MODULE 1: INTRODUCTION TO TRUENAT

Truenat® Tests for the Detection of TB and Rifampicin Resistance
Central-Level Training

ACKNOWLEDGEMENTS

These training modules were developed as a collaboration between the United States Agency for International Development (USAID) and its Infectious Disease Detection and Surveillance project (IDDS) and the Stop TB Partnership, as part of the *introducing New Tools Project* (iNTP). The content is based on the Stop TB/USAID/GLI *Practical Guide to Implementation of Truenat™ Tests for the Detection of TB and Rifampicin Resistance*, together with content provided by Molbio Diagnostics (Module 3).

Introduction

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

Module 1: Introduction to Truenat

Course Outline

01. **TB Context**

02. **Plenary Session**
Key Challenges in Diagnosing TB

03. **TB Laboratory Tests**
World Health Organization (WHO) Recommendations

04. **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

Global Context

- 10 million people fall ill with tuberculosis (TB) ever year.
- 1.5 million people die from TB each year – making it the world's top infectious killer.
- TB is the leading cause of death of people with HIV and a major contributor to antimicrobial resistance

Source: WHO 2021, https://www.who.int/health-topics/tuberculosis

Global TB Situation

To what extent do you think country is meeting these goals?

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)

Global TB Situation

WHO Guidelines on DST

- Updated WHO guidelines stress the importance of DST prior to treatment, especially for the medicines for which WHO-recommended rapid molecular tests are available.
- Rapid molecular tests are available for testing resistance to:
  - RIF
  - FQs
  - Isoniazid (INH)
  - Pyrazinamide (PZA)

WHO-recommended rapid molecular tests exist for detection of resistance to which medicines?

Country TB Context

TB Data

- Number of cases (TB and DR-TB), TB incidence
- Number of deaths
- Pediatric TB
- Treatment coverage and undiagnosed cases
  - Emphasize missing cases
- Access to molecular testing
- Spatial molecular test localization if possible and available

Country TB Context

National Priorities

- National priorities per the TB strategic plan, including scaling up molecular testing

Country TB Context

Overview of the Diagnostic Network

- Overview of the diagnostic network

Challenges with Diagnosing TB in Country

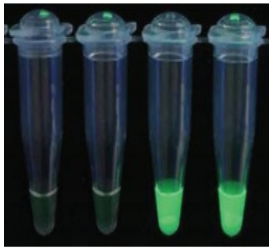
PLENARY SESSION

TB LABORATORY TESTS

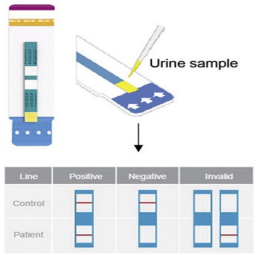
Menu of TB Lab Tests

MICROSCOPY

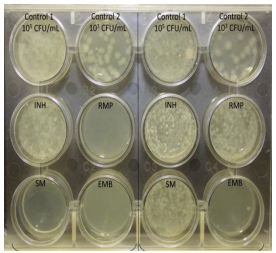
CULTURE



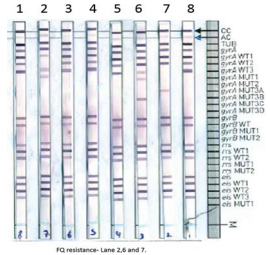
TB LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)



MYCOBACTERIAL LIPOARABINOMANNAN (LAM) ANTIGEN DETECTION



PHENOTYPE (CULTURE BASED) DRUG SUSCEPTIBILITY TESTING (DST)



LINE PROBE ASSAY (LPA)

XPERT MTB/RIF OR XPERT ULTRA

TRUENAT

Lab Tests for TB

AFB-Smear Microscopy

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 (50%), which is further reduced in HIV-positive individuals and children
- Limited specificity; can detect non-tuberculous mycobacteria(NTMs) and does not detect drug resistance.
- Can not detect DRTB in HIV+ individuals.

WHO RECOMMENDATION

WHO recommends TB programs transition to replacing microscopy as the initial diagnostic test with molecular WRDs that allow for the detection of MTBC.

For monitoring treatment progress, WHO recommends that in all settings LED fluorescence microscopy should be phased in to replace conventional bright-field microscopy and Ziehl-Neelsen staining.

Lab Tests for TB

Culture

Uses

- Used as an initial diagnostic for TB as well as to isolate cultures for DST
- Monitors MDR-TB treatment

Benefits

- High sensitivity and specificity test for the detection of MTBC
- Provides an isolate for DST (phenotypic testing)
- Can assess treatment progress

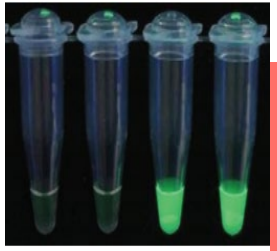
Limitations

- 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

WHO RECOMMENDATION

WHO recommends the use of liquid culture and rapid species identification within the context of country specific, comprehensive plans for laboratory capacity strengthening and based on a step-wise approach.

Lab Tests for TB



TB-LAMP

Uses
- Used as an initial diagnostic for TB

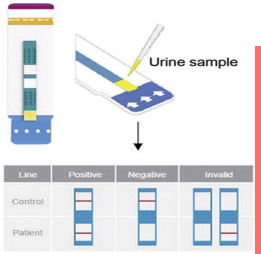
Benefits
- Requires minimal infrastructure and has biosafety requirements similar to smear microscopy
- Detection of amplified product is based on the turbidity visualized with the naked eye or under ultraviolet light
- Requires less than one hour to perform

Limitations
- Most suitable for settings with low prevalence of HIV and MDR-TB
- Cannot be used to monitor treatment
- Does not detect RIF resistance

WHO RECOMMENDATION

WHO suggests using TB-LAMP as a replacement test for sputum smear microscopy for the diagnosis of pulmonary TB.

Lab Tests for TB



LF-LAM Antigen Detection

Uses

- Rapid test to detect mycobacterial lipoarabinomannan(LAM) MTBC antigens in urine

Benefits

- Requires no equipment
- Point of care test
- Simple, easy and fast (25 mins)
- Easy to collect urine specimen
- Allows treatment to be started early
- Inexpensive

Limitations

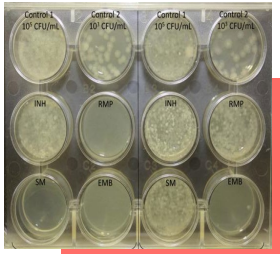
- Does not provide any information on drug resistance
- Cannot be used to monitor treatment
- Low sensitivity
- Require follow up with other diagnostic test
- Used in limited patient population
- Can not distinguish MTB from other non mycobacteria

WHO RECOMMENDATION

WHO recommends using Alere Determine™ TB LAM Ag for

1) Inpatient settings, to assist in the diagnosis of active TB in HIV-positive adults, adolescents and children with signs and symptoms of TB (pulmonary or extrapulmonary) with advanced HIV disease or who are seriously ill, or with a CD4 cell count of less than 200 cells/mm3 irrespective of signs and symptoms of TB.
2) Outpatient settings, to assist in the diagnosis of active TB in HIV positive adults, adolescents and children who: have signs and symptoms of TB (pulmonary or extrapulmonary); are seriously ill; or have a CD4 cell count of less than 100 cells/mm3 irrespective of signs and symptoms of TB

Lab Tests for TB



Phenotypic (culture-based) DST

Uses

- Detection of resistance to anti-TB drugs

Benefits

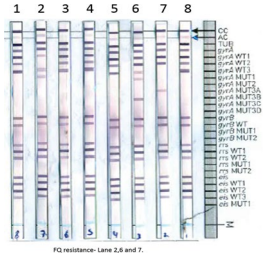
- Culture-based, phenotypic DST remains essential for drugs for which there are not yet reliable molecular tests
- Phenotypic DST for second-line agents is required to confirm or exclude XDR-TB

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.

Lab Tests for TB



FQ resistance- Lane 2,6 and 7.

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 test at once
- Fast and accurate results under 48 hrs
- 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
- Can not fully replace conventional culture methods
- Expensive
- Requires well trained staff

WHO RECOMMENDATION

- FL-LPA is recommended for use on smear-positive sputum specimens and M. tuberculosis isolates as the initial test instead of Phenotypic culture-based DST to detect resistance to RIF and INH.

- WHO suggests using SL-LPA as the initial test instead of phenotypic culture-based DST to detect resistance to FQs and AMK directly from patient specimens or cultures.

Lab Tests for TB

Xpert MTB/RIF or Xpert Ultra

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
- WHO recommendation on an MTB/XDR cartridge expected in Q3 2021
- Expensive

WHO RECOMMENDATION

WHO recommends the use of Xpert MTB/RIF or Ultra as an initial diagnostic test for TB and detection of rifampicin-resistance rather than smear microscopy/culture and phenotypic drug-susceptibility testing.

Lab Tests for TB

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 and minimally trained Lab technicians
- Battery-powered and uses room temperature stable reagents
- Can generate results for TB in one hour and for RIF resistance in one additional hour

Limitations

- RIF resistance test is a reflex test
- Electricity still required for charging the batteries
- More manual steps than the Xpert MTB/RIF test

Lab Tests for TB

Truenat

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.

MTB

For the detection of TB

MTB Plus

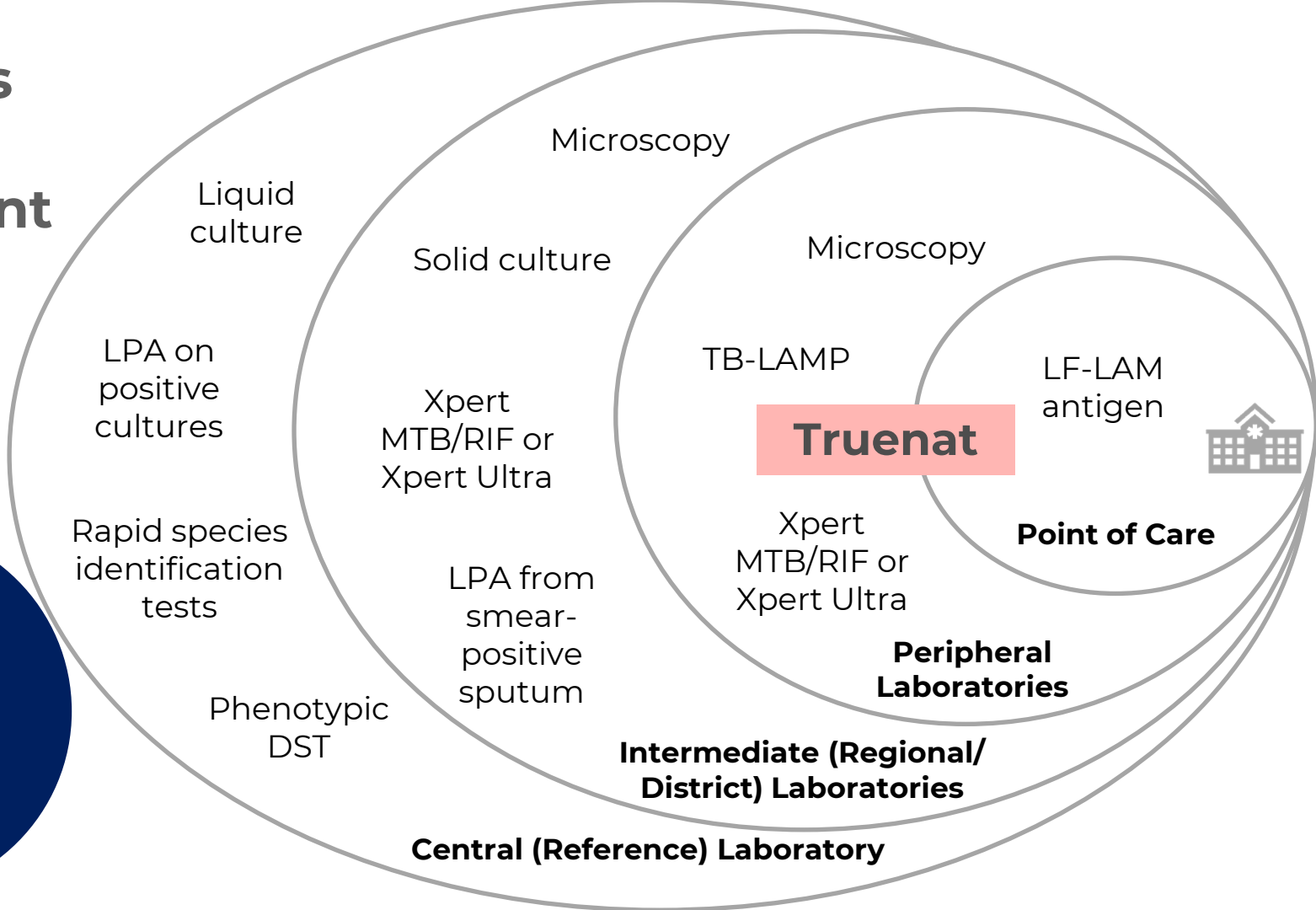
A more sensitive test for TB detection

MTB-RIF Dx

For the detection of RIF resistance

PLACEMENT OF TRUENAT IN DIAGNOSTIC NETWORKS

Lab Tests for TB: Placement in the Network



Where should we place Truenat in this diagram?

Placement of Truenat in Diagnostic Networks

- Truenat can be placed at peripheral health centres to replace microscopy as the initial diagnostic test for TB
- X-ray may be used as a screening tool for confirmatory testing with Truenat
- Specimen referral networks may be needed to allow for further DST

Positioning vs. Xpert or TB-LAMP

- Truenat and TB-LAMP can be placed at lower levels than Xpert
- Placement at lower levels can increase patient access to rapid molecular testing for TB, decentralize testing for RIF resistance, and reduce need for patient travel

****Truenat is not a replacement for existing Xpert networks**

A country can use more than one test for rapid testing

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	0.73	0.91	0.37	0.98
Truenat MTB Plus	0.80	0.96	0.46	0.97
Truenat MTB-RIF Dx	0.84	0.88	0.67	0.95

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	0.84	0.98	0.45	0.97
Truenat MTB Plus	0.87	0.99	0.55	0.95
Xpert MTB/RIF	0.85	0.99	0.48	0.97
Truenat MTB-RIF Dx	0.82	0.86	0.33	0.98
Xpert MTB/RIF	0.84	0.89	0.33	0.98

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Effect of prior treatment on specificity

	Specificity – No History of TB Treatment	Specificity – History of TB Treatment
Truenat MTB	0.97 (0.96-0.98)	0.92 (0.83-0.96)
Xpert MTB/RIF	0.97 (0.96-0.98)	0.90 (0.81-0.95)
Truenat MTB Plus	0.95 (0.94-0.97)	0.88(0.78-0.94)
Xpert MTB/RIF	0.97 (0.96-0.98)	0.90 (0.80-0.95)

Foundation for Innovative New Diagnostics, multicenter prospective clinical evaluation study in 19 clinical sites and 7 reference laboratories in 4 countries.

