Truenat™ Tests for the Detection of TB and Rifampicin Resistance

Training Guide

Contents

ACKNOWLEDGEMENTS

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MODULE 1: INTRODUCTION TO TRUENAT

Introduction

This module discusses the challenges of diagnosing TB and the World Health Organization's recommendations for TB testing.

Course Outline

1. **TB Context**
2. **Plenary Session**: Key Challenges in Diagnosing TB
3. **TB Laboratory Tests:** World Health Organization (WHO) Recommendations
4. **Truenat:** Placement in diagnostic networks Accuracy

Learning Objectives

By the end of this module, participants should be able to:

- Describe the global and country-specific context of TB
- List the different laboratory tests used to diagnose TB and drug resistance, and WHO's recommendations for each
- Describe the advantages of introducing Truenat within a TB diagnostic network
- Compare the diagnostic accuracy of Truenat to other TB laboratory tests

TB CONTEXT

Global TB Situation

- 10 million people fall ill with tuberculosis (TB) ever year.
- Newly diagnosed and reported TB fell from 7.1 million in 2019 to 5.8 million, a direct impact of COVID-19 pandemic
- 1.5 million people die from TB each year – making it the world's one of the top infectious killers.
- TB is the leading cause of death of people with HIV and a major contributor to antimicrobial resistance

WHO End-TB Strategy

- WHO-recommended rapid TB diagnostics (WRDs) should be available to all persons with signs or symptoms of TB
- All bacteriologically confirmed TB patients should receive drug-susceptibility testing (DST) at least for rifampin (RIF)
- All patients with RIF-resistant TB should receive DST at least for fluoroquinolones (FQs)

Bangladesh TB Context

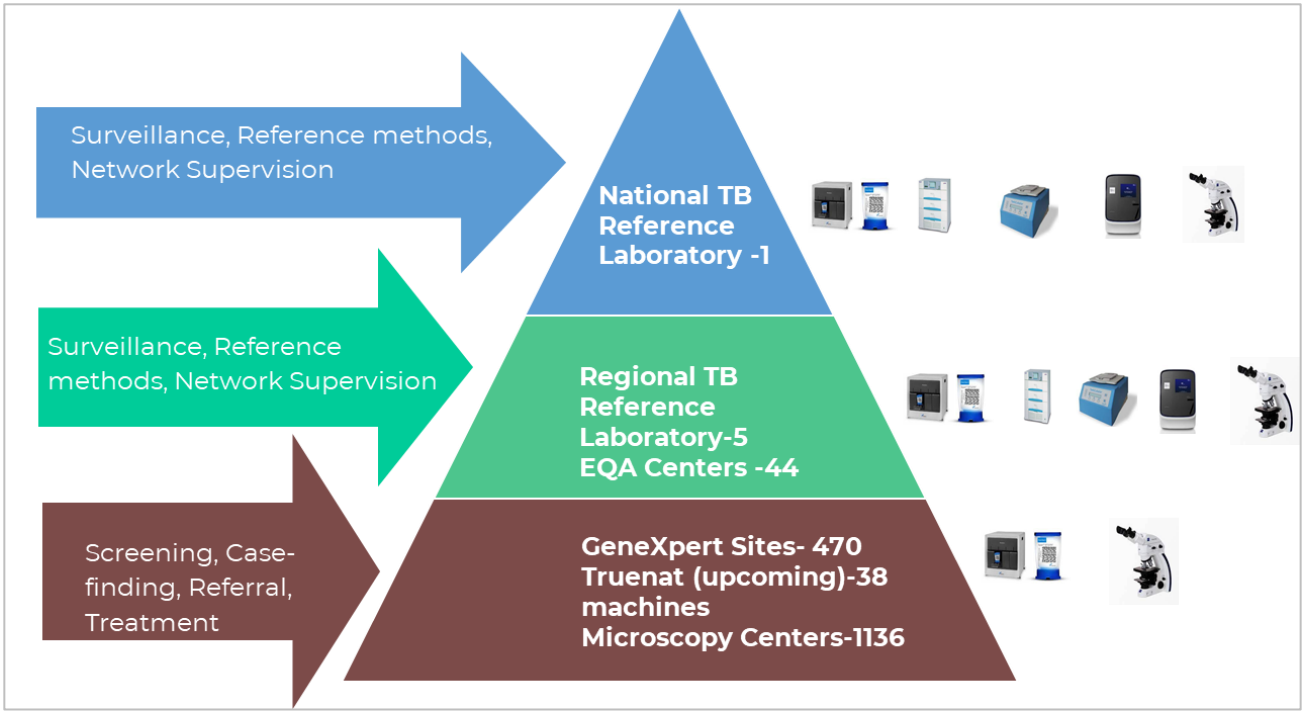
TB Data

- Estimated TB incidence in 2020: 360,000
- Total notification of TB in 2020 (new and relapse): 230,000
- Estimated DR-TB incidence in 2020: 3,300
- Total notification of DR-TB in 2020: 1,113
- Number of TB related deaths in 2020: 44,000
- Estimated incidence of pediatric TB: 23,000
- Notification of pediatric TB in 2020: 9,200
- Treatment coverage: 64%
- Estimated DR-TB incidence in 2022: 3297

National Priorities relevant to TB diagnostics

- Detection of all missing TB cases
- Expansion of rapid molecular diagnostics tools to increase detection of TB and RR-TB
- Increase detection of child TB
- Increase coverage for Drug Susceptibility Testing (DST) at least for all Rifampicin Resistant cases

TB Laboratory Network of Bangladesh NTP



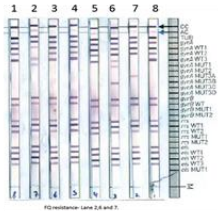
TB LABORATORY TESTS

Menu of TB Lab Tests

MICROSCOPY

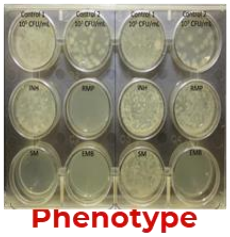
Gene Xpert

Truenat



LINE PROBE ASSAY (LPA)

Culture



Phenotype (Culture Based) drug susceptibility testing(DST)

AFB-Smear Microscopy (ZN & LED)

Uses

- Used as an initial diagnostic test for the detection of AFB in PTB
- Monitors response to therapy

Benefits

- Can be done safely with low risk level and minimal biosafety precautions
- Same day results
- Inexpensive and widely available

Limitations

- Low sensitivity (around 50%)
- Need high bacillary load 5000-10000/ml for ZN and 3000/ml for LED
- Limited specificity; can detect non-tuberculous mycobacteria (NTMs) and does not detect drug resistance.

Xpert MTB/RIF

Uses

- Detects both Mycobacterium tuberculosis complex bacteria (MTBC) and RIF resistance in sputum and EPTB specimens

Benefits

- Fast turnaround (<2 hours), high sensitivity, low risk in terms of biosafety
- Automated one step process
- High sensitivity and specificity
- Same machine can be used for multiple tests for diagnosis of HIV, Hepatitis C etc

Limitations

- Requires an uninterrupted and stable electrical power supply, yearly calibration of the modules and an ambient temperature of 15-30 °C.
- Cannot be used to monitor treatment
- Does not detect resistance to anti-TB agents other than RIF

Truenat

Uses

- First WHO-recommended molecular test for TB and RIF resistance that can be used in peripheral settings with limited infrastructure
- Used as an initial diagnostic test for TB

Benefits

- Designed to be operated in peripheral laboratories with minimal infrastructure.
- Portable
- Battery-powered and uses room temperature stable reagents
- Can generate results for TB in one hour and for RIF resistance in one additional hour
- Can diagnose many other diseases eg: Covid, HIV, Hepatitis etc.

Limitations

- RIF resistance test is a reflex test
- Electricity still required for charging the batteries
- More manual steps than the Xpert MTB/RIF test
- Need training for MT-Lab (technical for data entry and test performance)
- Cannot be used for treatment monitoring

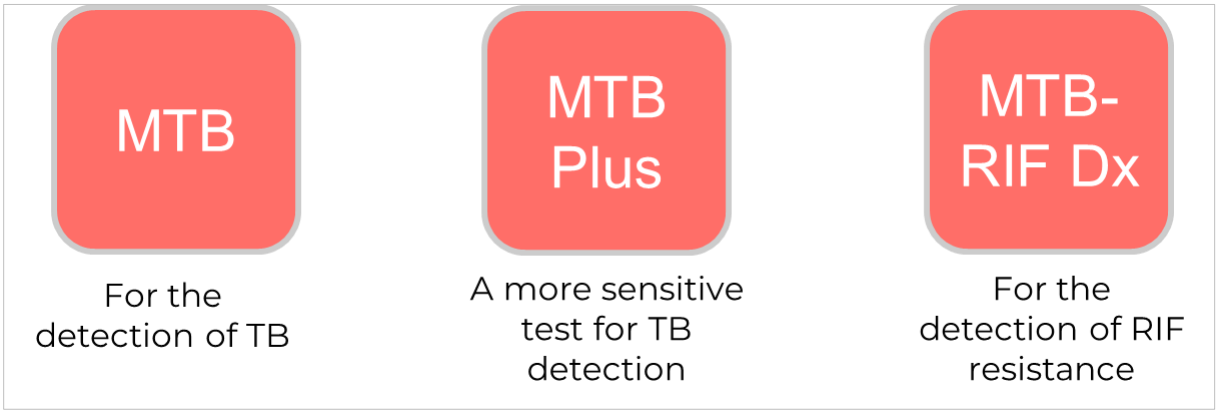
WHO Recommendation

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/culture.

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 an initial test for RIF resistance rather than culture and phenotypic DST.

Truenat Chips

Truenat has three types of chips for three different tests.



Culture

Uses

- Monitors MDR-TB treatment

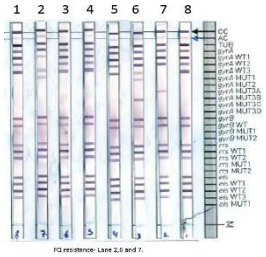
Benefits

- High sensitivity and specificity test for the detection of MTBC (only 10-100/ml bacilli can be detected in culture).
- Provides an isolate for DST (phenotypic testing)
- Can assess treatment progress

Limitations

- Requires a high level of biosafety precautions
- Requires trained staff
- Automated liquid culture is more expensive than solid culture
- Solid culture is slow; takes 4–8 weeks to detect MTBC
- High contamination chance in liquid culture
- Equipment maintenance and supply are costly.

LPA



Uses

- Detection of resistance to anti-TB drugs

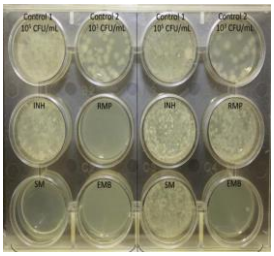
Benefits

- Able to rapidly detect resistance to RIF, INH, FQs and second line injectables.
- Detects Mycobacterium Tuberculosis complex (MTBC) and determines its drug sensitivity to RIF and INH.
- Can perform multiple tests at once
- Fast and accurate results within 48-72 hours
- Able to provide guidance on treatment decision

Limitations

- Requires at least 3 separate rooms to avoid cross-contamination and moderate to high levels of biosafety precautions (biosafety containment level3)
- Cannot be used to monitor treatment
- Cannot fully replace conventional culture methods
- Requires well trained staff
- Costly reagent & equipment also calibration kit.

Phenotypic (Liquid culture-based) DST



Uses

- Detection of resistance to anti-TB drugs

Benefits

- Culture-based, phenotypic DST remains essential for drugs for which there are not yet reliable molecular tests
- Phenotypic DST for second-line agents is required to confirm or exclude XDR-TB

Limitations

- Requires a high level of biosafety precautions, highly skilled staff and strict quality control
- Culture-based phenotypic DST can take weeks to months to generate results
- Reliable phenotypic DST methods are not available for all anti-TB drugs

*****DST remains essential for drugs for which there are not yet reliable molecular tests.***

Placement of Truenat in Diagnostic Networks

- Truenat can be placed at peripheral health centers to replace microscopy as the initial diagnostic test for TB.
- Microscopy will be used for follow-up of DS TB treatment.
- Truenat sites will be linked with referral network for specimen collection.
- Truenat sites will be linked with referral network to send specimens to referral labs for LPA, culture and DST.

***** Molecular test (Truenat, Xpert) cannot be used for follow-up of treatment***

DIAGNOSTIC ACCURACY OF TRUENAT

Diagnostic accuracy relative to culture, in microscopy center settings

	Sensitivity (All patients)	**Sensitivity (SS + patients)**	**Sensitivity (SS – patients)**	**Specificity (All patients)**
Truenat MTB Plus	0.80	0.96	0.46	0.97
Truenat MTB-RIF Dx	0.84	0.88	0.67	0.95
Foundation for Innovative New Diagnostics, multicenter prospective clinical evaluation study in 19 clinical sites and 7 reference laboratories in 4 countries.				

Diagnostic accuracy relative to culture among individuals being evaluated for TB, reference laboratory settings

	Sensitivity (All patients)	**Sensitivity (SS + patients)**	**Sensitivity (SS – patients)**	**Specificity (All patients)**
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
Foundation for Innovative New Diagnostics, multicenter prospective clinical evaluation study in 19 clinical sites and 7 reference laboratories in 4 countries.				

Advantages of Truenat

1. Patient access- Use of Truenat at primary healthcare level can reduce the need for sample transport for detection of RIF resistance
2. Time taken for the assay- MTB detection is completed in 1 hour and the RIF assay is done only as a reflex test
3. Cost effectiveness- Low equipment and test costs
4. Availability of DNA- With Truenat, DNA is available for repeat testing and any further investigation and quality control purposes
5. In – built connectivity allows for use of digital data including rapid reporting of results to clinicians.
6. Near Point of care technology (POC), battery operated and portable
7. Can be used for active case finding strategies remotely in rural areas

SUMMARY

- **Truenat is a promising new TB diagnostic tool**- More sensitive and specific than microscopy
- **Truenat has minimal infrastructure requirements and can be used at POC/near-POC**- Results are rapidly available allowing for same-day diagnosis
- **Truenat can detect RIF resistance within two hours**- Can be used as initial resistance test

Knowledge Check

1. What are the main TB diagnostic test available in Bangladesh?
2. Which TB diagnostic test suitable for your setting/rural area?
3. List three advantages of Truenat compared to other tests.

MODULE 2: DIAGNOSTIC STEPS AND RESULTS INTERPRETATION

Introduction

This module introduces the TB diagnostic steps for Truenat and how to interpret results.

Course Outline

1. WHO Recommendations
2. Truenat Steps
3. Patient flow

Learning Objectives

By the end of this module, participants should be able to:

- Understand the WHO recommendations for using Truenat.
- Follow the Truenat steps to use Truenat.
- Understand patient flow within the TB diagnostic network and describe procedures for patient referral.

Learning contents

WHO Recommendations

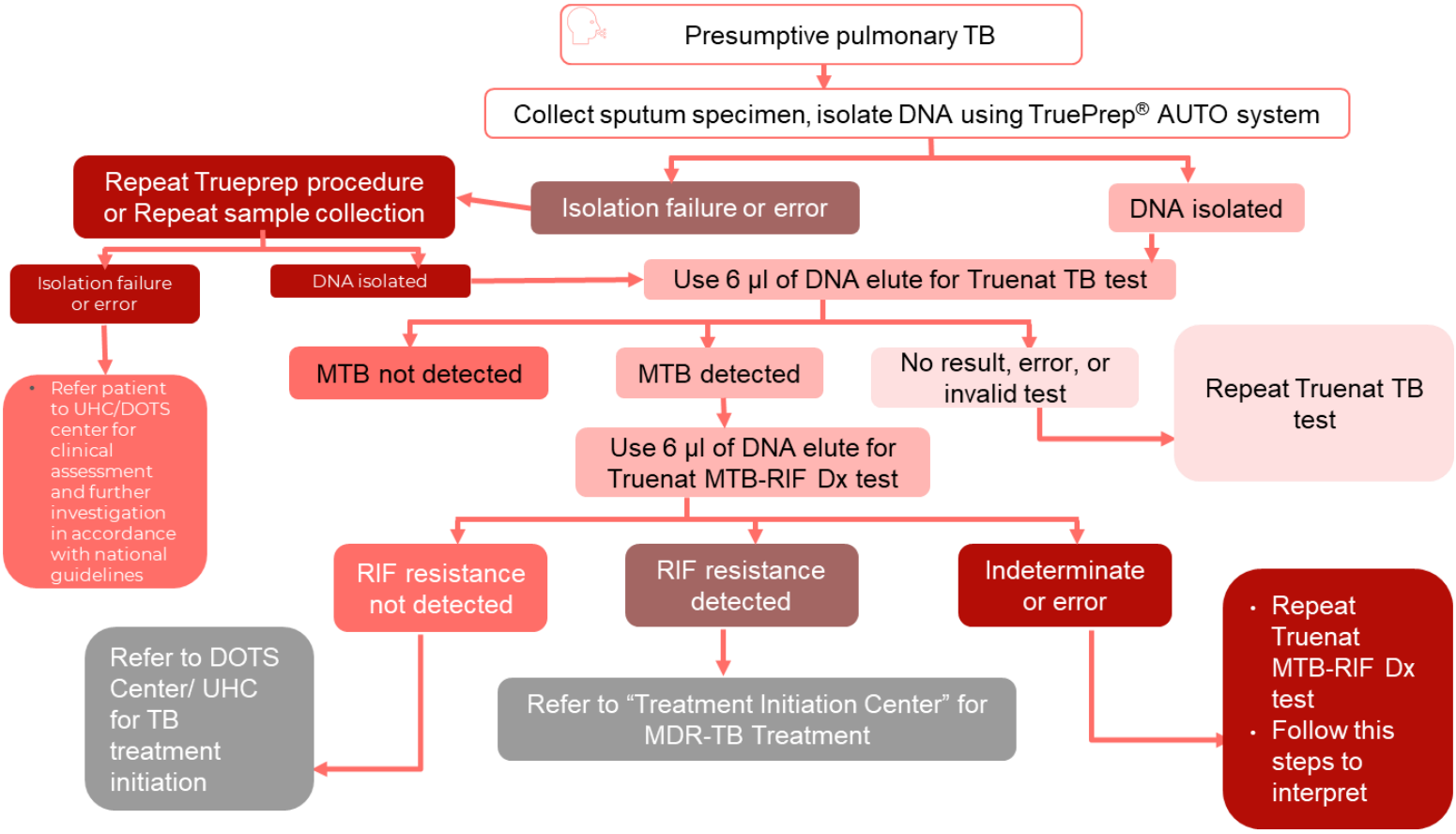
Truenat MTB or MTB Plus- May be used as an initial diagnostic test for TB rather than smear microscopy/culture

