or other NAAT are possible, although countries may decide to develop and use test-specific forms.

The revisions to the test requisition forms, reporting forms and registers should ensure that the relevant patient and test data are captured and that the essential information (e.g., test result) is provided in an easy-to-read format to facilitate the interpretation of the test results and decision making by end users (clinicians or NTP staff).

Activities at a glance

- Review and revise request for examination and reporting forms
- Review and revise laboratory and clinical registers

9. Training and competency assessment

The Truenat TB test procedures require multiple hands-on steps as well as precision micro-pipetting. Laboratory technicians should be properly trained on all procedures and in Good Molecular Biology practices. For laboratory staff conducting the Truenat TB tests, a training curriculum may include:

- Background on the scientific basis of Truenat TB testing
- The Truenat TB testing algorithm
- Use of test requisition forms, results reporting forms and laboratory registers
- Operation of testing instruments
- Standard operating procedures
- Hands-on experience with sample preparation, DNA extraction and chip processing
- Using and evaluating quality controls
- External quality assessment programmes
- Good laboratory practices including equipment maintenance and cleaning, reagent storage, waste disposal and chemical and biological safety
- Troubleshooting, including interpretation of errors

Activities at a glance

- Develop and implement a training curriculum and strategy
- Assess and document the competency of staff

Early implementers using the test at peripheral level health centers have not cited significant problems in training microscopists to use the Truenat TB tests. One procedure that requires special care in training technicians is the micropipetting/dispensing of 6µl of DNA eluate solution into the well of the Truenat chip: a "steady hand" may also be an asset. The flatscreen digital interface on the Truelab micro PCR analyzer has not

been cited as being problematic for technicians without prior experience with similar technologies.

In addition to the manufacturer-provided manuals, on-site SOPs and other job aids should be provided as reference materials for technicians. Job aids are available from the manufacturer; see [Annex 11](#) for examples.

Clinician training or sensitization must be done in parallel with training of laboratory staff to ensure all clinicians involved in screening and care of TB patients understand the benefits and limitations of the Truenat TB test, are sensitized to the Truenat TB testing algorithm, test requisition process, specimen referral procedures, interpretation of results and necessary follow-up testing. For clinical staff including clinicians, nurses and other health care workers, training curricula may include:

- Background on the scientific basis of Truenat TB testing
- The diagnostic cascade and Truenat TB testing algorithms
- Guidelines for selection of patients for Truenat TB testing
- Procedures for specimen collection, labelling, storage, packaging and transport
- Use of test requisition forms and clinical registers
- Recording and reporting of results
- Guidelines for interpreting Truenat TB test results for patient care decisions
- Follow-up testing that may be needed

Competency assessments of laboratory technicians should be performed after training and periodically (e.g., annually) thereafter and should include assessment of the knowledge and skills for performing each of the tasks involved in a diagnostic test. The positive and negative controls in the Truenat™ Positive Control Kit-Panel I can be used for competency testing.

10. Monitoring and evaluation

During the initial planning phase, countries should establish a set of key indicators and milestones that can be used to monitor the implementation process. The high-level checklist in [Annex 2](#) may be useful for monitoring implementation. Once launched, utilization of the testing services should be tracked. For example, the rate of ordering of Truenat TB tests could be monitored to determine if clinical staff at all sites that should offer the test, actually order the test.

A framework for monitoring and evaluation of the impact of a diagnostic test is essential to inform decision-making. See [Annex 10](#) for an example framework of indicators that can be collected to monitor and evaluate impact.

Activities at a glance

- Monitor implementation of the Truenat TB tests
- Monitor and evaluate impact of the Truenat TB tests

PART

C



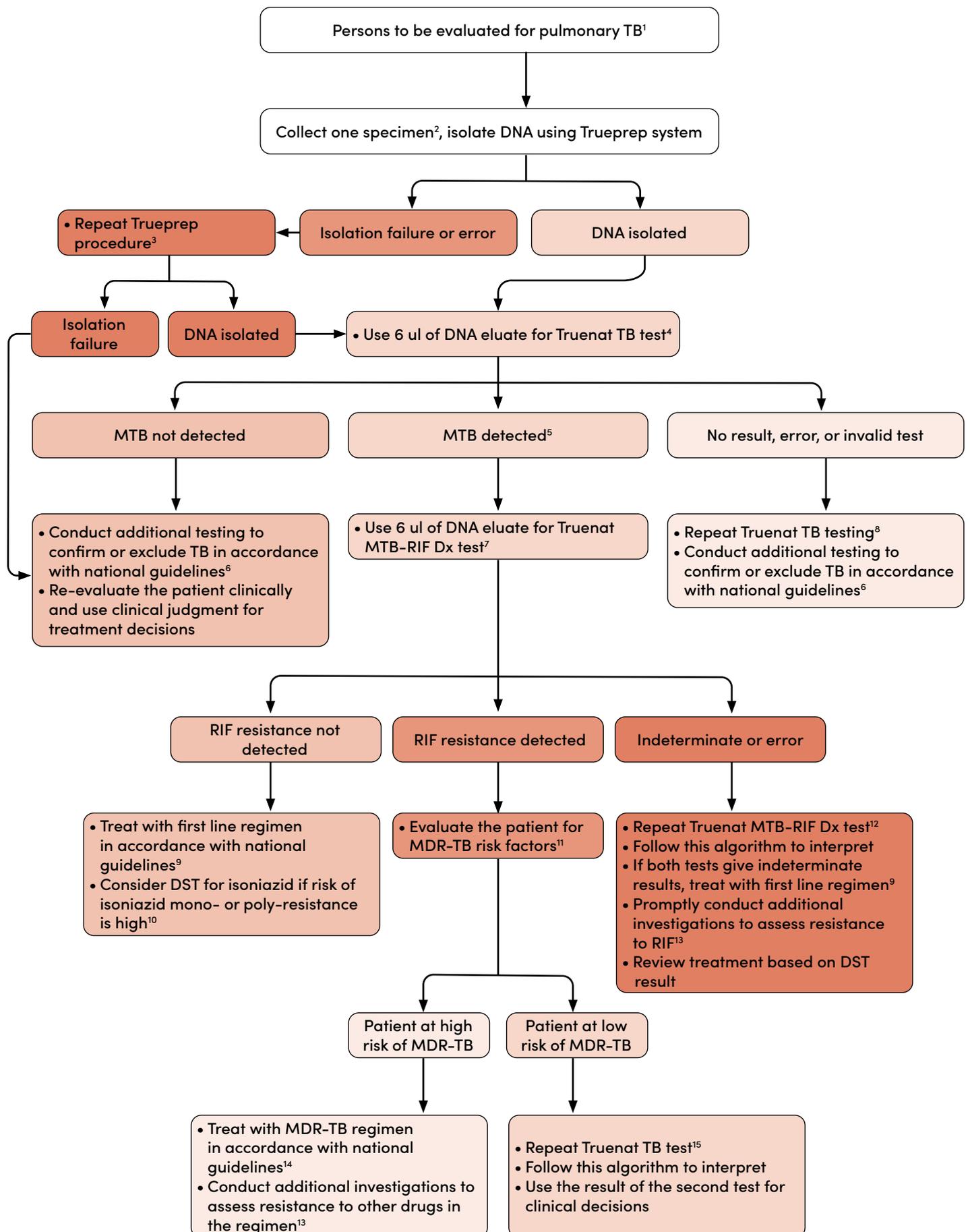
Image: Tuberculosis Reference Laboratory Bamenda, Cameroon

Truenat TB Testing Algorithm

Effective and efficient TB diagnostic algorithms are key components of a diagnostic cascade which ensures that patients with TB are accurately and rapidly diagnosed and are promptly placed on appropriate therapy. Both laboratory and clinical staff must be trained in the diagnostic algorithm to ensure that the testing is optimally used. The following algorithm and decision tree describe the use of the Truenat TB tests as the initial diagnostic test for persons being evaluated for having pulmonary TB. This algorithm is adapted from the model WHO algorithm on use of a rapid molecular diagnostic as the initial test for TB depicted in the 2020 *WHO operational handbook on tuberculosis, Module 3: Diagnosis (Algorithm 1)*¹⁵.

15 WHO operational handbook on tuberculosis. Module 3: diagnosis - rapid diagnostics for tuberculosis detection. Geneva: World Health Organization; 2020. <https://www.who.int/publications/i/item/who-operational-handbook-on-tuberculosis-module-3-diagnosis---rapid-diagnostics-for-tuberculosis-detection>

Algorithm for the use of the Truenat TB tests as the Initial Diagnostic Test for pulmonary TB



1. Persons to be evaluated for pulmonary TB include adults and children with signs or symptoms suggestive of TB or with a chest X-ray with abnormalities suggestive of pulmonary TB.
2. Programmes may consider collecting two specimens upfront. The first specimen should be promptly tested using the Truenat test. The second specimen may be used for the additional testing described in this algorithm. For persons being evaluated for pulmonary TB, sputum is the preferred specimen.
3. Repeat the DNA isolation using the same sputum specimen and a second Trueprep cartridge. Unsuccessful isolations are typically related to cartridge errors. If both attempts fail, conduct additional testing to confirm or exclude TB in accordance with national guidelines.
4. Truenat TB test refers to either the Truenat MTB test or MTB Plus test.
5. MTB detected includes 'detected' on Truenat MTB and 'detected high', 'moderate', 'low', or 'very low' on Truenat MTB Plus.
6. Further investigations for TB may include chest X-ray, additional clinical assessments, clinical response following treatment with broad-spectrum antimicrobial agents, repeat Truenat TB testing, testing with other WHO-approved rapid diagnostics (e.g., Xpert MTB/RIF Ultra) or culture.
7. For samples that are positive in the Truenat MTB or MTB Plus test, a portion of the DNA elute used for the Truenat TB tests and the Truelab Real Time micro PCR Analyzer are used for the Truenat MTB-RIF Dx test to determine RIF resistance.
8. Repeat the Truenat TB test using the same DNA eluate or a fresh sample. Tests with an Invalid result should be repeated using a fresh specimen and processed starting with the sample preparation step. If both tests give indeterminate inconclusive results, conduct additional testing to confirm or exclude TB in accordance with national guidelines.
9. Patients should be initiated on a first-line regimen according to national guidelines unless the patient is at very high risk of having MDR-TB. Such patients at very high risk should be initiated on an MDR-TB regimen.
10. A sample may be sent for molecular (preferred) or phenotypic drug susceptibility testing (DST) for isoniazid (INH) if there is a high prevalence of INH resistance not associated with RIF resistance (i.e., INH mono- or poly-resistance) in this setting or if required by national guidelines. Do not delay initiation of therapy to wait for the results of additional DST.
11. Patients at high risk for MDR-TB include previously treated patients including those who had been lost to follow-up, relapsed, and failed a treatment regimen; non-converters (smear positive at end of intensive phase); MDR-TB contacts; and any other MDR-TB risk groups identified in the country.
12. Repeat the Truenat MTB-RIF Dx test using the same DNA eluate or a fresh specimen. Interpret the result of the repeat test as shown in this algorithm. Use the result of the second test for clinical decisions. Inconclusive results are usually related to very low numbers of bacilli in the sample such as samples with the Truenat MTB Plus test result of 'MTB Detected very low'. Repeat testing with the same DNA isolate only produces interpretable results in about 30% of retests.
13. Phenotypic (culture and DST) and molecular (line-probe assays, DNA sequencing, high-throughput assays, etc.) methods are available to evaluate drug resistance. Rapid molecular methods are preferred.
14. Patients should be promptly initiated on an MDR-TB regimen in accordance with national guidelines and WHO recommendations.
15. Repeat the Truenat MTB-RIF Dx test using a DNA eluate from a fresh specimen. Interpret the result of the repeat test as shown in this algorithm. Use the result of the second test for clinical decisions.

Decision Tree for the Truenat TB testing algorithm

Note: 'Truenat TB test' designates either the Truenat MTB test or the Truenat MTB Plus. The individual tests are named when describing test-specific features.