Sensitivity and Specificity Trade-Offs

- In deciding whether to select Truenat MTB or MTB Plus, countries will need to consider the possible trade-offs between higher or lower sensitivity and higher or lower specificity based on the prevalence of:
  - TB
  - DR-TB
  - TB/HIV

Why might a country with a a high DR-TB burden choose not to use MTB Plus?

High HIV Burden Settings

- In a population with a high prevalence of HIV, a more sensitive test (i.e., Truenat MTB Plus) may be the more appropriate test because of its increased sensitivity for the detection of MTBC in smear-negative samples.

Advantages of Truenat

In your opinion, which of these advantages is the most important in your country?

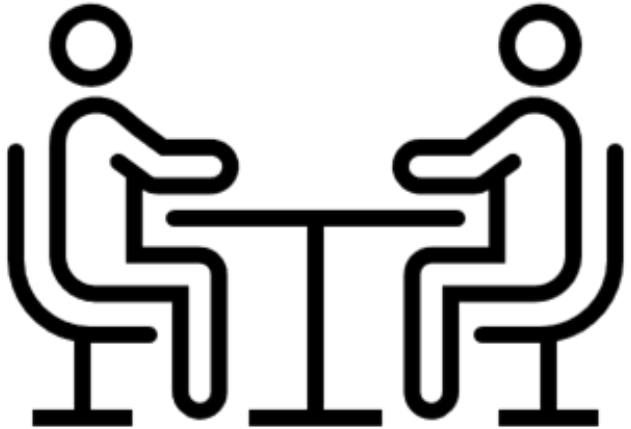
- Cost-effectiveness
  - Low equipment and test costs
- Patient access
  - Use of Truenat at primary healthcare level can reduce the need for sample transport for detection of RIF resistance
- Time taken for the assay
  - MTB detection is completed in 1 hour and the RIF assay is done only as a reflex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

Activity: Which Test Should I Use?

Scenario 1:

You are working in a primary care clinic. A 33-year-old woman presents with a low-grade fever and a persistent cough. The woman informs you that she has been coughing for the last two weeks.

Which test(s) should be performed?

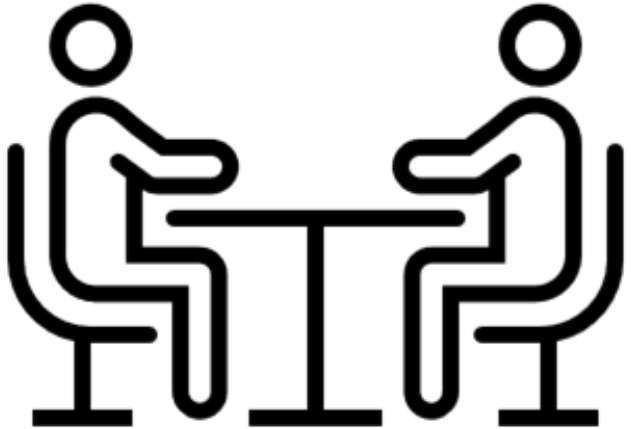


Activity: Which Test Should I Use?

Scenario 2:

A patient presents with trouble breathing and coughing that she has experienced for the last week. She tells you that last year she was treated for an illness but she doesn't know what it was, only that she had to take medicine every day. You do not have access to her health record.

Which test(s) should be performed?

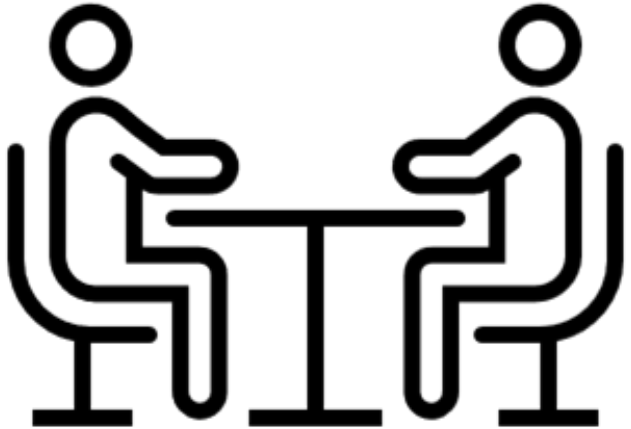


Activity: Which Test Should I Use?

Scenario 3:

A patient enters your clinic with a bad cough that has lasted for two months. Sometimes the cough produces blood. He is also suffering from malaise. You work in a city where there is a high prevalence of HIV.

Which test(s) should be performed?



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 – Question 1

1. What are some of the main challenges with diagnosing TB in country?

Knowledge Check – Question 2

2A. _____, _____, _____, _____, _____, and _____ are tests that can be used to diagnose TB.

2B. _____ and _____ are tests that can be used to monitor TB treatment.

2C. _____, _____, _____, and _____ are tests that can be used to diagnose RIF resistance.

2D. _____ and _____ are tests that can be used to diagnose other types of drug resistance.

Knowledge Check – Question 3

3. List three advantages of Truenat compared to other tests.

Knowledge Check – Question 4

4. Why is Truenat promising as a new TB diagnostic tool at primary health care settings?

THANK YOU


UNITED STATES AGENCY INTERNATIONAL DEVELOPMENT
USAID
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FROM THE AMERICAN PEOPLE


Stop TB Partnership

MODULE 2: DIAGNOSTIC ALGORITHM AND RESULTS INTERPRETATION

Truenat® Tests for the Detection of TB and Rifampicin Resistance
Central-Level Training

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Introduction

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

COURSE OUTLINE

01. WHO Recommendations

02. Truenat Algorithm

03. Digital Results Reporting

04. 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 algorithm and decision tree to use Truenat.
- Understand patient flow within the TB diagnostic network and describe procedures for patient referral.

WHO RECOMMENDATIONS

WHO Recommendations

Truenat MTB or MTB Plus

May be used as an initial diagnostic test for TB rather than smear microscopy/culture

MTB

MTB Plus

Truenat MTB-RIF Dx

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

MTB-RIF Dx

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

When to Use Truenat

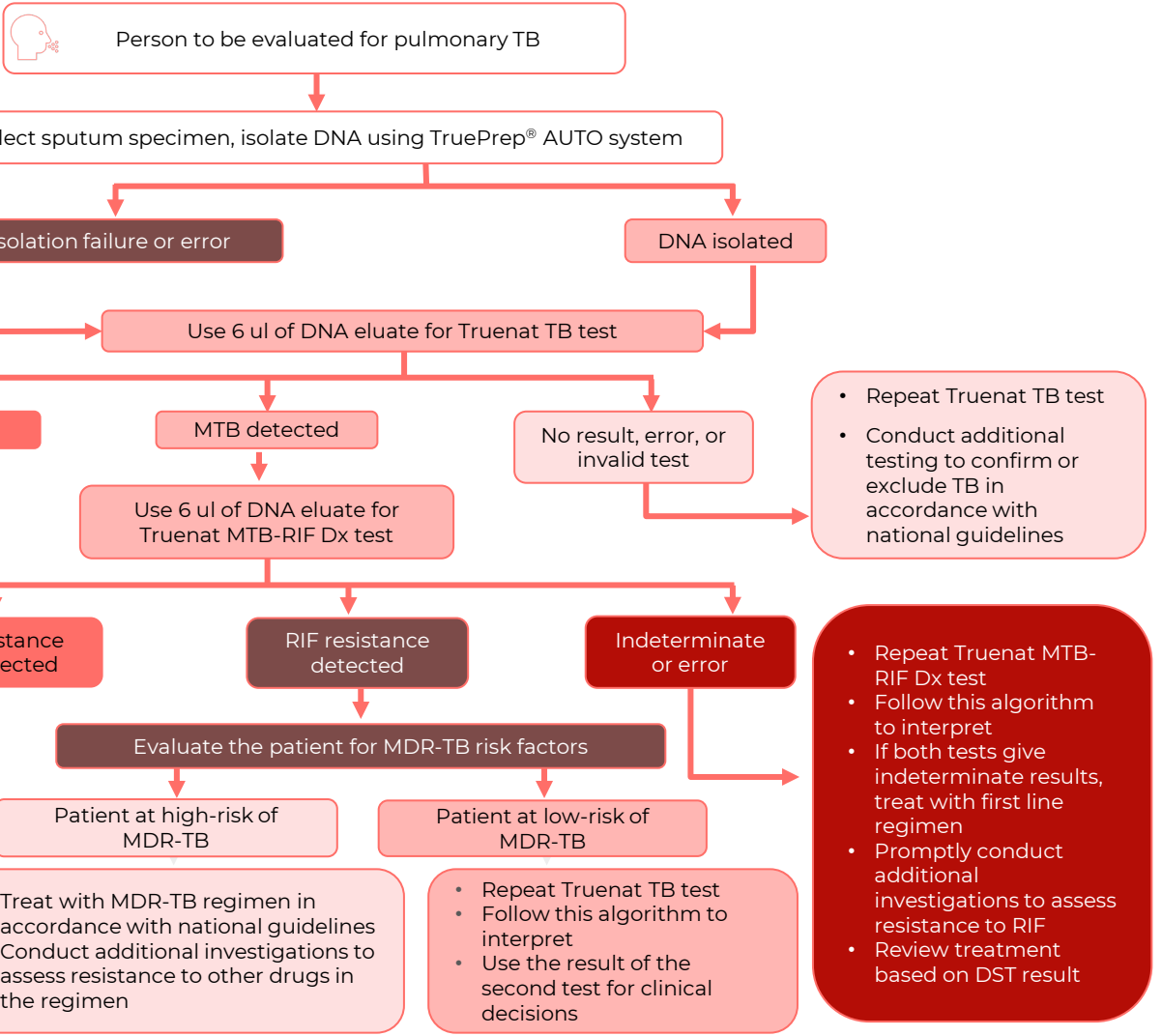
- To detect MTBC in specimens from persons newly presenting with signs and symptoms of pulmonary TB
- To detect RIF resistance in persons found to be positive from a Truenat MTB or MTB Plus test, and in persons with TB who may have developed RIF resistance.

When NOT to Use Truenat

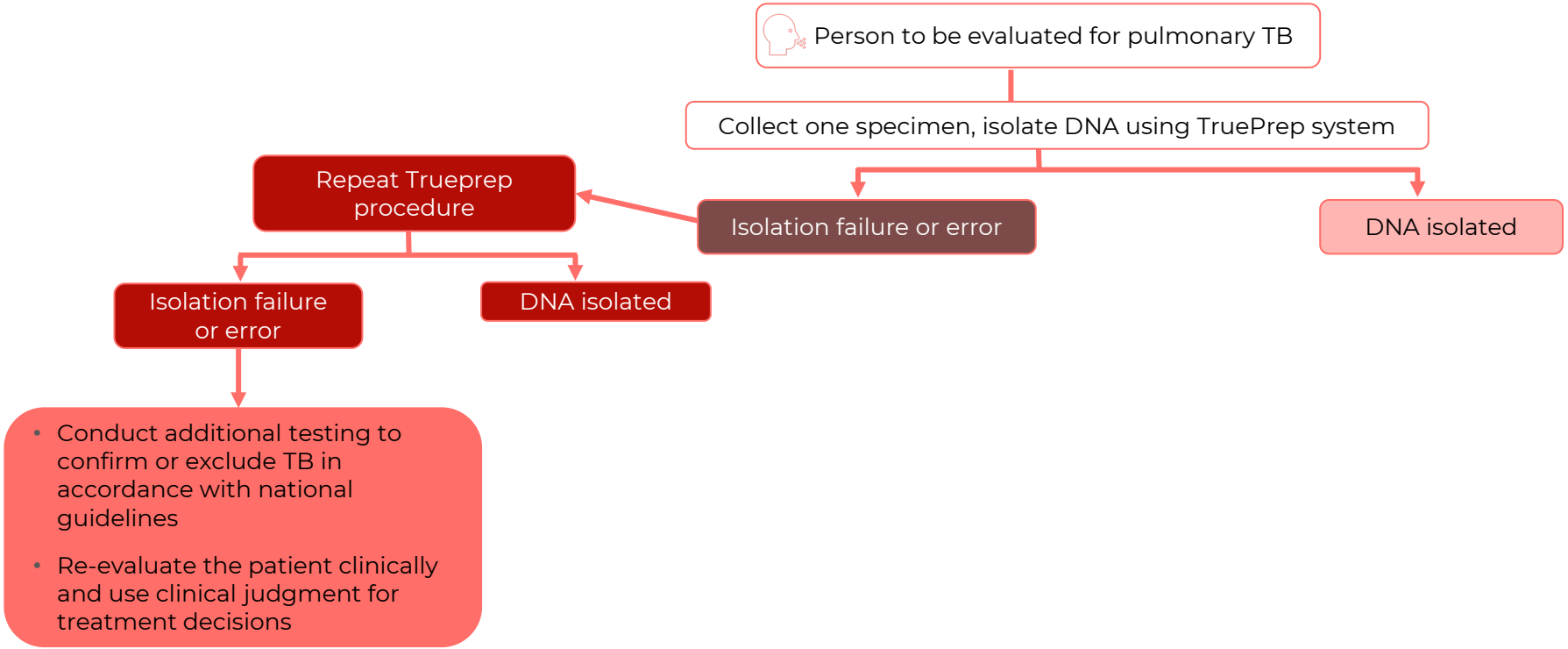
- Truenat is not yet recommended for suspected extrapulmonary TB (due to insufficient evidence)
- Do **NOT** USE Truenat for treatment monitoring

TRUENAT ALGORITHM

Truenat Algorithm



Algorithm Part 1: Isolate DNA



Algorithm Part 1: Isolate DNA

Programs may consider collecting two specimens upfront

- 1st specimen: Should be promptly tested using Truenat TB test
- 2nd specimen: Use for additional testing in the algorithm

Sputum is the recommended specimen

Prepare the sample using the Trueprep AUTO MTB Sample Pre-treatment Pack

Isolate DNA using:

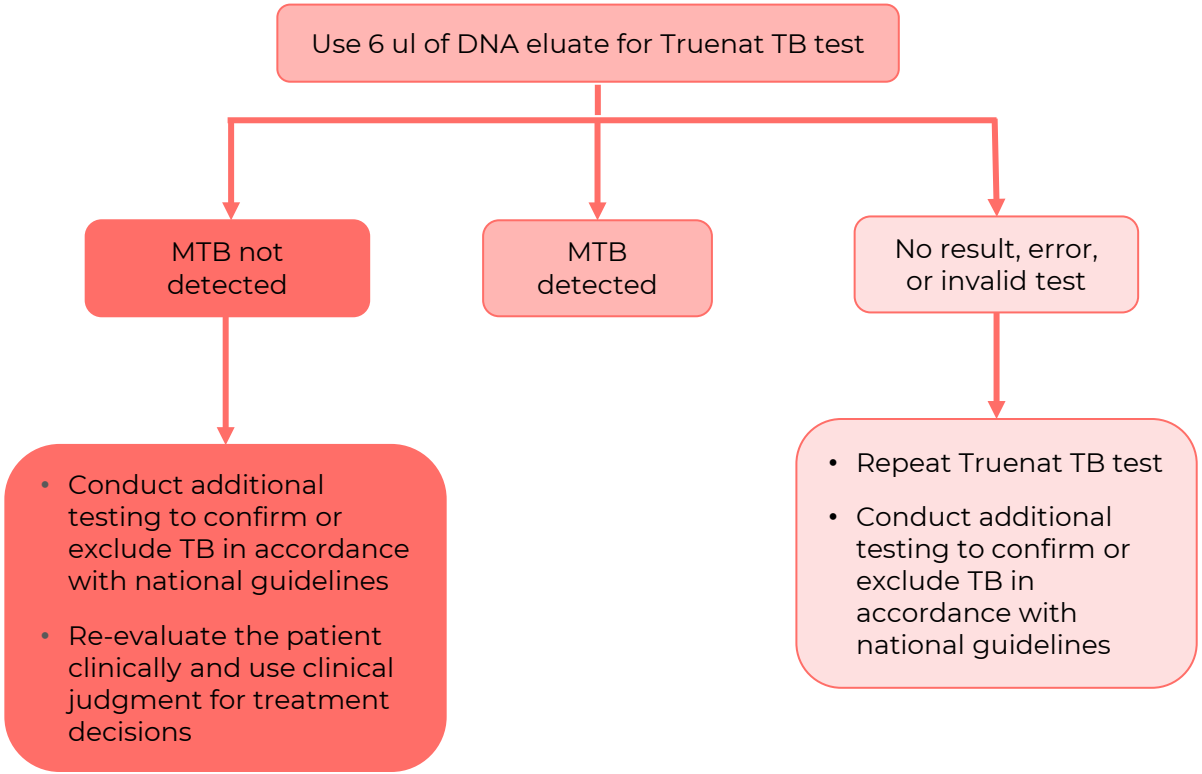
- Trueprep AUTO v2 Universal Cartridge Based Sample Prep Kit
- Trueprep AUTO v2 Universal Cartridge Based Sample Prep Device

Algorithm Part 1: Isolate DNA

If DNA isolation is unsuccessful

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

Algorithm Part 2: TB Test



Algorithm Part 2: TB Test

If TB test result is MTB not detected

Re-evaluate the patient and conduct additional testing in accordance with national guidelines.

Consider the possibility of clinically defined TB (TB without bacteriological confirmation)

- Use clinical judgement for treatment decisions

Algorithm Part 2: TB Test

If TB test result is MTB detected

MTB detected results appear as follows:

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

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

Algorithm Part 2: TB Test

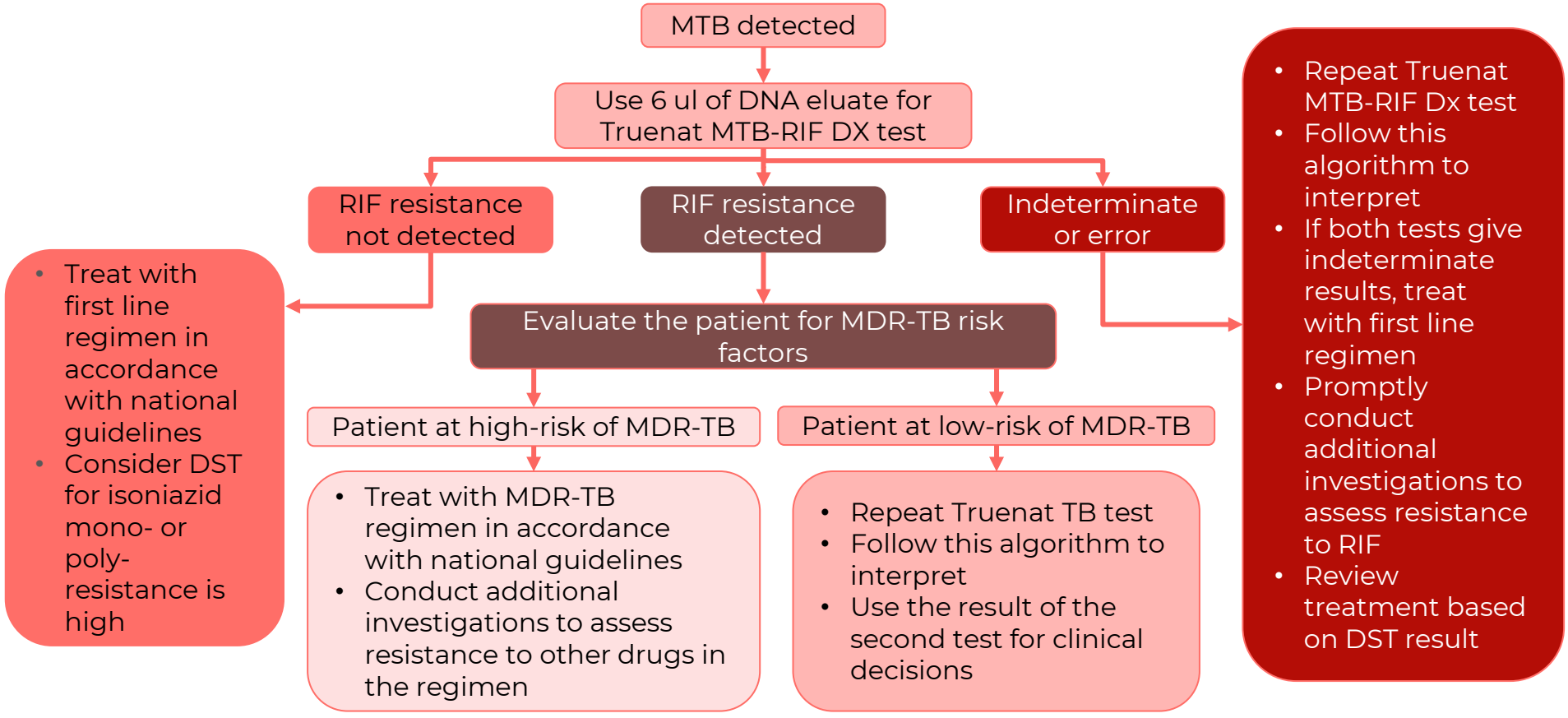
If TB test result is inconclusive

- Test result reads “Error” or “No result:”
  - Repeat Truenat TB test with second portion of the remaining DNA or new sample
- Test result reads “invalid:”
  - Repeat Truenat TB test with new specimen
  - Start anew with the sample preparation and DNA isolation
- If repeated test has a valid result, continue with algorithm

If second attempt is also inconclusive

Next Step:
Conduct additional testing to confirm or exclude TB in accordance with the national guidelines.

Algorithm Part 3: RIF Resistance Test



Algorithm Part 3: RIF Resistance Test

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

- Initiate patient on appropriate regimen using first-line TB drugs in accordance with national guidelines
- Consider requesting additional DST in accordance with national algorithms

Algorithm Part 3: RIF Resistance Test

If MTB-RIF Dx test result is RIF Resistance Detected:

- Assess whether patient is at high risk for MDR-TB

If Patient is High-Risk	If Patient is Low-Risk
Consider the RIF-resistant test result definitive	Repeat the Truenat TB test and MTB-Rif Dx test on a fresh sample
Initiate the patient on a regimen for RR-TB or MDR-TB in accordance with national guidelines and WHO recommendations	If second test also indicates RIF resistance, initiate an MDR-TB regimen in accordance with national guidelines
	If second test indicates RIF resistance not detected, assume false-positive and initiate treatment with a first-line regimen in accordance with national guidelines

Algorithm Part 3: RIF Resistance Test

For all patients with RR-TB or MDR-TB

- Conduct additional investigations to assess resistance to the other drugs in the treatment regimen.
- Rapid molecular methods are preferred
- Reminder: WHO recommends rapid DST for FQs for all persons with RR-TB

Algorithm Part 3: RIF Resistance Test

If MTB-RIF Dx test result is RIF indeterminate

- Initiate patient on TB treatment regimen using the first-line TB drugs in accordance with national guidelines
    - Unless the patient is very high risk for MDR-TB, in which case initiate on an MDR-TB regimen

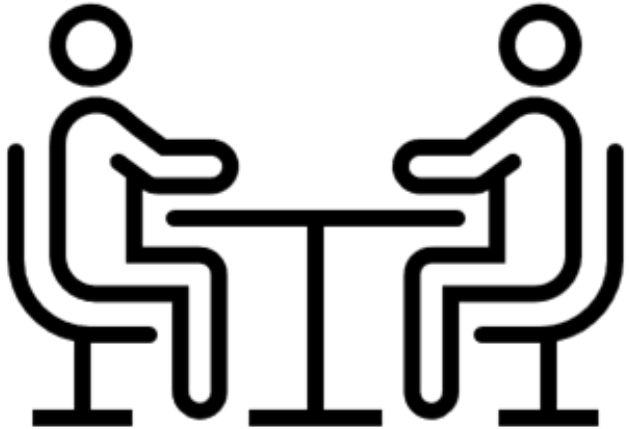
RIF resistance indeterminate result is usually caused by a paucibacillary TB load in the sample

- In this case, repeat the MTB-RIF Dx test using an aliquot from the same DNA eluate. If the repeated test is again indeterminate, run the the Truenat MTB-RIF Dx test using a DNA eluate from a fresh specimen.
- Follow steps previously described based on the second result.

Activity: Truenat Algorithm

Scenario:

Rebekah comes to your clinic and has a severe cough and malaise. Her X-ray shows abnormalities on her lungs that suggest pulmonary TB. Follow the algorithm to determine what steps you should follow.



PATIENT FLOW

Patient Flow

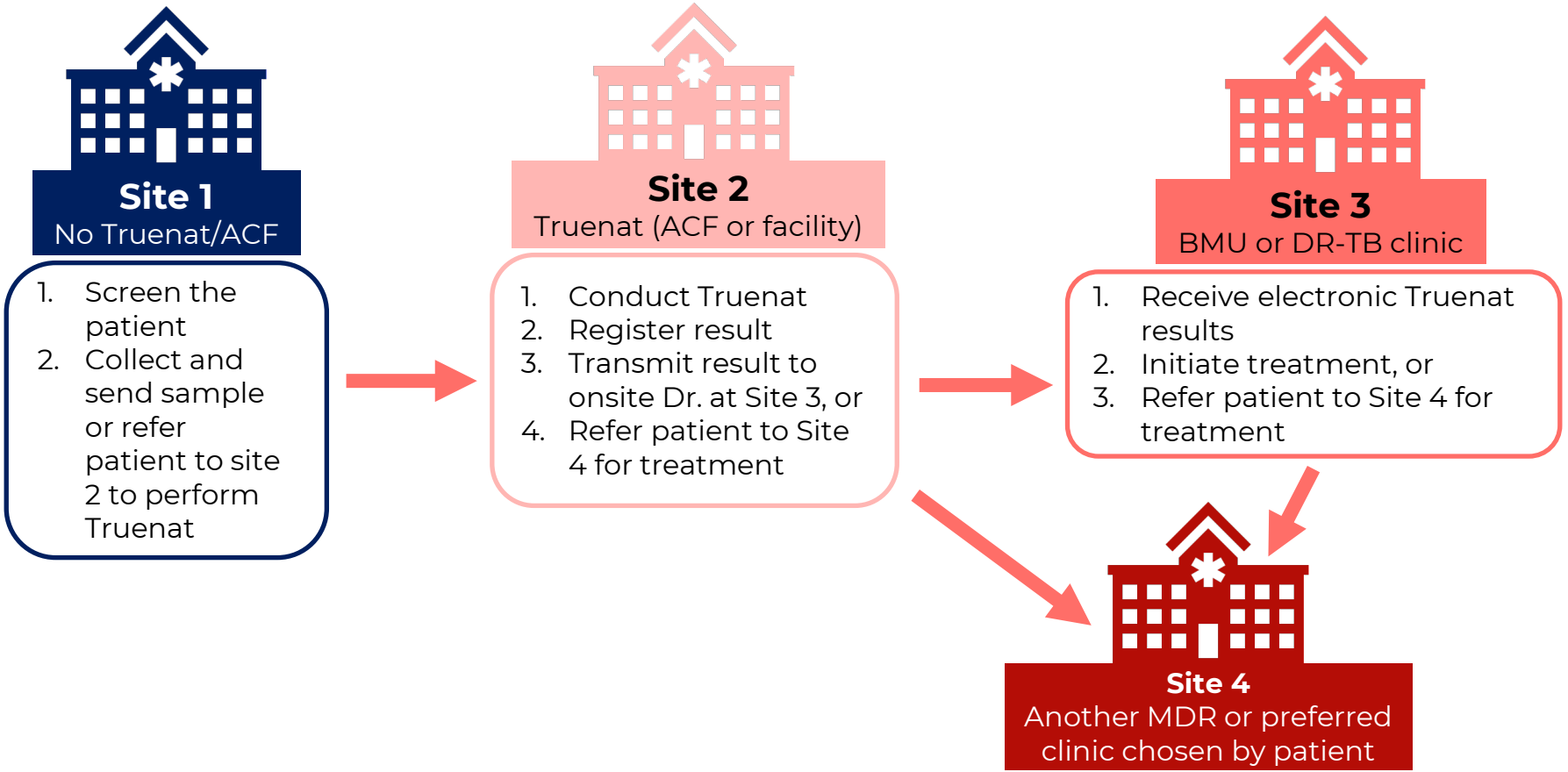
- Patient flow will vary based on where the Truenat is placed in the diagnostic network, i.e., at point of care or in peripheral labs.

- Extremely important that patients are linked to additional testing as needed, treatment and care.

Questions to Consider About Patient Flow

1. How are patients referred for TB screening and testing?
2. Are patients referred from the community to the facility for TB testing?
    - Or are samples collected at the community level and transported to the facility for testing?
    - Or is testing conducted at the community level and TB patients referred to the facility for TB/DR-TB treatment initiation?
3. How are results transmitted back to the clinician/facility/patient?
4. How are diagnosed patients referred for TB/DR-TB clinical monitoring?