Truenat MTB-RIF Dx- May be used as the initial test for detection of RIF resistance rather than culture and phenotypic DST

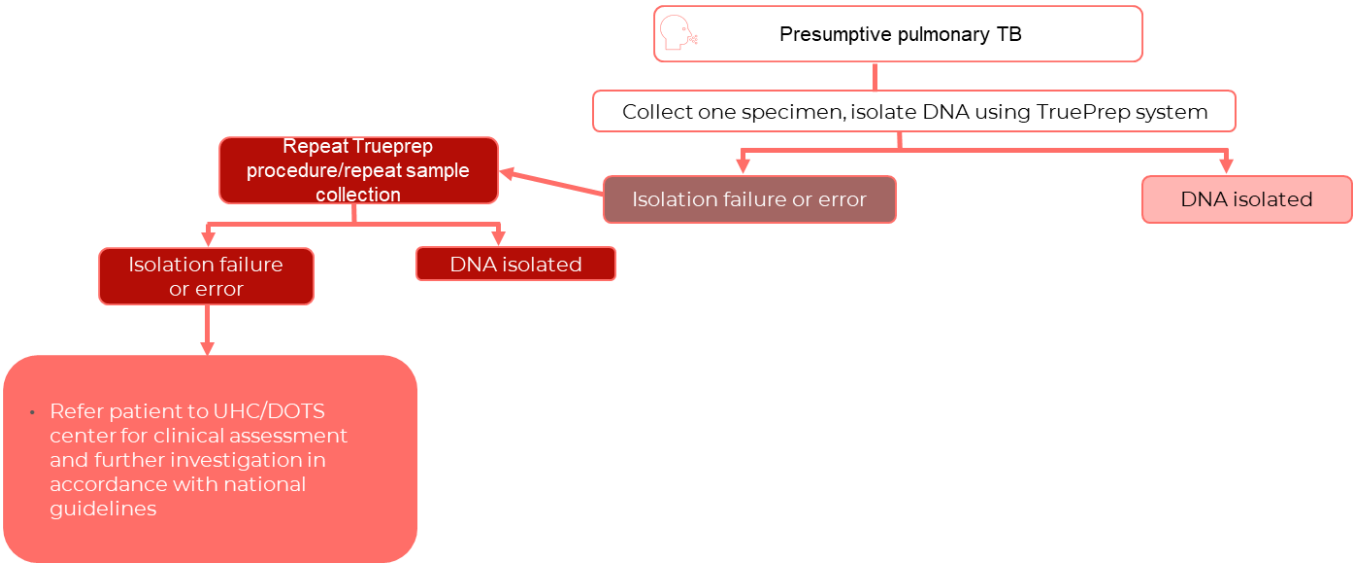
Truenat can be used for all adults and children with signs and symptoms of pulmonary TB

When to Use Truenat	When NOT to Use Truenat
• To detect MTBC in specimens from persons newly presenting with signs and symptoms of pulmonary TB	• Truenat is not yet recommended for suspected extrapulmonary TB (due to insufficient evidence)
• To detect RIF resistance in persons found to be positive from a Truenat MTB or MTB Plus test, and in persons with TB who may have developed RIF resistance	• Do **NOT** USE Truenat for treatment monitoring (follow-up of treatment)

TRUENAT STEPS



Part 1: Isolate DNA



- Sputum is the recommended specimen
- Prepare the sample using the Trueprep AUTO MTB Sample Pre-treatment Pack

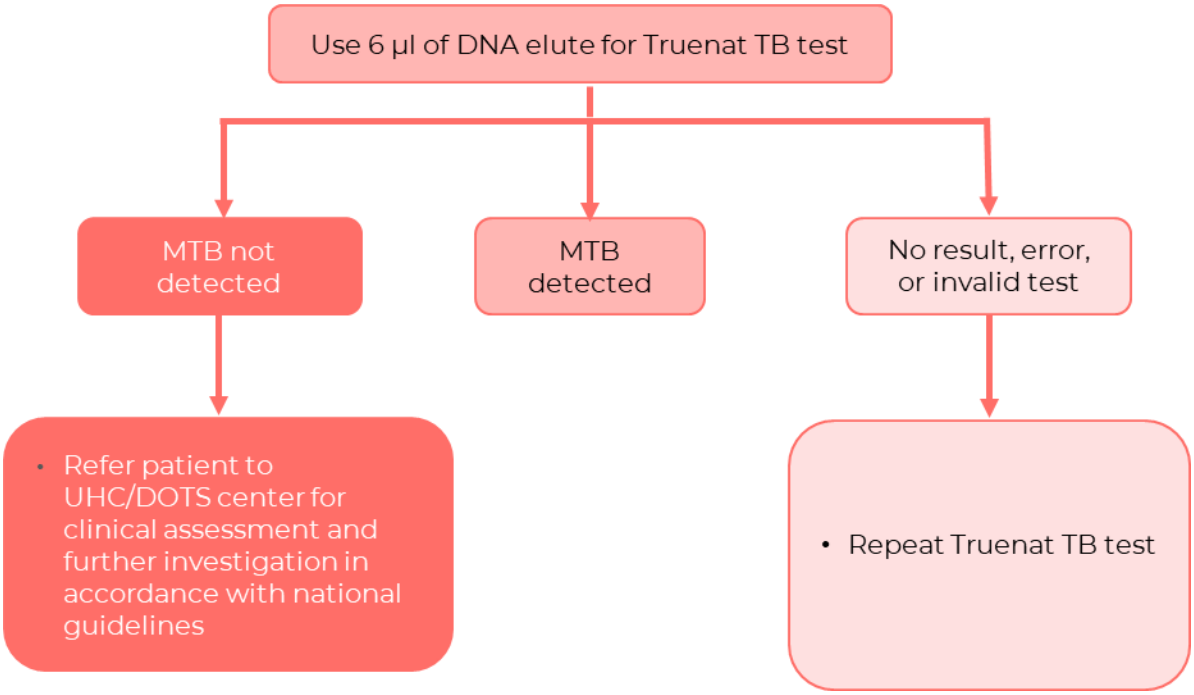
Isolate DNA using:

- Trueprep AUTO v2 Universal Cartridge Based Sample Prep Kit
- Trueprep AUTO v2 Universal Cartridge Based Sample Prep Device

If DNA isolation is unsuccessful

- Repeat the DNA isolation with Trueprep device using the same prepared sample and a second Trueprep cartridge.

Part 2: TB Test



If TB test result is MTB not detected

- Refer patient to UHC/DOTS center for clinical assessment and further investigation in accordance with national guidelines

If TB test result is MTB detected

MTB detected results appear as follows:

- Truenat MTB Plus: 'detected high,' 'medium,' 'low,' or 'very low

Next Step: Conduct RIF resistance testing with Truenat MTB-RIF-Dx (Part 3 of steps)

If TB test result is not conclusive

- Test result reads "Error" or "No result:"

- Repeat Truenat TB test with second portion of the remaining DNA

- Test result reads “invalid:”
  - Repeat Truenat TB test
  - Start a new with the sample preparation and DNA isolation

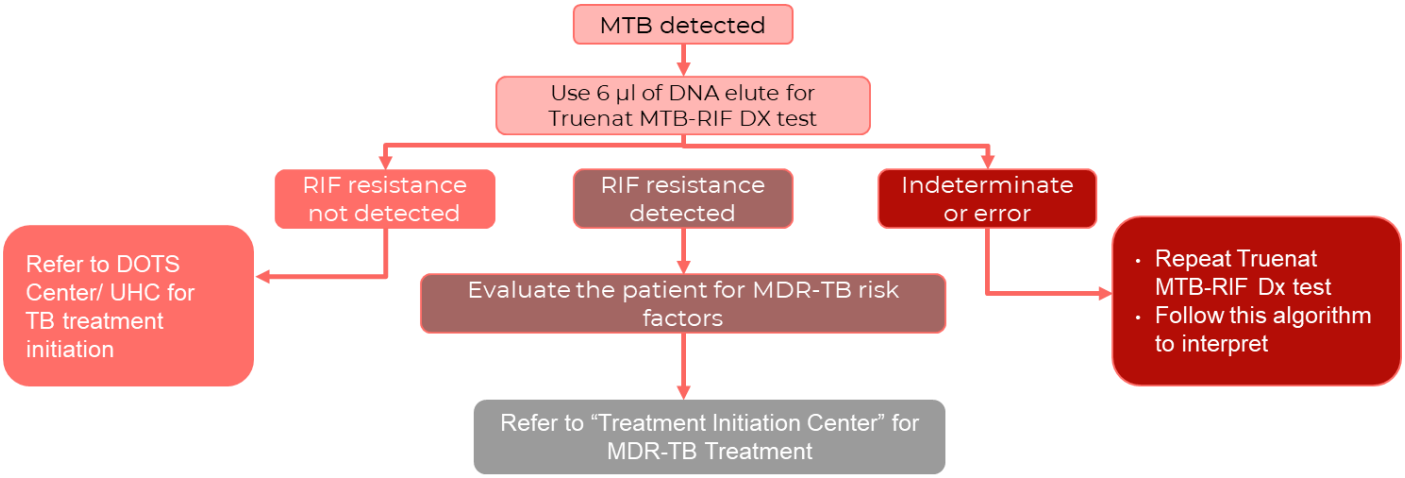
- If repeated test has a valid result, continue with steps

If second attempt is also inconclusive

Next Step:

- Refer patient to UHC/DOTS center for clinical assessment and further investigation in accordance with national guidelines

Part 3: RIF Resistance Test



If MTB-RIF Dx test result is RIF Resistance Not Detected

- Refer to DOTS Center/ UHC to Initiate patient on appropriate regimen using first-line TB drugs in accordance with national guidelines

If MTB-RIF Dx test result is RIF Resistance Detected

- Refer to DR TB Treatment Initiation Center

For all patients with RR-TB

Refer the patient to RR TB treatment initiation center to:

- Conduct clinical assessment
- Perform DST for at least FQs by 2nd line LPA and/or liquid culture
- Initiate treatment

If MTB-RIF Dx test result is RIF indeterminate

- Repeat the MTB-RIF Dx test using an aliquot from the same DNA elute.
- If the repeated test is again indeterminate, run the Truenat MTB-RIF Dx test using a DNA elute from a fresh specimen.
- Follow steps previously described based on the second result.
- If RIF NOT DETECTED, refer the patient to UHC/ DOT center
- If RIF DETECTED, refer the patient to DR TB initiation center

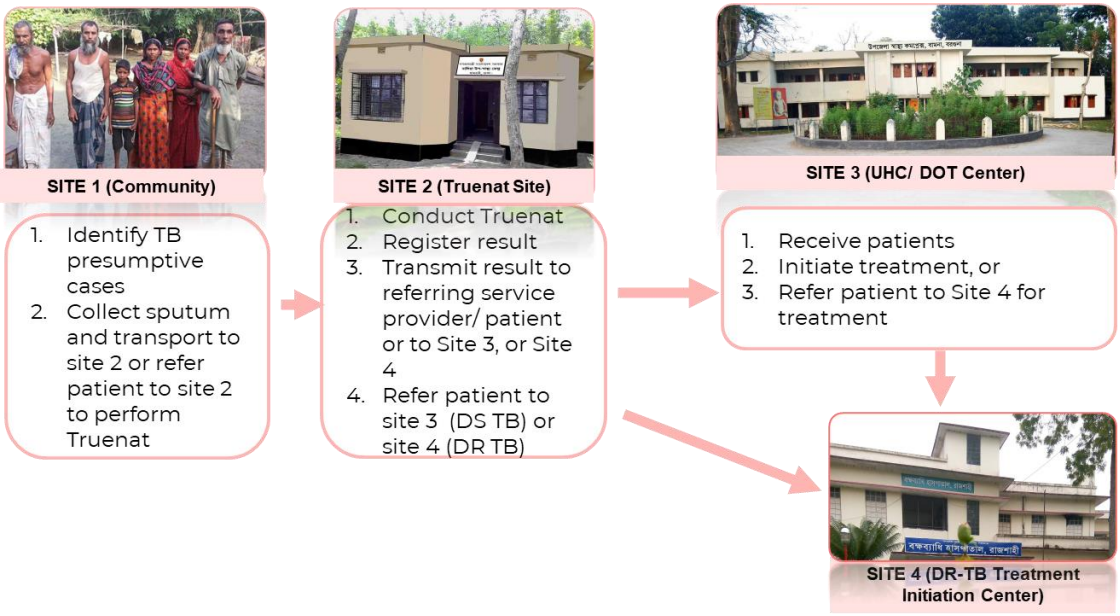
***** RIF resistance indeterminate result is usually caused by a paucibacillary TB load in the sample***

Activity: Truenat Steps

Scenario:

Nasima comes to your center with TB symptoms. You have done MTB plus test and result is MTB detected but RIF indeterminate. What steps you will follow?

PATIENT FLOW



SUMMARY

WHO Recommendation

- WHO recommends using Truenat MTB or MTB Plus for all adults and children with signs and symptoms of pulmonary TB. Truenat MTB-RIF Dx can be used as the initial diagnostic test for RIF resistance.

Truenat test steps

- The test step shows how to use Truenat.

Referral Pathways

- In cases of TB-positive and RIF positive results the patient must be referred to TB or DR TB treatment initiation center.

Knowledge Check

1. What are the two tests that can be done by Truenat?
2. What are the three main parts of Truenat test steps?
3. What two things should happen after a patient test positive for TB with a Truenat TB test?

MODULE 3: OPERATIONAL ASPECTS

Introduction

This module provides information on how to use the Truenat equipment, procedures for conducting tests, and explains infrastructure requirements.

Learning Objectives

By the end of this module, participants should be able to:

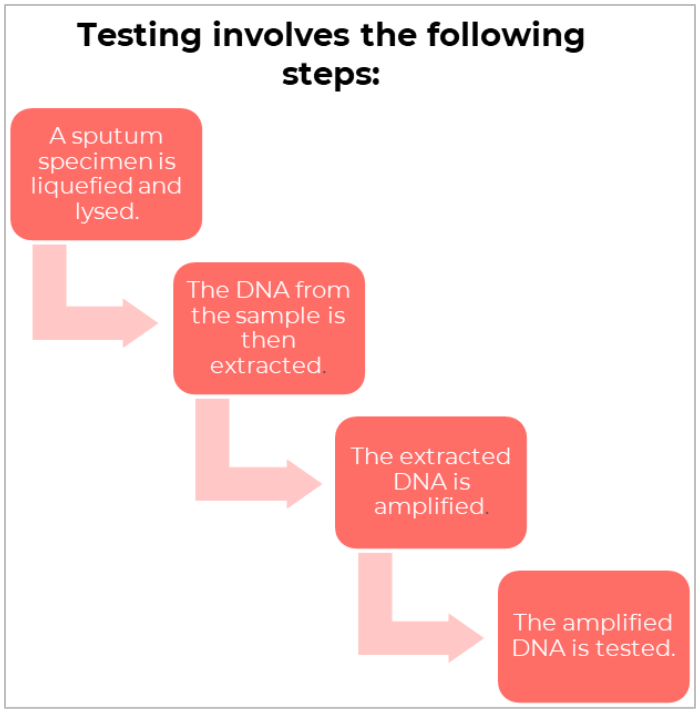
- List the equipment and supplies needed to run Truenat tests
- Describe the procedures for running a Truenat test
- Describe the infrastructure requirements for using Truenat equipment

Course Outline

- Truenat TB PCR Testing
- Equipment and Reagents
- Truenat Test Procedures
- Infrastructure Requirements

Learning contents

Truenat TB PCR Testing



Truenat TB test is a **chip-based real time polymerase chain reaction (PCR)** test for the semi-quantitative detection and diagnosis of ***Mycobacterium tuberculosis*** **complex bacterial (MTBC)** in human sputum samples. Also, can diagnosis of MTB-RIF resistance.

EQUIPMENTS AND SUPPLIES

Equipment

1. **Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Device (Trueprep)** for automated extraction and amplification of DNA.