General considerations:

- WHO recommends the use of a Truenat TB test as the initial diagnostic test rather than microscopy, culture and drug-susceptibility testing (DST) for all persons with signs and symptoms of TB who are being evaluated for pulmonary TB. This includes all newly presenting symptomatic persons and may also include patients who are on treatment or have been previously treated if the patient is being evaluated for possible RR-TB (e.g., non-converters at the end of the intensive phase of treatment) or for a new or continuing episode of TB (e.g., relapse cases or previously treated patients including those who had been lost to follow-up).
- The Truenat TB tests are recommended for use in testing adults and children with signs and symptoms of pulmonary TB. These tests only detect MTBC. A second test (Truenat MTB-RIF Dx test) on DNA isolated for the Truenat TB test is conducted to assess RIF resistance. Considerations for these tests include:
 - There is uncertainty about use of this test in PLHIV, because at the time of the WHO recommendations there were no data available on the performance of these tests in PLHIV. The indirect data on test performance in smear-negative patients were used by WHO to extrapolate the recommendation to use in PLHIV. Because of its increased sensitivity for detecting MTBC in smear-negative samples, the Truenat MTB Plus test may be the better test to use in populations with a high prevalence of HIV or for persons known to be HIV-positive.
 - In children, sufficient data were available to recommend the use of these tests with sputum samples only. There were no data on how these tests performed with other specimens.
 - The performance of these tests for the detection of extrapulmonary TB is unknown.
- The higher sensitivity of the Truenat MTB Plus test compared to the Truenat MTB test is accompanied by a slight loss of specificity (i.e., an increase in the number of patients incorrectly identified as having active TB). This is because the Truenat MTB Plus assay can detect very small numbers of bacilli which may be non-viable or non-replicating, particularly in patients with a history of TB treatment. Such non-viable bacteria may also be detected by the Truenat MTB test, albeit less frequently. The Xpert Ultra test (the more sensitive test) and the Xpert MTB/RIF test (the less sensitive test) behave similarly.¹⁶
- The Truenat TB tests are not recommended as tests to monitor treatment because the presence of dead bacilli may generate a positive result. Microscopy and culture should be used in accordance with national guidelines and WHO recommendations.
- The algorithm describes the collection of one initial specimen to be used for Truenat TB testing and the collection of additional specimens as needed. For operational issues, programmes may consider collecting two specimens (e.g., two

¹⁶ WHO consolidated guidelines on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection. Geneva: World Health Organization; 2020. <https://www.who.int/publications/i/item/who-consolidated-guidelines-on-tuberculosis-module-3-diagnosis---rapid-diagnostics-for-tuberculosis-detection>

spot specimens, or a spot and morning specimens) from each patient routinely instead of only collecting a second specimen when additional testing is needed. If so, the first specimen should be promptly tested using a Truenat TB test. The second specimen may be used for the additional testing described in the algorithm (e.g., repeat testing, further DST) or for smear microscopy or culture as a baseline for treatment monitoring.

- For the Truenat MTB test, if MTB is detected, the estimated number of bacteria in terms of colony forming units per ml (CFU/ml) in the original sample is also reported. For the Truenat MTB Plus test, semi-quantitative results are reported as 'MTB not detected'; 'MTB detected (high, medium, low or very low)'; 'no result'; 'error'; or 'invalid'. Each of the semi-quantitative categories of MTB detected is considered as bacteriological confirmation of TB.
- The Trueprep AUTO v2 system produces approximately 100 microliters of high-quality DNA.¹⁷ While more research is needed, the DNA preparation is likely suitable for use in many molecular procedures including PCR and line-probe assays. Upon confirmation of its suitability for other testing, laboratories may be able to ship the DNA eluate rather than a specimen to other laboratories for additional testing (e.g., Step 6b or Step 7c below).

Decision Tree:

1. Collect a good quality specimen and transport it to the testing laboratory. For persons being evaluated for pulmonary TB, induced or expectorated sputum samples may be used. Little or no published data are available on the performance of the Truenat TB test with non-sputum or extrapulmonary TB samples, and WHO did not make a recommendation regarding the use of the Truenat TB tests with such specimens.
2. Prepare the sample (Trueprep AUTO MTB Sample Pre-treatment Pack) and isolate DNA using the Trueprep AUTO v2 Universal Cartridge Based Sample Prep Kit and the Trueprep AUTO v2 Universal Cartridge Based Sample Prep Device.
 - a. If DNA isolation is unsuccessful, repeat the DNA isolation with another portion of the prepared sample. Unsuccessful isolations are typically associated with cartridge errors (damaged cartridge valve, clogged cartridge, a leak in the cartridge, heater failure, etc.), and the Trueprep AUTO v2 device instrument will provide an error message identifying the category of error. The manufacturer states that sputum may be stored in lysis buffer for up to 1 week at 30°C or 3 days at 40°C with no degradation of DNA. If both tests fail, consider collecting a fresh specimen and processing as well as conducting additional testing to confirm or exclude TB in accordance with national guidelines.
 - b. If DNA isolation is successful, use 6 µL for the Truenat TB test.
3. If the Truenat TB test result is 'MTB not detected', re-evaluate the patient and conduct additional testing in accordance with national guidelines.
 - a. Further investigations for TB may include chest X-ray, additional clinical assessments, clinical response following treatment with broad-spectrum

17 Beall SG, Cantera J, Diaz MH, Winchell JM, Lillis L, White H, et al. (2019) Performance and workflow assessment of six nucleic acid extraction technologies for use in resource limited settings. PLoS ONE 14(4): e0215753. <https://doi.org/10.1371/journal.pone.0215753>

antimicrobial agents, additional Truenat TB testing, testing with other WHO-approved rapid diagnostic test (e.g., Xpert MTB/RIF) or culture.

- b. Consider the possibility of clinically defined TB (i.e., TB without bacteriological confirmation). Use clinical judgement for treatment decisions.
4. If the Truenat TB test does not give a result or gives a result of error, the Truenat TB test should be repeated at the same testing site with a second aliquot of the remaining DNA or with a fresh sample. Tests with an 'invalid result' should be repeated using a fresh specimen starting with the sample preparation step. In the FIND study, repeat testing reduced inconclusive results from 6.2% to 1.7% for the Truenat MTB test and from 9.2% to 3.9% for the Truenat MTB Plus test.
 - a. Follow this algorithm if the second test gives a valid result (MTB not detected or MTB detected).
 - b. If both tests give inconclusive results, conduct additional testing to confirm or exclude TB in accordance with national guidelines.
 5. If the Truenat TB test result is 'MTB detected'
 - a. The result of 'MTB detected' should be considered as bacteriological confirmation of TB.
 - b. Use 6 µL of the DNA eluate and the Truelab Real Time micro PCR Analyzer for the Truenat MTB-RIF Dx test to determine RIF resistance.
 - c. While any delay should be avoided, if for some reason there is a significant delay in conducting the Truenat MTB-RIF Dx test (expected assay time is 1 hour), the patient should be initiated on an appropriate regimen using first-line TB drugs in accordance with national guidelines unless the patient is at very high risk of having MDR-TB. Such patients should be initiated on an MDR-TB regimen. Note that in most settings, a history of prior TB treatment is not sufficient to indicate that the patient is at very high risk of having MDR-TB for the purpose of making treatment decisions.
 6. If the Truenat MTB-RIF Dx test result is 'RIF resistance not detected'.
 - a. The patient should be initiated on an appropriate regimen using first-line TB drugs in accordance with national guidelines unless the patient is at very high risk of having MDR-TB. Such patients should be initiated on an MDR-TB regimen.
 - b. Programmes may request additional DST in accordance with national algorithms.
 - i. Molecular (genotypic) or phenotypic DST for INH is particularly indicated:
 - * if the patient has been previously treated with INH or is a contact of a known Hr-TB patient;
 - * if there is high prevalence of INH resistance that is not associated with RIF resistance (i.e., INH mono-resistance or poly-resistance, not MDR-TB) in this setting;
 - * to determine whether there is a mutation associated with resistance in the *InhA* gene (for Ethionamide) to guide decision-making around continuation of a shorter MDR-TB regimen; or
 - * if national guidelines require DST for INH
 - ii. Molecular (genotypic) or phenotypic DST for resistance to RIF may be requested if the patient is considered to be at risk of having RR-TB despite the initial Truenat MTB-RIF Dx result. False RIF-susceptible results are rare

with WHO-approved rapid diagnostics but have been observed in 1–5% of TB cases tested in various epidemiologic settings. In contrast, phenotypic DST for RIF, especially using liquid culture, is associated with a higher proportion of false-susceptible results.¹⁸

7. If the Truenat MTB-RIF Dx test result is 'RIF resistance detected', an MDR-TB risk assessment is needed. Patients at high risk for MDR-TB include previously treated patients including those who had been lost to follow-up, relapsed or failed a treatment regimen; non-converters (e.g., smear positive at end of intensive phase of treatment for drug-susceptible TB); contacts of MDR-TB patients; and any other MDR-TB risk groups identified in the country. In high MDR-TB burden countries, every TB patient is considered to be at high risk of having MDR-TB.
 - a. If the patient is at high risk of having MDR-TB, the RIF-resistant test result is definitive and the patient should be initiated on a regimen for RR-TB or MDR-TB in accordance with national guidelines and WHO recommendations¹⁹.
 - b. If the patient is at low risk of having MDR-TB, repeat the Truenat TB test and MTB-Rif Dx test on a fresh sample.
 - i. Initiate an MDR-TB regimen in accordance with national guidelines if the second test also indicates RIF resistance.
 - ii. Initiate treatment with a first-line regimen in accordance with national guidelines if the Truenat MTB-RIF Dx result for the second sample is RIF resistance not detected. While in most situations false-positive RIF-resistant results due to technical performance of the assay are rare, false-positive RIF-resistant results due to laboratory or clerical errors may be more likely. It is assumed that repeat test is performed with more caution and the result of the second test is correct and the result of the first test may have been due to a laboratory or clerical error.
 - c. For all patients with RR-TB or MDR-TB, conduct additional investigations to assess resistance to the other drugs being used in the treatment regimen. Phenotypic (culture and DST) and molecular (line-probe assays, DNA sequencing, centralized molecular assays, etc.) methods are available to evaluate drug resistance.²⁰ Rapid molecular methods are preferred.
 - i. For MDR-TB regimens that rely on the use fluoroquinolones (e.g., the WHO recommended 18 to 20-month regimen with BDQ, LZD and a later generation fluoroquinolone), a sample should be submitted for molecular testing for fluoroquinolone resistance.
 - ii. Ideally, a specimen from each patient should be submitted for DST for each of the drugs used in the regimen for which there is a reliable testing method. However, treatment initiation should not be delayed to await DST results (e.g., phenotypic DST can take weeks to months to provide results).
 - iii. Any positive culture recovered during treatment monitoring that is suggestive of treatment failure should undergo DST for the drugs used in the treatment regimen.

18 A. Van Deun et al. Rifampin drug resistance tests for tuberculosis: Challenging the gold standard. *J Clin Microbiol.* August 2013; 51 (8): 2633–2640. DOI: <http://dx.doi.org/10.1128/JCM.00553-13>

19 Consolidated guidelines on drug-resistant tuberculosis treatment. WHO/CDS/TB/2019.7. Geneva: World Health Organization, 2019. <https://www.who.int/tb/publications/2019/consolidated-guidelines-drug-resistant-TB-treatment/en/>

20 Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis. WHO/CDS/TB/2018.24. Geneva: World Health Organization, 2018. https://www.who.int/tb/publications/2018/WHO_technical_drug_susceptibility_testing/en/

8. If the Truenat MTB-RIF Dx test gives a result of 'RIF indeterminate', the initial result of 'MTB detected' should be considered as bacteriological confirmation of TB. The patient should be initiated on an appropriate regimen using first-line TB drugs in accordance with national guidelines unless the patient is at very high risk of having MDR-TB. Such patients should be initiated on an MDR-TB regimen. Note that in most settings, a history of prior TB treatment is not sufficient to indicate that the patient is at very high risk of having MDR-TB for the purpose of making treatment decisions.
 - a. The 'RIF resistance indeterminate' result with most Truenat MTB-RIF Dx tests is usually caused by a paucibacillary TB load in the sample. In this case, repeating the Truenat MTB-RIF Dx test using a DNA eluate from a fresh specimen or another aliquot of the DNA eluate may be useful. However, in the FIND study, repeat testing with the same DNA eluate produced interpretable results in only about 30% of retests. Interpret the result of the repeat test as shown in this algorithm. Use the result of the second test for clinical decisions.
 - i. If the result of the second Truenat MTB-RIF Dx test is 'RIF resistance not detected' follow step 6. If it is 'RIF-resistance detected' follow step 7.
 - ii. If both tests generate results of 'RIF resistance indeterminate', additional investigations such as culture and phenotypic DST or genotypic DST may be needed to confirm or exclude resistance to RIF because the indeterminate result provides no information on resistance.
 - b. Culture and DST or testing with other rapid diagnostics (e.g., Xpert MTB/RIF, line probe assays) may be performed for follow up testing to confirm or exclude RIF resistance.