Example Patient Referral Pathway



Procedures for Patient Transfers and Referrals

If TB services are available at the same facility

- Escort patient to care and treatment
- Provide care and treatment site with test results

If TB services are not available at the same facility

- Provide TB patient with a written referral to care and treatment facility
- Counsel patient on need for immediate treatment
- Call TB care and treatment facility to alert them of referral and transmit test results electronically
- Provide name and contact information for patient and date of positive test result
- Follow up with patient and treatment facility

Digital Results Reporting

- The Truelab instrument has built-in software for digital results reporting, when a SIM card is used with a data bundle

- Digital results reporting can be used to:
  - Send test results to clinicians
  - Send information about performance and issues to Molbio (error reads, sample processing information)
  - Send data to national servers for surveillance purposes

- Third-party connectivity software platform (Aspect and DataToCare) companies are currently working to allow for smooth flow of data to these platforms

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 Algorithm

- The algorithm shows how to use Truenat.

Patient Transfer or Referrals

- The procedures for patient transfer or referrals will be country specific. In cases of TB-positive results the patient must be referred to TB care and treatment services.

Knowledge Check – Question 1

1. What are the three Truenat tests and how should they be used?

Knowledge Check – Question 2

2. What are the three main parts of Truenat's algorithm?

Knowledge Check – Question 3

3. What two things should happen after a patient tests positive for TB with a Truenat TB test?

THANK YOU


UNITED STATES AGENCY INTERNATIONAL DEVELOPMENT
USAID
USAID
FROM THE AMERICAN PEOPLE


Stop TB Partnership

MODULE 3: OPERATIONAL ASPECTS

Truenat® Tests for the Detection of TB and Rifampicin Resistance
Central-Level Training

ACKNOWLEDGEMENTS

These training modules were developed as a collaboration between the United States Agency for International Development (USAID) and its Infectious Disease Detection and Surveillance project (IDDS) and the Stop TB Partnership, as part of the *introducing New Tools Project* (iNTP). The content is based on the Stop TB/USAID/GLI *Practical Guide to Implementation of Truenat™ Tests for the Detection of TB and Rifampicin Resistance*, together with content provided by Molbio Diagnostics (Module 3).

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

MODULE 3: OPERATIONAL ASPECTS- COURSE OUTLINE

01. Truenat TB PCR Testing

02. Equipment and Reagents

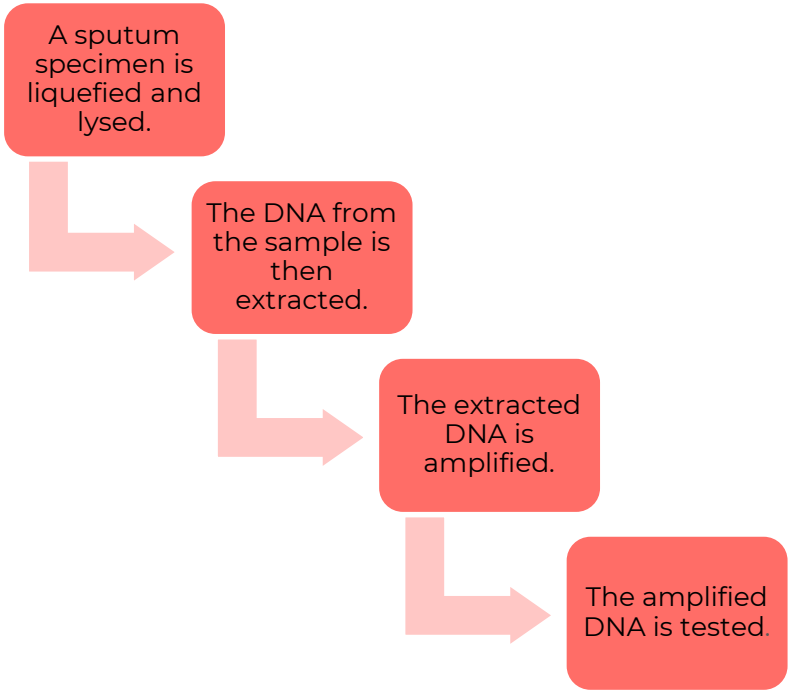
03. Truenat Test Procedures

04. Infrastructure Requirements

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.

Testing involves the following steps:

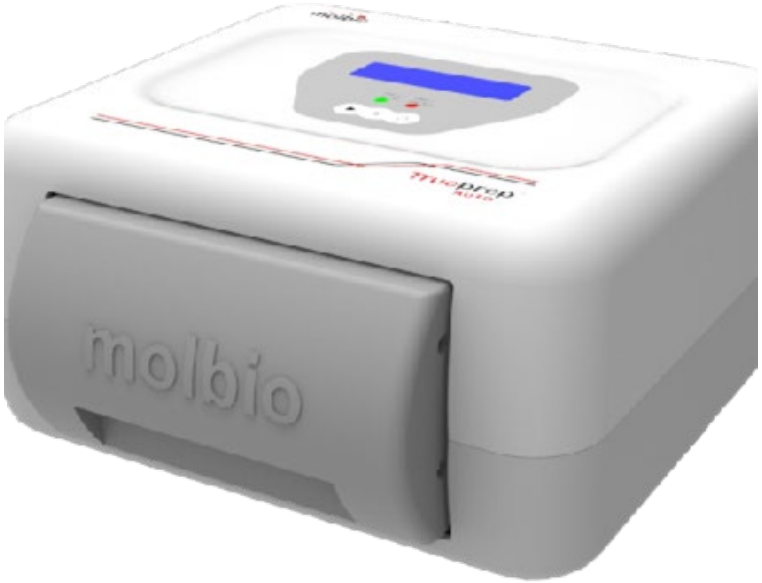


EQUIPMENT AND SUPPLIES

Equipment

Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Device (Trueprep)

- For automated extraction and amplification of DNA



Equipment

Truelab Real Time micro–PCR Analyzer

- For performing PCR
- Comes in three models:

Truelab® Uno Dx

Truelab® Duo

Truelab® Quattro

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

Equipment

Truelab micro-PCR printer

- Bluetooth printer, wirelessly prints the results of the PCR tests performed by Truelab® Uno Dx/ Duo / Quattro

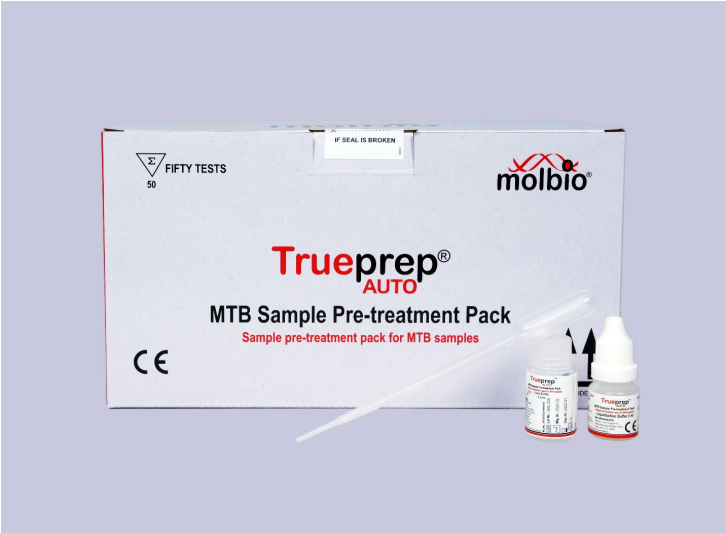
Reagents and Consumables

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

Trueprep® AUTO MTB Sample Pretreatment Pack

Contents:

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



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

Contents:

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

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

Contents:

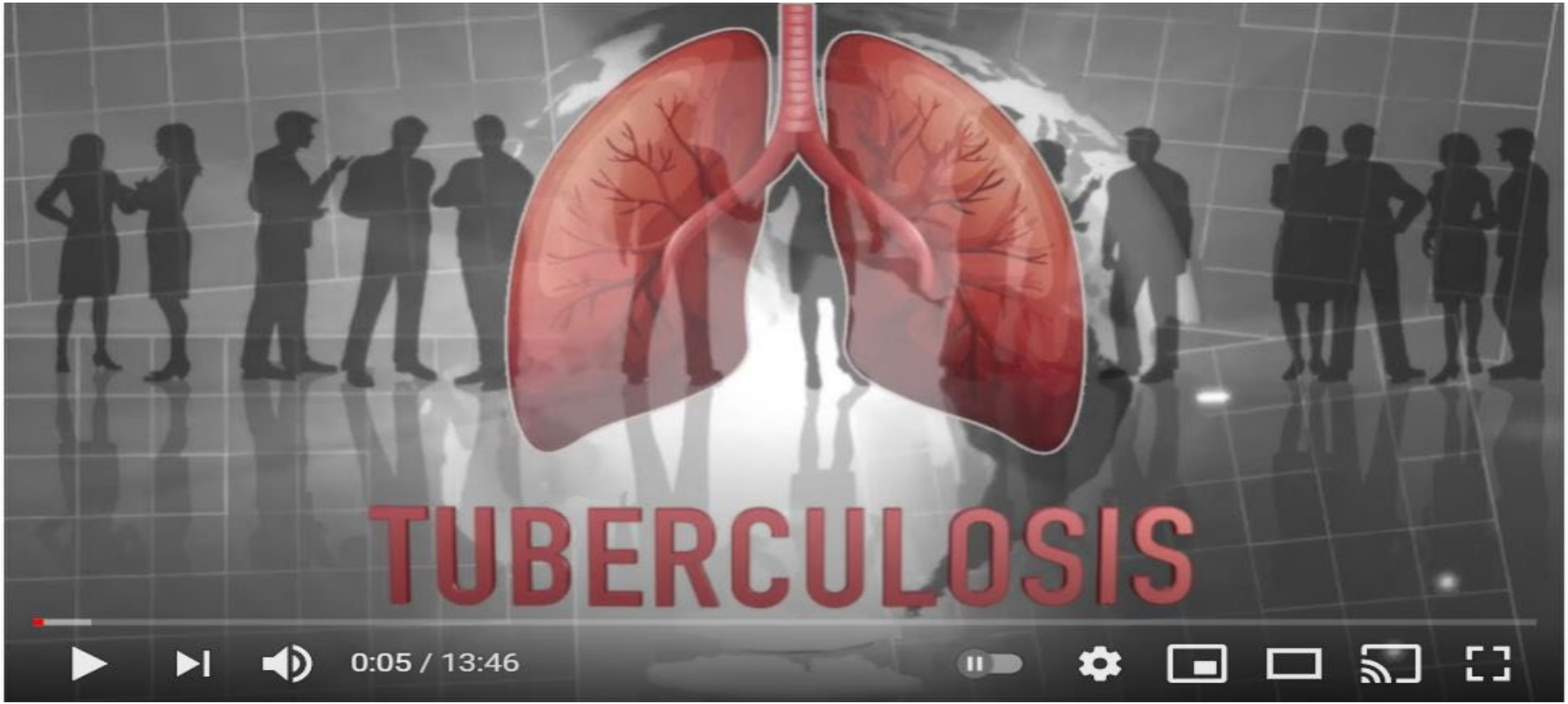
- Truenat chip pouches
- Microtube containing freeze-dried PCR reagents
- Filter barrier pipette tip
- Desiccant pouch

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

MTB	MTB Plus	MTB -RIF Dx
For the detection of TB	A more sensitive test for TB detection	For the detection of RIF resistance

TRUENAT TEST PROCEDURES – Prepare Samples and Extract DNA

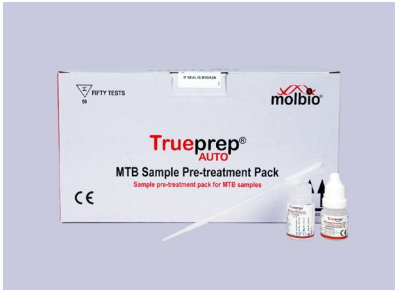
Video: Prepare Samples and Extract DNA



Prepare Sample and Extract DNA

Equipment

- Trueprep® AUTO MTB Sample Pretreatment Pack
  - Liquification buffer
  - Lysis Buffer
  - Disposable transfer graduated pipette - 1ml
  - Package Insert.



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

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)


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

Prepare Sample and Extract DNA

6 Transfer 0.5 ml of liquefied sputum sample into the lysis buffer bottle using a 1 ml transfer pipette (Figure 3).

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)

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.

Prepare Sample and Extract DNA

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)

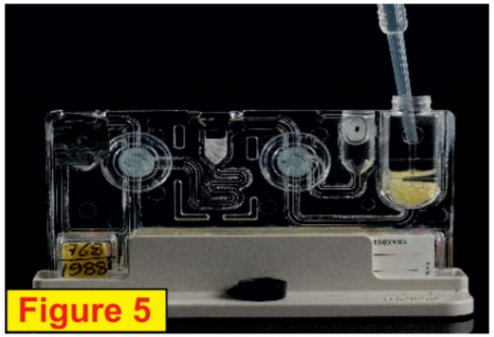


Figure 5

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


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.”

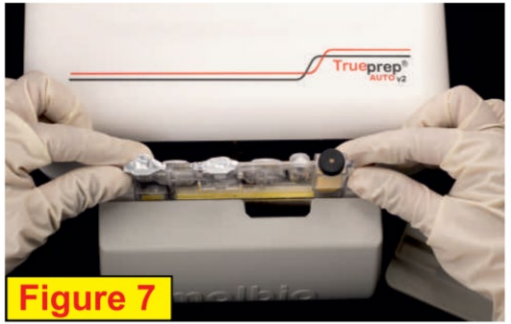


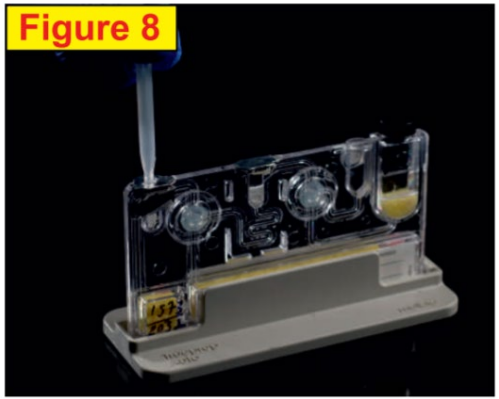
Figure 7

Prepare Sample and Extract DNA

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.



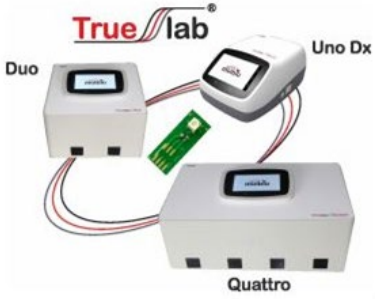
TRUENAT TEST PROCEDURES – Run a PCR TB Test

Video: Running a PCR TB Test

Equipment & Supplies for Running a PCR TB Test

Equipment

- Truelab microPCR Analyzer (Uno, Duo or Quattro)



molbio
Molbio Diagnostics Pvt. Ltd.