2. **Truelab Real Time micro–PCR Analyzer**

- For performing PCR
- Comes in three models

Truelab® Uno Dx

Truelab®

Truelab®

Different Models of Truelab Analyzers to Match Anticipated Throughput

	Throughput per 8-Hour Shift (1 Workday) with Optimized Workflow	Estimated Throughput per 8-Hour Shift (1 Workday) with "Real World" Conditions
1 Truelab® Uno Dx Analyzer + 1 Trueprep extractor	10-12 specimens	7-9 specimens
1 Truelab® Duo Analyzer + 1 Trueprep extractor	20-24 specimens	**15-18 specimens**
1 Truelab® Quattro Analyzer + 2 Trueprep extractors	40-48 specimens	30-36 specimens

3. **Truelab micro-PCR printer**
   - Bluetooth printer, wirelessly prints the results of the PCR tests performed by Truelab® Uno Dx/ Duo / Quattro
   - Printer roll/ paper

SUPPLIES

- PPE (gloves. Mask, gown)
- Sputum cup/ collection tube
- Beaker
- Biohazard bag
- Disinfectant (Ethanol/Bleach)
- Cartridge stand
- Printer roll/ paper
- Refrigerator

Reagents and Consumables

There are **three** packets that include the reagents and consumables needed to run Truenat

1. **Trueprep® AUTO MTB Sample Pretreatment Pack**

Contents:

- Graduated transfer pipettes (1 ml)- 20 pcs
- Lysis buffer bottle (2.5 ml of buffer)- 20 pcs
- Liquefaction buffer bottle (3 ml)- 1 pcs- 2 drops per sample

2. **Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Kit (for 25 or 50 tests)**

Contents:

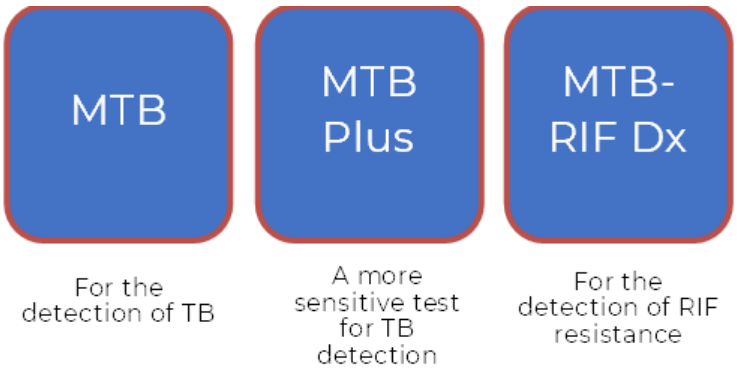
- Reagent pack (25 pcs)
- Transfer pipettes (3 ml) (25 pcs)
- Cartridge pouch (25 pcs)
    - Cartridge
    - Elute collection tube (ECT)
    - Transfer pipette
    - Label for ECT

3. **Truenat Chip Pack (for 5, 20, or 50 tests)**

Contents:

- Truenat chip pouches
- Microtube containing freeze-dried PCR reagents
- Filter barrier pipette tip
- Desiccant pouch (SILICA GEL)

Truenat has three types of chips, so three different types of chip packs



TRUENAT TEST PROCEDURES – Prepare Samples and Extract DNA

Video: Prepare Samples and Extract DNA:

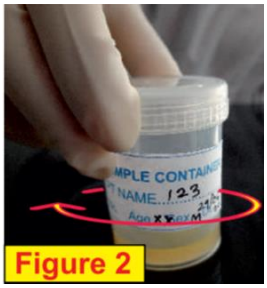
https://www.youtube.com/watch?v=ydR2I5S2v3c&ab_channel=MolbioDiagnostics

Prepare Sample and Extract DNA

1. Wear the appropriate gloves for specimen handling
2. Collect 2-5ml adult pulmonary sputum sample in sputum cup and label with patient details
3. Add 2 drops of liquefication buffer to the sputum cup (Figure 1)


Figure 1

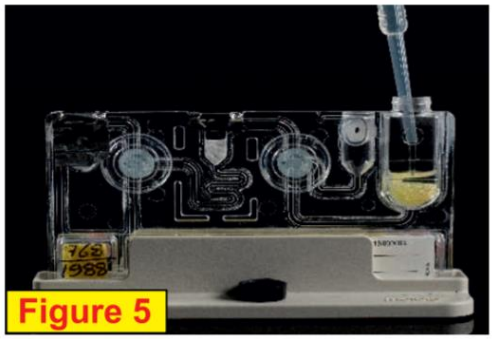
4. Close the cap and swirl gently to mix (Figure 2)



5. Incubate for 10 minutes at room temperature. If sample is not pipetteable after 10 minutes, incubate for another 5 minutes with swirling at 2-minute intervals
6. Transfer 0.5 ml of liquefied sputum sample into the lysis buffer bottle using a 1 ml transfer pipette (Figure 3).

7. Add 2 drops of liquefication buffer into the lysis buffer bottle, swirl gently to mix and incubate for 3-5 minutes (Figure 4)

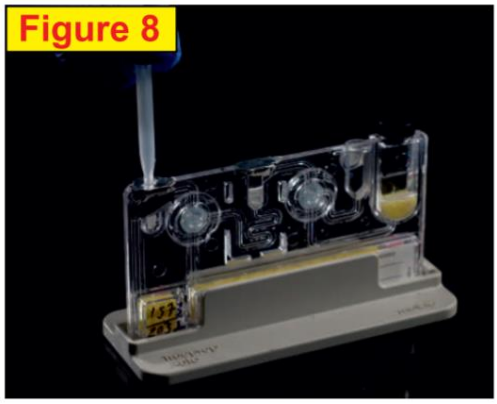
8. Remove the cartridge from the pouch, label it and place it on the cartridge stand. Take out the elute collection tube (ECT) and label it appropriately. Keep it aside for later use. Keep the elute transfer pipette in the pouch for later use.
9. Transfer the entire contents of the lysis buffer tube to the sample chamber (black cap) of cartridge using 3 ml transfer pipette (Figure 5)



10. Switch “on” the Trueprep® AUTO v2 device. Press “eject” button to open and gently pull out the cartridge holder (Figure 6).

11. Place the cartridge in the tray in the orientation shown (Figure 7), and gently push to close the cartridge holder. Press “start.”

12. The device will beep at the end of the DNA extraction process (20 minutes), and the cartridge holder will eject automatically.
13. Gently pull out the cartridge holder, remove cartridge, and place it on the cartridge stand.
14. Carefully pierce the elute chamber with the provided elute transfer pipette (Figure 8), and transfer the entire elute into the ECT. Discard the transfer pipette and cartridge.



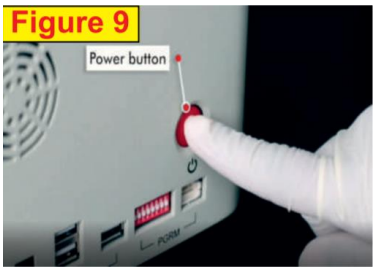
TRUENAT TEST PROCEDURES – Run a PCR TB Test

Video: Running a PCR TB Test:

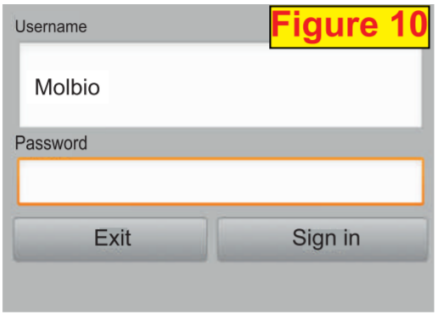
https://www.youtube.com/watch?v=ydR2I5S2v3c&ab_channel=MolbioDiagnostics

Process Flow- Running a PCR TB Test

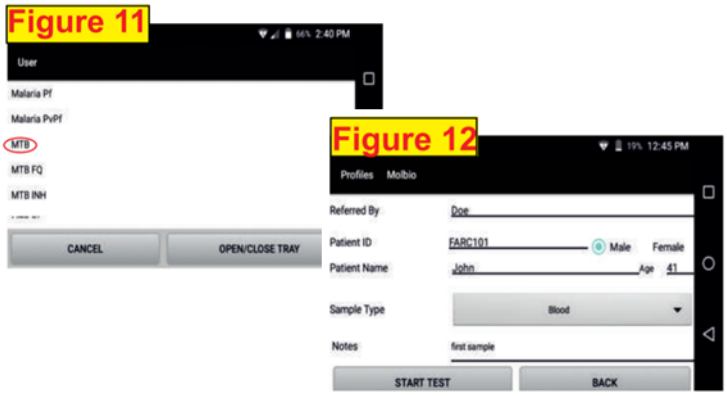
1. Switch "**on**" the Truelab microPCR analyzer by pressing the red button in the back right corner for **2 seconds**. LED will glow in Green (Figure 9). Wait for 30-50 seconds for "boot-up screen" to appear followed by "home screen."



2. Select USER ID, enter password. Press "Sign in" to Log in (Figure 10).



3. Select test profile “MTB” or “MTB Plus” (Figure 11). To confirm selection tap “PROCEED” and enter patient details (referred by, patient ID, gender, patient name & age) (Figure 12).



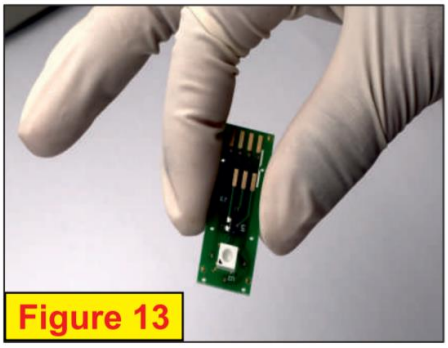
4. Select sample type (sputum).

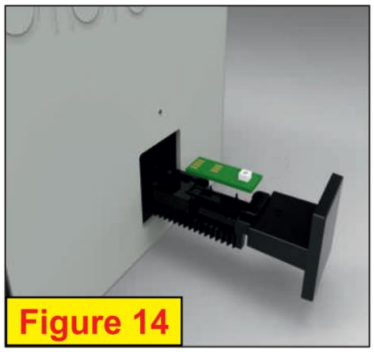
5. Press “START TEST” on the screen. Chip tray opens. “Please Load Sample” will appear. (Don’t press “YES” until chip loading is complete.}

6. Open a TRUENAT™ MTB Plus chip pouch.

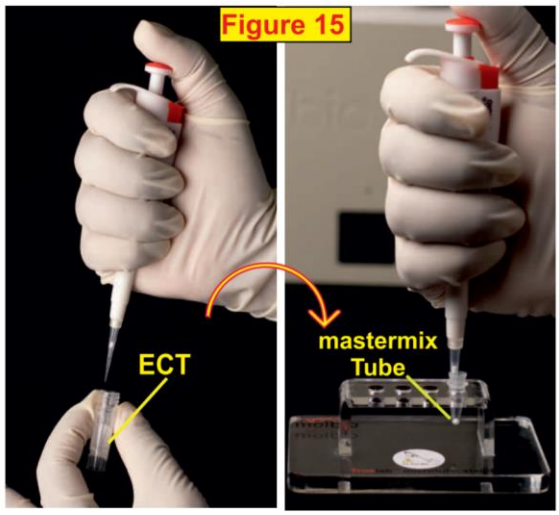
   *Pull out the orange desiccant pouch and confirm that it is orange in color.

7. Gently take out the chip (Figure 13) without touching white well portion and place it on the chip tray by aligning it in the slot provided (Figure 14)





8. Open the mastermix tube, discard the stopper and place the tube in the microtube stand.
   *Check for white cake at the bottom of the microtube.
9. Attach the 6µl micro tip provided in the pouch to the single push pipette.
10. Transfer 6µl of the elute from ECT into the mastermix tube (Figure 15).



11. Allow the mastermix to stand for 30 SECONDS to get a clear solution.

*Do not mix by tapping, shaking or reverse pipette.

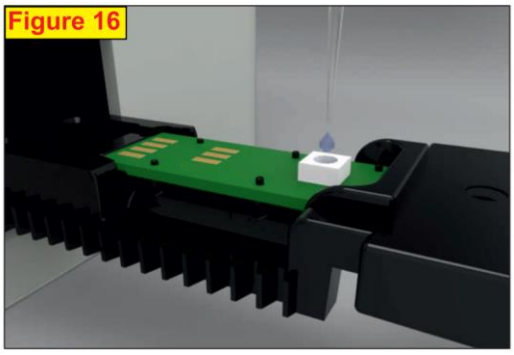
*Do not discard the pipette tip.

12. Transfer the elute from the mastermix tube to the white reaction well of the chip (Figure 16).

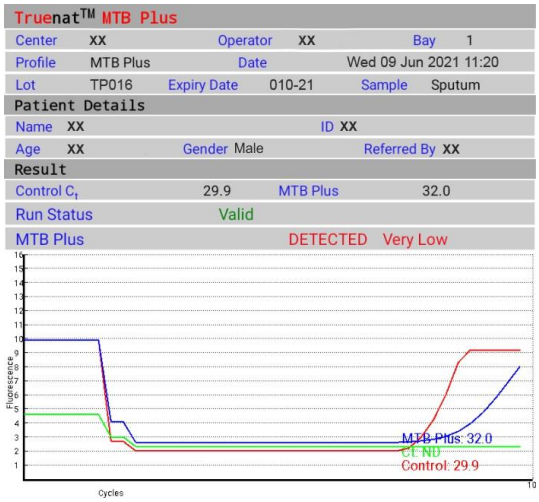
*Avoid spillage of the clear solution outside the white reaction well.

*__Discard__ the pipette tip and mastermix tube.

13. Click “YES” on the device screen to start the test. The PCR will be completed in **35 minutes**.



14. Tap the “Open/Close Tray” button to eject the chip tray and discard the used chip immediately after the reaction.
15. If MTB is detected (Figure 17) test the same elute for RIF resistance using the Truenat MTB RIF Dx chip as a follow-on test. The test takes about 55 minutes. Optional: Press “**Print**” to print result page using Truelab® microPCR printer.



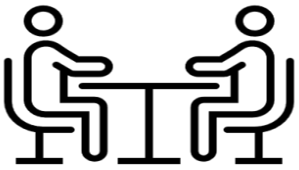
TRUENAT TEST PROCEDURES – Run a RIF Resistance Test

Process flow- Running a RIF-Resistance Test

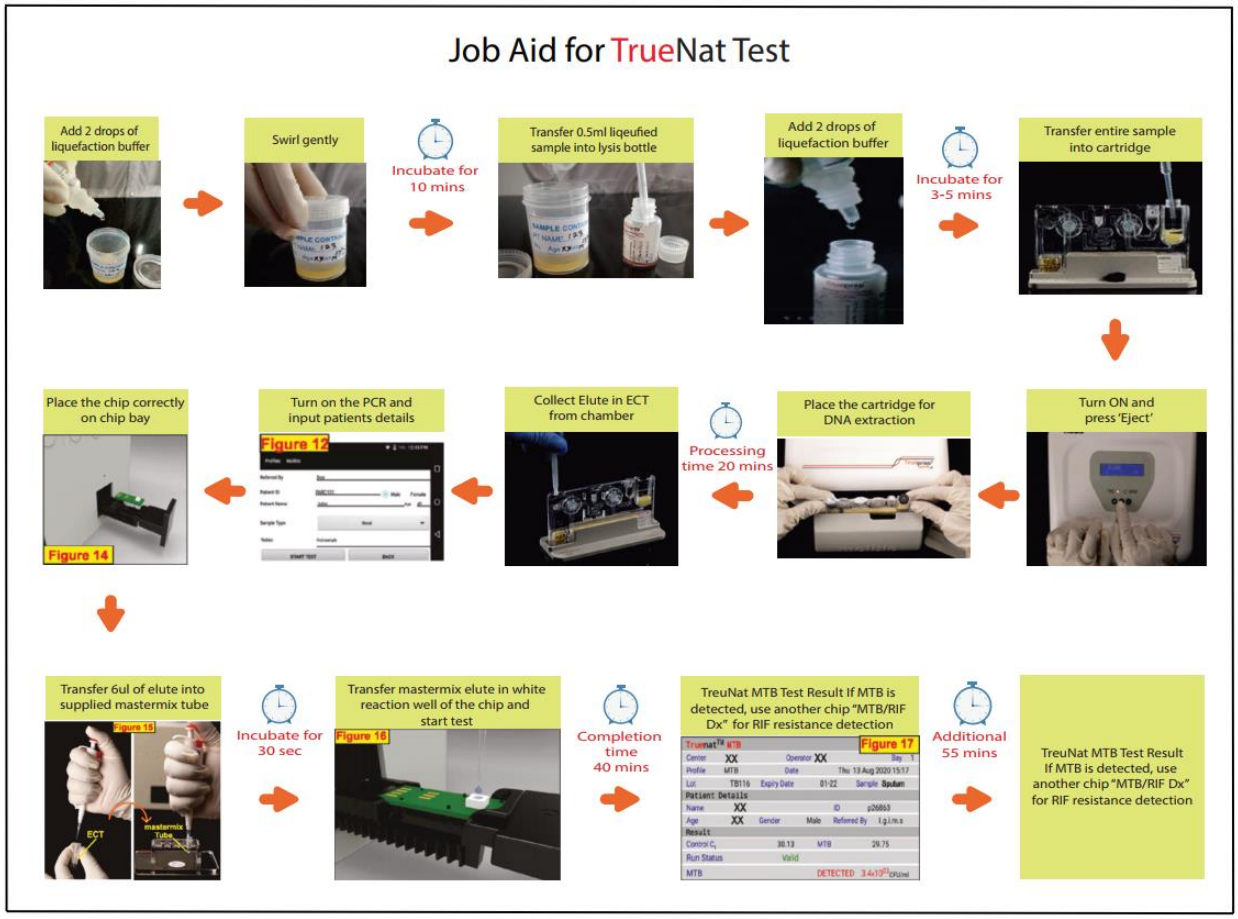
- If MTB is detected in a sample, you should run a RIF resistance test.
- A portion of the same DNA eluate can be used to test for RIF resistance using a Truenat MTB-RIF Dx chip.
- Start by returning to Step 3 in the PCR TB test process and repeat for RIF-resistance
    - Select “MTB RIF” as the test type in the Truelab micro PCR Analyzer.
- RIF-resistance testing takes an additional **60 minutes**

Activity: Let's Practice

- Practice on equipment
- Observe and assist



JOB AID (See Annex 7)



WASTE MANAGEMENT

- Truenat tests generate a significant amount of plastic waste
- Dispose or incinerate per national guidelines
- Decontaminate samples and consumables prior to disposal

Notes on Truenat Procedures – Waste Disposal

- The following items should be disinfected in freshly prepared 1% sodium hypochlorite solution and processed as plastic waste:
    - Transport media tubes
    - Lysis buffer tubes
    - Transfer pipettes (1ml and 3ml)
    - Cartridges
    - Microtubes
    - Elute transfer pipettes
    - Microchips
    - Gloves (even if contaminated) should also be disposed of as hazardous waste

- PPE made of fiber material or other materials except disposable plastic should be disposed of as infectious waste, including:
    - Face masks
    - Gowns
    - Caps

- Other items should be disposed of as general waste, including:
    - Cartridge pouches
    - Chip pouches
    - Transfer pipette wrappers
    - Desiccant pouches
    - Sleeves

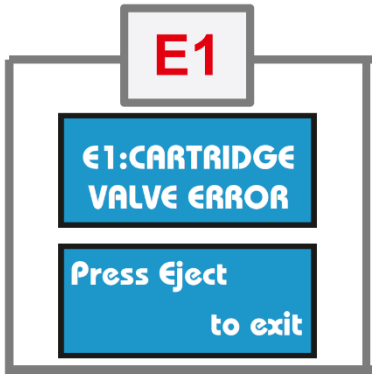
ERRORS AND TROUBLESHOOTING

- Truenat machines will prompt you in case of hardware malfunction or errors encountered when performing a test
- Truelab device automatically records data within the system whenever it encounters an error.
    - Users can generate a log file to send to Molbio to help resolve errors – instructions can be found in the user guide
    - If a test is in progress when the error occurs, you should create the log file before beginning the next test

Trueprep Error Messages

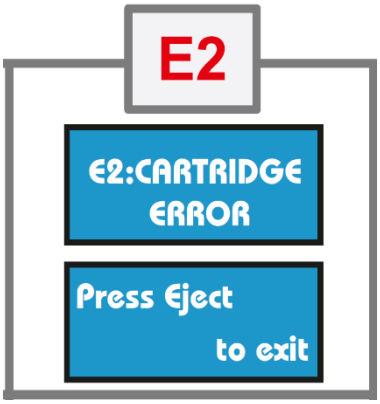
Error 1: Cartridge Valve Error

- Meaning: Cartridge valve is damaged
- Solution: Start over. Process remainder of sample in lysis buffer and load into new cartridge.