Suggested Reading



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- WHO operational handbook on tuberculosis. Module 3: diagnosis - rapid diagnostics for tuberculosis detection. Geneva: World Health Organization, 2020. <https://www.who.int/publications/i/item/who-operational-handbook-on-tuberculosis-module-3-diagnosis---rapid-diagnostics-for-tuberculosis-detection>
 - WHO consolidated guidelines on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection. Geneva: World Health Organization, 2020. <https://www.who.int/publications/i/item/who-consolidated-guidelines-on-tuberculosis-module-3-diagnosis---rapid-diagnostics-for-tuberculosis-detection>
 - GLI Practical Guide to TB Laboratory Strengthening. Geneva: Global Laboratory Initiative, 2017. <http://stoptb.org/wg/gli/gat.asp>
 - GLI Practical Guide to Implementing a Quality Assurance System for Xpert MTB/RIF Testing. Geneva: Global Laboratory Initiative, 2019 . <http://www.stoptb.org/wg/gli/pgiqas.asp>
 - GLI Planning for country transition to Xpert® MTB/RIF Ultra Cartridges. Geneva: Global Laboratory Initiative, 2017. http://www.stoptb.org/wg/gli/assets/documents/GLI_ultra.pdf
 - WHO Consolidated guidelines on drug-resistant tuberculosis treatment. WHO/CDS/TB/2019.7. Geneva: World Health Organization, 2019. <https://www.who.int/tb/publications/2019/consolidated-guidelines-drug-resistant-TB-treatment/en/>
 - GLI Quick Guide to TB Diagnostics Connectivity Solutions. Geneva: Global Laboratory Initiative, 2016. http://www.stoptb.org/wg/gli/assets/documents/gli_connectivity_guide.pdf
 - WHO Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis. WHO/CDS/TB/2018.24. Geneva: World Health Organization, 2018. https://www.who.int/tb/publications/2018/WHO_technical_drug_susceptibility_testing/en/
 - Package inserts and manuals for the instruments and reagent kits are available on the manufacturer’s website. <http://www.molbiodiagnostics.com/products-listing.php>
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Annexes



Annex 1: Example of an SOP for Truenat™ MTB Plus using a Truelab® Duo analyzer

Courtesy of the Bamenda TB Reference Laboratory, Cameroon, with minor adaptations for this guide

| | | |
|---------------------------|---|--------------|
| Name of laboratory | Standard Operating Procedure (SOP) Truenat™ MTB Plus assay | Code: TN-01 |
| | | Version: 1.0 |
| | | Date: X |
| | | Page: X of X |

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| | Compiled by | Examined by | Approved by | Replace version | New version |
|----------------------|-------------|-------------|------------------|--------------------|-------------|
| Name | | | | | |
| Date | | | | | |
| Signature | | | | | |
| Laboratory area: | | | N° of copies: 01 | Reason for change: | |
| Date of next review: | | | | | |

1. Scope

This SOP describes the use of the Truenat™ MTB Plus assay, a chip-based Real Time Polymerase Chain Reaction (PCR) test, for the semi-quantitative, detection and diagnosis of Mycobacterium tuberculosis complex bacteria (MTBC) in human sputum samples.

2. Definitions and abbreviations

- PCR: Polymerase Chain Reaction
- DNA: Deoxyribonucleic Acid
- MTB: Mycobacterium tuberculosis complex
- IPC: Internal Positive Control
- Ct: Cycle threshold
- LCD: Liquid-crystal Display
- ECT: Elute Collection Tube

3. Education and training

All lab staff performing this procedure must have successfully completed training in the following areas: potential risks to health (symptoms of TB disease and transmission), precautions to be taken to minimize aerosol formation and prevent exposure, hygiene requirements, wearing and use of protective equipment and clothing, handling of potentially infectious materials, prevention of incidents and steps to be taken by workers in the case of incidents (biohazard incidents, chemical, electrical, post exposure prophylaxes and fire hazards), good laboratory practice and good microbiological techniques, organization of work flow from clean to dirty areas, use of chemical and biological indicators, waste management, use of equipment (operation, identification of malfunctions, maintenance).

The training is given before a staff member takes up his/her post, strictly supervised, adapted to take account of new or changed conditions.

4. Procedure

4.1 Principle

The Truenat™ MTB Plus works on the principle of Real Time Polymerase Chain Reaction. A sputum specimen is first liquefied and lysed using the Trueprep™ AUTO MTB Sample Pre-treatment Pack. The DNA from the sample is then extracted using the Trueprep™ AUTO v2 Universal Cartridge Based Sample Prep Device and Trueprep™ AUTO v2 Universal Cartridge Based Sample Prep Kit. The extracted DNA is then amplified by the Truelab Real Time micro-PCR analyzer. The Truenat™ MTB Plus chip is placed on the chip tray of the Truelab™ Real Time micro PCR Analyzer. Six (6) µL of the purified DNA is then dispensed into the reaction well of the Truenat™ MTB Plus chip and the test is started.

4.2 Sample

- Sputum samples

4.3 Equipment and Materials

4.3.1 Extraction of DNA

- Trueprep AUTO v2 Universal Cartridge Based Sample Prep Device
- Trueprep AUTO MTB Sample Pre-treatment Pack
- Trueprep AUTO v2 Universal Cartridge Based Sample Prep Kit

4.3.2 Amplification of purified DNA

- Truelab Duo Real Time Quantitative micro PCR Analyzer
- Truenat™ MTB Plus micro PCR chip
- Truelab™ micro PCR printer
- Truepet SPA fixed volume (6 µl) Precision micropipette
- DNase and RNase-free pipette tips with filter barrier

4.3.3 Others

- Truenat™ Positive Control Kit – Panel I
- Powder free disposable gloves
- Two waste disposal containers, with lids, containing bleach solutions
- Timer
- Two waste bags
- Microtube Stand
- Cartridge Holder
- Two cryovial racks

4.4 Reagents and Solutions

- Liquefaction buffer
- Lysis buffer
- Conc. bleach and 70% alcohol

4.5 Detailed procedure

4.5.1 Specimen collection

Spot and morning sputum samples are collected from each patient.

4.5.2 Sample storage and transportation

Samples collected for testing on the Truenat instruments should be stored in the fridge between 2°C to 8°C and transported to the testing lab. During transportation, the samples should be well parceled in a

sample flask/sample transportation box at 2°C to 8°C containing ice packs.

4.5.3 Installation of Trueprep AUTO v2 Universal Cartridge Based Sample Prep Kit Reagent Pack

1. Connect a new reagent pack to the Trueprep Auto v2 device by inserting the Plug-in Connector into the slot provided (**Figure 1**).
2. One reagent pack is sufficient to conduct 50 extractions.
3. When the instrument is off, press the “Power” button to switch on the Trueprep™ AUTO v2 device. Power in use indicator LED glows red.

Note:

- **Trueprep™ AUTO v2 device will not let you begin a run if the battery is low.**
 - For charging or to use Trueprep™ AUTO v2 on direct electricity line, connect the AC Adapter to the charging port on the left side of the back panel of the device and the other end to the lightning protected distributor.
4. When new buffer is loaded using a new Reagent Pack of color-coded reagent bottles, perform a buffer count reset. When prompted to change the Reagent Pack and reset, press ‘start’ and ‘eject’ simultaneously to reset.

4.5.4 Sample Processing procedure

Prior to sample processing on the Truelab instrument, the sputum sample should be homogenized and pipettable.

1. Put on personal protective equipment.
2. Clean the working surfaces with freshly prepared 10% bleach then with 70% alcohol.



Figure 1



Procedure to change Trueprep AUTO v2 Reagent Pack after completion of 50 extractions

- a. After completion of 50 extractions, the Trueprep AUTO v2 device will prompt the user to change the reagent pack and reset the buffer count.
- b. Disconnect the used reagent pack by removing the Plug-in connector.
- c. Take a new reagent pack. Hold the reagent pack's connector and remove the cap.
- d. Plug in the connector into the socket of Trueprep AUTO v2.
- e. Press Eject button to open the cartridge holder and gently pull out the door.
- f. Insert the Reagent card as shown and gently push to close the cartridge holder.
- g. Press start button.
- h. It will display New Reagent Pack Registered and Ejects the Reagent Reset Card.
- i. Remove Reagent Reset card and proceed with further testing

3. Clean the instruments with paper towel wet with 70% alcohol.
4. Empty the two liquid waste containers and fill the two waste containers ½ way with concentrated bleach solution.
5. Open an Trueprep AUTO MTB sample pre-treatment kit, which contains a graduated 1 mL transfer pipette, lysis buffer bottle and liquefaction buffer bottle. Bring all refrigerated samples or reagents to room temperature before using.
6. Arrange the items needed to run a complete batch of 2 samples.
 - i. Liquefaction buffer bottle
 - ii. Graduated 1ml and 3ml transfer pipette
 - iii. Lysis buffer bottle. Visually check for any damage and that the volume is 2.5ml. If the volume is less than 2.5ml due to damage, do not use that lysis buffer.
 - iv. Cartridge pouch and the cartridge holder.
 - v. Result register
7. Ensure the Trueprep AUTO v2 sample prep device is ON.
8. Arrange the cryovials containing the 0.5ml pipettable sputum in ascending order of sample numbers on the sample rack.
9. Record sample information (serial number, sample number, date of test) in the Truenat register.
10. Label lysis buffer bottle with corresponding sample number and date of extraction.
11. Place labelled lysis buffer bottle in front of the corresponding sample.
12. Add 2 drops of liquefaction buffer to sputum container containing 1st sample in the batch.
13. Swirl container to allow buffer to mix with sample.
14. Incubate for 10 minutes at room temperature. If sample is not pipettable after 10 minutes, incubate for another 5 minutes with swirling at 2 minutes intervals.
15. Transfer 0.5 ml of liquefied sputum sample from the sample container to the corresponding lysis buffer bottle using the 1ml graduated transfer pipette provided.
16. Dispose the transfer pipette into the container filled with concentrated bleach.
17. Add 2 drops of liquefaction buffer into the lysis buffer bottle.

Note: To avoid cross contamination, DO NOT bring the nozzle of the liquefaction buffer bottle near the sample container.
18. Swirl gently to mix and incubate lysis buffer bottle at room temperature for 3-5 minutes and observe to ensure that the sample has completely liquified.

Note: Sputum may be stored in lysis buffer for up to 1 week at 30°C with no degradation of DNA

4.5.5 Nucleic acid extraction

19. While incubating the sputum in the lysis buffer, tear open the cartridge pouch. Each pouch contains a cartridge, an eluate collection tube (ECT) and a transfer pipette.
20. Take out the cartridge and place on the cartridge stand and keep the transfer pipette and the ECT in the pouch for later use.
21. Observe the sample chamber and visually confirm that the reddish IPC is present. If absent, discard that cartridge and take a different one and report this issue.
22. Label the cartridge pouch with the patient number.
23. Label the cartridge with number of the sample and the date of test.
24. Open the sample chamber of the cartridge by gently pulling the black cap upward.

25. Transfer **ALL** the contents of the lysis buffer bottle (3 ml) into the sample chamber of the cartridge using the 3ml transfer pipette provided.

Note: Sample should not be stored inside the cartridge. Therefore, only load the cartridge when ready to run the test.

26. Dispose the transfer pipette and the used lysis buffer bottle into the waste container filled with concentrated bleach.
27. Recap the cartridge sample chamber with the black cap and put on a fresh pair of gloves.

28. Press "Eject" button to eject cartridge holder (**Figure 2**).

29. Gently pull out after ejection and insert the cartridge into the cartridge holder of the instrument (**Figure 3**).

Note: The orientation of the cartridge is to be placed as in Figure 3. Ensure that the site containing the sample is to the right, when looking at the front of the instrument.

30. Gently push to close the cartridge holder. You will hear 2 **clicking sounds** when the cartridge is properly loaded in the instrument.

Caution: Placing the cartridge in the wrong orientation will cause the cartridge holder to remain open, and the cartridge will not be inserted in the device.

31. Press the "Start" button to begin the DNA extraction process.
32. The reagents from the bottles in the back will be automatically added to the cartridge based on the pre-programmed protocol.
33. While the test is in progress, prepare the next sample in the batch as in steps 1 to 12.
34. After 18–20 minutes, the device will give a beeping sound indicating completion of the extraction process with a displayed message.
35. The device will automatically eject the cartridge holder.

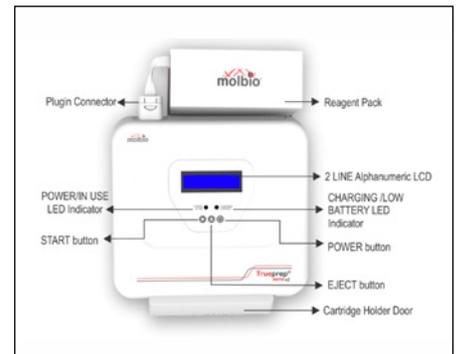


Figure 2



Figure 3

36. **IMPORTANT:** To avoid the elute from being evaporated by heat generated during the extraction process, remove the cartridge as soon as the cartridge holder is ejected.
37. Lift the cartridge up and place on the cartridge stand.
38. Inspect the tray in the cartridge holder for any spilled liquid.
Note: In the event of a spill, dispose of the tray in container filled with concentrated bleach for 30 minutes and spray the cartridge holder with 70% isopropyl alcohol. After 5 minutes, place a new tray in the cartridge holder.
39. Take out the ECT tube from the test pouch, and label the tube using the sticker provided in the pouch with patient number, age, sex and date.
40. With the precision and filter barrier pipette tip, pierce the covering of elute compartment in the cartridge, and aspirate the entire amount of the elute.
41. Dispense the eluate into the labelled ECT tube and close the ECT tube cap tightly.
42. Put the ECT tube in the labelled ECT holder.
43. If eluate is not to be amplified immediately, store in a fridge at 4°C for up to 24 hours or at -20°C for up to 1 year.
44. Dispose the pipette tip and used cartridge into the container filled with concentrated bleach.
45. Dispose your gloves in dedicated waste container.
46. Load the next sample in the batch into the Trueprep instrument.
47. Transfer the ECT tube containing the eluate to the Truelab micro PCR analyzer for amplification.