Supplies (Chip pack)

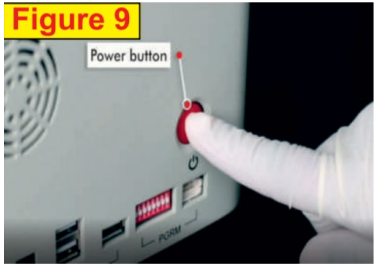
- MTB or MTB Plus Truenat chip
- Truepet 6µl Precision Micropipette

Process Flow

Running a PCR TB Test

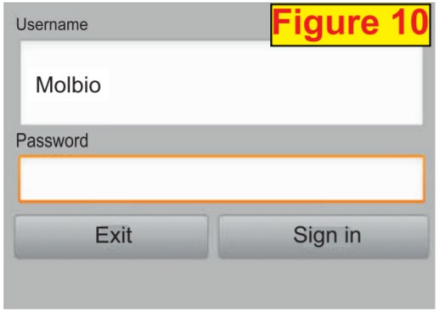
1 Switch “on” the Truelab microPCR analyzer by pressing the red button in the back right corner for 2 seconds. LED will glow in Green (Figure 9). Wait for 30-50 seconds for “boot-up screen” to appear followed by “home screen.”

Figure 9



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

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).

Figure 11

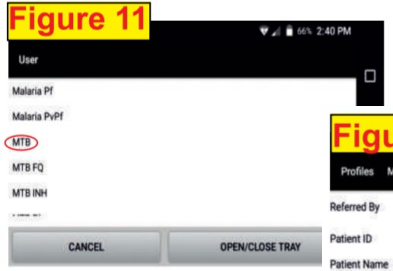
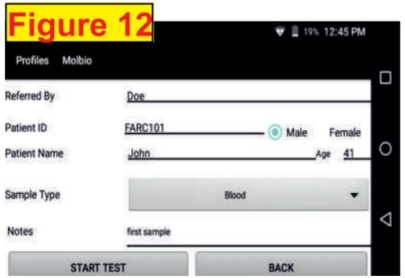


Figure 12



Process Flow

Running a PCR TB Test

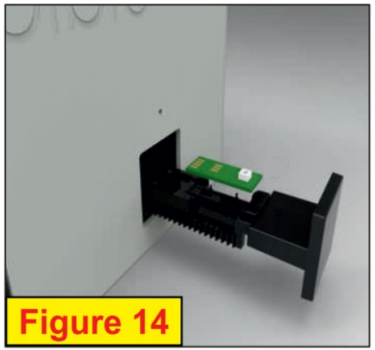
4 Select sample type (sputum).

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)



Process Flow

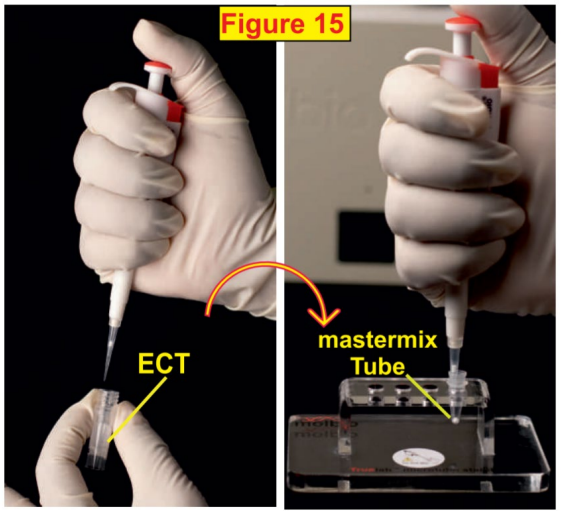
Running a PCR TB Test

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 6ul micro tip provided in the pouch to the single push pipette.

10 Transfer 6ul of the elute from ECT into the mastermix tube (Figure 15).



Process Flow

Running a PCR TB Test

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.

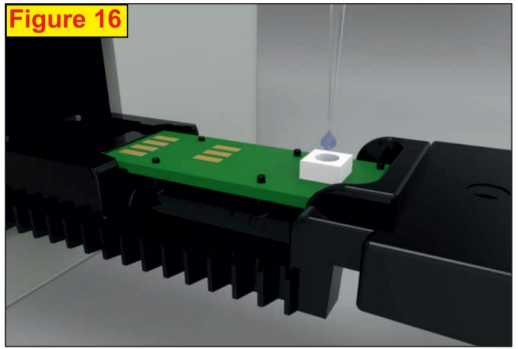
12 Transfer the elute from the mastermix tube to the white reaction well of the chip (Figure 16).

*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.



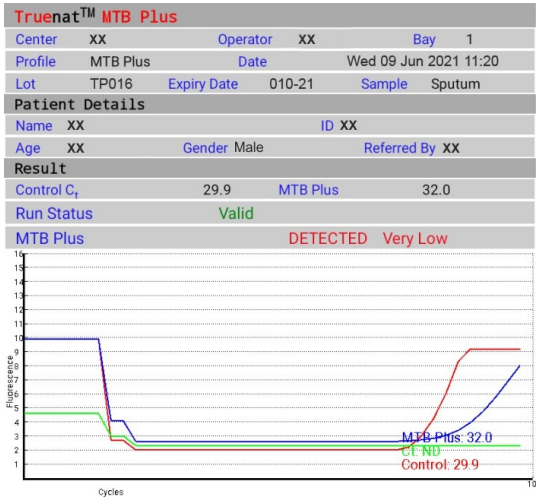
Process Flow

Running a PCR TB Test

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.

16 Optional: Press “Print” to print result page using Truelab® microPCR printer.

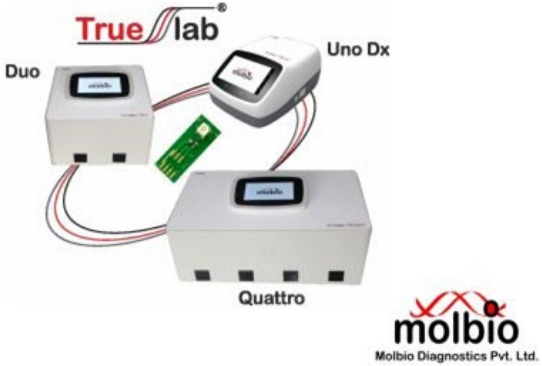


TRUENAT TEST PROCEDURES – Run a RIF Resistance Test

Running a RIF Resistance Test - Equipment

Equipment

- Truelab micro PCR Analyzer (Uno, Duo or Quattro)



Supplies (Chip pack)

- Truenat MTB-RIF Dx chip
- Truepet 6µl Precision Micropipette

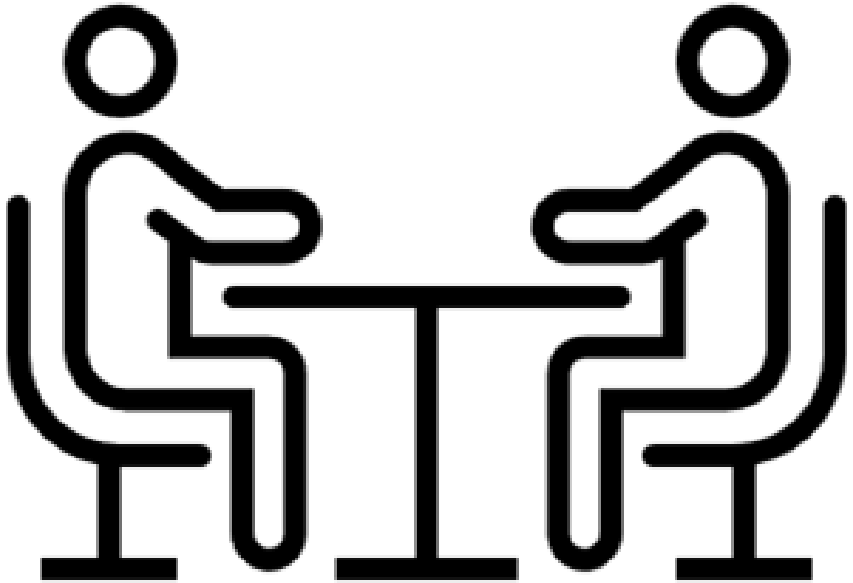
Process Flow

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

- Pair off
- One practice on equipment
- One observe and assist



WASTE MANAGEMENT

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

Waste disposal color code:

RED

Notes on Truenat Procedures – Waste Disposal

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

Waste disposal color code:

YELLOW

Notes on Truenat Procedures – Waste Disposal

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

Waste disposal color code:

BLACK

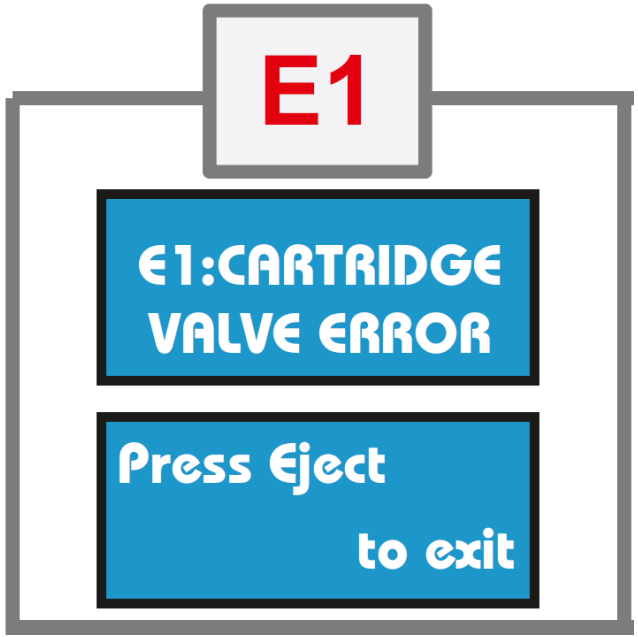
ERRORS AND TROUBLESHOOTING

Errors and Troubleshooting

Molbio has created a Troubleshooting, Alerts and Errors handout for Trueprep and Truelab (included)

- 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.

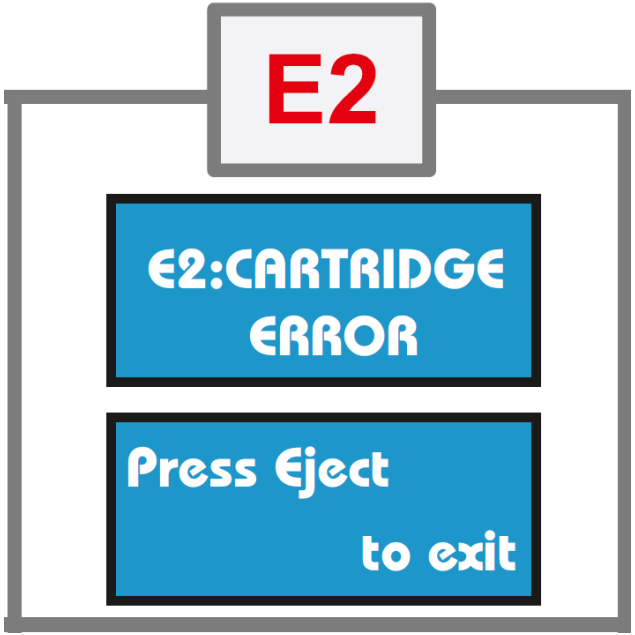
Trueprep Error Messages



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.

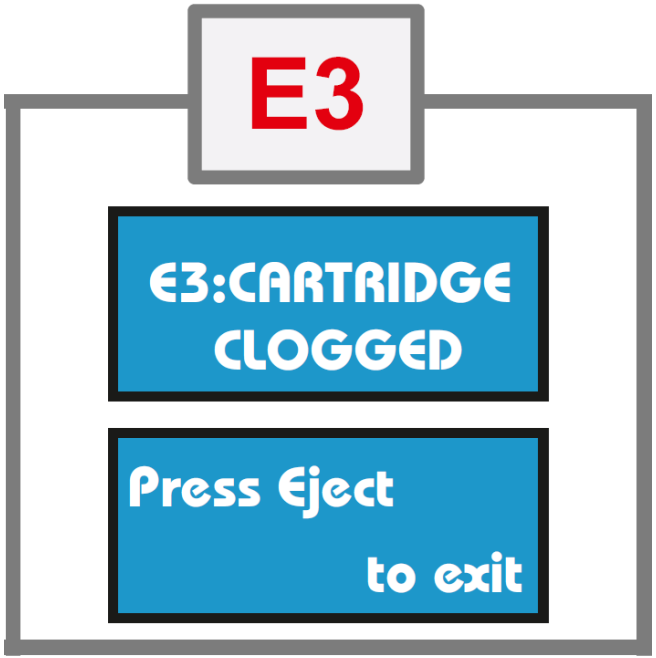
Trueprep Error Messages



Error 2: Cartridge Error

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

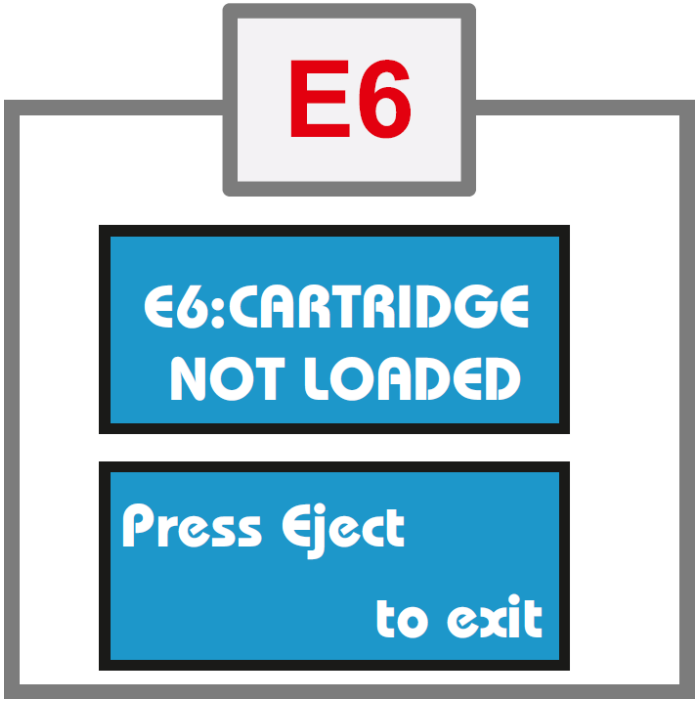
Trueprep Error Messages



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.