Error 2: Cartridge Error

- Meaning: Pressure drop error
- Solution: Start over. Process remainder of sample in lysis buffer and load into new cartridge.

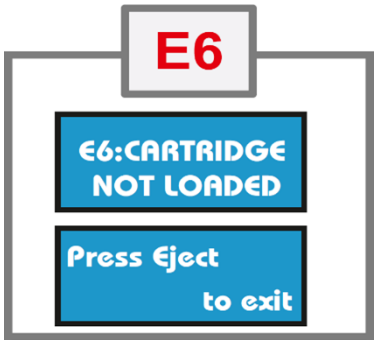


Error 3: Cartridge Clogged

- Meaning: Sample/specimen is too thick
- Solution: Ensure sample is liquefied and pipettable. Repeat extraction with new cartridge/request for new sample.

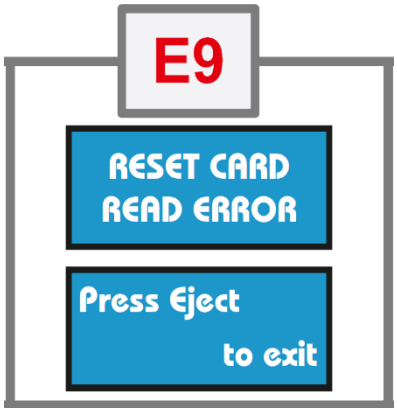
Error 6: Cartridge not Loaded

- Meaning: Cartridge not detected
- Solution: Ensure cartridge is loaded properly in correct orientation



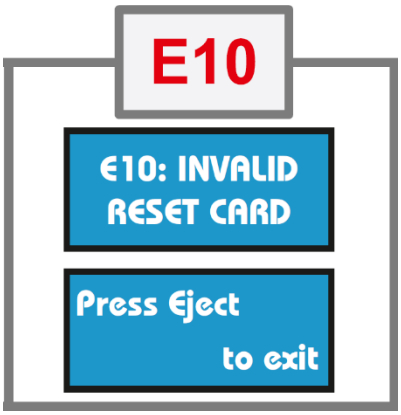
Error 9: Reset Card Error

- Meaning: Problem with the reset card or QR code reader
- Solution: Contact Molbio support



Error 10: Invalid Reset Card

- Meaning: Problem with the reset card or QR code reader
- Solution: Contact Molbio support

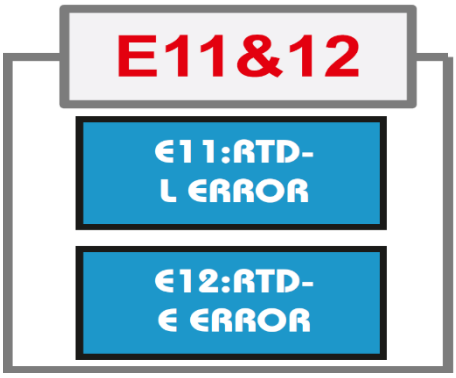


Error 11: RTD-L Error

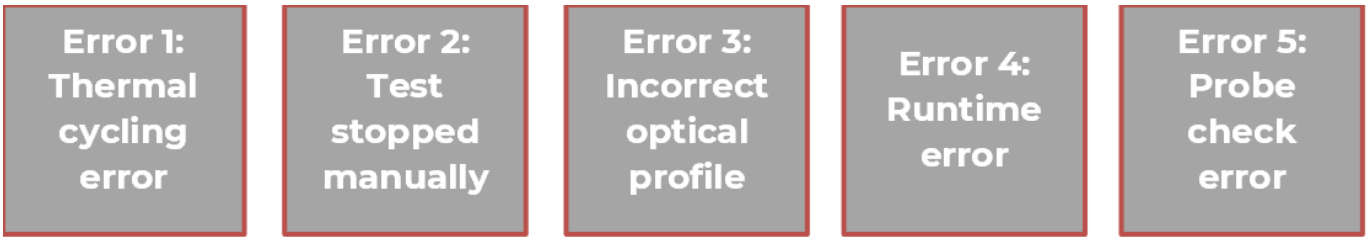
- Meaning: Device heater plates not working
- Solution: Contact Molbio support

Error 12: RTD-E Error

- Meaning: Device heater plates not working
- Solution: Contact Molbio support



Truelab Error Messages



Solution: Repeat the run using a fresh chip and reload the elute by pressing the repeat button. Follow user guidelines for proper loading of elute onto the white reaction well of the chip. Contact the Molbio support team if the problem persists.

Meaning: Internal control did not amplify in PCR or improper sample extraction.

Solution: Rerun the same elute using another chip. If you receive another Invalid result, process the sample again and run elute using another chip. Contact the Molbio support team if the problem persists.

Truelab Alert Messages

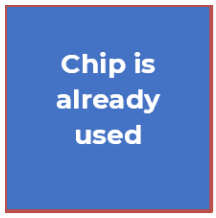
Meaning: Analyzer was unable to read chip memory.
Solution: Check if chip was loaded properly into the tray.
Remove the chip and re-select the profile from Status Screen and repeat the steps.
If message reappears, load a new chip and re-load the elute again

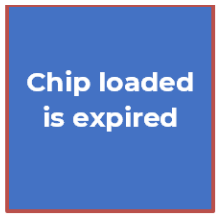
Meaning: The system was unable to establish an internal connection.

Solution: Attempt the test again by using a new chip and re-loading the elute again.

Meaning: User loaded a used chip or expired chip in the tray.

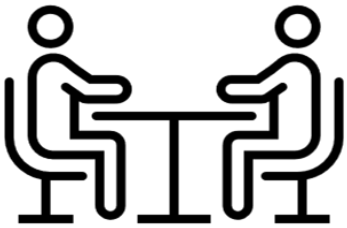
Solution: Use a fresh chip and re-load the elute.





Activity: Fix the Error

Read through each scenario and determine the best way to fix the error.



INFRASTRUCTURE REQUIREMENTS

Power

- Equipment is battery operated for up to 8 hours
- Electric or solar power required for re-charging.
- Electric power allows charging and testing at the same time
- Devices are able to operate within the 100-240 voltage range
- Electrical power may be needed to cool storage rooms in areas where temperature exceeds 30^{o} C
- Chips can be stored at up to 45^{o} C for up to 1 month and up to 40^{o} C for 6 months

Solar Power (Optional)

- 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

Room Layout

Trueprep and Truelab instruments should be installed on a flat, stable surface

Minimum surface dimensions of 1.2 m by 0.6 m

Install away from instruments that cause vibrations or electromagnetic interference

Install away from machines that generate or radiate heat and out of direct sunlight

Three well-grounded electrical outlets are recommended for operating or charging the instruments at once

Ambient Temperature

Equipment	Ambient Condition
Trueprep® AUTO v2 Device and Truelab Analyzers	Temperature: 15°C – 40°C
Chips	Storage temperature is up to 45°C for up to one month; 40°C up to six months; and up to 2 years when at 30°C
Reagent packs	Storage temperature: 2°C – 40°C for 2 years

Dust

- The Truelab® Real Time microPCR Analyzer does not require air intake to allow for the PCR process, so Truenat use will not be compromised in dusty settings.
- The manufacturer recommends installing the instruments in a dust-free environment when possible.

Biosafety

Truenat TB tests require the same biosafety precautions as microscopy, Xpert MTB/RIF

Take standard precautions in handling sputum samples

Security

Equipment should be kept in a secure, lockable facility

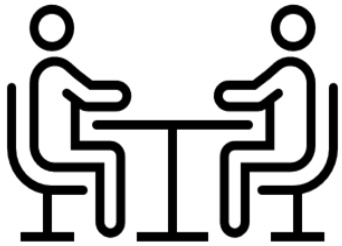
Equipment can be transported in the portable Truelab Real Time PCR Workstation Field case

Preventive Maintenance

Daily maintenance	Monthly maintenance	Monthly maintenance
• Clean work area • Discard used chips and cartridges	• Disinfect instrument surfaces • Clean Truelab bays • Temperature calibration • Verification of the fixed 6µl pipette	• Flush protocol for the Trueprep instrument • Spillage tray or linear motion guide tray replacement • Slider glass replacement - indicate bay

ACTIVITY –PREVENTIVE MAITENANCE

How would you carry out daily, monthly and as necessary preventive maintenance when performing tests with a Truelab platform?



WARRANTY

WARRANTY CONDITION

- To activate the warranty, the customer must fill and sign the installation report & warranty certificate and return the slip to Molbio Diagnostics Private Limited(?)
- Molbio Diagnostics Private Limited, guarantees that all its instruments are free from manufacturing defects or faults.
- Molbio undertakes repair or free of charge substitution/replacement of spare part which may be found to have manufacturing defects.
- Repair and interventions carried out during the period of the warranty do not extend or renew the period of warranty.
- The repairs of the instrument will be carried out onsite (except in case of major repairs where the instruments will have to be shipped to Molbio's head-office or Country Partner's location) by Molbio's authorized engineer/country partner representatives only.
- In the event Molbio is unable to repair the instruments onsite, it reserves the right to recall the instrument for repair at the head office/country partner locations if major/frequent problem has been observed in the instrument.

PREINSTALLATION REQUISITES

Check for the following parameters with respect to location of installation:

1. Workstation should be positioned on the workplace/table/workbench in an upright position on flat and dry surface.
2. Installation site should be away from direct sunlight or any radiating or heating apparatus.
3. Installation area should be free of devices which may cause vibrations or electromagnetic interference.
4. Installation site should be free from any atmosphere of potentially explosive liquids, vapors and gas.
5. Room temperature should be between 15ºC and 40ºC.

6. Relative humidity (RH) should be between 10% - 80% (non-condensing).
7. Power Supply minimum requirement is 100 to 240V/5Amps AC for all the devices as AC to DC adapters which are provided along with the device for charging an Inbuilt Battery can function.
8. Check for Earthing Voltage which should be less than 5V
9. Dimensions of our Devices are as Follows:

   Trueprep Autov2: 215 mm x 235 mm x 115 mm

   Truelab Quattro: 400 mm x 242 mm x 159 mm

   Truelab Duo: 240 mm x 242 mm x 159 mm

   Truelab UnoDX: 248 mm x 185 mm x 112 mm
10. Table space requirement should be after taking other accessories like MicroTube stand, Cartridge stand, Thermal Printer (small Size) and MicroPipette (6uL) Stand also into consideration. Minimum table dimensions:

    Truelab Duo Workstation with Accessories: 118 cm X 60 cm

    Truelab Quattro Workstation with Accessories: 148 cm x 60 cm

OTHER WARRANTY INFORMATION

Validity and duration:

- This warranty shall be considered valid only on the condition that this certificate is accompanied by the installation report.
- The warranty is valid for a period of 12 months from the date of successful installation or 14 months from the date of invoice whichever is earlier.

The following damage & faults are not covered under this warranty:

- Damage deriving &/or originating from an insufficient or inadequate electric circuit or from the area where the instrument is set up & used.
- Breakdowns caused by careless handling, imprudence, lack of expertise & in any case caused by lack of skill or any degree of negligence on the part of the operator.
- Damage, defects & faults deriving from unexpected events, accidents during transport by the purchaser, due to FORCE MAJEURE & in any case, due to situation which can in no way be attributed to manufacturing &/or material defects.
- Molbio shall accept no responsibility whatsoever for damage either directly or indirectly to persons or materials from the use of the instrument.

SUMMARY

- Truenat is a chip-based real-time polymerase chain reaction (PCR) test that involves four steps:
    - A liquefied lysed sputum specimen
    - Extracting DNA from the sample
    - Amplifying the extracted DNA
    - Testing the amplified DNA
- Three pieces of equipment are used for Truenat:
    - Trueprep
    - Truelab (Uno, Duo, or Quattro)
    - Optional microPCR printer
- Procedures for operating Truenat are summarized in an easy-to-follow job aid
- Truenat equipment requires minimal infrastructure and minimal preventive maintenance

Knowledge Check

1. There are ____ packets that include the reagents and consumables needed to run Truenat. What are they?
2. What are the three versions of the Truelab Real Time micro–PCR Analyzer? What is the difference between them?
3. What are the monthly maintenance requirements for Truelab and Trueprep equipment?

MODULE 4: ORDER PLANNING AND QUALITY ASSURANCE

Introduction

This module provides details on how to forecast and plan supply orders and how to develop and follow quality assurance procedures at test sites.

Learning Objectives

By the end of this module, participants should be able to:

- Explain how to forecast for Truenat supplies
- List the key elements of good stock management
- Identify some quality assurance procedures for Truenat testing

Course Outline

1. **Forecasting and Quantification**
2. Quality Assurance
3. Monitoring Quality
4. **Summary**

Learning contents

FORECASTING AND QUANTIFICATION

Reminder: There are three packets that include the reagents and consumables needed to run Truenat

Reagents and Consumables

- Trueprep® AUTO MTB Sample Pre-treatment Pack
- Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Kit (for 25 or 50 tests)
- Truenat Chip Pack (MTB, MTB Plus, or MTB-RIF Dx)

Ordering Reagents and Consumables

- What to order?
- From where?
- How much?
- How often?
- How to assess the correctness of an order?
- What is the lead time needed?
- What is the reserve (buffer) stock needed?
- Who is responsible for placing orders?

Storage Conditions and Shelf-Life of Consumables

Equipment	Recommended Storage Condition	Shelf Life
Chips	Storage temperature: 2°C – 30°C	2 years shelf under recommended storage conditions Up to 6 months at a temperature under 40°C, if conditions do not allow for storage under 30°C, and up to 1 month at temperatures up to 45°C
Reagent packs (Sample Pre-treatment Pack and Prep Kit)	Storage temperature: 2°C – 30°C	2 years under recommended storage conditions Please note that 2 years shelf is from the date of manufacture. The shelf life will be less upon arrival in the country and upon distribution to the sites.

Quantities for an Initial Order of Reagents

Average number of tests per day	Needed Instruments		
		1 Truelab Analyzer Duo + 1 Trueprep AUTO v2 Device	
2			
4			
6			
8		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	
24			
32			

Order Quantities

The number of MTB-RIF Dx kits to order will depend on the anticipated proportion of people tested that will be MTB positive, and therefore in need of a test for RIF resistance.

Note: In addition, by default, Molbio provides 20 free MTB-RIF Dx tests for every 100 MTB or MTB Plus tests bought through GDF.