4.5.6 Nucleic acid amplification

Important note: The Truelab Duo device can run a maximum of 2 tests at a time.

1. Clean the working surfaces with 10% bleach followed by 70% alcohol.
2. Clean the instruments with paper towel wet with 70% alcohol.
3. Put on a fresh pair of gloves
4. Before starting the amplification process for a maximum of 2 samples, ensure you have the following arranged on your work station:
 - i. PCR chip set which contains the chip, micro tube with freeze dried PCR reagent micropipette tip and a blue desiccant
 - ii. The white, fixed-volume micropipette
 - iii. The microtube stands to hold the microtubes
 - iv. ECT tubes containing the extracted DNA
5. Switch on the Truelab Duo device by pressing the red button in the back left corner for 2 seconds. The power/ in use indicator will glow green. In 30-50 seconds, the boot screen will appear followed by the home screen. Ignore the insert sim pop up message.
6. Click on Molbio and select a user name from the drop-down menu.

7. Tap on the password text box to pull up the on-screen keyboard.
8. Enter password and press “sign in “to log in” the selected user.
9. Inspect the two-chip bay to ensure there are no used chip in the instrument by clicking on each bay. Select ‘open/close’ to close the bay.
10. Choose any of the test bay 1 or 2 and select MTB plus.
11. A pop up will appear. Confirm selection by pressing “PROCEED”.
12. Enter the information required (referred by, patient ID, patient name, age and gender).
13. Select the sample type SPUTUM.
14. Press ‘start test’ and the cartridge bay selected will automatically open.
Note: When the “please load sample” prompt appears, DO NOT press “YES” until the chip is loaded.
15. Tear open the chip pouch.
16. Pull out the desiccant pouch and confirm that it is blue.
Note: If the desiccant pouch is white or pink in color, do not use the contents of that pouch. Take another one. (This means the chip has been exposed to excess moisture.)
17. Pull out the chip enclosed in the chip sleeve.
Note:
-NEVER touch the white reagent well
-Minimize the exposure of the chip to light by preparing and running the test immediately after opening
18. Label the chip with the participant ID using a marker at the space provided on the back side of the chip. Avoid writing on the QR code.
19. Place the chip on the tray by aligning the registration holes with the tray pins.
Note: The white reaction well should face upward and away from the device
20. Take out the microtube containing the freeze-dried PCR reagent and remove the lid
21. Place the microtube containing the freeze-dried PCR reagents in the microtube stand provided.
22. Inspect to be sure the PCR reagent is at the bottom of the tube.
23. Take the 6µl precision micropipette and attach the micropipette filter barrier tip enclosed in the chip pouch.
24. Pipette out 6µl of purified DNA from the ECT and put into the microtube. Confirm visually that the pipetted solution is 6µl.
Note: DO NOT mix it by tapping, shaking or by reverse pipetting.
25. Do not dispose the pipette tip. Keep it attached to the fixed volume micropipette but ensure the tip is retain into the sleeve.
26. Cap the remaining extracted DNA in the ECT tube and move it one step behind.
27. Allow mixture of eluate and PCR reagent to stand for 30 to 60 seconds to get a clear solution.

28. Pipette out 6µl of treated DNA from the microtube and put into the reaction well of the chip.
Note: DO NOT spill eluate on the outsides of the well. Take care not to scratch the internal well surface
29. On the instrument screen, select “YES” when the “please load sample” pops up.
30. Chip tray will close automatically and reaction will start.
31. Dispose the microtube, microtip and used gloves into waste container containing concentrated bleach.
32. Truelab will verify chip and commence test.
Note:
–If desired, press “PLOT” to view test progress in real time. No user intervention or interpretation is required. Amplification profile is visible in “optical” view.
–DO NOT touch or shake the instrument while the test is in progress
33. Follow steps 9–29 to load the second sample in the batch but this time selecting the other test bay.
34. At the end of the run (35 minutes), press “RESULT” to go to the result screen.
35. Possible result:
 - i. Valid/invalid and
 - ii. MTB result Detected/not detected/Errors
36. Record the result in the lab register.
37. If result is MTB detected, test same eluate for rifampin resistance using MTB–RIF chip. In this case, select the MTB Rif assay.
38. If test gives an invalid or error result, record result and repeat the amplification using the same extracted DNA and a different chip. If valid result cannot still be obtained, run test with a different sample and eluate.
39. Press “print” to print results.
Note: Test results are automatically stored and can be retrieved any time later.
40. Lift the chip from the instrument tray and directly dispose into waste container filled with concentrated bleach.
Caution! DO NOT put the chip down on table or any other place. Do not discard chip anywhere else. The amplicons may contaminate another test and give a false positive result.
41. Dispose your gloves in the dedicated waste container.
42. Switch off the Truelab analyzer and Trueprep device at the end day.
43. Cover each of these instruments with the instrument plastic covers.
44. Clean the surfaces using bleach, at the end of the day

4.6 Reading and interpretation results

At the end of the test run, the result screen will display “DETECTED” for Positive result or “NOT DETECTED” for Negative results. The result screen will also display the MTB load as “HIGH”, “MEDIUM”, “LOW” or “VERY LOW” for positive

specimens. The result screen also displays the validity of the test run as “VALID” or “INVALID”.

1. Two amplification curves are displayed on the Truelab Analyzer screen when optical plot is selected to indicate the progress of the test.
2. The target and the internal positive control (IPC)* curves will take a steep, exponential path when the fluorescence crosses the threshold value in case of POSITIVE samples.
3. The target curve will remain horizontal throughout the test duration and the IPC curve will take an exponential path in case of NEGATIVE samples.
4. If the IPC curve remains horizontal in a negative sample, the test is considered as INVALID. This may be due to inhibitors in the sample or issues with the reagents used. Tests with an Invalid result should to be repeated using a fresh specimen and processed starting with the sample preparation step.

Note: IPC will co-amplify in most positive cases. In some specimens having a high target load, the IPC may not amplify, however the test run is still considered valid.

4.7 Storage of DNA

Store the rest of the eluate after extraction and amplification in the ECT tube at -20°C.

4.8 Quality control

To ensure that the Truelab Analyzer is working accurately, positive and negative controls may be run one time per month. The Truenat Positive Control Kit- Panel I containing Positive Control and Negative Control may be used in running these controls; alternatively, PBS may be used as a negative control and a known positive sample (e.g., from culture) as a positive control.

- Quality control will also be performed if the temperature of the storage area falls outside of 2-30°C.

Acceptable criteria: The result will be acceptable if the positive controls give positive results while negative controls give negative results.

Corrective action: Repeat the control and/or inform the Lab Director.

Documentation: Controls should be recorded in the result register.

5. Waste management and other safety precautions

- Submerge the used cartridges, replaceable trays, reagent bottles and other consumables in freshly prepared 10% bleach for 30 minutes before disposal as per the standard medical waste disposal guidelines.
- Samples and reagents of human origin, as well as contaminated materials, disposables, neutralized acids and other waste materials must be discarded after decontamination by immersion in a freshly prepared 0.5% of sodium hypochlorite for 30 minutes (1 volume of 5% sodium hypochlorite for 10 volumes of contaminated fluid or water).
- Do not autoclave materials or solutions containing bleach.

- Chemicals should be handled in accordance with good laboratory practice and disposed according to the Biosafety Manual.
- Check all packages before using the kit. Damage to the packaging does not prevent the contents of the kit from being used. However, if the outer packaging is damaged the user must confirm that individual components of the kit are intact before using them.
- Do not perform the test in the presence of reactive vapours (e.g., from sodium hypochlorite, acids, alkalis or aldehydes) or dust.
- While retrieving the Truenat™ MTB micro PCR chip and the DNase & RNase free pipette tip from the pouch, ensure that neither bare hands nor gloves that have been used for previous tests run are used.
- All pipetting steps should be performed with utmost care and accuracy in order to prevent cross-contamination between reagents and samples which may lead to invalid results.

6. References

- Truenat MTB Plus package insert version 5.
- The Trueprep™ AUTO v2 Universal Cartridge Based Sample Prep Device user manual.
- TBRL Bamenda Biosafety manual, Version 4.0, section 10.
- Truenat™-A Point-of-care Real Time PCR Test for Tuberculosis, video by Molbio available at <https://youtu.be/ydR2l5S2v3c>

7. List of changes to SOP

| Date | Change(s) made | Page number | Initial(s) |
|------|----------------|-------------|------------|
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

8. Reading and understanding list for documents

I acknowledge having read and understood this SOP

| Date | Change(s) made | Page number |
|------|----------------|-------------|
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |

Annex 2: Truenat TB test implementation - high level checklist

1. Policy and planning

- Have roles and responsibilities for coordinating the Truenat adoption process been clearly defined?
- Which national guidelines, policies and other materials will need to be updated to include the Truenat TB tests (e.g., NTP policies and guidelines, diagnostic algorithm, TB-HIV policies and guidelines, case definitions, etc.)?
- Has a stakeholder mapping process been conducted, including all key internal (within government) and external stakeholders (local and international)?
- What support can partners provide for the implementation process?
- Has the intended use and setting of the Truenat TB tests been decided? Have projections been made for the number of samples to be tested per year or per site?
- Has a costed implementation plan been developed?
- Have adequate financial resources for capital investments, implementation and projected on-going costs been secured?

2. Regulatory

- What are the importation requirements for instruments, reagents and supplies for the Truenat TB tests?
- What is the regulatory process required for the Truenat TB tests?
- Is country verification of the Truenat TB tests needed for regulatory approval?
- If so, what type of protocol and number of samples are required? Timeline? Where will verification studies be conducted?
- Is the designated authority (NTP, procurement agency or partners) engaged with manufacturers to support regulatory processes?

3. Site readiness

- Are adequate laboratory facilities, space and infrastructure available?
- Do facilities, equipment, policies and practices meet TB biosafety standards?
- Are appropriately trained and competent staff available to conduct the Truenat TB tests?
- Are adequate specimen referral and results reporting systems available?

4. Procurement and supply chain

- Which partners procure instruments and consumables?
- Has the manufacturer engaged a country-level distributor or other service provider to support implementation, equipment maintenance (warranties or service contracts) and commodity importation?
- Is a procurement system available to ensure the availability of reagents and supplies that takes into account procurement times, consumption rates and shelf-life of reagents?
- What is the planned procurement by MOH and partners for this year?

5. Procedures

- Which SOPs, forms and registers will need to be updated or developed?

6. Data

- What applications of electronic data connectivity will be pursued? (e.g., results to clinicians by SMS/email, results to electronic patient registers or other data systems, results to third party connectivity systems, remote monitoring of data on manufacturer-provided dashboard)
- What modifications or upgrades to existing data systems (e.g., electronic patient registers, laboratory information management systems, third party connectivity systems) are required to make use of Truenat data?
- Are procedures in place to define data sharing protocols and ensure the confidentiality?

7. Quality assurance

- Are the essential elements of a quality assurance system in place at the testing site?
- Are protocols in place to conduct and document quality checks of each step of the Truenat TB test process and ensure the use of positive and negative controls?
- Is an external quality assessment programme in place?
- Which partners can assist with proficiency testing, supervisory visits and re-checking of samples?
- Have quality (performance) indicators been defined and appropriate data collection tools developed?

8. Recording and reporting

- Is a national request form in use? If no, review all request forms being used to Truenat TB tests and revise as needed.
- Is revision of the current request for examination form required for introduction of Truenat TB tests?
- Is revision of reporting forms needed?
- Is a revision of laboratory or clinical registers needed?
- If an electronic laboratory information system is in use, what updates will be required?
- If an electronic recording and reporting system is in place, what updates will be required? If not, what system will be used to inform clinicians promptly about Truenat TB test results?

9. Training

- Is a national approved training curriculum available?
- Who is responsible and what is the process for updating training materials for laboratory and clinical staff?
- Is the approved curriculum used for all trainings, including those delivered by partners?

- Are standard procedures used to assess and document the competence of all staff involved in Truenat TB testing?

10. Monitoring the transition

- What changes to M&E tools and processes would be required to enable monitoring of additional indicators (i.e., progress indicators, laboratory indicators and clinical impact indicators)?
- What support can partners provide in monitoring of new algorithms and adherence to guidelines at sites?
- What support can partners provide for operational research to monitor the impact of Truenat TB testing?