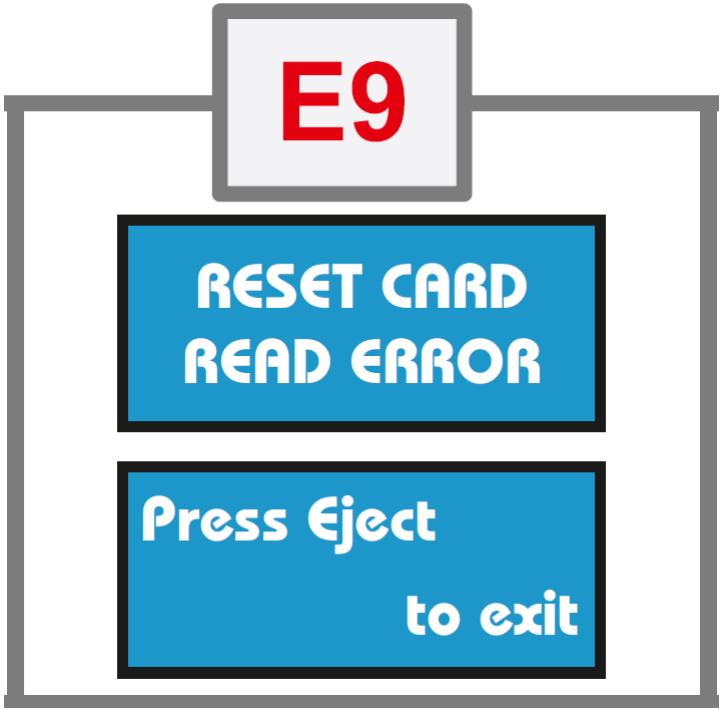
Trueprep Error Messages



Error 6: Cartridge not Loaded

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

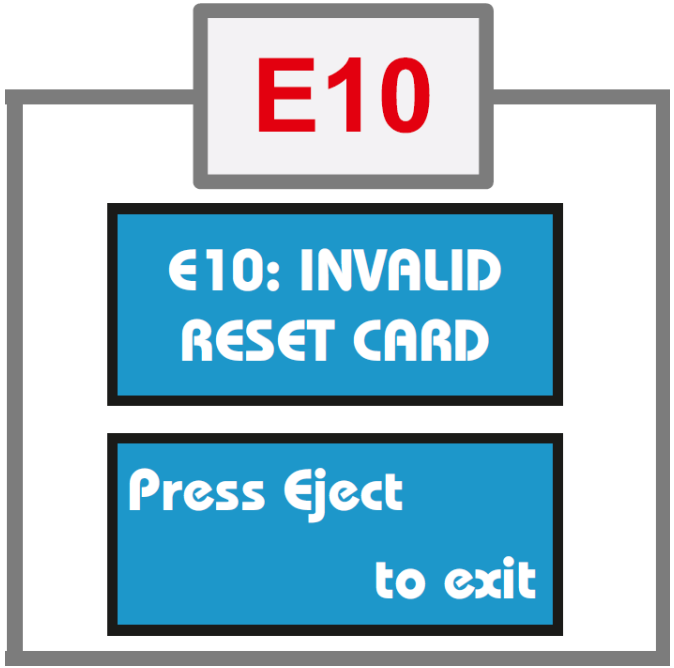
Trueprep Error Messages



Error 9: Reset Card Error

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

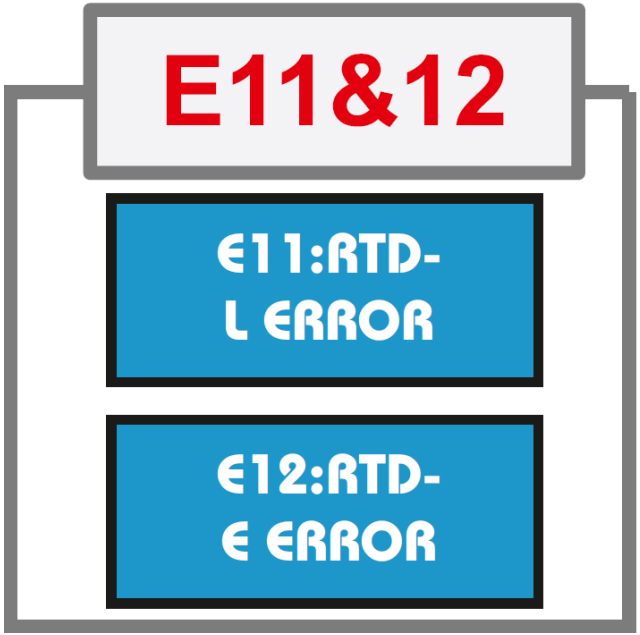
Trueprep Error Messages



Error 10: Invalid Reset Card

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

Trueprep Error Messages



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

Error 1: Thermal cycling error	Error 2: Test stopped manually	Error 3: Incorrect optical profile	Error 4: Runtime error	Error 5: Probe check error

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.

Truelab Error Messages

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

Unable to read chip information

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.

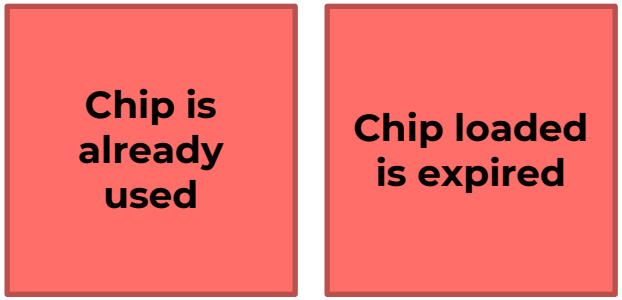
Truelab Alert Messages

Could not initialize. Please try 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.

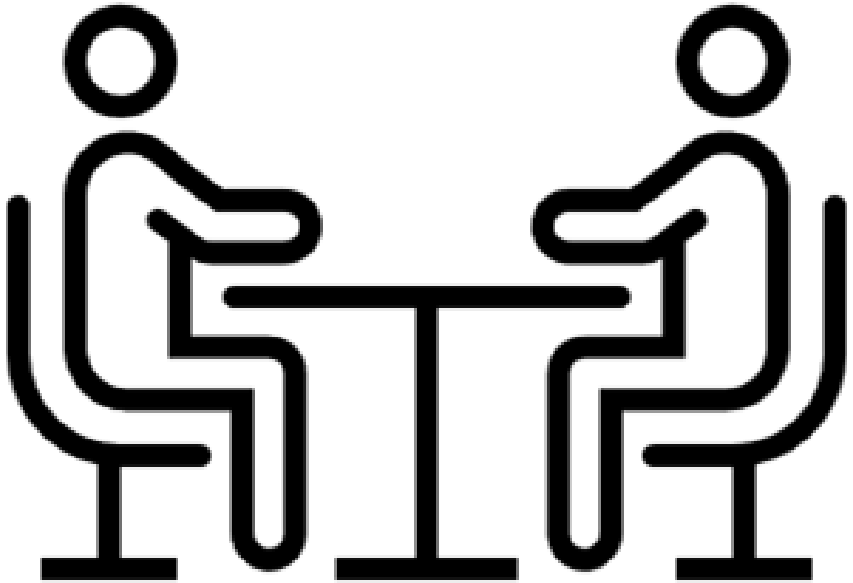
Truelab Alert Messages



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



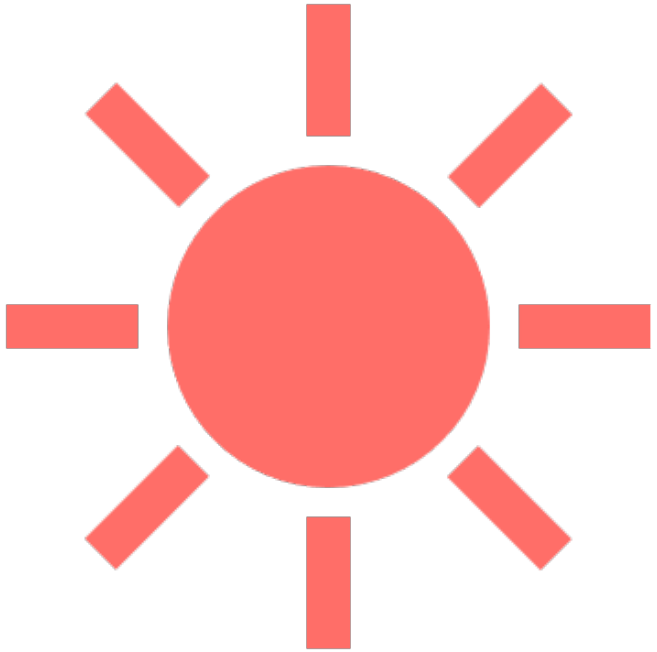
INFRASTRUCTURE REQUIREMENTS

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^{0} C
- Chips can be stored at up to 45^{0} C for up to 1 month and up to 40^{0} 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 or TB-LAMP

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

- Clean work area
- Discard used chips and cartridges

Monthly maintenance

- Disinfect instrument surfaces
- Clean Truelab bays
- Temperature calibration
- Verification of the fixed 6µl pipette

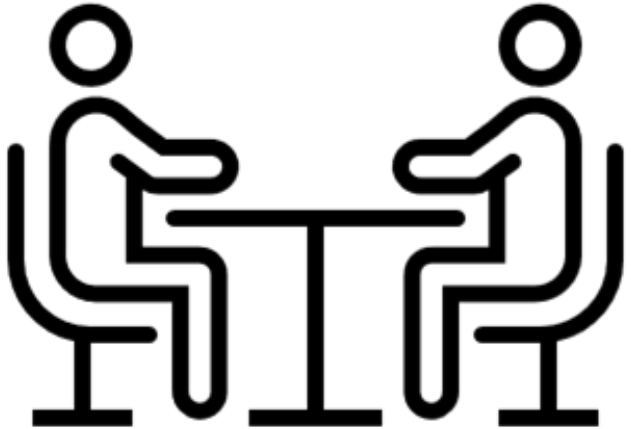
As necessary

- Flush protocol for the Trueprep instrument
- Spillage tray or linear motion guide tray replacement
- Slider glass replacement - indicate bay

An example of a Preventive Maintenance Log can be found in Annex 4 of the Truenat Implementation Guide

ACTIVITY –PREVENTIVE MAITENANCE

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



RECORDING TESTING ACTIVITIES

Recording Testing Activities

- May be necessary to make revisions to requisition forms (specimen examination request) for Truenat
- Laboratory and clinical registers may also need to be modified to record the results of Truenat tests
  - Forms and registers being used for the Xpert MTB/RIF test may be suitable for use with the Truenat TB tests.
    - Truenat tests generate the same type of information as Xpert (e.g., MTB detected or not detected)

Recording Testing Activities

**If lab request forms and registers, rejection logs, or other recording forms have already been developed – this is a placeholder for explaining them

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.

TERMINATION OF WARRANTY

The warranty shall be terminated at the end of the warranty period & also in the following cases:

- Where attempts to make repairs or alterations have been made by unauthorized person &/or with spare parts which are not originals.
- Alteration have been made to the serial number of the product on either the certificate or on the instrument.
- **The instrument is transferred to a new location without following the appropriate processes as per Installation Qualification (IQ)/ Operational Qualification (OQ)/ Performance Qualification (PQ) or prior written approval from Molbio Diagnostics Private Limited or Molbio Country Partners**
    - **In order to transfer an instrument to a new location without termination of the warranty, contact Molbio/Molbio local partners to inform them and take their assistance. Molbio/Molbio local partners will request that you confirm that the new site will conform with the pre-installation requisites (see next slide). The pre-installation requisites will need to be checked again once the transfer has taken place.**
    - **This point about transferring to a new location does not apply to a situation when an end user has initially installed the instruments in a mobile vehicle that moves from place to place, and the instruments remain installed on the same bench in the mobile vehicle**

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 15°C and 40°C.
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:
  1. A liquefied lysed sputum specimen
  2. Extracting DNA from the sample
  3. Amplifying the extracted DNA
  4. Testing the amplified DNA
- Three pieces of equipment are used for Truenat:
  1. Trueprep
  2. Truelab (Uno, Duo, or Quattro)
  3. 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 – Question 1

1. There are _____ packets that include the reagents and consumables needed to run Truenat. What are they?

Knowledge Check – Question 2

2. What are the three versions of the Truelab Real Time micro–PCR Analyzer? What is the difference between them?

Knowledge Check – Question 3

3. What are the monthly maintenance requirements for Truelab and Trueprep equipment?

THANK YOU


UNITED STATES AGENCY INTERNATIONAL DEVELOPMENT
USAID
USAID
FROM THE AMERICAN PEOPLE


Stop TB Partnership

MODULE 4: ORDER PLANNING AND QUALITY ASSURANCE (QA)

Truenat® Tests for the Detection of TB and Rifampicin Resistance
Central-Level Training

ACKNOWLEDGEMENTS

These training modules were developed as a collaboration between the United States Agency for International Development (USAID) and its Infectious Disease Detection and Surveillance project (IDDS) and the Stop TB Partnership, as part of the *introducing New Tools Project* (iNTP). The content is based on the Stop TB/USAID/GLI *Practical Guide to Implementation of Truenat™ Tests for the Detection of TB and Rifampicin Resistance*, together with content provided by Molbio Diagnostics (Module 3).

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

Module 4: Order Planning and QA and Control

Course Outline

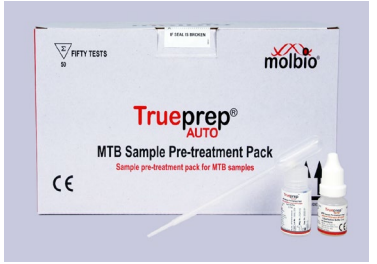
01. Forecasting and Quantification
02. Quality Assurance
03. Monitoring Quality
04. Summary

FORECASTING AND QUANTIFICATION

Reagents and Consumables

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

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?

Pricing

Pricing of equipment, reagents and service packages through Stop TB Partnership's Global Drug Facility (GDF) is outlined in the GDF Diagnostics Catalog

PRODUCT CODE	DESCRIPTION	NUMBER OF UNITS PER PACK	PRICE (IN USD)
EQUIPMENT			
106689	Truelab Uno Dx Workstation	1	10,000.00
106690	Truelab Duo Workstation	1	14,000.00
106691	Truelab Quattro Workstation	1	18,000.00

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

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: Regional Reference Laboratory							
Region: Western Region	Supply Quarter 3						
District: Urban	**Year:** 2021						
Total tests performed in previous quarter, including failed tests (A): 159							
Items	**Quantity Needed per Test (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

Stock Management

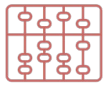
- 6 key components to maintain adequate stock of Truenat supplies

1. Do a monthly “stock count”
2. Maintain inventory records
3. Determine how much to order
4. Place orders in accor-dance with local ordering practices
5. Inspect and verify supplies received
6. Ensure proper storage of stock

Stock Management: Stock Log

Date and amount ordered

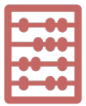
Date of receipt of item



Amount received

Lot number

Expiration date



Amount of used items

Balance of items still in stock

What fields should be included in a stock log for Truenat?