Data Needed for Regular Forecasting

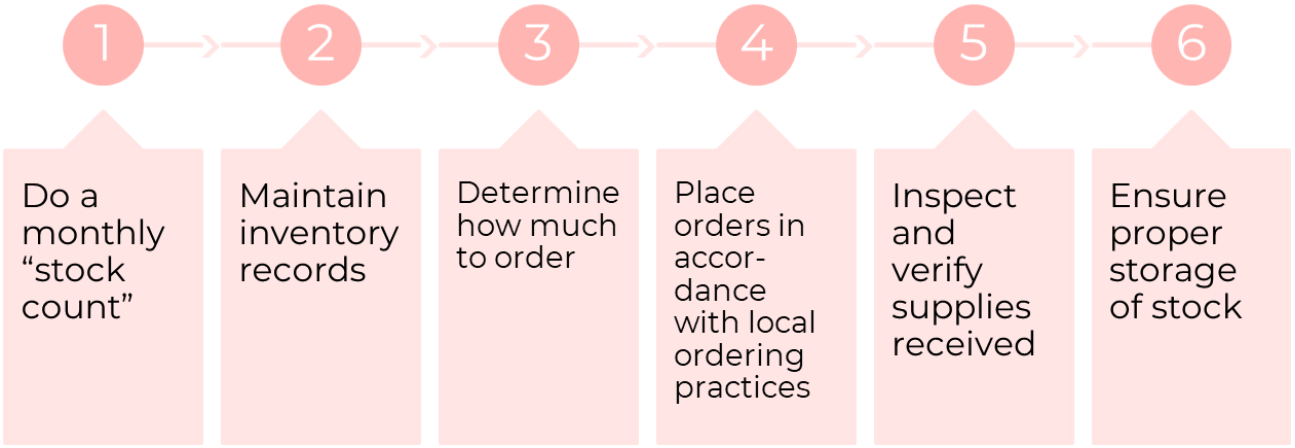
A: Average number of tests performed for the order period	B: Quantity of items needed per test	C: Quantity of an item required for one month	D: Buffer/ reserve stock
E: Stock currently available	F: Amount of stock currently needed by the lab, plus buffer	G: Unit in which stock can be ordered	H: Amount to be requested (considering pack size)

Activity: Regular Forecasting

Quarterly Supply Requirements for Truenat Testing							
Laboratory name: XX							
Bangladesh	Supply Quarter:						
District: XX	**Year:** 20__						
Total tests performed in previous quarter, including failed tests (A):							
Items	**Quantity Needed per Site (B)**	**Stock for one month © = (A/3) * B**	**Stock for quarter with 1 month buffer (D)= C*4**	**Stock on hand (E)**	**Calculated request (F) = D-E**	**Order unit (G)**	**Actual order (H) = F/G and round up**
Trueprep® AUTO MTB Sample Pre-treatment Pack (20 tests per kit)							
Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Kit (20tests)							
Truenat Chip Pack							
Truenat MTB Plus Chip Pack							
Truenat MTB-RIF-Dx Chip Pack							

STOCK MANAGEMENT

6 key components to maintain adequate stock of Truenat supplies



Stock Log

Temperature and Shelf Life

- Recommended storage conditions for the Truenat TB chips: 2°C–30°C
- Shelf-life of reagents under recommended storage conditions: 2 years (at date of manufacture)
  - GDF-negotiated minimum shelf life at time of readiness for delivery is 19 months

Storage and Expiry

- Organize existing and new shipments by the expiry date

QUALITY ASSURANCE (QA)

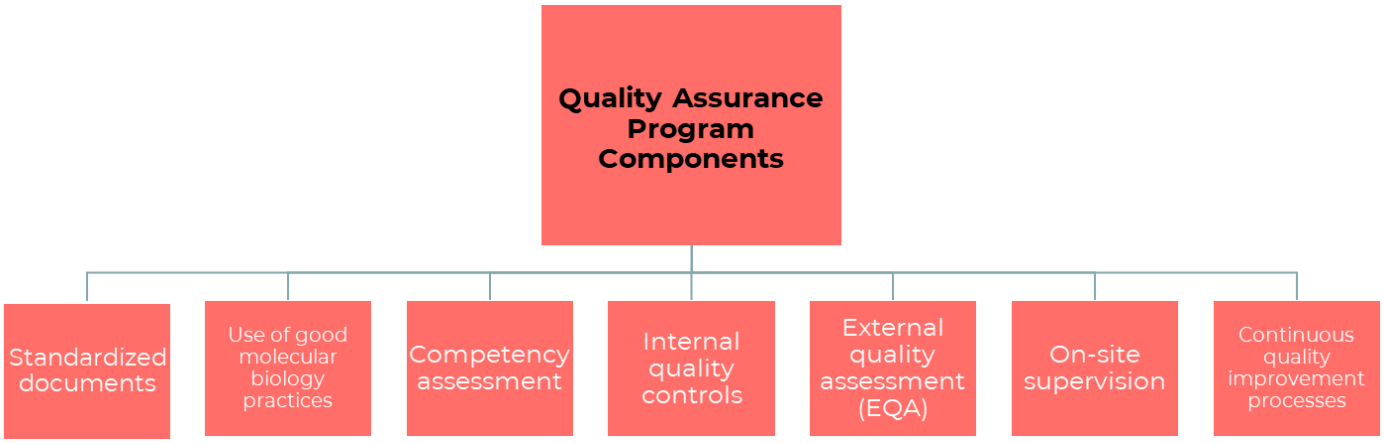
QA and Control

Roles in Ensuring Quality

- Laboratory staff is responsible for overseeing QA activities

Quality Assurance Program

A comprehensive discussion of the essential elements of a quality assurance system for any rapid TB diagnostic test may be found in the GLI



Good Molecular Biology Practices

- The Truenat TB test procedures require multiple hands-on steps as well as precision micro-pipetting.
- Laboratory technicians should be trained on good molecular biology practices before operating Truenat.
- 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.
- At least 10–15 specimens per week should be tested to maintain proficiency of staff conducting the Truenat TB tests.

Competency Assessment

- Competency assessments of laboratory technicians should be performed after training and periodically (annually).
- The positive and negative controls in the Truenat Positive Control Kit Panel-1 can be used for competency testing during hands-on training

Standardized Documents (SOPs)

- SOPs should be provided as reference materials for technicians
- Printed Job aids would be distributed to Truenat sites. (See annex)

Internal Quality Controls (IQC)

- Internal quality controls are designed to detect, prevent, and minimize erroneous results in laboratories' internal processes from pre-analytical, analytical and post-analytical phases.
- Truenat TB assays incorporate an internal positive control that undergoes the same processes as the specimen; from extraction to amplification, thereby assessing the validity of the test run from sample to result.
- The positive and negative controls in the Truenat control kit panel 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.

MTB External Quality Assessment (EQA)

- Truenat MTB/RIF assays being introduced at the peripheral level for the first time. IDDS has contracted with SmartSpot to provide EQA for Truenat sites (FY22 only).
- EQA provides valuable information regarding success of Truenat implementation:
  - Assess competency and performance of the laboratory and compare to peers.
  - Provides ongoing verification of performance of the test system
  - Data can be used to identify labs in need of additional support & direct technical assistance.
  - Helps ensure quality of lab results.
  - Inform NTP on performance of Truenat in the field and help direct resources.
  - Identify problems in the pre-analytical, analytical and post-analytical phases of testing.
  - Essential component of the Quality Management System (QMS)

SmartSpot MTB External Quality Assessment (EQA)

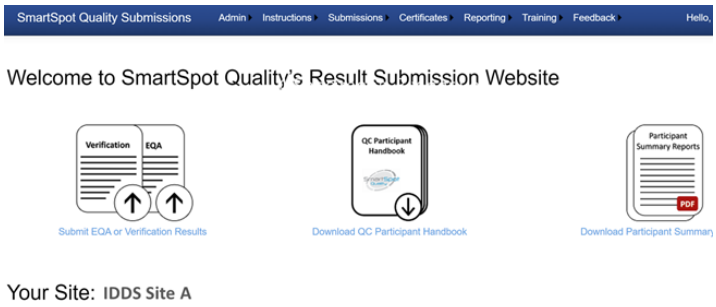
- MTB EQA Panel consists of 4 samples with QR codes.
- 4 Dry Culture Spots (DCS) sample per panel consisting of a combination of MTB and non-tuberculosis mycobacteria (NTM) and/or MTB negative material
- Panels DCS are inactivated (non-infectious), quantified and intact organisms.
- There are 3 cycles of EQA (each cycle with 4 samples) for 2022.
- Cycle 2&3 will dispatch in one shipment (18 July 2022).
- DCS panels can be stored at ambient temperatures

EQA Reporting

- Results are due within 30 days of the cycle start date.
- Report EQA results via SmartSpot Quality Monitor (SSQM) web-portal.
- All sites, NTP and IDDS will have access to the SSQM web-based dashboard to view performance.
- Log in to www.SSQmonitor.com

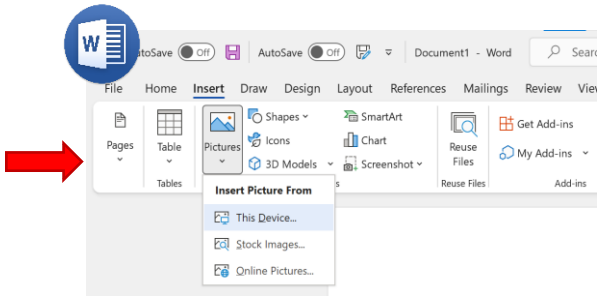
EQA Reporting: How to Export Results

- Capture an image of each result OR transfer the results of all four EQA specimens to a PC via Bluetooth or email
- Open a Microsoft Word Document and select INSERT PICTURE FROM THIS DEVICE
- Insert the 4 EQA result images

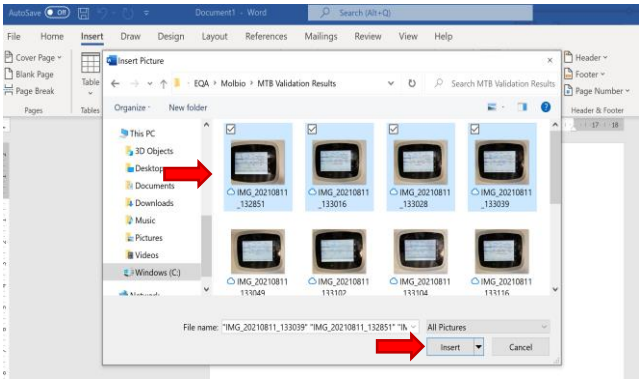


Insert image

1. Capture an image of each result OR transfer the results of all four EQA specimens to a PC via Bluetooth or email
2. Open a Microsoft Word Document and select *INSERT PICTURE FROM THIS DEVICE*

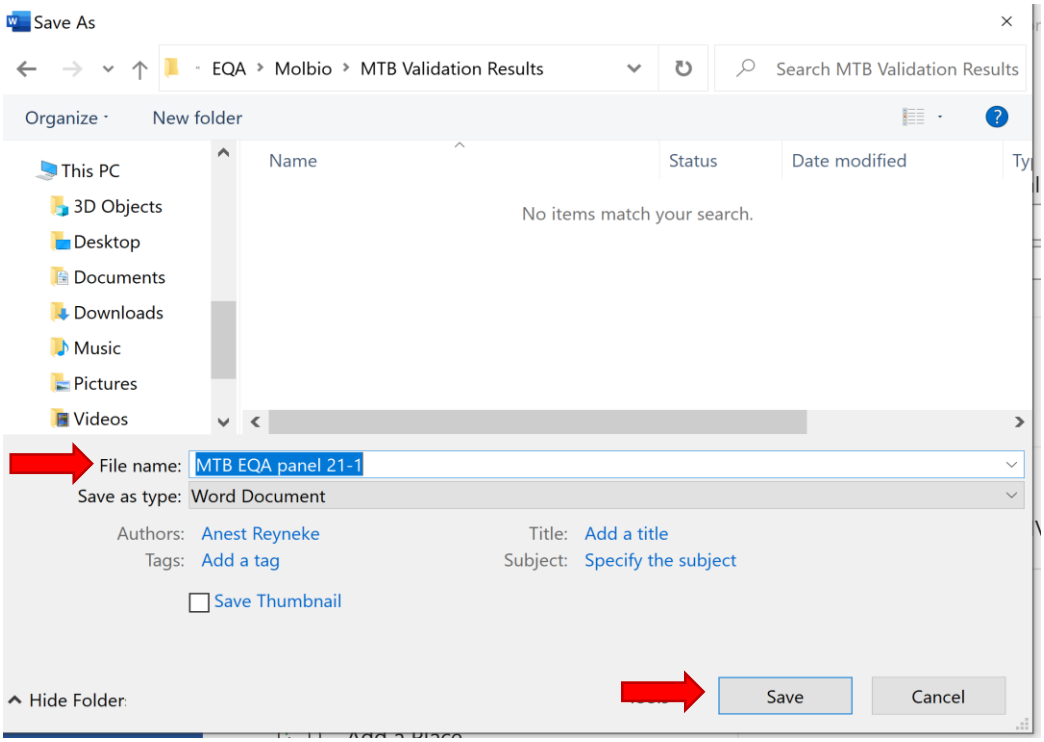


3. Insert the 4 EQA result images



Save the Word Document.

Change the file name to the EQA type and EQA panel number and Click SAVE



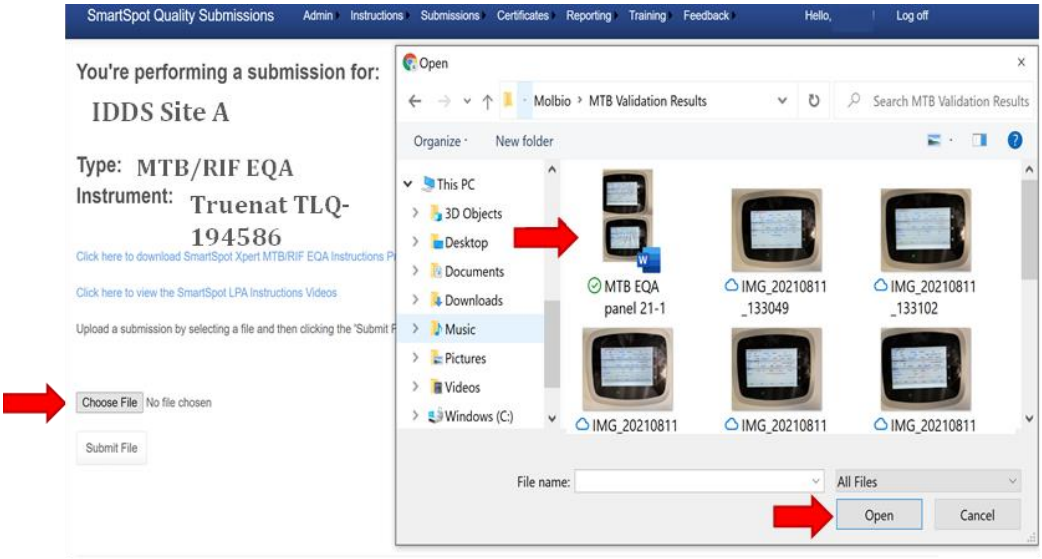
Go to SUBMISSIONS > PERFORM NEW SUBMISSION

Upload the saved Word document containing all 4 of the EQA results

1. Select CHOOSE FILE

2. Select the saved Word document

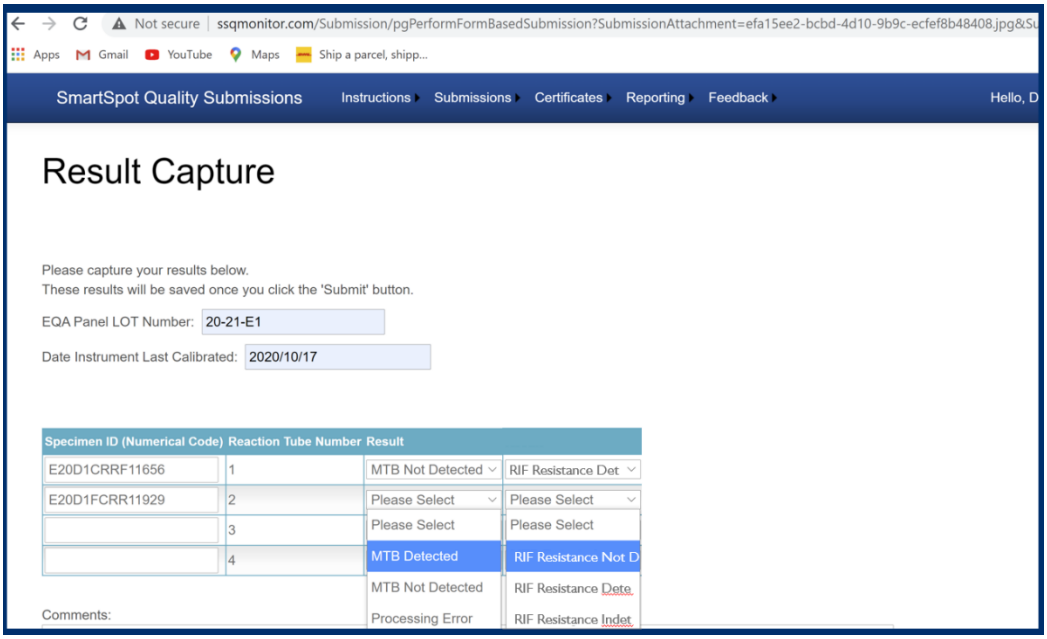
3. Click OPEN



Capture final result interpretation for each sample

1. Fill in the EQA Specimen IDs

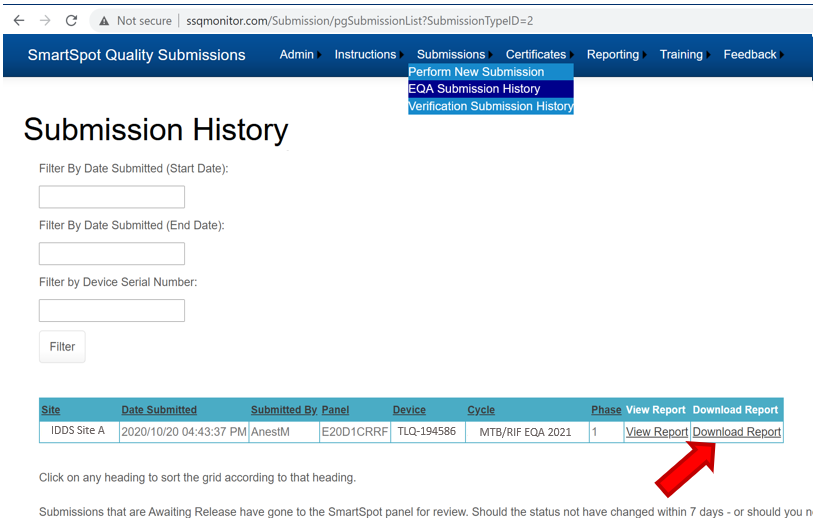
2. Select the final result from the drop-down menus



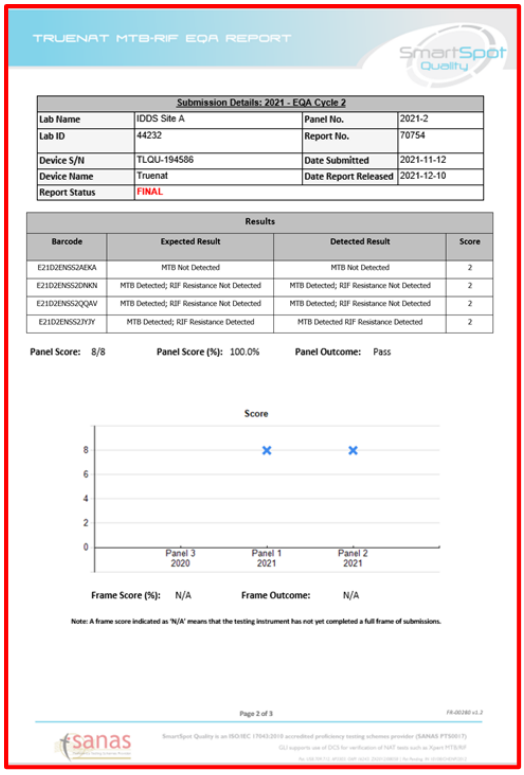
EQA Final Report

A final EQA performance report will be available for download for each Truenat site.