Annex 3: Budget considerations for implementation

| Budgetary considerations | |
|---|--|
| Policy and Planning | <ul style="list-style-type: none"> • Workshop for stakeholder engagement and planning • Technical workshop for guideline and algorithm update • Situational analysis cost – HR, travel and report writing • Printing and distribution costs for revised guidelines and algorithms • Development of a costed operational plan • Cost of external technical assistance, if needed |
| Regulatory | <ul style="list-style-type: none"> • Regulatory submission costs, if applicable (borne by manufacturer) • Local travel costs to regulatory authority • Importation processes and costs • Verification study – samples, reagents, HR |
| Procedures | <ul style="list-style-type: none"> • Workshop and HR for the development of SOPs • Printing and dissemination of revised procedures • Development, printing and dissemination of revised clinical protocols and guidance for the selection of patients to be tested, ordering tests, interpreting test results, and making patient care decisions |
| Truenat TB testing laboratory | <ul style="list-style-type: none"> • Costs of assessing site readiness – travel, HR • Costs of upgrading laboratory facilities and infrastructure |
| Purchase and installation of Truelab instruments | <ul style="list-style-type: none"> • Purchase (or lease) of instruments and ancillary equipment • Delivery and importation costs • Installation by manufacturer or authorized service provider • Training • Extended warranty or service contract • Costs of routine preventive and annual maintenance |
| Diagnostics connectivity | <ul style="list-style-type: none"> • Costs of data transmission (e.g., 3G data transfer, SMS) • HR and costs of remote monitoring • Cost of technical assistance • Optional: purchase or acquisition (e.g., subscription fee) of third-party diagnostics connectivity solution to complement manufacturer's system |
| Procurement and supply chain | <ul style="list-style-type: none"> • Workshop for stakeholders involved in procurement planning • Cost of maintaining centralized stores and costs of distribution • Material cost per test, including but not limited to test reagents, consumables, sample collection items, printing paper, etc. Additional equipment costs that include additional equipment requirements (printer, computer, printer cartridges), shipping and courier costs • Costs of new lot testing |

| Budgetary considerations | |
|--|--|
| Recording and reporting | <ul style="list-style-type: none"> • Workshop and HR to update recording and reporting forms, registers • Printing and distribution of updated materials |
| Training | <ul style="list-style-type: none"> • Workshop and HR to update training packages • Training of training workshop, on-site trainings/sensitization meetings for laboratory technicians and for clinicians • Printing and distribution of updated training manuals and clinician sensitization materials |
| Quality assurance, control and assessment | <ul style="list-style-type: none"> • Preparation and regular review of all testing and quality assurance documents (SOPs, checklists, etc.) based on national requirements • Cost of conducting quality controls (e.g., testing known positives or negatives) • Costs of HR for routinely collecting and analysing quality indicators • Costs of conducting on-site visits – travel, HR, preparation of checklists and reports • Costs associated with hosting an on-site visit and preparation of documents • Costs associated with providing PT panels and overseeing PT, reporting results and corrective actions and costs associated with testing PT panels at each site • Costs associated with re-testing of samples at a higher-level laboratory (e.g., shipment of samples, testing, reporting, etc.), if applicable • Costs associated with annual competency testing of staff |
| Monitoring | <ul style="list-style-type: none"> • Meetings to update M&E system and regular meetings to review impact of transition and re-plan • M&E refresher training • Operational research study to measure clinical impact |
| Annual on-going costs | <ul style="list-style-type: none"> • Consumables • Human resources • Equipment maintenance and servicing • Digital data systems and connectivity solutions • Specimen referral and results reporting system • External quality assessment |

Annex 4. Example of a Truenat Preventive Maintenance Log

(Courtesy of the Bamenda TB Reference Laboratory, Cameroon)

| Truelab and Trueprep maintenance log | | Version: 1.1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|--------------------|--------------|---|---|---|---|---|---|---|--------------|----|----|----|----|----|----|----|----|----|--------------|----|----|----|----|----|----|----|----|----|----|----|--|
| | | Date: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Compiled by: | | | | | | | | | | Reviewed by: | | | | | | | | | | Approved by: | | | | | | | | | | | | |
| Month: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Days of the month | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Maintenance activity | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | |
| Daily maintenance | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Clean work area | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Discard used chips and cartridges | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Monthly maintenance | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Disinfect instrument surfaces | Date: Initials: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Clean Truelab bays | Date: Initials: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Temperature calibration | Date: Initials: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Verification of the fixed 6µl pipette | Date: Initials: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| As necessary | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Flush protocol for the Trueprep instrument | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Spillage tray or linear motion guide tray replacement | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Slider glass replacement - indicate bay | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Notes:

Indicate completion of an activity by writing your initials in the corresponding date box.

- Slider glass should be replaced after running at most 50 tests and/or when related errors occur.
- Temperature calibration of the Truelab should be done on a monthly basis and/or when error related to temperature occurs and/or when temperature curve is abnormal, i.e having blips. Normal values: 3.39–3.42.
- Flush protocol for the Trueprep instrument should be done if no test will be run for the next 10 days and/or when errors relating to extraction process occur.
- Spillage tray and linear motion guide trays should be replaced when there is sample spillage on the trays during extraction or when cross contamination is suspected.

Annex 5. Example roadmap for implementation

| | Planning and Assessing | | | | Preparation, installation and Training | | | | Testing | |
|---|------------------------|---|---|---|--|---|--|--|---------|--|
| Policy and planning | | | | | | | | | | |
| Establish and define roles and responsibilities of a TWG | ■ | | | | | | | | | |
| Review WHO policies and any available technical or implementation guide | ■ | ■ | | | | | | | | |
| Define immediate and future purposes of Truenat TB testing | ■ | ■ | | | | | | | | |
| Update national diagnostic algorithm and guidelines | ■ | ■ | | | | | | | | |
| Perform a situational analysis | | | ■ | ■ | | | | | | |
| Identify laboratories that will perform Truenat TB testing | | | ■ | ■ | | | | | | |
| Assess Truenat TB testing site suitability | | | ■ | ■ | | | | | | |
| Develop a costed operational plan for implementation | | | | | ■ | | | | | |
| Regulatory | | | | | | | | | | |
| Determine importation requirements | | | ■ | ■ | | | | | | |
| Conduct country verification if required | | | | | ■ | ■ | | | | |
| Complete national regulatory processes | | | | | ■ | ■ | | | | |
| Equipment | | | | | | | | | | |
| Equipment selection | | | ■ | ■ | | | | | | |
| Procure and Install equipment | | | | | ■ | ■ | | | | |
| Perform necessary upgrades to testing site infrastructure | | | | | ■ | ■ | | | | |
| Assess site readiness | | | | | | | | | ■ | |

| | Planning and Assessing | | | | Preparation, installation and Training | | | | Testing | |
|---|------------------------|--|---|---|--|---|---|---|---------|--|
| Supplies | | | | | | | | | | |
| Review forecasting, ordering and distribution procedures | | | | | ■ | ■ | | | | |
| Develop procedures to monitor reagent quality and shelf-life | | | | | ■ | ■ | | | | |
| Procedures | | | | | | | | | | |
| Develop standard operating procedures | | | | | ■ | ■ | | | | |
| Update clinical procedures and strengthen the clinical-laboratory interface | | | | | ■ | ■ | | | | |
| Data | | | | | | | | | | |
| Plan for use of digital data and diagnostics connectivity | | | ■ | ■ | | | | | | |
| Acquire and install any necessary hardware and/or SIM cards to allow for diagnostics connectivity | | | | | ■ | ■ | | | | |
| Training in use of digital data | | | | | | | ■ | ■ | | |
| Establish data monitoring dashboards and systems | | | | | ■ | ■ | | | | |
| Develop procedures for data backup, security and confidentiality | | | | | ■ | ■ | | | | |
| Quality assurance | | | | | | | | | | |
| Strengthen quality assurance programmes in Truenat TB testing laboratories | | | | | ■ | ■ | | | | |
| Develop policies and procedures for quality control of Truenat TB testing | | | | | ■ | ■ | | | | |
| Develop and implement an external quality assessment (EQA) programme | | | | | ■ | ■ | | | | |
| Develop tools and system for routine monitoring of quality indicators | | | | | ■ | ■ | | | | |

| | Planning and Assessing | | | | | Preparation, installation and Training | | | | | Testing | |
|---|------------------------|--|--|--|---|--|---|---|---|---|---------|---|
| Recording and reporting | | | | | | | | | | | | |
| Revise and disseminate test requisition, recording and reporting forms | | | | | | ■ | ■ | | | | | |
| Modify and disseminate laboratory and clinical registers as needed | | | | | | ■ | ■ | | | | | |
| Training and competency assessment | | | | | | | | | | | | |
| Develop training curricula for different staff cadres | | | | | | ■ | ■ | | | | | |
| Conduct training | | | | | | | | ■ | ■ | | | |
| Conduct competency assessments after training and periodically thereafter | | | | | | | | ■ | ■ | | | |
| Monitoring and evaluation | | | | | | | | | | | | |
| Develop key indicators or milestones for implementation | | | | | ■ | | | | | | | |
| Monitoring implementation | | | | | | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| Develop a framework to monitor and evaluate impact | | | | | ■ | ■ | ■ | | | | | |
| Monitor and evaluate impact | | | | | | | | | | | ■ | ■ |
| Begin testing | | | | | | | | | | | | |
| | | | | | | | | | | | ■ | ■ |

Annex 6: Checklist to confirm suitability of a testing site

This checklist is used to assess the suitability of a laboratory to conduct Truenat TB testing. The checklist focuses on the key operational and environmental requirements for conducting Truenat TB testing as well as basic laboratory procedures for ensuring quality. Once selected, preparations for Truenat TB testing should be implemented as described in the *Stop TB / USAID / GHI Practical Guide to Implementation of Truenat Tests for the Detection of TB and Rifampicin Resistance*. A separate checklist is available to assess the readiness of a site to begin testing clinical samples for patient care. Most questions are to be answered with a 'Yes', 'No' or 'Partial'. Space is provided to provide comments for the responses for each question.

| | |
|---|---|
| Name of laboratory | |
| Location of site (City/town, District, State) | |
| Laboratory Level | () National () Intermediate (supervisory) () Peripheral |
| How does the laboratory receive specimens for testing? | () Collect specimens. Average number per month: _____ () Receive referred specimens. Average number per month: _____ |
| TB tests performed at this site (check all that apply) | Average number of tests conducted per month |
| () AFB Smear-microscopy for diagnosis | () |
| () Xpert MTB/RIF or () Xpert MTB/RIF Ultra | () |
| () Culture | () |
| () DST (FL or SL) | () |
| () LPA (FL or SL) | () |
| () LF-LAM | () |
| () Other: _____ | () |
| Anticipated monthly number of Truenat TB tests based on planned algorithm and number of specimens expected to be received including from referring labs | () |
| Persons interviewed | |
| Name | Position and contact details |
| | |
| | |
| Assessor name: | |
| Assessor contact details: | |
| Date of assessment: | |

| | Yes | No | Partial | Comments |
|--|-----|----|---------|----------|
| Human resources | | | | |
| a. Are a sufficient number of qualified staff available for the anticipated Truenat TB testing workload? | | | | |
| b. Is a staff member (part-time or full-time) available to oversee the implementation of Truenat TB testing? | | | | |
| A safe and functional testing site | | | | |
| a. Does the laboratory have a lockable door and secure windows | | | | |
| b. Is there adequate space for receiving and processing specimens? | | | | |
| c. Is there adequate amount and quality of bench space for the Truelab instruments and ancillary equipment? <ul style="list-style-type: none"> • flat, stable surfaces? • vibration-free or vibration-dampened? • away from instruments that cause electromagnetic interference? (note: Molbio recommends placing micro PCR instruments at least one meter away from other instruments or equipment) • out of direct sunlight? • away from to any radiating or heating apparatus? | | | | |
| d. Does the testing site provide sufficient ventilation and biosafety for Truenat TB testing procedures? | | | | |
| e. Does the testing site ensure an optimal working temperature (15°C to 40°C) and environment (humidity [10-80%], dust-free) for the Truelab instruments? | | | | |
| f. Are existing workstations clean, free of clutter, and organized for efficient operation? | | | | |
| g. Is available electricity adequate for Truenat TB testing and battery charging? Note: For sites that would rely on Truelab equipment in-built batteries, up to 10 hours of electricity is required to recharge the batteries, in order to be able to run tests for 8 hours. | | | | |
| h. Would a solar panel system need to be procured to provide the required electricity? | | | | |
| i. Are there 3 available sockets on the lab bench for the Trueprep and Truelab instruments? | | | | |
| j. Is there sufficient, secured, and organized storage space for chips (2°C–30°C) and reagent kits (2°C–40°C)? | | | | |