Stock Management: 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

Stock Management: Storage and Expiry

- Organize existing and new shipments by the expiry date

QUALITY ASSURANCE (QA)

QA and Control

What types of quality control processes do you currently institute in your lab that would be relevant to Truenat?

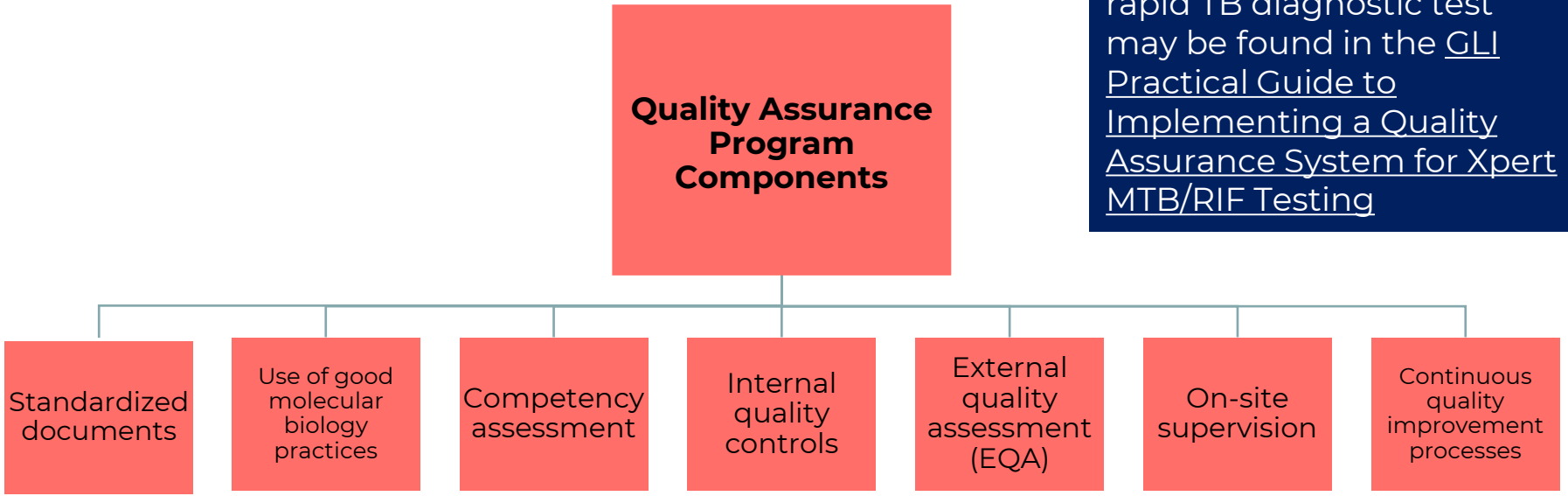
Roles in Ensuring Quality

- Laboratory manager is responsible for overseeing QA activities at larger facilities.

- The Health Facility Quality Committee (HFQC) may provide oversight and coordination for QA activities at larger facilities.

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 Practical Guide to Implementing a Quality Assurance System for Xpert MTB/RIF Testing



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

- Job aids are available in Annex 11 of the Truenat Implementation Guide:

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)

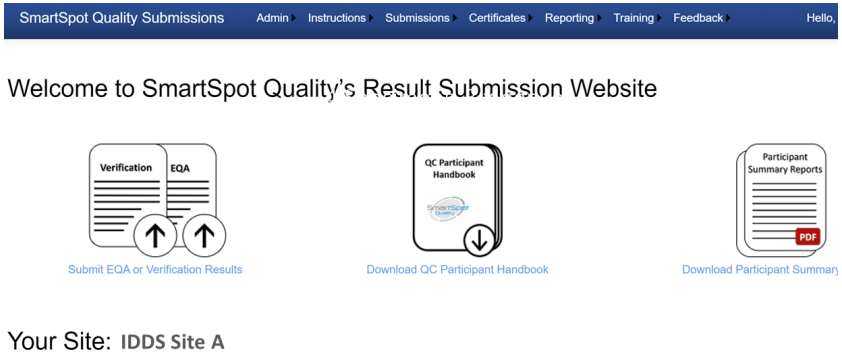
SmartSpot MTB External Quality Assessment (EQA)

- 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.

Cycle	Dispatch Date	Results Due
Cycle1	23 Feb 2022	March 1-31, 2022
Cycle 2	18 July 2022	August 1-31, 2022
Cycle 3		November 1-30, 2022

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

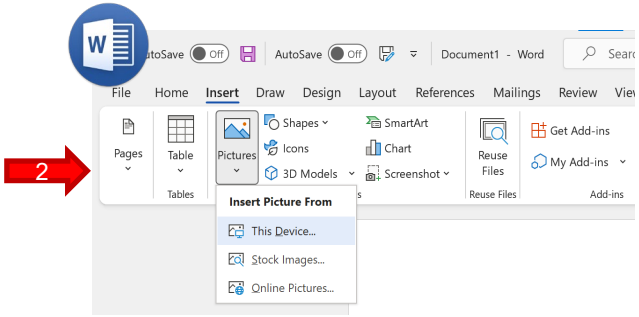
EQA Reporting: How to Export Results

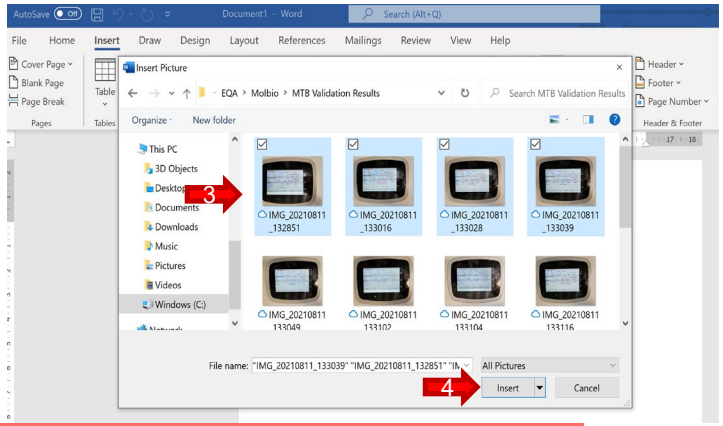
1. Capture an image of each result OR transfer the results of all four EQA specimens to a PC via Bluetooth or email

2. Open a Microsoft Word Document and select *INSERT PICTURE FROM THIS DEVICE*

3. Select the 4 EQA result images

4. Insert the 4 EQA result images



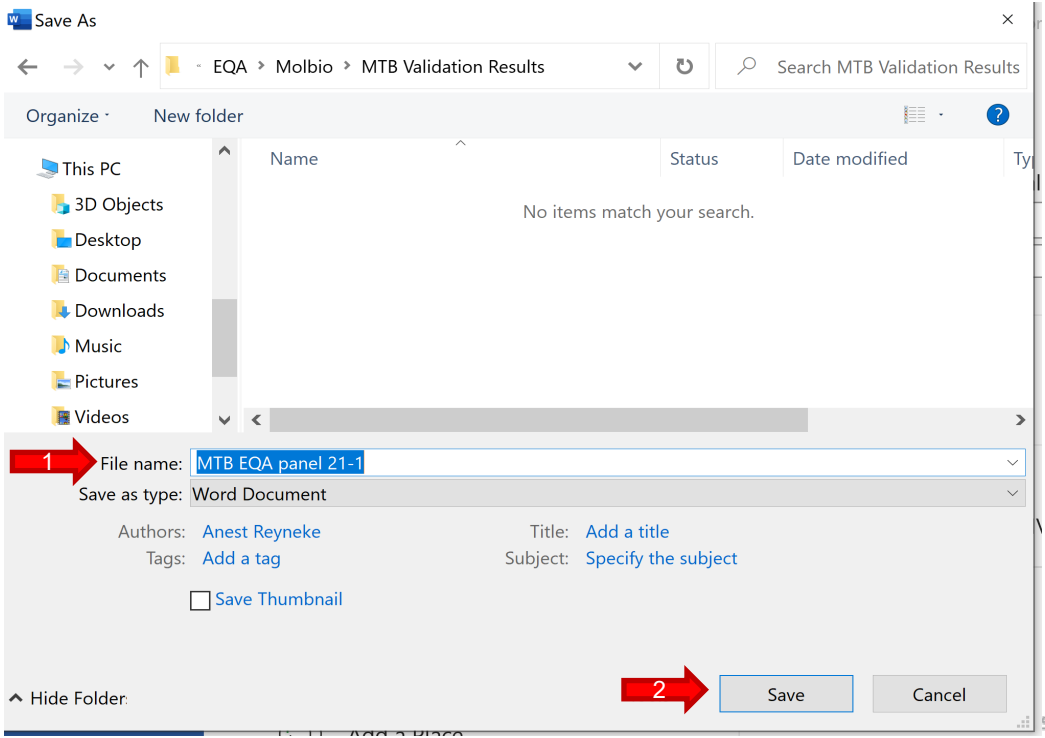


EQA Reporting: How to Export Results

Save the Word Document.