Summary report available for NTP and IDDS



Go to SUBMISSIONS > EQA SUBMISSION HISTORY to view your EQA report



Things to be ensured to run the Truenat sites

1. Correct placement of the equipment
2. Ensure the security of the equipment
3. Ensure uninterrupted power supply

4. Ensure optimum environment and safety
5. Ensure timely supply of the logistics
6. Ensure proper bio risk management
7. Finally, staff is properly trained.

Initial Calibration / Verification Panels

Positive and Negative Controls

Controls can be purchased as part of the Truenat™ Positive Control Kit- Panel I

New Lot Testing (Lot-to-Lot Verification)

- Run positive and negative controls whenever:
- A new shipment of Truenat TB test kits is received
- To assess reagents if temperature of storage areas falls outside recommended ranges

Maintaining QC Records of Lot Testing

- Review by testing site manager
- Retention onsite for a period according to local or national policy

Regular Maintenance

1. Perform negative control tests monthly

2. Record preventative maintenance in a log

MONITORING QUALITY

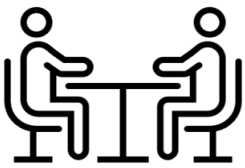
Performance Indicators for Truenat TB Tests

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%
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 pattens
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 pattens
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%

Indicator	Description	Target
Truenat MTB-RIF Dx		
Number and proportion of specimens with RIF resistance not detected	**Number of specimens with RIF 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 RIF resistance detected	**Number of specimens with RIF 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 RIF resistance indeterminate	**Number of specimens with RIF resistance indeterminate / Total number of specimens tested for RIF 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 for RIF resistance. 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

Class Activity



- Group discussion: Forecasting, QA, Targets
- 10 minutes to work through
- Present your discussions and answers

SUMMARY

Forecasting and Quantification

- It is important to monitor inventory, ensure supplies have not expired and forecasting future needs.

Quality Assurance

- There are procedures and programs that allow for oversight and coordination of QA activities.

Monitoring Quality

- Performance indicators help track and monitor each testing site to ensure quality tasks are carried out appropriately.

Knowledge Check

1. What information is needed to determine how many supply packs to order each quarter?
2. What are some practices to ensure good stock management?
3. What are the main components of a quality assurance program?

MODULE 5: BIOSAFETY AND SPECIMEN COLLECTION AND REFERRAL

Introduction

This module introduces procedures for specimen collection and referral. Orientation on bio-safety will be covered in this module.

Course Outline

- Specimen Collection
- Specimen Storage and packaging
- Biosafety
- Specimen Referral
- Summary

Learning Objectives

By the end of this module, participants should be able to:

- List the equipment needed to collect a quality sputum specimen
- List the steps for collecting a quality sputum specimen
- List the steps for packaging sputum specimens
- Describe storage requirements for collected specimens
- Maintain appropriate bio-safety measures in laboratory
- Understand the process for specimen referral

Learning Contents

SPECIMEN COLLECTION

SPUTUM SPECIMEN COLLECTION: CONTAINER SPECIFICATIONS

- 30-50 ml capacity
- Translucent or clear material
- Sides and walls that allow easy labelling
- Single-use combustible material
- Leak-proof with a screwcap
- Wide mouth

Sputum Cup

Falcon Tube

INFORMATION TO BE GIVEN TO PATIENTS BEFORE SPUTUM COLLECTION

- The importance of good quality sputum sample
- Morning Sample is recommended
- How to open and close sputum cup
- Difference between sputum and saliva
- How to produce good sputum from inside the lung
- To rinse mouth with plenty of water to remove any food debris or betel leaf (paan) residual

- Importance of sputum collection in airy and daylight area
- Importance of sending sputum sample to laboratory as soon as possible

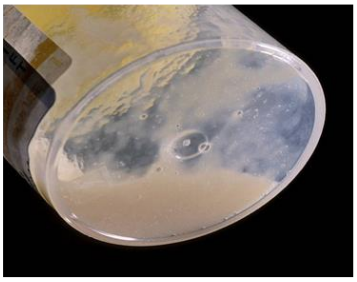
SPUTUM SPECIMEN COLLECTION: SAFETY

- When providing a sputum specimen, a patient may produce infectious aerosols and therefore biosafety precautions are needed:
    - Instruct the patient to cover his or her mouth when coughing
    - Never collect sputum in the laboratory
    - Collect sputum away from other people in a well-ventilated space following the NTP's guidelines
    - Do not stand in front of the patient during specimen collection!

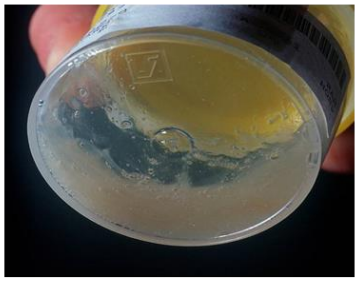
COLLECTING A GOOD QUALITY SPECIMEN

- Obtaining an adequate quantity of good quality sputum is critical to ensure accurate test results
- Induced or expectorated sputum specimens may be used
- Spot and morning sputum samples can be collected from each patient
- Proposed algorithm describes the collection of at least one initial specimen to be used for Truenat testing and the collection of additional specimens as needed
- For best results, obtain >1ml of purulent/mucoid sputum (see below)

Purulent



Mucoid



Blood-stained

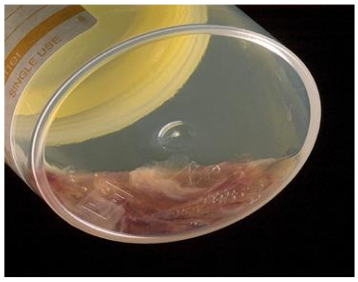
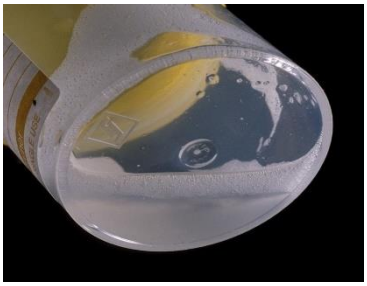


Photo credit A. van Deun

SPUTUM SPECIMEN COLLECTION: POOR QUALITY

- Poor quality specimens will give poor quality results



Salivary (thin, watery, or comprised mainly of bubbles)

SPUTUM SPECIMEN COLLECTION: LABELLING

- Label the sputum cup with the patient's identification number
- Label the outer sides of the container with permanent ink
- Never label the lid
- Complete the Laboratory Request Form according to NTP guidelines for the country
- Once specimens are collected and labelled, sample is ready for Truenat testing.

SPECIMEN STORAGE AND PACKAGING

Packaging and Storing the Specimen

- Specimens should be tested in the same day. If it is not possible then it has to be preserved in a refrigerator or a cool box between 2°C to 8°C for a maximum of 5 days.
- Specimens should be well packed in a specimen flask or transportation box
- During transportation, specimens should be kept between 2°C to 8°C.

Once specimens reach Truenat sites, specimens need to be brought to room temperature before processing with Trueprep® AUTO MTB sample pre-treatment pack.

List of materials needed for sputum sample transportation

1. Sputum cup (20-30 ml capacity)
2. Kit (includes following :)
    - Transport box
    - Zip lock poly bag (small)
    - Zip lock poly bag (medium)
    - Zip lock poly bag (large)
    - Biohazard sticker
    - Address sticker
    - Scotch tap
    - Tissue paper roll
3. Marker pen
4. Laboratory Request Form & Shipment form

Steps of labeling and packing samples before transportation

Step 1: Label sample ID number/patient number to the falcon tube

Step 2: Transfer Sputum from Sputum cup to falcon tube

Step 3: Tightening the cap and insert the falcon tube into small poly bag

Step 4: Insert the tube to the Zip lock poly bag and close properly

Step 5: Take the transport box and put a biohazard sticker

Step 6: Put and address sticker to the opposite side of the box

Step 7 Insert the tube inside the transport box

Step 8 Insert the tissue paper into the empty places (if any) inside the box

Step 9: Close the box carefully

Step 10: Complete box

Step 11: Insert the complete box inside the large poly bag

Step 12 Insert the request form inside the large poly bag

Step 13: Ready for shipment

Step 14: Transport to nearest LPA lab/ culture lab

Step 1:Label sample ID number to the falcon tube

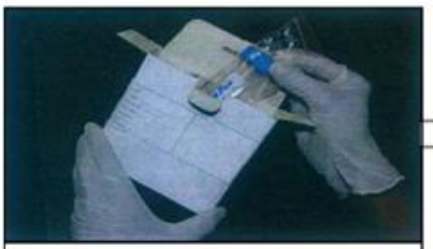
Step 2: Transfer Sputum from Sputum cup to falcon tube

Step3: Tightening the cap and insert the falcon tube into small poly bag

Step 4: Insert the tube to the Zip lock poly bag and close properly

Step 5:Take the transport box and put a biohazard sticker

Step 6: Put an address sticker to the opposite site of the box



Step 7: Insert the tube inside the transport box

Step 8: Insert the tissue paper into the empty places (if any) inside the box

Step 9: Close the box carefully

Step 10: Complete box

Step 11: Insert the complete box inside the large poly bag

Step 12: Ready for shipment

Responsibilities MT Lab during transportation of samples

- Ensure that enough quantity of specimen should be collected.
- The request form has been properly filled in.
- The sample is matching with the request form.
- The cap of falcon tube is tightly screwed.
- The sample kept in the transport box after with Liquid absorbent paper/ tissue box.
- Address sticker and bio-hazard sticker are properly tagged on the transport box.
- Always mark " Home delivery" on courier delivery challan.

Class Activity

- Group work
- Place the index cards in order based on the steps in the specimen collection process.
- Facilitator will observe and assist

BIO-SAFETY

Safe working practice

- Reduce the risk of infection to you, co-workers, and the community
- Protect the patient from incorrect results

Laboratory symbols

Warning

Failure to follow these instructions may harm your health or cause immediate damage to equipment.

Warning

Failure to follow these instructions may affect test results, or cause equipment damage over time

Do not do this

Wash your hands

Wear a laboratory gown for this procedure

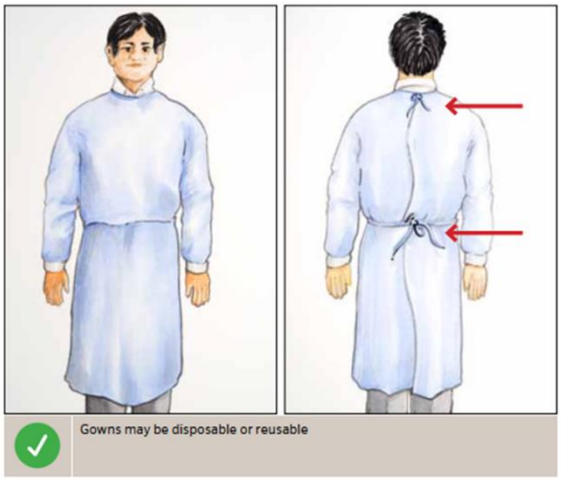
Wear gloves for this procedure

This substance is flammable

This substance is toxic

Gowns & Coats

- Laboratory gowns fasten at the back, provide protection across the front.
- Gowns should be changed weekly or after an obvious spill occurs.
- Gowns must be available in appropriate size.

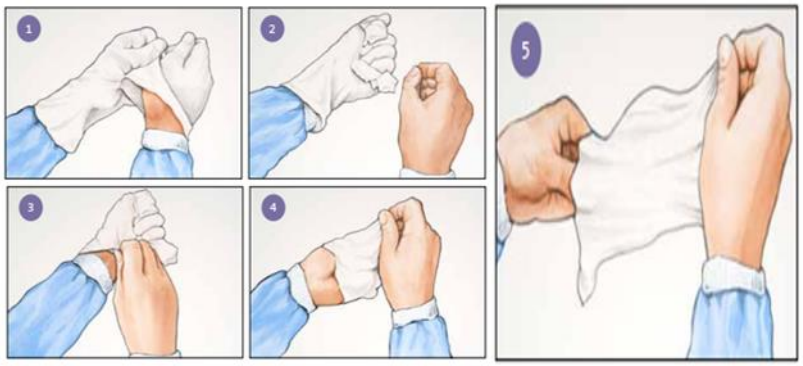


Wearing gloves

- Different sizes of gloves must be available (small, medium, large).
- Too small and they are easy to tear
- Too large and fine motor skills are lost

Gloves removing technique

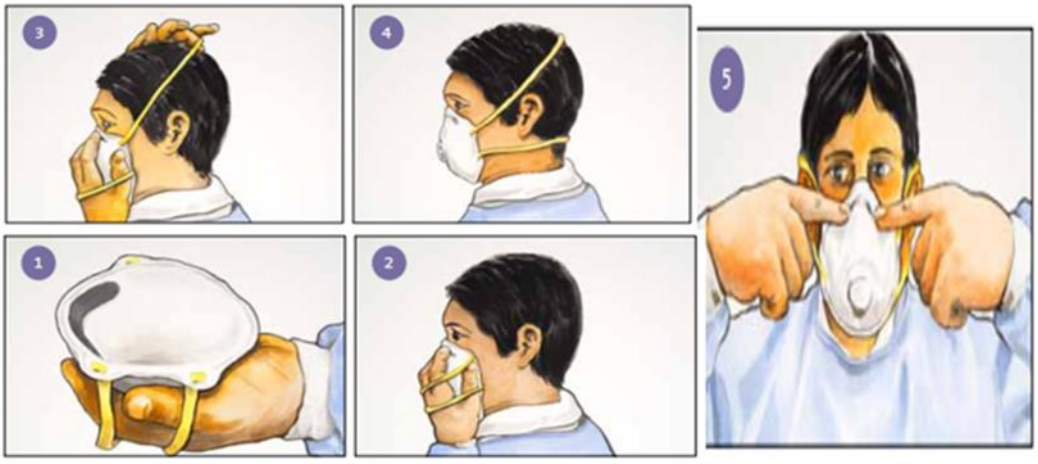
- Hold the cuff of the gloves on the other hand and slowly peel the glove from the hand.
- Gather the used glove into the palm of the other hand and close fingers.
- Carefully slip ungloved fingers under the cuff of the gloved hand; be careful no to touch the outer surface of the glove
- Peel the glove off such that the held glove is not inside of the glove being removed
- Dispose of the gloves into an infectious waste bin.



Once gloves are removed, wash your hands immediately.

How to wear a respirator correctly

- Hold the respirator in the palm of your hands, straps underneath and aligned appropriately.
- Position the respirator under the chin, place the respirator gently over the face
- Place the upper headband over the head, locate it higher at the back of the head and over the ears
- Pull the lower headband over the head and locate under the ears.
- Place the finder trips at both hands on the metal noise piece and mould the metal strap to the nose. Exhale and inhale to confirm the pressure and detect possible leakage.



Donning

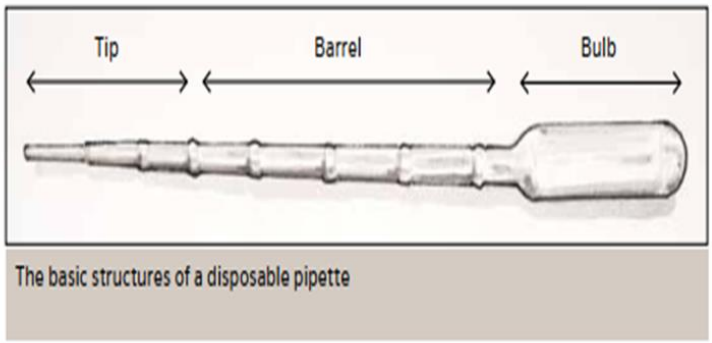
https://www.youtube.com/watch?v=H4jQUBAlBrI&ab_channel=CentersforDiseaseControlandPrevention%28CDC%29

Doffing

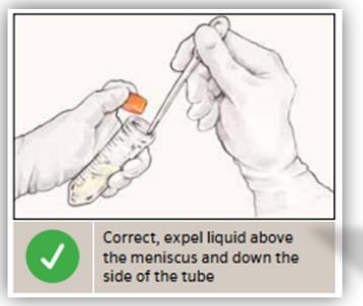
https://www.youtube.com/watch?v=PQxOc13DxvQ&ab_channel=CentersforDiseaseControlandPrevention%28CDC%29

Pipetting technique

- Slowly expel liquid from the pipette
- Direct it down the inside wall of the container
- Ensure the pipette tip is above the meniscus
- Never pour a solution directly onto another
- These principles apply to all pipette types.