| | Yes | No | Partial | Comments |
|--|-----|----|---------|----------|
| k. Is there documented monitoring of environmental temperatures at the testing and storage areas? | | | | |
| l. Does the laboratory have a refrigerator for storing sputum samples? | | | | |
| m. Does the testing site use appropriate disinfectants and are they prepared correctly? | | | | |
| n. Does the testing site use appropriate disinfectants and are they prepared correctly? | | | | |
| o. Is suitable personal protective equipment (PPE) provided at the testing site and are staff trained in its correct use? | | | | |
| p. Does the testing site segregate waste and dispose of it by incineration or as per national regulations or guidelines? | | | | |
| q. Does the testing site have adequate capacity for safely and properly disposing of the anticipated significant amount of plastic waste generated by Truenat testing? | | | | |
| Equipment service and maintenance | | | | |
| a. Are routine maintenance (daily, weekly, and monthly) procedures performed and recorded for existing instruments? | | | | |
| b. Is there an SOP in place to obtain repairs or service for existing instruments? | | | | |
| Supply chain | | | | |
| a. Would the current supply chain be adequate to continuously supply Truenat reagents? | | | | |
| b. How long is the average lead time from ordering to receiving stock | | | | |
| c. Does the testing site monitor consumption of consumables? | | | | |
| d. Are testing supplies available, in-date, labeled with receive date, organized and stored at recommended conditions? | | | | |
| e. Is quality control testing performed on new lots of reagents prior to their use for testing patient samples? | | | | |
| f. Are supplies inventoried (physical count) at least monthly? | | | | |

| | Yes | No | Partial | Comments |
|--|-----|----|---------|----------|
| Procedures | | | | |
| a. Are all of the needed standardized forms related to Truenat TB testing readily accessible to all staff? <ul style="list-style-type: none"> • TB test requisition form • Sample collection and transport forms • Laboratory register • Instrument maintenance log • Stock cards • Temperature monitoring records • Result reporting form | | | | |
| b. Are the following standard operating procedures (SOPs) approved and accessible at the testing site? <ul style="list-style-type: none"> • Specimen collection • Specimen processing and storage • Specimen referral • Specimen receipt and accessioning • Laboratory testing • Recording and reporting • External quality assessment • Quality indicator monitoring and data analysis • Waste management • Spill management | | | | |
| c. Is there evidence that all SOPs, documents and forms have been read by the personnel? | | | | |
| Digital data and diagnostics connectivity | | | | |
| a. Is an electronic laboratory management system in use? If yes, which? | | | | |
| b. Does the testing site have WIFI or at least 3G coverage, to allow for data transfer? | | | | |
| c. Are any diagnostics connectivity solutions in use? If yes, which? | | | | |
| d. Are procedures in place that ensure the confidentiality of patient information? | | | | |
| e. Is suitable secure storage available for laboratory test data? | | | | |
| Quality Assurance | | | | |
| a. Are protocols in place that ensure the routine use of positive and negative controls in laboratory testing? | | | | |

| | Yes | No | Partial | Comments |
|---|-----|----|---------|----------|
| b. Is an external quality assessment programme in place? If yes, describe <ul style="list-style-type: none"> • Proficiency testing (PT)? • Re-checking of samples? • On-site supervisory visits? | | | | |
| c. Are laboratory statistics and performance indicators are currently reported to the TB programme and how? | | | | |
| Recording and reporting | | | | |
| a. How are results currently returned to clinicians? | | | | |
| b. If an electronic laboratory information system is in use, can Truenat TB testing results be entered into it? | | | | |
| c. If an electronic recording and reporting system is in place, can Truenat TB testing results be recorded and reported using it? | | | | |
| Training | | | | |
| a. What are the anticipated training needs for the laboratory staff? | | | | |
| Partner support (optional) | | | | |
| a. Are any partners available that could support Truenat TB implementation testing and how can they contribute? | | | | |

Annex 7: Checklist to confirm readiness of a Truenat TB testing site

This checklist is used to assess the readiness of a site to conduct Truenat TB testing of clinical specimens and for clinicians to use the results for patient care. The checklist may also be used at the beginning of the implementation process to identify areas in need of improvement. The numbering of the sections in this checklist corresponds with the numbering of the sections in the *Stop TB / USAID / GLI Practical Guide to Implementation of Truenat Tests for the Detection of TB and Rifampicin Resistance*. Most questions are to be answered with a 'Yes', 'No' or 'Partial'. Some questions will have text answers. Space is provided to provide comments for the responses for each question.

Note: not all sections or questions must be answered yes for a site to be ready to begin testing. For example, developing systems to transmit digital data and use the data to maximum potential may require a phased approach that would not be expected to be fully executed before testing begins. Likewise, a fully functional EQA programme is not a requirement to initiate testing.

| | |
|---|---|
| Name of laboratory | |
| Location of site (City/town, District, State) | |
| Laboratory Level | () National () Intermediate (supervisory) () Peripheral |
| How does the laboratory receive specimens for testing? | () Collect specimens. N°. per month: _____ () Receive referred specimens. N°. per month: _____ |
| TB tests performed at this site (check all that apply) | Average number of tests conducted per month |
| () AFB Smear-microscopy for diagnosis | () |
| () Xpert MTB/RIF or () Xpert MTB/RIF Ultra | () |
| () Culture | () |
| () DST (FL or SL) | () |
| () LPA (FL or SL) | () |
| () LF-LAM | () |
| () Other: _____ | () |
| Anticipated monthly number of Truenat TB tests based on planned algorithm and number of specimens expected to be received including from referring labs | () |
| Persons interviewed | |
| Name | Position and contact details |
| | |
| | |
| Assessor name: | |
| Assessor contact details: | |
| Date of assessment: | |

| | Yes | No | Partial | Comments |
|--|-----|----|---------|----------|
| 1. Policies and planning | | | | |
| a. Are the following national guidelines, policies and plans accessible at the testing site: <ul style="list-style-type: none"> National TB diagnostic algorithm that includes the use of Truenat TB testing? National plan for Truenat TB test implementation? | | | | |
| b. Has a staff member (part-time or full-time) been appointed in the laboratory to oversee the start-up of implementation of Truenat TB testing? | | | | |
| c. Are partners available that are supporting readiness for Truenat TB testing and how are they contributing? | | | | |
| d. Are adequate resources (e.g., funding, staff, laboratory infrastructures, etc.) available to support on-going costs of Truenat TB testing including: <ul style="list-style-type: none"> Equipment maintenance and service contracts? Diagnostics connectivity costs? On-going training and competency assessments? Quality assurance (proficiency testing, monitoring indicators etc.)? Projected on-going costs related to Truenat TB testing (staff, reagents, consumables, etc.)? | | | | |
| e. Are a sufficient number of qualified staff available for the anticipated Truenat TB testing workload? | | | | |
| 2. Equipment, service and maintenance | | | | |
| a. Are copies of the manuals and package inserts for Truelab instruments and reagents readily available and accessible at the testing site? | | | | |
| b. Were the Truenat TB instruments verified on site prior to routine use for patient testing? | | | | |
| c. Is a routine maintenance log available indicating daily, weekly, and monthly tasks? | | | | |
| d. Is there an SOP in place to obtain repairs or service for all Truenat TB instruments? | | | | |
| e. Is a service contract in place to provide comprehensive service and maintenance? | | | | |
| 3. Site readiness | | | | |
| a. Does the laboratory have a lockable door and secure windows | | | | |

| | Yes | No | Partial | Comments |
|--|-----|----|---------|----------|
| b. Is there adequate space for receiving and processing specimens? | | | | |
| c. Is there adequate amount and quality of bench space for the Trueprep and Truelab instruments and ancillary equipment? <ul style="list-style-type: none"> • flat, stable surfaces • vibration-free or vibration-dampened • away from instruments that cause electromagnetic interference (note: Molbio recommends placing the micro PCR instruments at least one metre away from other instruments or equipment) • out of direct sunlight • away from to any radiating or heating apparatus | | | | |
| d. Are the Truenat TB instruments placed correctly in the laboratory? | | | | |
| e. Are Truenat TB workstations clean, free of clutter, and organized for efficient operation? | | | | |
| f. Does the testing site provide sufficient ventilation and biosafety for Truenat TB testing procedures? | | | | |
| g. Does the testing site ensure an optimal working temperature (15°C to 40°C) and environment (humidity [10-80%], dust-free) for the Truelab instruments? | | | | |
| h. Is available electricity adequate for Truenat TB testing and battery charging? Note: For sites that would rely on Truenat equipment in-built batteries, up to 10 hours of electricity is required to recharge the batteries in order to be able to run tests for 8 hours. | | | | |
| i. Are there 3 available sockets on the lab bench for the Truenat instruments? | | | | |
| j. If needed, is a solar powered generator available for charging of Truelab instruments? | | | | |
| k. Is there sufficient, secured, and organized storage space for chips (2°C–30°C) and reagent kits (2°C–40°C)? | | | | |
| l. Is there documented monitoring and review of environmental temperatures at the testing and storage areas? | | | | |
| m. Does the laboratory have a refrigerator for storing sputum samples? | | | | |
| n. Does the testing site use appropriate disinfectants and are they prepared correctly? | | | | |
| o. Is suitable personal protective equipment (PPE) provided at the testing site and are staff trained in its correct use? | | | | |

| | Yes | No | Partial | Comments |
|--|-----|----|---------|----------|
| p. Does the testing site segregate waste and dispose of it by incineration or as per national regulations or guidelines? | | | | |
| q. Does the testing site have adequate capacity for safely and properly disposing of the anticipated significant amount of plastic waste generated by Truenat testing? | | | | |
| 4. Supply chain | | | | |
| a. Is procurement system adequate for Truenat TB reagents and consumables? | | | | |
| b. Will the testing site monitor consumption of Truenat TB reagents and monitor inventory (physical count)? | | | | |
| c. Are Truenat TB testing supplies available at the testing site, in-date, labeled with receive date, organized and stored at recommended storage conditions? | | | | |
| d. Is quality control testing (QC) performed on new lots of Truenat TB reagents prior to their use for testing patient samples to ensure that they perform as expected? | | | | |
| e. Does the testing site adequately store Truenat TB reagents? | | | | |
| 5. Procedures | | | | |
| a. Are all of the needed standardized documents, records and forms related to Truenat TB testing readily accessible to all staff? <ul style="list-style-type: none"> • Truenat TB test requisition form • Sample collection and transport forms • Laboratory register • Truenat TB instrument maintenance log • Stock cards • Temperature monitoring records • Truenat TB performance indicator reporting form • Truenat TB test reporting form • Truenat TB PT results form & failure follow-up form | | | | |

| | Yes | No | Partial | Comments |
|--|-----|----|---------|----------|
| <p>b. Are the following Truenat TB-related standard operating procedures (SOPs) approved and accessible at the testing site?</p> <ul style="list-style-type: none"> • Truenat TB test requisition • Specimen collection • Specimen processing and storage • Specimen referral • Specimen receipt and accessioning • Sample processing for DNA extraction • DNA extraction (Trueprep) • PCR amplification (Truenat micro PCR) • Recording and reporting • External quality assessment • Quality indicator monitoring and data analysis • Waste management • Spill management | | | | |
| c. Is there evidence that all SOPs, documents and forms have been read by the personnel? | | | | |
| d. Is there an established sample transportation system from clinical sites to the Truenat TB testing laboratory? if yes, describe the current system, its adequacy, efficiency and coverage | | | | |
| e. Is a sample referral system in place for additional testing for TB samples at referral laboratories (e.g., second-line DST)? If yes, describe the current system, its adequacy, efficiency and coverage | | | | |
| 6. Digital data and diagnostics connectivity | | | | |
| a. Are laboratory staff familiar with use of the digital flatscreen interface of the Truelab analyzer? | | | | |
| b. Is an electronic laboratory management system in use? If yes, which? | | | | |
| c. Are any third-party diagnostics connectivity solutions in use? If yes, which? | | | | |
| d. Is a mechanism in place for data transfer (via 3G or WiFi)? | | | | |
| e. Is an online dashboard available from the manufacturer to allow for monitoring of testing? | | | | |
| f. What support are partners able to provide for implementing diagnostics connectivity applications? | | | | |
| g. Are procedures in place to define data sharing protocols and ensure the confidentiality of patient information? | | | | |

| | Yes | No | Partial | Comments |
|--|-----|----|---------|----------|
| h. Is suitable secure storage available for the archived or back-up copies of Truenat TB test data? | | | | |
| i. Are adequate resources available for projected on-going costs of data transfer, storage and analysis? | | | | |
| 7.1. Quality Assurance | | | | |
| a. Are the essential elements of a quality assurance system in place? <ul style="list-style-type: none"> • SOPs, training and competence assessment • Quality control (QC) • Lot testing (also known as incoming quality control or new batch testing) • External quality assessment (EQA) • Quality indicator monitoring | | | | |
| 7.2. Establish and monitor quality controls | | | | |
| a. Are protocols in place that ensure the use of positive and negative controls? | | | | |
| 7.3. External quality assessment | | | | |
| a. Is an external quality assessment programme in place? <ul style="list-style-type: none"> • Proficiency testing (PT)? • Re-checking of samples? • On-site supervisory visits? | | | | |
| b. Will the site participate in a PT programme for Truenat TB testing if yes, <ul style="list-style-type: none"> • who will provide the PT panels? • who will oversee the PT programme and provide feedback on the performance of testing site? | | | | |
| c. Does the laboratory participate in inter-laboratory comparisons (re-checking of samples)? If yes, with which laboratories? | | | | |
| d. Does the laboratory receive on-site supervisory visits? If yes, <ul style="list-style-type: none"> • who conducts the supervisory visits? • is feedback provided to the testing site following a supervisory visit? • when was the last supervisory visit and what was the feedback? | | | | |
| 7.4. Monitor and analyze quality indicators | | | | |
| a. Are appropriate data collection tools for Truenat TB test quality indicators available? | | | | |

| | Yes | No | Partial | Comments |
|---|-----|----|---------|----------|
| b. Which laboratory statistics and performance indicators are currently reported to the TB programme and how? | | | | |
| c. Are the following quality indicators monitored and analysed by the Truenat TB testing site and reported: <ul style="list-style-type: none"> • Number of specimens tested (Disaggregated by HIV status, MDR risk, extra-pulmonary TB, pediatric) • Number and proportion of Truenat TB tests that generated a result of MTBC detected • Number and proportion of Truenat TB tests that generated a result of MTBC not detected • Number and proportion of Truenat TB tests that generated non-determinate results • Number and proportion of Truenat MTB-RIF Dx tests that generated a result of RIF-resistance detected • Number and proportion of Truenat MTB-RIF Dx tests that generated a result of RIF-resistance not detected • Number and proportion of Truenat MTB-RIF Dx tests that generated non-determinate results • Number and proportion of specimens for which a Truenat TB test result was reported within the target turnaround time (i.e., time from receipt of specimen to reporting of results) | | | | |
| 8. Recording and reporting | | | | |
| a. How will results be returned to clinicians? Via paper and/or electronically? <ul style="list-style-type: none"> • Are laboratory staff aware of how to send results to clinicians electronically via SMS and/or email? | | | | |
| b. Is an approved request for examination form available to request Truenat TB testing? | | | | |
| c. Is an approved reporting form available to report results of Truenat TB testing? | | | | |
| d. Are the laboratory and clinical registers suitable for recording the results of Truenat TB testing? | | | | |
| e. If an electronic laboratory information system is in use, can Truenat TB testing results be entered into it? | | | | |
| f. If an electronic recording and reporting system is in place, can Truenat TB testing results be recorded and reported using it? | | | | |

| | Yes | No | Partial | Comments |
|---|-----|----|---------|----------|
| 9. Training and competency assessment | | | | |
| a. Are terms of reference and competency -based job descriptions available for key staff involved in Truenat TB testing? | | | | |
| b. Are records in place documenting that all staff have been trained on assigned work processes, procedures, and tasks? | | | | |
| c. Are standard procedures used to assess and document the competence of all staff involved in Truenat TB testing? | | | | |
| d. Are competency assessments for Truenat TB users conducted annually? | | | | |
| e. Are records in place documenting that all Truenat TB users have been assessed for competency? | | | | |
| f. Has the site provided Truenat TB training for on-site clinicians and healthcare workers on: <ul style="list-style-type: none"> • Diagnostic algorithm • Ordering tests? • Sample requirements for testing? • Sample transport? • Interpretation of test results? | | | | |
| g. Have clinicians and healthcare workers in sites that will be referring specimens to this site been trained on all of the above components? | | | | |
| h. Are there any additional training needs for clinical staff? | | | | |
| 10.1. Monitoring and evaluation of implementation of Truenat TB testing | | | | |
| a. Will the accomplishment of key indicators and milestones for Truenat TB implementation be reported to the national programme? | | | | |
| 10.2. Monitoring and evaluation of impact of Truenat TB testing | | | | |
| a. Will the following indicators be monitored and reported to the national programme at least annually <ul style="list-style-type: none"> • Number and proportion of notified bacteriologically confirmed TB cases with reported Truenat TB test results • Number and proportion of notified bacteriologically confirmed TB cases with reported Truenat MTB-RIF Dx test results for rifampicin susceptibility | | | | |

Annex 8: Checklist to confirm readiness of a clinical site

This checklist is used to assess the readiness of a clinical site to participate in a Truenat TB testing programme and use the Truenat TB test results for patient care decisions. Clinical sites to assess should include all sites with Truenat instruments on the premises as well as all sites that will be referring samples to other health care facilities with Truenat instruments. The checklist may also be used at the beginning of the implementation process to identify areas in need of improvement. Most questions are to be answered with a 'Yes', 'No' or 'Partial'. Some questions will have text answers. Space is provided to provide comments for the responses for each question.

| | |
|---|---|
| Name of laboratory | |
| Location of site (City/town, District, State) | |
| Type of Facility | <input type="checkbox"/> Central hospital <input type="checkbox"/> Regional hospital <input type="checkbox"/> District hospital <input type="checkbox"/> Primary health facility Other: _____ |
| TB activities performed at this site (check all that apply) | Average number of procedures conducted per month |
| <input type="checkbox"/> Screen presumptive TB patients | <input type="checkbox"/> |
| <input type="checkbox"/> Chest X-ray | <input type="checkbox"/> |
| <input type="checkbox"/> Refer patients to testing laboratory | <input type="checkbox"/> |
| <input type="checkbox"/> Collect specimens | <input type="checkbox"/> |
| <input type="checkbox"/> Package and ship samples to testing laboratory | <input type="checkbox"/> |
| <input type="checkbox"/> Initiate patients on TB therapy | <input type="checkbox"/> |
| Other: _____ | <input type="checkbox"/> |
| Persons interviewed | |
| Name | Position and contact details |
| | |
| | |
| Assessor name: | |
| Assessor contact details: | |
| Date of assessment: | |

| | Yes | No | Partial | Comments |
|---|-----|----|---------|----------|
| <p>a. Is the National TB diagnostic algorithm that includes the use of Truenat TB testing available at the clinical site?</p> <ul style="list-style-type: none"> • Have clinical staff been trained on the national TB testing algorithm? • Are clinical staff aware of intended uses of the Truenat TB tests? • Are clinical staff aware of criteria for selecting which patients should get a Truenat TB? | | | | |
| <p>b. Are all of the needed standardized forms related to Truenat TB testing readily accessible to all staff at the clinical site?</p> <ul style="list-style-type: none"> • An approved request for examination form for requesting Truenat TB testing? • Sample collection and transport forms? • An approved reporting form to report results of Truenat TB testing? | | | | |
| <p>c. Are the following Truenat TB-related standard operating procedures approved and accessible at the clinical site?</p> <ul style="list-style-type: none"> • How to order a Truenat TB test? • How to complete the Truenat TB test requisition form? • How to collect specimens suitable for Truenat TB testing? • How to store specimens if testing cannot be done immediately? • How to package TB specimens for safe transport? • How to arrange for transport to the Truenat TB testing site? • How to record Truenat TB test in clinical registers? • How to interpret Truenat TB tests and use results for patient care decisions? | | | | |
| <p>d. Have all clinicians and healthcare workers at this clinical site been trained in all procedures in question 'c'? If no, describe training needs.</p> | | | | |
| <p>e. Is there an established sample transportation system from clinical sites to the Truenat TB testing laboratory?</p> <ul style="list-style-type: none"> • if yes, describe the current system, its adequacy, efficiency and coverage • Are appropriate packaging materials available? | | | | |
| <p>f. How will results be returned to clinicians? Via paper and/or electronically?</p> | | | | |
| <p>g. Are the clinical registers suitable for recording the results of Truenat TB testing?</p> | | | | |
| <p>h. If an electronic patient management system is in use, can Truenat TB testing results be entered into it?</p> | | | | |

Annex 9: Performance indicators for Trueprep and Truenat TB tests

General laboratory performance indicators*

| Indicator | Target |
|--|---|
| Number of tests performed, by type of test | - |
| Service interruptions | No interruptions |
| Stock outs | No stock outs leading to service interruption |
| Equipment down time | No equipment downtime leading to service interruption |
| Turnaround time (TAT) | 90% of results meet test-specific TAT |
| Test statistics (quality indicator) report | 100% reports completed by defined due date |
| EQA results | >90% EQA panels are passed |
| QC results | >90% QC results meet expected criteria |
| Specimen rejection | <1% specimens rejected |
| Customer satisfaction | >80% surveyed customers are satisfied |
| Technician productivity | Report average number of tests performed per month per technician |

*General indicators are from the GLI Practical Guide to TB Laboratory Strengthening. Geneva, Global Laboratory Initiative, 2017. <http://stoptb.org/wg/gli/gat.asp>

Performance indicators for Truenat TB tests

The performance indicators are modeled after the GLI-recommended performance indicators for Xpert MTB/RIF testing that should be monitored monthly by each testing site. For some indicators (e.g., proportion of specimens that are rifampicin resistant), targets are setting-specific. Laboratories should monitor indicators and establish local targets and acceptable ranges. Deviations from expected values should be investigated.

| Indicator | Description | Target |
|--|--|---------------------------------------|
| Trueprep | | |
| Number and proportion of specimens for which DNA extraction was unsuccessful | Number of specimens for which DNA could not be extracted / Total number of specimens processed Errors should be stratified by type, to enable troubleshooting | Initial test: <3% Repeat test: <1% |

| Indicator | Description | Target |
|--|---|--|
| Truenat TB | | |
| Number and proportion of specimens with MTBC detected | Number of specimens with MTBC detected/ Total number of specimens tested with successful results | Dependent on population tested and country drug-resistance prevalence and patterns |
| Number and proportion of specimens with MTBC not detected | Number of specimens with MTBC not detected / Total number of specimens tested with successful results | Dependent on population tested and country drug resistance prevalence and patterns |
| Number and proportion of specimens with unsuccessful results (errors, invalid, no results) | Number of specimens with unsuccessful results/ Total number of specimens tested. Errors should be stratified by type, to enable troubleshooting. | <3% Initial test: <10% Repeat test: <3% |
| Truenat MTB-RIF Dx | | |
| Number and proportion of specimens with rifampicin resistance not detected | Number of specimens with rifampicin resistance not detected / Total number of specimens tested with successful results | Dependent on population tested and country drug-resistance prevalence and patterns |
| Number and proportion of specimens with rifampicin resistance detected | Number of specimens with rifampicin resistance detected / Total number of specimens tested with successful results | Dependent on population tested and country drug-resistance prevalence and patterns |
| Number and proportion of specimens with rifampicin indeterminate | Number of specimens with rifampicin indeterminate / Total number of specimens tested for rifampicin resistance | Dependent on population tested (e.g., proportion of patients with smear-negative TB) |
| Number and proportion of specimens with unsuccessful results (errors, invalid, no result) | Number of specimens with unsuccessful results / Total number of specimens tested. Errors should be stratified by type, to enable troubleshooting. | <3% for Truenat MTB or MTB Plus test Initial RIF-Dx test: <7% if reflexed from Truenat MTB Initial RIF-Dx test: <15% if reflexed from Truenat MTB Plus |
| Laboratory turnaround time | | |
| Laboratory turnaround time | Time between receipt of specimen at the laboratory and result reporting | 2-24hrs |

Annex 10: Impact measures for Truerep and Truenat TB tests

A framework for monitoring and evaluation of the impact of a diagnostic test is essential to inform decision-making. New or improved TB diagnostic tests often have the objective to improve the laboratory confirmation of TB or the detection of drug resistance. For each objective of a test, indicators should be developed to assess its impact. For example, implementation of the Truenat TB tests should increase the ability to detect TB and RIF-resistant TB as measured by the WHO targets for laboratory strengthening under the End TB Strategy. The WHO indicators described in the Table below reflect the use of any WRD. Programmes will need to assess the contribution of Truenat TB tests, Xpert MTB/RIF tests and any other WRD used in the country to determine the impact of Truenat TB testing on the accomplishment of these indicators.

| WHO indicators for Laboratory Strengthening | WHO Target |
|--|---------------------------|
| Percentage of notified new and relapse TB cases tested with a WHO-approved rapid diagnostic test (WRD) as the initial diagnostic test (End TB Strategy Laboratory Indicator 2) ²¹ | 80% (2020) |
| Percentage of notified new and relapse TB cases with bacteriological confirmation (Indicator 3) | 80% [relapse: 90%] (2020) |
| Percentage of testing sites using a WRD at which a data connectivity system has been established that transmits results electronically to clinicians and to an information management system (Indicator 4) | 100% (2020) |
| Percentage of notified bacteriologically confirmed TB cases with DST results for rifampicin (Indicator 7) | 100% (2020) |
| Percentage of notified rifampicin-resistant TB cases with DST results for fluoroquinolones and second-line injectable agents (Indicator 8) | 100% (2020) |

The implementation of Truenat TB tests at facilities closer to point-of-care may lead to improvements in the entire patient care cascade from screening to initiation of appropriate treatment. In addition to WHO indicators 2 and 3 above, possible impact measures may include:

- Number and proportion of presumptive TB patients that have been tested with a WRD
- Proportion of the population that has access to WRD within a 5-kilometer distance
- Number and proportion of estimated TB patients that are evaluated for TB (i.e., reach a diagnostic center)
- Number and proportion of presumptive TB patients that reach a diagnostic center and for whom a TB test is ordered
- Number and proportion of presumptive TB patients for whom a test is ordered and who provide a specimen for testing
- Number and proportion of presumptive TB patients for whom a specimen is collected and whose specimen is received at the testing laboratory
- Number and proportion of presumptive TB patients whose specimen is received at the testing laboratory and for whom a test is conducted
- Number and proportion of presumptive TB patients for whom a test is conducted and for whom test results are reported to the clinician

²¹ The indicator number in parentheses refers to the number of the global indicators in the WHO Framework of indicators and targets for laboratory strengthening under the End TB Strategy. <http://www.who.int/tb/publications/labindicators/en/>

- Number and proportion of presumptive TB patients for whom test results are reported to the clinician and that are notified of the result
- Proportion of specimens collected for WRD testing for which a result was received by the ordering clinician within the specified target time (i.e., time from collection of a specimen to receipt of results). Note that the 'specified time' should be determined for each laboratory taking into account testing schedules and specimen transport schedules.