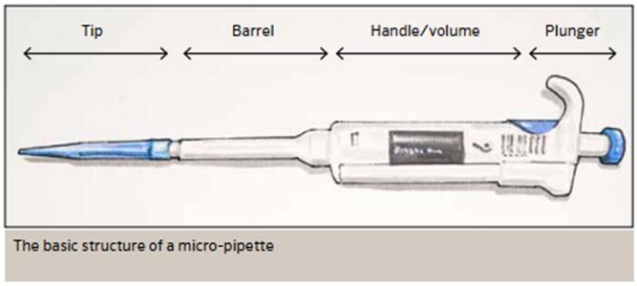


The basic structures of a disposable pipette

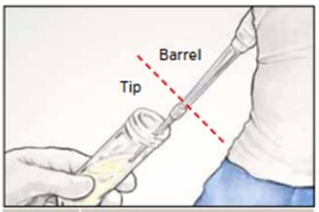


Using micro-pipette

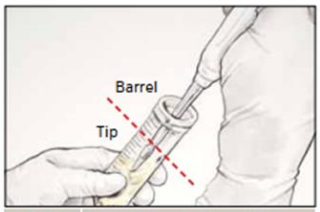
- Always ensure that the tip is placed inside a container and above the meniscus, before slowly releasing the contents. Never insert the barrel into the container



The basic structure of a micro-pipette



Insert the tip only

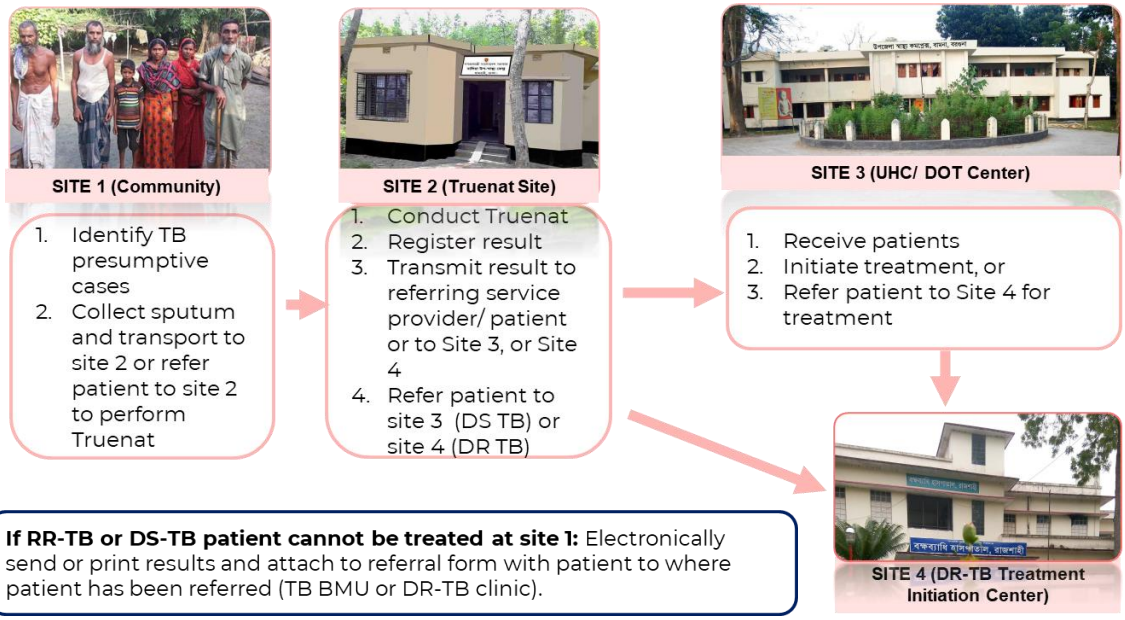


Never insert the barrel into the tube

Commonly used disinfectants

- Sodium hypochlorite should be used at a concentration of 10% in water.
- Phenolic compound used for decontaminating equipment and single –use items prior to disposal
- Alcohol do not leave residues on treated items

SPECIMEN REFERRAL



Questions to Consider about Sample Flow

- How are patients referred for TB screening and testing?
- Are patients referred from the community to the facility for TB Testing?
- Are specimens collected at the community level and transported to the facility for testing or are they testing at the community level and TB patients referred to the facility for TB/DR-TB Rx initiation?
- How are diagnosed patients referred for TB-DR-TB clinical monitoring?

Summary

Specimen Collection

- Procedures for completing lab request forms, sputum collection, packaging, storage, and transportation is by collecting good quality specimens, using the algorithm to describe the collection of specimen and results reporting.

Specimen Referral

- When a specimen is not collected on site, you must store at the collection site following the appropriate temperature and transportation process.

Biosafety

- Safe working practice can reduce the risk of infection to you, co-workers, and the community.
- Gowns should be changed weekly or after an obvious spill occurs
- Appropriate sizes of gloves must be available
- Slowly expel liquid from the pipette
- Sodium hypochlorite should be used at a concentration of 10% in water.

Knowledge Check

1. What equipment is needed for specimen collection?
2. At what temperature / where should specimens be stored before being pre-treated?
3. At what temperature should specimens be stored after being pre-treated?
4. A sputum specimen is collected at a primary health care facility and tested using Truenat for MTBC and RIF-resistance. The specimen is revealed to be positive for both.
    - Where should the specimen be referred next?
    - Where should results be sent?
    - Where should the patient be referred?

MODULE 6: RECORDING AND REPORTING

Introduction

This module introduces different templates will be used for recording & reporting at Truenat sites.

Course Outline

- Learning Objective
- Recording Template
- Reporting Template

Learning Objectives

By the end of this module, participants should be able to:

- Know the about the different templates
- Learn how to fill up the templates

Learning Contents

Recording templates

- Request forms- TB 05, DR TB- 06 (See Annex 1 & 2)
- Register (See Annex-3)

Reporting templates

- Monthly reporting forms- DR 10A (See Annex-4)
- Stock register (See Annex 6)
- Maintenance & service log (See Annex 8)

SUMMARY

- TB 05/06 form is used for sending patient to UHC & treatment initiation center

Knowledge Check

1. What is name of form for refer MTB patient for the treatment?
2. What is the name of the form for referring DRTB patient to treatment initiation center?
3. What is the name of the form for referring DRTB patient to treatment initiation center?

MODULE 7: MONITORING AND EVALUATION

Introduction

Monitoring and evaluating Truenat's implementation and the impact of Truenat on TB-related targets and goals is critical.

This module will provide participants with an overview of how to establish an M&E plan for Truenat by detailing indicators that can be incorporated into national systems and plans.

Course Outline

- M&E for Truenat
- Indicators

Learning Objectives

By the end of this module, M&E and program staffs should be able to:

- Outline a general approach to monitoring and evaluating the impact of Truenat on implementation plan wise targets.

Learning Contents

M&E for Truenat

- Integration of Truenat should help a country meet its existing targets for case detection, bacteriological confirmations, drug resistance testing, etc.
- If adding Truenat does not help to achieve these targets, then either the targets need to be revised or a different solution is needed.

Quality Assurance Versus Monitoring & Evaluation (QA vs. M&E)

- The QA indicators described in the previous module should be used to monitor performance of the instruments.
- Impact indicators described in this module should be used to monitor and evaluate progress towards achieving broader goals of the health system related to TB.
- Both sets of indicators should be considered when developing a recording and reporting system and plans for reviewing data.

INDICATORS

Impact 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)	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 RIF (Indicator 7)	100% (2020)
Percentage of notified RIF-resistant TB cases with DST results for fluroquinolones and second-line injectable agents (Indicator 8)	100% (2020)

Other Possible Impact Indicators

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

9. Number and proportion of presumptive TB patients for whom test results are reported to the clinician and that are notified of the result
10. 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

Monitoring Outcomes and Impact

Diagnostics Connectivity

- Software can rapidly and automatically calculate many of the key performance indicators and facilitate the M&E process.
- Third-party connectivity software platforms (e.g., System One's "Aspect" and Savics's "DataToCare") allow for smooth flow of data..
- Digital results reporting can be used to send data to national servers for M&E and surveillance purposes.

Key Indicators

- Key indicators and milestones to monitor the implementation process of Truenat:
- Laboratory Performance Indicators
- Performance Indicators for Truenat TB Tests

M&E Forms for Truenat

- Template for Truenat site assessment
- Template for Supportive Supervision (See Annex- 9)

SUMMARY

Monitoring and Evaluating the Impact of Truenat

- Progress toward achieving WHO indicators for laboratory strengthening should be measured to assess the impact of Truenat.
- Other indicators may also be selected by national programs to supplement WHO indicators.

Annex 1: DRTB 06 request form

Government of the People's Republic of Bangladesh
National TB Control Programme
Programmatic Management of Drug Resistant Tuberculosis (PMDT)
Request and Reporting form for Diagnosis/Follow up of Drug Resistant TB

DR TB 06

A. Patient Identification (ID) :

TB registration No. (Current) : _____ Previous TB registration No. (if any) : _____ DR TB registration No : _____

e-TB registration No : _____ Name of patient : _____ Age : _____ Sex : _____ * HIV status : Pos / Neg / Unknown

Address of patient : _____

_____ Cell Phone # : _____

B. TB Disease Type and Treatment History :

Type : A) Pulmonary ☐ B) Extra Pulmonary ☐ (Specify site) .....

History :

1) Failures of Category I (remain positive at month 5 or later and smear negative patients who became smear positive at month 2)
2) Failures of Re-treatment (remain positive at month 5 or later)
3) Non-converters of Re-treatment (remain positive at month 2)
4) Non-converters of category I (remain positive at month 2)
5) Relapses - a) Category I b) Re-treatment
6) Treatment after loss to follow up - a) Category I b) Re-treatment
7) Close contacts of DR TB patient with symptoms, a) Unknown history ☐ b) New ☐ c) Prev. treated ☐
8) HIV infected person, with TB S/S a) Unknown history ☐ b) New ☐ c) Prev. treated ☐
9) Others (Specify) i. Pulmonary, clinically diagnosed, a) Unknown history ☐ b) New ☐ c) Prev. treated ☐
ii. Extra Pulmonary, a) Unknown history ☐ b) New ☐ c) Prev. treated ☐
iiii. Pulmonary, Bacteriologically Confirmed a) Unknown history ☐ b) New ☐
10) Presumptive Pulmonary Smear Negative TB cases a) Unknown history ☐ b) New ☐ c) Prev. treated ☐
11) Presumptive TB - a) Unknown history ☐ b) New ☐

C. Origin of request :

Division name & ID : _____ District name & ID : _____ Local laboratory name & ID : _____

Local laboratory registration / serial no : _____ Date of test : ...../...../..... Smear result : 1st _____ 2nd _____ specimen _____

Microscopy technique used : Ziehl-Neelsen (ZN) ☐ LED Flourescence microscopy (FM) ☐

D. Request for test at the reference laboratory : NTRL/RTRL _____ / GeneXpert/Truenat MTB/RIF Site _____

1) Diagnosis 2) Follow up : Month of _____

Date of specimen(s) collected _____/_____/20_____ Specimen Identification no.(s) : _____

Specimen : Sputum ☐ Sputum in preservative, type ☐ specify _____ ☐ Other (specify) : _____

Requested tests : ☐ Microscopy (Type : ZN/LED ☐ Culture (L-J/MGIT) ☐ GeneXpert ☐ DST Conventional ☐ Line Probe Assay (LPA) ☐ Truenat MTB/RIF ☐ Others (Specify) _____

Person requesting examination : Name : _____ Position : _____ Cell No. _____

Organization : Govt. / Non Govt. (specify) : _____ Signature (with official seal) and Date : _____

* Information that can be disclosed optionally

E. Reference laboratory results :

Date of specimen recieved/Collected in the reference laboratory : NTRL/RTRL _____ / GeneXpert/Truenat MTB/RIF Site : _____

Reference laboratory specimen ID : _____

1. Microscopic examination : Date reported _____ Previous report and date : (if any) _____

ID #	Negative	Scanty	1+	2+	3+

Ziehl-Neelsen ☐ LED flourescence ☐ Others (specify) _____

Direct smear ☐ Concentrated smear ☐

2. GeneXpert result : Date reported _____ Previous report and date : (if any) _____

ID #	T = MTB detected, RIF resistance not detected	RR = MTB detected, RIF resistance detected	TI = MTB detected, RIF resistance indeterminate	N = MTB not detected	I = invalid / no result / error

3. Culture result : Method used : Solid (LJ) ☐ Liquid (MGIT) ☐ Date reported _____ Previous report and date : (if any) _____

ID #	Contaminated	Negative	Positive	A typical Mycobacteria (species)	Mycobacterium tuberculosis complex			
					<20 = 1-19 colonies Actual count	1 + = 20 - 100 colonies	2 + = > 100 - 200 colonies	3 + = > 200 colonies

4. Results of M. tuberculosis susceptibility testing : Date reported _____

Method used : ☐ Proportion method (LJ) ☐ Liquid (MGIT) ☐ Line Probe Assay (LPA) ☐ GeneXPert

Legend : S = Susceptible; R = Resistant; C = Contaminated; ND = Not Done

ID #	INH (H)	Rifampicin (R)	Ethambutol (E)	Streptomycin (S)	Pyrazinamide (Z)	FQ : Ofloxacin / Levofloxacin	Kanamycin (KM)	Others	
								(specify)	(specify)

5. Truenat MTB/RIF result : Date reported _____ Previous report and date : (if any) _____

ID #	T = MTB detected, RIF resistance not detected	RR = MTB detected, RIF resistance detected	TI = MTB detected, RIF resistance indeterminate/ unsuccesful	N = MTB not detected	I = invalid / no result / error

Result
Date : ___ / ___ / 20___

Name :
Designation :
Cell No.
Signature with Official seal

Annex 2: Truenat Register

Government of the People's Republic of Bangladesh
National TB Control Programme
Programmatic Management of Drug Resistant Tuberculosis (PMDT)
Laboratory Register for Truenat MTB/RIF

Lab Serial No.	Date & Time of sample		Patient Name, Address & Cell phone no.	Age	Sex (M/F)	DNA Extraction Successful		* Type of error (Trueprep) (If AT-1 is error)	** Criteria for Presumptive TB / DR TB	*** Truenat Result (High/Medium/Low)	**** Type of Error (Truelab)	Date & Time when specimen is tested	Date & Time when test result was reported (to patient / Referring Service Provider)	***** Referred by Name: Address: Mobile No.	No. of Used			Signature	Remarks
	Collected	Recieved				AT-1	AT-2								Cartridge	MTB chip	MTB RIF Dx chip		

* **Error Code for Trueprep** : Fail to power up, Not charging, Error 1 : Catridge valve error, Error 2 : Cartridge error, Error 3 : Cartridge clogged, Error 4 : Used cartridge, Error 5 : Expired cartridge, Error 6 : Cartridge not loaded, Error 7 : Expired reset card, Error 8 : Used reset card, Error 9 : Reset card read error, Error 10 : Invalid reset card, Error 11 : RTD-L error, Error 12 : RTD-E error, Error 14 : Cartridge error (elution)

** **Criteria for Presumptive TB/DR TB** :
1. Failures of Category I (remain positive at month 5 or later and smear negative patients who become smear positive at month 2), 2. Failures of re-treatment (remain positive at month 5 or later), 3. Non-converters of Re-treatment (remain positive at month 2), 4. Non-converters of Category I (remain positive at month 2) 5. Relapses - a) Category I, b) Re-treatment, 6. Treatment after loss to follow up - a) Category I, b) Re-treatment,7. Close contacts of DR TB patient with symptoms, a) Unknown history b) New c) Prev. treated, 8. HIV infected person, with TB S/S a) Unknown history b) New c) Prev. treated, 9. Others (Specify) i. Pulmonary, clinically diagnosed, a) Unknown history, b) New, c) Prev. treated, ii. Extra Pulmonary, a) Unknown history, b) New, c) Prev. treated, iii. Pulmonary, Bacteriologically confirmed, a) Unknown history, b) New 10. Presumptive Pulmonary Smear Negative TB Cases, a) Unknown history b) New c) Prev. treated, 11. Presumptive TB- a) Unknown history, b) New

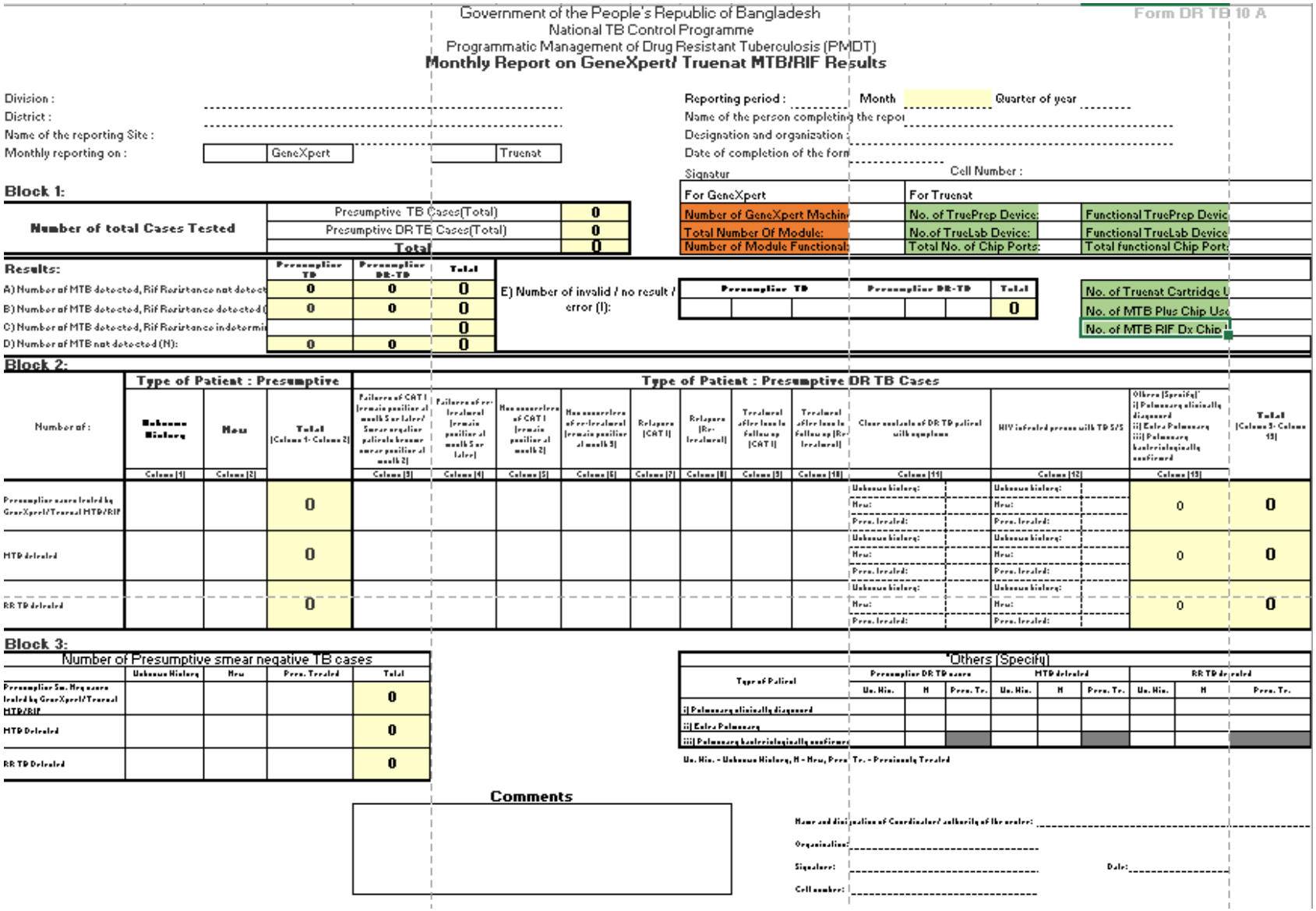
*** **Truenat MTB/RIF test result reporting as follows** : T = MTB detected, Rif resistance not detected, RR = MTB detected, Rif resistance detected, TI = MTB detected, Rif resistance indeterminate/ unsuccessful, N = MTB not detected, I = Invalid/No result/Error