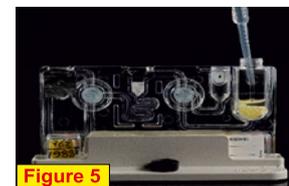
It should be noted that a detailed analysis of the impact measures (time and completion rate) for each step in the patient care pathway is an intensive process that may require data from laboratory, clinical and programme personnel. Such information may be collected using a once a year survey or through operational research. Programmes should establish baseline performance and monitor the impact of Truenat TB tests to improvements in performance over time.

Molbio Diagnostics Private Limited
VERNA-GOA



Truenat™ RT-PCR PROCESS FLOW- MTB - SAMPLE TO RESULT

- Collect 2-5 ml adult pulmonary sputum sample in 50 ml Falcon tube/sputum cup**
- ↓
- Label the sputum cup with patient details
- ↓
- Add 2 drops of liquefaction buffer to the Sputum cup (Figure 1)
- ↓
- Close the cap and swirl gently to mix (Figure 2)
- ↓
- Incubate for 10 minutes at room temperature. If sample is not pipettable after 10 minutes, incubate for another 5 minutes with swirling at 2 minutes intervals
- ↓
- Transfer 0.5 ml of liquefied sputum sample into the Lysis Buffer bottle using a 1ml transfer pipette (Figure 3)
- ↓
- Add 2 drops of liquefaction buffer to the lysis buffer bottle, swirl gently to mix and incubate for 3-5 minutes (Figure 4)
- ↓
- Remove the cartridge from a pouch and place it in the cartridge stand. Keep the Elute Collection Tube (ECT), label and elute transfer pipette for later use
- ↓
- Transfer the entire content of the lysis buffer into the sample chamber (black cap) of the cartridge using a 3ml transfer pipette (Figure 5)
Discard* the transfer pipette
- ↓
- Turn 'ON' the Trueprep® AUTO Sample Prep device, press 'eject' button to open and gently pull out the cartridge holder (Figure 6)
- ↓
- Place the cartridge in the tray (Figure 7) and gently push to close the cartridge holder
- ↓
- The device will beep at the end of the DNA Extraction process (20 minutes) and the cartridge holder will eject automatically
- ↓
- Gently pull out cartridge holder, remove cartridge and place it on the stand
- ↓
- Carefully pierce the elute chamber with the elute transfer pipette (Figure 8) and transfer the entire elute into the ECT
Discard* the transfer pipette and cartridge

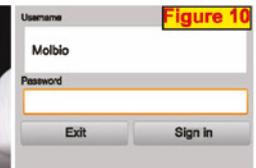


1

ANY WHERE, ANY TIME, REAL TIME PCR

Truenat™ RT-PCR PROCESS FLOW- MTB - SAMPLE TO RESULT

Switch 'ON' the Truelab® Real Time micro PCR Analyzer (Figure 9)



Select User ID and enter the login password (Figure 10)

Select test profile 'MTB'(Figure 11) and enter the patient details(Figure 12)



Press 'Start test' on the screen

Open a Truenat™ MTB chip pouch

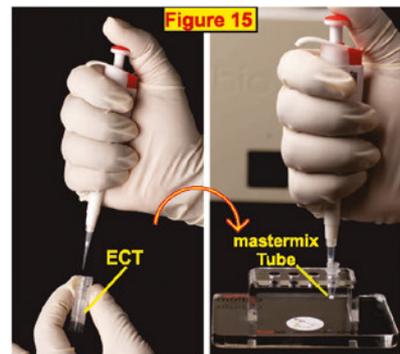
Gently take out the chip (Figure 13) and place it on the chip tray by aligning it in the slot provided (Figure 14)



Open the mastermix tube, discard the stopper and place the tube in the microtube stand

Attach the micro tip to the single push pipette

Transfer 6µL of the elute from ECT into the mastermix tube (Figure 15)



Allow the mastermix to stand for 30 seconds to get a clear solution

Transfer the elute from the mastermix tube to the white reaction well of the chip (Figure 16).Discard* the pipette tip and mastermix tube



Click 'Yes' on the device screen to start the test

The PCR will be completed in 35 minutes

Open the chip tray and discard the used chip immediately after the reaction

If MTB is detected (Figure 17), test the same elute for Rifampicin resistance using the Truenat™ MTB-RIF Dx chip
The test takes 55 minutes to complete

| Truenat™ MTB | | | |
|------------------------|-------|-------------|------------------------------|
| Center | XX | Operator | XX |
| Profile | MTB | Date | Thu 13 Aug 2020 15:17 |
| Lot | TB116 | Expiry Date | 01-22 |
| Patient Details | | Sample | Sputum |
| Name | XX | ID | p26853 |
| Age | XX | Gender | Male |
| Result | | Referred By | I.g.i.m.s |
| Control C _t | 30.13 | MTB | 29.75 |
| Run Status | Valid | | |
| MTB | | DETECTED | 3.4x10 ^{9.9} CFU/ml |

Truenat™ MTB test Result

* Discard in freshly prepared 1% Sodium Hypochlorite Solution

** Sputum sample with Pan masala, gutkha, tobacco or any food particles and blood stained samples should be rejected

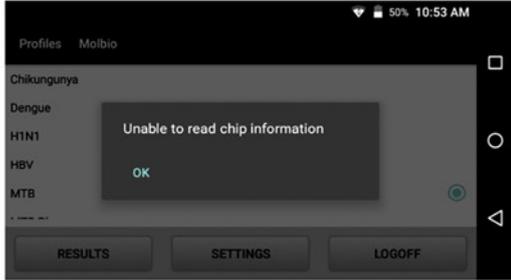
Chips and cartridges are categorized as 'plastic waste' and these should be discarded as per biomedical waste management rules

Troubleshooting, Alerts and Errors

| Trueprep® AUTO v2 Universal Cartridge based Sample Prep Device | | | | | | |
|--|----------------------------|-------------------------------|---------------------------------|---|--------------------------------|----------------------------------|
| E1 | E2 | E3 | E6 | E9 | E10 | E11&12 |
| E1: CARTRIDGE VALVE ERROR | E2: CARTRIDGE ERROR | E3: CARTRIDGE CLOGGED | E6: CARTRIDGE NOT LOADED | RESET CARD READ ERROR | E10: INVALID RESET CARD | E11: ATD- L ERROR |
| Press Eject to exit | Press Eject to exit | Press Eject to exit | Press Eject to exit | Press Eject to exit | Press Eject to exit | E12: ATD- E ERROR |
| Cartridge valve is damaged | Pressure drop error | Sample/ specimen is too thick | Cartridge not detected | Problem with the Reset card or QR code reader | | Device heater plates not working |
| <p>SOLUTION</p> <p>E1/E2*: Please start afresh by processing remainder of sample in lysis buffer and load into new cartridge.</p> <p>E3*: Ensure sample is liquefied and pipettable. Repeat extraction with new cartridge/request for new sample.</p> <p>E6*: Ensure cartirdge is loaded properly in correct orientation</p> <p>E9/E10/E11/E12: Contact Molbio support <i>*If error persists, contact Molbio support</i></p> | | | | | | |

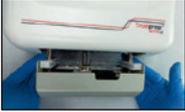
| Truelab® Real Time Quantitative micro PCR Analyzer | | | | | |
|--|-----------------------|--|----------------|--|---|
| ERROR 1 | ERROR 2 | ERROR 3 | ERROR 4 | ERROR 5 | INVALID |
| Thermal cycling error | Test stopped manually | Incorrect optical profile | Runtime Error | Probe check Error | Internal Control did not amplify in PCR or improper sample extraction |
| <p>SOLUTION</p> <p>ERROR1/2/3/4/5**: Repeat the run using a fresh chip and re-load*** the elute by pressing Repeat button</p> <p>INVALID**: Re-run the same elute using another chip. If Invalid repeats, process the sample again and run elute using another chip.</p> <p>**Contact the Molbio support team if the problem persists</p> <p>***Follow User Guidelines for proper loading of elute onto the white reaction well of the chip</p> | | | | | |
| Truelab® Alert Messages | | | | | |
| “Unable to read chip information” | | “Could not initialize. Please try again” | | “Chip is already used” OR “Chip loaded is expired” | |
| Analyzer was unable to read chip memory. Check if chip was loaded properly into the tray. Remove the chip and re-select the profile from Status Screen and repeat the steps. If message reappears, load a new chip and re-load the elute again. | | The system was unable to establish an internal connection. attempt the test again by using a new chip and re-loading the elute again | | User loaded a used chip/expired chip in the Tray. Use a fresh chip and re-load the elute | |

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| Description | Reason for Error and Possible | Solution Screenshot of Error |
|--|--|--|
| <p>Login attempt failure, with system message “Login Invalid, Incorrect Password.”</p> | <p>Reason: The password you entered was incorrect.</p> <p>Solution:</p> <ul style="list-style-type: none"> • Tap the OK Button This will take you back to the login screen • Re-enter the correct password (password is case-sensitive) |  <p>Figure 153 : Login invalid prompt</p> |
| <p>System message “Invalid Patient Name” in Sample Details Screen.</p> | <p>Reason: The “Patient Name” field in the Sample Details Form is blank. This field is mandatory.</p> <p>Solution: You need to fill in mandatory information to continue with the test.</p> |  <p>Figure 154 : Sample Details Screen</p> |
| <p>System Message “Unable to read chip information.”</p> | <p>Reason: The Analyzer was unable to read chip memory.</p> <p>Solution:</p> <ol style="list-style-type: none"> 1. Tap 'OK' on the Read Error Prompt. 2. Check if chip was loaded properly into the tray. If so, remove the chip and select the profile from Status Screen and repeat the steps. 3. Load a different chip or the same chip in case not loaded before. <p>If the same message reappears, please contact Molbio support team.</p> |  <p>Figure 155 : Chip information read error</p> |

Do's and Dont's

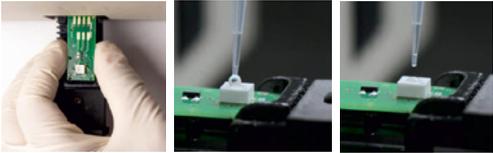
Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Device

- ✓ Place the device in a dust free environment
- ✓ Wipe the surface of the device regularly with 1% Sodium hypochlorite solution followed by distilled water
- ✓ Replace the cartridge holder tray in case of a spillage in the device 
- ✓ If the device is kept idle for more than 10 days, carry out the flushing protocol (Refer manual section 8.2.3) before resuming operation

- ✗ Do not leave a used cartridge inside the device. Remove the cartridge after extraction and collect the elute in the ECT
- ✗ Do not keep used material on the work bench and on the device 
- ✗ Do not tilt or shake the device while connecting charger, during extraction or while removing cartridge from device
- ✗ Do not touch the nozzle of the liquefaction buffer bottle to the lysis buffer tube during sample preparation 

Truelab® Uno Dx/Duo/Quattro Real Time Quantitative micro PCR Analyzer

- ✓ Place the analyzer on a flat surface in a dust free environment, away from direct sunlight and vibration causing instruments

- ✓ Wipe the surface of the device regularly with 1% Sodium hypochlorite solution followed by distilled water
- ✓ Ensure that the elute is loaded in the center of the reaction well


- ✗ Do not move the analyzer when a test is in progress
- ✗ Do not spill water or any other solution on the surface of the analyzer
- ✗ Do not touch the white reaction well or the connector pads of the chip. Hold the chip by the edges only

- ✗ Do not leave a used chip inside the device
- ✗ Do not keep used material on the work bench and on the device


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USAID
FROM THE AMERICAN PEOPLE



Stop TB Partnership

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