**** **Error code for Truelab : Fail to power up, Not charging, Bay 1 failed, Bay 2 failed, Error 1 : Thermal cycling error, Error 2 : Test stopped manually, Error 3 : Incorrect optical profile, Error 4 : Runtime error, Error 5 : Probe check error, Other**

***** **Referred by** :
GPP = Graduate Private Practitioner, NGPP = Non-Graduate Private Practitioner, GSF = Government Field Staff, SS = Shastha Shebika, NGFS = Non-Government Field Staff, VD = Village Doctor, CV = Community, Volunteer, GH = Government Hospital, PH = Private Hospital, CHCP = Community Health Care Provider, TBP = TB Patient, Self = Self, Other (Specify)

Abbreviation: AT-1 = Attempt 1, **AT-2** = Attempt 2

Annex 3: Monthly reporting template



Government of the People's Republic of Bangladesh
National TB Control Programme
Programmatic Management of Drug Resistant Tuberculosis (PMDT)

Monthly Report on GeneXpert/ Truenat MTB/RIF Results

Form DR TB 10 A

Division :
District :
Name of the reporting Site :
Monthly reporting on : GeneXpert / Truenat

Reporting period : Month Quarter of year
Name of the person completing the repo
Designation and organization :
Date of completion of the form
Signatur Cell Number :

Block 1:

Number of total Cases Tested		
	Presumptive TB Cases(Total)	0
	Presumptive DR TB Cases(Total)	0
	Total	0

For GeneXpert	For Truenat	
Number of GeneXpert Machin	No. of TruePrep Device:	Functional TruePrep Devic
Total Number Of Module:	No.of TrueLab Device:	Functional TrueLab Device
Number of Module Functional:	Total No. of Chip Ports:	Total functional Chip Port:

Results:	Presumptive TB	Presumptive DR-TB	Total
A) Number of MTB detected, Rif Resistance not detect	0	0	0
B) Number of MTB detected, Rif Resistance detected (	0	0	0
C) Number of MTB detected, Rif Resistance indetermi			0
D) Number of MTB not detected (N):	0	0	0

E) Number of invalid / no result / error (I):

Presumptive TB			Presumptive DR-TB			Total
						0

No. of Truenat Cartridge U
No. of MTB Plus Chip Use
No. of MTB RIF Dx Chip

Block 2:

Number of :	Type of Patient : Presumptive			Type of Patient : Presumptive DR TB Cases											
	Unknown History	New	Total [Column 1+ Column 2]	Failures of CAT I [remain positive at month 5 or later/ Smear negative patients become smear positive at month 2]	Failures of re-treatment [remain positive at month 5 or later]	New smear+ve of CAT I [remain positive at month 2]	New smear+ve of re-treatment [remain positive at month 5]	Relapses [CAT I]	Relapses [Re-treatment]	Treatment after loss to follow up [CAT I]	Treatment after loss to follow up [Re-treatment]	Close contacts of DR TB patient with symptoms	HIV infected persons with TB S/S	Others [Specify]* i] Pulmonary clinically diagnosed ii] Extra Pulmonary iii] Pulmonary bacteriologically confirmed	Total [Column 3+ Column 13]
	Column [1]	Column [2]		Column [3]	Column [4]	Column [5]	Column [6]	Column [7]	Column [8]	Column [9]	Column [10]	Column [11]	Column [12]	Column [13]	
Presumptive smear+ve/ by GeneXpert/Truenat MTB/RIF			0									Unknown history: / New: / Prev. treated:	Unknown history: / New: / Prev. treated:	0	0
MTB detected			0									Unknown history: / New: / Prev. treated:	Unknown history: / New: / Prev. treated:	0	0
RR TB detected			0									Unknown history: / New: / Prev. treated:	Unknown history: / New: / Prev. treated:	0	0

Block 3:

Number of Presumptive smear negative TB cases

	Unknown History	New	Prev. Treated	Total
Presumptive Sm. Neg cases tested by GeneXpert/Truenat MTB/RIF				0
MTB Detected				0
RR TB Detected				0

*Others (Specify)

Type of Patient	Presumptive DR TB cases			MTB detected			RR TB detected		
	Un. His.	N	Prev. Tr.	Un. His.	N	Prev. Tr.	Un. His.	N	Prev. Tr.
i] Pulmonary clinically diagnosed									
ii] Extra Pulmonary									
iii] Pulmonary bacteriologically confirmed									

Un. His. – Unknown History, N – New, Prev. Tr. – Previously Treated

Comments

Name and designation of Coordinator/ authority of the center:
Organization:
Signature: Date:
Cell number:

Annex 4: IDDS reporting template

Infectious Disease Detection and Surveillance (IDDS) Project
Quarterly Report on Case Finding of TB/DR TB

Division : .....
District : .....
Name of the reporti .....

Reporting period : From ..... **To** ..... Year .....
Name of the person completing the repo .....
Designation and organization : .....
Date of completion of the forr .....
Signature ..... Cell Number : .....

Block 1: TB Case Notificatons

TB CASE TYPE:				SEX				HIV STATUS				Comment
New	Relapse	Unknown	Sub Total	Male	Female	Other	Sub Total	Positive	Negative	Unknown	Sub Total	

Block 2: DR TB Notificatons

Lab-confirmed drug resistance dissagregate						TOTAL	SEX			AGE		Comment
MDR [multi-drug resistant]	RR [Rifampicin Resistant]	Hr [Isoniazid mono-resistant]	Pre-XDR patients [Pre-Extensively drug resistant]	XDR patients [Extensively drug resistant]	Other DR	Total Drug Resistance	Male	*Female*	*Others*	*0-14 Years*	14 Years above	

Block 3: Childhood TB Notificatons

CHILD TB PATIENT TYPE: NEW +RELAPSE+ UNKNOWN				SEX (cases with unknown previous TB				HIV STATUS				AGE		
New	Relapse	Unknown	Sub Total	Male	Female	Other	Sub Total	Positive	Negative	Unknown	Sub Total	0-4 years old	5-14 years old	Sub Total

Block 4. Bacteriological Diagnosis Coverage (Pulmonary TB)

Male	Female	Other	Total	Age			Male	Female	Other	Total	Age		
Bacteriologically confirmed pulmonary TB (includes smear positive or culture positive or positive by WRD test)			Pulmonary TB (new and relapse pulmonary TB patients without extrapulmonary TB)	0-14 Years	14 years above	Total	Pulmonary TB (includes smear positive or culture positive or positive by WRD test)+ Clinically Diagnosed			Pulmonary TB (new and relapse pulmonary TB patients without extrapulmonary TB)+ Clinically Diagnosed	0-14 Years	14 years above	Total

Block 5: Rapid diagnostic testing coverage

WRD test type dissagregate				Sex		Total	Age			Presumptive TB patients					
GeneXpert MTB/RIF	*Ultra*	*Truenat*	*LPA*	Male	Female	Presumptive TB patients tested with WRD during reporting period	0-14 Years	14 yerars above	Total	Male	Female	Total presumptive TB patients tested during reporting period	0-14 Years	14 yerars above	Total

WHO-recommended rapid diagnostic (WRD)

Block 6: Notified RR-TB/MDR-TB cases with DST results for second-line TB drugs

DST dissagregates			TOTAL	Gender			TOTAL
Genotypic LPA Assay	Phenotypic AssayMGIT	Phenotypic solid media LJ	Second-line drug testing (MDR/RR)	Male	Female	Other	Second-line drug testing (MDR/RR)

Comments

Annex 5: Stock register

STOCK CARD FOR GeneXpert and Truenat

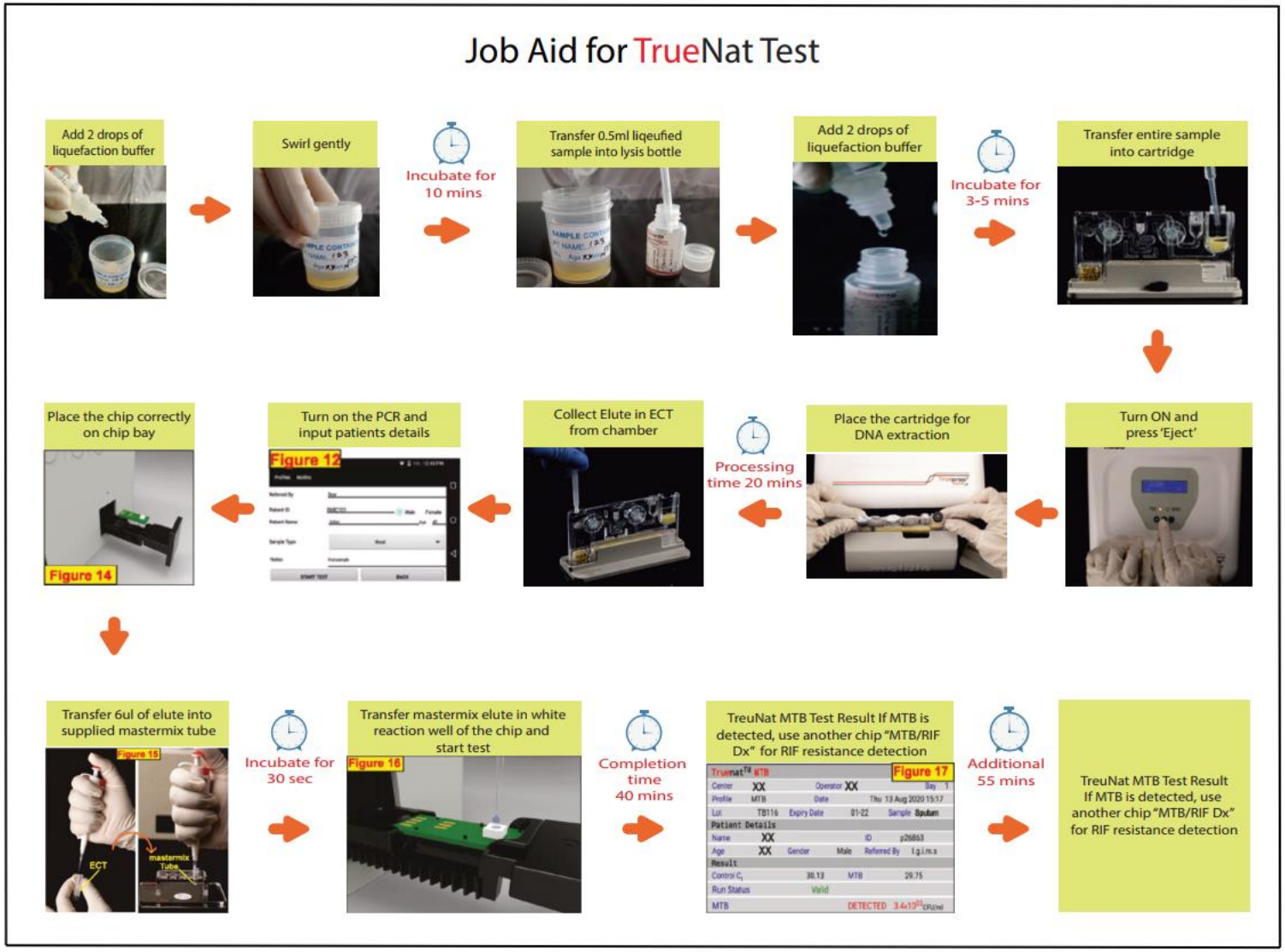
Name of the Reporting Site: _____ **Address:** _____

Item name: **GeneXpert:** ☐ MTB/RIF Cartridge **Truenat:** ☐ Universal Cartridge

(Use separate sheet for each item) ☐ MTB/RIF Ultra Cartridge ☐ MTB/XDR Cartridge ☐ MTB plus chip ☐ MTB RIF Dx chip

Date	Opening Balance (a) or Final Balance (e)	Chalan No.	Received From	Quantity Received (b)	Lot Number	Expiry Date	Used/ Out (c)	No. of Successful Test	No. of Unsuccessful Test	Damage	Final Balance (a+b-c) =e	Signature	Remarks

Annex 6: Job aid



Annex 7: Service and Maintenance log

TrueLab and TruePrep Maintenance Log				Month & Year:	
Laboratory Name:			Lab Contact Person Name & Mobile no.:		
TruePrep Machine Serial no.:		TrueLab Analyzer Machine Serial No.:		Technical Service Representative Name & Mobile No.:	

	Days of Month																												
Maintenance	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 Working Space																															
Discard Used Chips & Cartridges																															
Battery Backup (Trueprep) (hour)																															
Battery Backup (Truelab) (hour)																															

Monthly Maintenance	As Necessary (Please Specify)		
Disinfect Instrument Surface	Date: Signature:	Flush protocol for the Trueprep instrument	Date: Specify reason:
Clean TrueLab bays	Date: Signature:	Spillage tray or linear motion guide tray replacement	Date: Specify reason:
Temperature Calibration	Date: Signature:	Slider glass replacement	Date: Indicate tray:
Verification of the fixed 6µl pipette	Date: Signature:		

Failure Events				Notes
Date	Event	Corrective Action Taken	Operator/ Technical Service Representative	Indicate completion of the 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 monthly 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 the 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 8: Monitoring and Supportive supervision Checklist

Monitoring & Supportive supervision checklist for Truenat

Name of Truenat site	Upazilla	District
Name & contact of site	Name	+880
Name and designation of evaluated technician	Name	
Name and designation of filling in this form	Name	Designation

Instructions for the evaluator:

- Please fill-up one form per Truenat site as well as technician.
- In the presence of the evaluator, the technician will prepare two sputum sample and conduct the PCR on the Truenat Micro-PCR analyzer system. No further instructions will be given during this time. If any answer is "No", technician will be re-trained on-the-spot using respective SOPs. Please indicate whether re-training was done using checkbox.
- Please review the record keeping documents such as relevant registers, reporting templates for last month/ quarter.
- Please visit the storeroom and match consumables with the stock register.
- Debrief the key findings with the technician.

Please observe & fill-up accordingly-

1. Sample collection and storage

a)	Are samples free of obvious food particles and solid particles?	☐ Yes	☐ No	☐ Retraining

2. Sample Preparation

a)	Did technician record sample information (serial number, sample number, date of test) in the Truenat register?	☐ Yes	☐ No	☐ Retraining
b)	Did technician incubate the sample for 10 minutes at room temperature?	☐ Yes	☐ No	☐ Retraining
c)	Did technician swirl gently to mix and incubate lysis buffer bottle at room temperature for 3-5 minutes?	☐ Yes	☐ No	☐ Retraining

3. DNA Extraction

a)	Did technician label the cartridge with number of the sample of test?	☐ Yes	☐ No	☐ Retraining

b)	Did technician dispense the elute into the labelled ECT tube and close the ECT tube cap tightly?	☐ Yes	☐ No	☐ Retraining

4. Amplification process

a)	Did technician allow elute for 30-60 Sec in master mix tube to get a clear solution?	☐ Yes	☐ No	☐ Retraining
b)	Did technician label the chip with the patient ID?	☐ Yes	☐ No	☐ Retraining

5. Adhering Biosafety Standards

a)	Did technician wear PPE properly?			
	☐ Yes	☐ No	Gown	Comments
	☐ Yes	☐ No	Face Mask	Comments
	☐ Yes	☐ No	Hand gloves	Comments
b)	Are PPE supplies adequate?			
	☐ Yes	☐ No		Comments
c)	Are biosafety equipment in place?			
	☐ Yes	☐ No	Waste bin/bucket	Comments
	☐ Yes	☐ No	Bio-Hazard Bug	Comments
d)	Does the lab have appropriate waste disposal system in place?			
	☐ Yes	☐ No	Incinerator	Comments
	☐ Yes	☐ No	Burner	Comments
	☐ Yes	☐ No	None	Comments
e)	Did technician handle the liquid waste properly and dispose (submerge used cartridges, reagent bottles and other consumables in freshly prepared 10% bleach before disposal) in a safe manner to prevent environmental contamination?			
	☐ Yes	☐ No		Comments

6. Supply chain management

a)	Does the laboratory maintain the stock register?	☐ Yes	☐ No
b)	Any stock out in last month	☐ Yes	☐ No
c)	Buffer stock of reagents and consumables items	☐ Yes	☐ No

7. Record keeping

a)	Printed record keeping documents available (Lab register, Sample referral form, maintenance and service log, stock register, result copy)?	☐ Yes	☐ No
	Comments if any:		

b)	Service interruption	☐ Yes	☐ No	Reason
c)	Number of sputum specimen collected/received			
d)	Specimen rejection	☐ Yes	☐ No	Reason
e)	Number of DNA extraction done			
f)	Number of specimens for which DNA extraction was unsuccessful			
g)	Number of specimens with MTB detected			
h)	Number of specimens with MTB not detected			
i)	Number of patient with MTB detected referred to the UHC/ DOTS center			
j)	Number of specimens with unsuccessful results (Error, Invalid, no result)			
k)	Number of specimens with RIF resistance detected			
l)	Number of patients with RR detected referred to the Treatment initiation center			
m)	Number of MTB positive specimens with unsuccessful RIF test results (Error, Invalid, no result)			

8. Maintenance

a)	Maintenance & Service log sheet properly filled up	☐ Yes	☐ No

9. Documents Availability

a)	Is printed SOP, Job aid, Troubleshooting manual, national algorithm available?	☐ Yes	☐ No
b)	Comments (If any)		

Key findings and recommendation

Findings	Recommendations
• ------ • ------ • ------ • ------ • ------	• ------ • ------ • ------ • ------

Feedback & mentoring

- After completion of observations and documents review, please list down your key findings and prioritize those.
- Please discuss those key findings with the medical technologist; first discuss about the positive findings you found to motivate and appreciate the technician, later discuss about those findings that need improvement.
- Finally listen to the technologist whether he/she have any challenges and feedback for improvement and note accordingly if needed.