1. Change the file name to the EQA type and EQA panel number

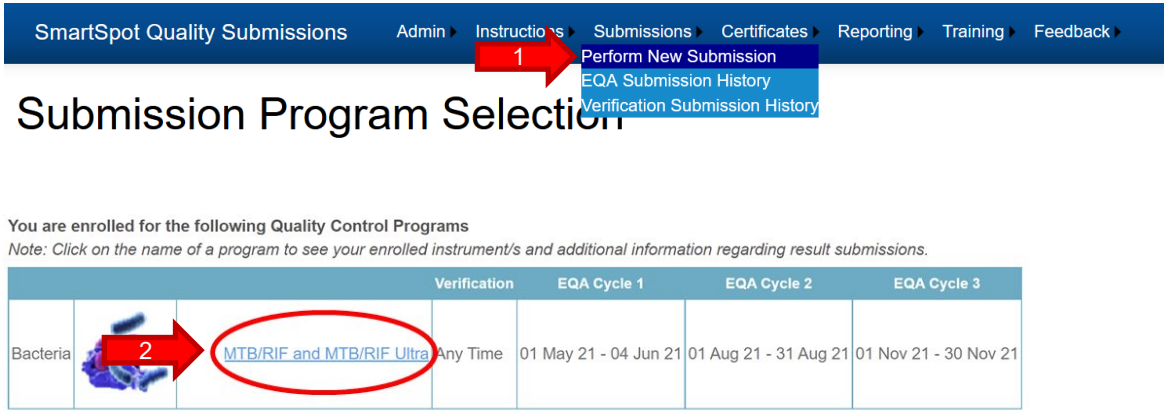
2. Click SAVE



EQA Reporting: How to Submit Results

1. Go to SUBMISSIONS > PERFORM NEW SUBMISSION

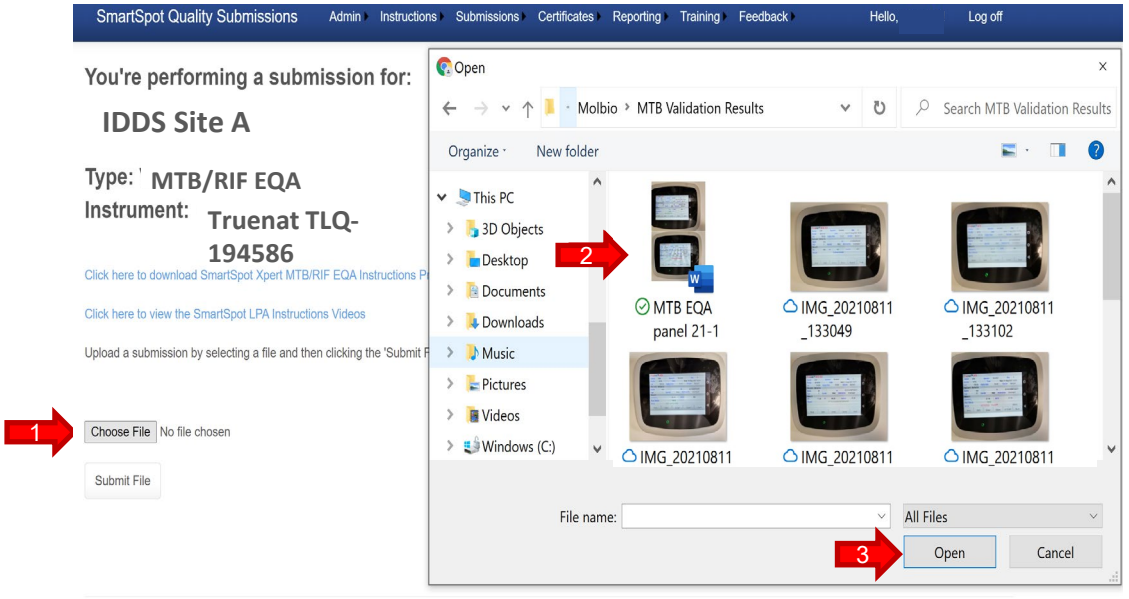
2. Select MTB/RIF Assay



EQA Reporting: How to Submit Results

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

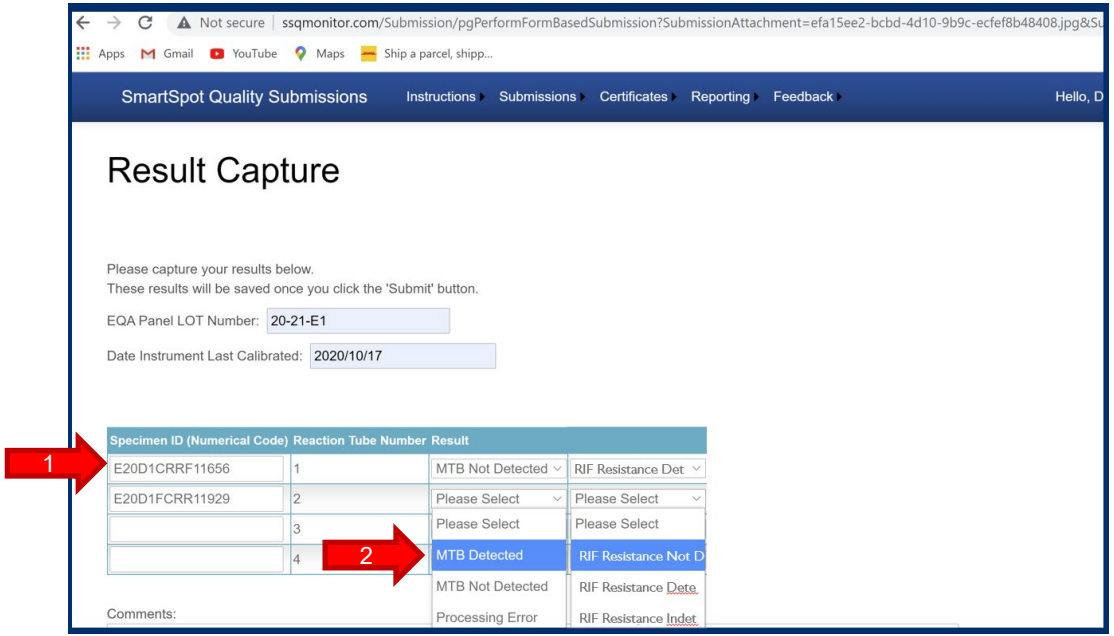


EQA Reporting: How to Submit Results

Capture final result interpretation for each sample

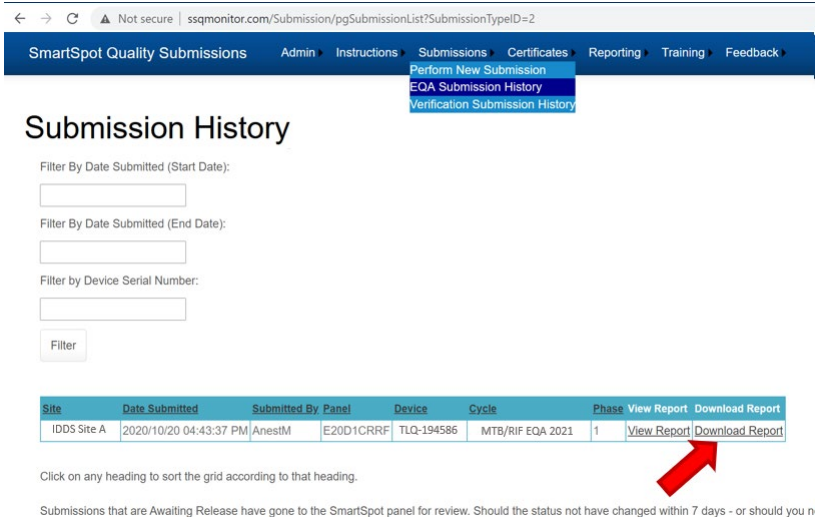
1. Fill in the EQA Specimen IDs

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

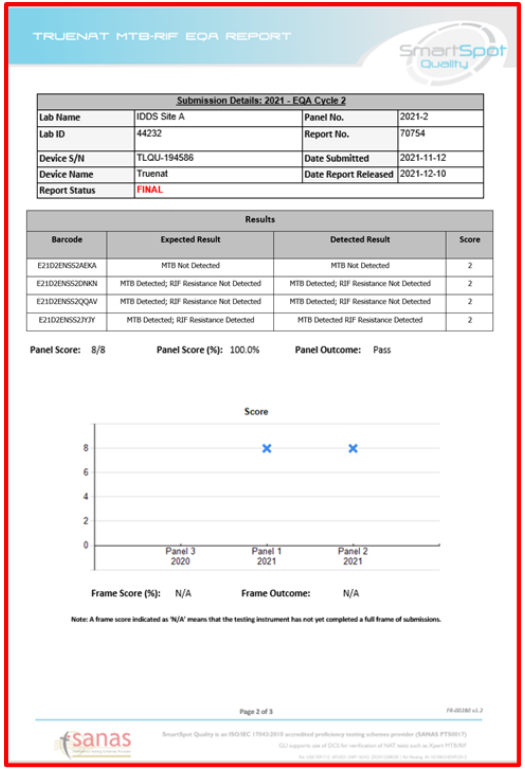


EQA Final Report

- 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



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
- For each new test kit lot
- 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. Swab testing of work surfaces and instruments

3. Record preventative maintenance in a log

Warranty and Repair

- 1-, 2-, 3-, 4-, and 5-year extended warranties are available from Molbio for US $1,220 per year through GDF
- Comprehensive maintenance contract includes:
  - Remote assistance/visit of service engineer
  - Repair and replacement of parts
  - In country travel and labor of company’s local agent
  - Calibration chips and material which is used as service items

MONITORING QUALITY

General Laboratory Performance Indicators

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

Which of these indicators do you already track for other tests?

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%

Performance Indicators for Truenat TB Tests

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

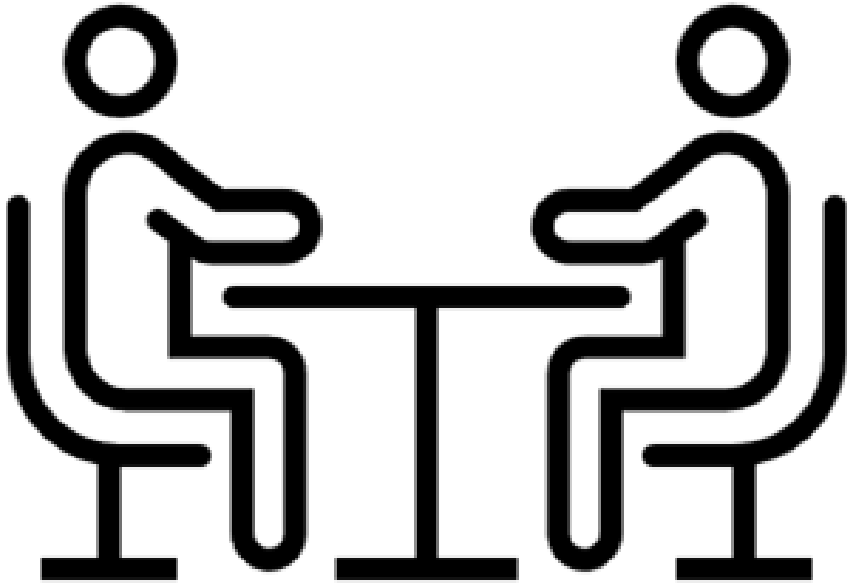
Performance Indicators for Monitoring Patient and Sample Flow

Indicator	Description	Target
Laboratory Turnaround Time		
Laboratory turnaround time	Time between receipt of specimen at the laboratory and result reporting	2-24 hours
Turn-around time: sample collection to sample testing		
Turn-around time: results availability to treatment initiation		
Linkage to Care		
Proportion of TB and RR-TB patients linked to appropriate care		

What targets would you set for these?

Class Activity

- Team up
- 10 minutes to work through
- Present your 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 – Question 1

1. What information is needed to determine how many supply packs to order each quarter?

Knowledge Check – Question 2

2. What are some practices to ensure good stock management?

Knowledge Check – Question 3

3. What are the main components of a quality assurance program?

Knowledge Check – Question 4

4. What valuable information can be provided when participating in an EQA program?

THANK YOU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Stop TB Partnership

MODULE 5: MONITORING & EVALUATION

Truenat® Tests for the Detection of TB and Rifampicin Resistance
Central-Level Training

ACKNOWLEDGEMENTS

These training modules were developed as a collaboration between the United States Agency for International Development (USAID) and its Infectious Disease Detection and Surveillance project (IDDS) and the Stop TB Partnership, as part of the *introducing New Tools Project* (iNTP). The content is based on the Stop TB/USAID/GLI *Practical Guide to Implementation of Truenat™ Tests for the Detection of TB and Rifampicin Resistance*, together with content provided by Molbio Diagnostics (Module 3).

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.

Module 5 Learning Objectives

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

- Outline a general approach to monitoring and evaluating the impact of Truenat on TB-related targets and goals.

Module 5: Monitoring & Evaluation

Course Outline

01. M&E for Truenat

02. Indicators

M&E FOR TRUENAT

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

Number and proportion of presumptive TB patients that have been tested with a WRD

Proportion of the population that has access to WRD within a 5-kilometer distance

Number and proportion of presumptive TB patients that are evaluated for TB (i.e., reach a diagnostic center)

Number and proportion of presumptive TB patients that reach a diagnostic center and for whom a TB test is ordered

Number and proportion of presumptive TB patients for whom a test is ordered and who provide a specimen for testing

Other Possible Impact Indicators

Number and proportion of presumptive TB patients for whom a specimen is collected and whose specimen is received at the testing laboratory

Number and proportion of presumptive TB patients whose specimen is received at the testing laboratory and for whom a test is conducted

Number and proportion of presumptive TB patients for whom a test is conducted and for whom test results are reported to the clinician

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

Proportion of specimens collected for WRD testing for which a result was received by the ordering clinician within the specified target time (i.e., time from collection of a specimen to receipt of results

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

Monitoring Outcomes and Impact

Key Indicators

- Key indicators and milestones to monitor the implementation process of Truenat should be identified at the outset.

- High-level checklist in Annex 2 of the Truenat Implementation Guide may be useful for monitoring implementation and introduction of Truenat.

- Utilization (the rate or number of tests ordered) of Truenat testing services should be tracked once the instruments are introduced to determine if clinical staff at all sites that should offer the test, actually order the test.

M&E Forms for Truenat

- Placeholder for countries to describe changes to M&E processes that are being introduced as a result of Truenat introduction.

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.

Knowledge Check – Question 1

Complete the chart with the associated WHO target.

WHO Indicators for Laboratory Strengthening	WHO Target
Percentage of notified bacteriologically confirmed TB cases with DST results for RIF	
Percentage of notified RIF-resistance TB cases with DST results for fluoroquinolones and second-line injectable agents	
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	
Percentage of notified new and relapse TB cases tested with a WHO-approved rapid diagnostic test (WRD) as the initial diagnostic test	
Percentage of notified new and relapse TB cases with bacteriological confirmation	

Knowledge Check – Question 2

Name one other impact indicator that you think is important to monitor and explain why.

THANK YOU


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MODULE 6: BIOSAFETY AND SPECIMEN COLLECTION AND REFERRAL

Truenat® Tests for the Detection of TB and Rifampicin Resistance Central-Level Training

ACKNOWLEDGEMENTS

These training modules were developed as a collaboration between the United States Agency for International Development (USAID) and its Infectious Disease Detection and Surveillance project (IDDS) and the Stop TB Partnership, as part of the *introducing New Tools Project* (iNTP). The content is based on the Stop TB/USAID/GLI *Practical Guide to Implementation of Truenat™ Tests for the Detection of TB and Rifampicin Resistance*, together with content provided by Molbio Diagnostics (Module 3).

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In no event shall the authors be liable for damages arising from its use. Development of this document was made possible with financial support from the United States Agency for International Development. The views expressed herein are those of the authors and do not necessarily reflect those of the U.S. Agency for International Development or the U.S. Government.

Introduction

This module introduces biosafety measures and procedures for specimen collection and referral.

These measures are specific to use of the Truenat rapid molecular test

Information in this module is informed by the Global Laboratory Initiative's "Laboratory Safety" handbook.

Module 6: Specimen Collection and Referral

Course Outline

01. Introduction
02. Biosafety Measures and Risk
03. Specimen Collection
04. Specimen Referral
05. Summary

Learning Objectives

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

- Demonstrate good biosafety practices and risks when performing Truenat testing
- 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
- Understand the process for specimen referral

BIOSAFETY MEASURES AND RISK

GENERAL PRINCIPLES OF BIOSAFETY

Biosafety has three key parts, all of which are needed to handle TB bacilli safely:

- **Primary:** Safe working practices to minimise creation of infectious aerosols and prevent spills Equipment that is ‘fit for purpose’, correctly used and maintained
- **Secondary**: Infrastructure and layout to support the primary activities
- **Tertiary**: Buildings to contain the laboratory and its activities

IMPORTANCE OF BIOSAFETY

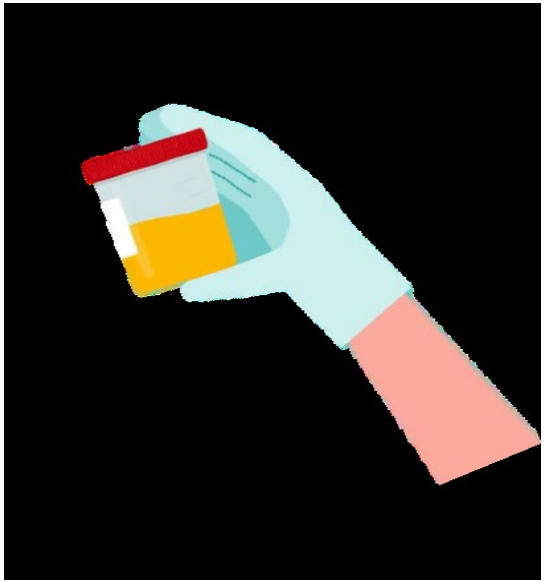
Laboratories present numerous hazards to staff, and not all are immediately obvious. Safe working practices are designed to

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

The main procedural risk in a TB laboratory is the generation of aerosols

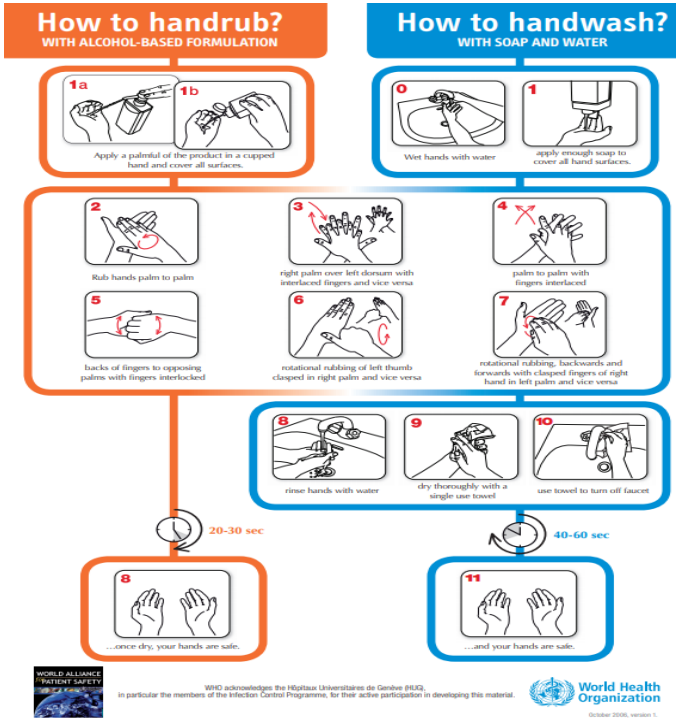
The level of biosafety risk depends on:

- Type of procedure
- Workload
- Consistency
- Bacillary load



STANDARD MICROBIOLOGICAL PROCEDURES

- Practices that are common to all laboratories
- These practices must include
  - Not eating, drinking, or applying cosmetics in the laboratory
  - Washing hands after working with infectious materials and before leaving the laboratory
  - Routinely decontaminating work surfaces



ADDRESSING BIOSAFETY

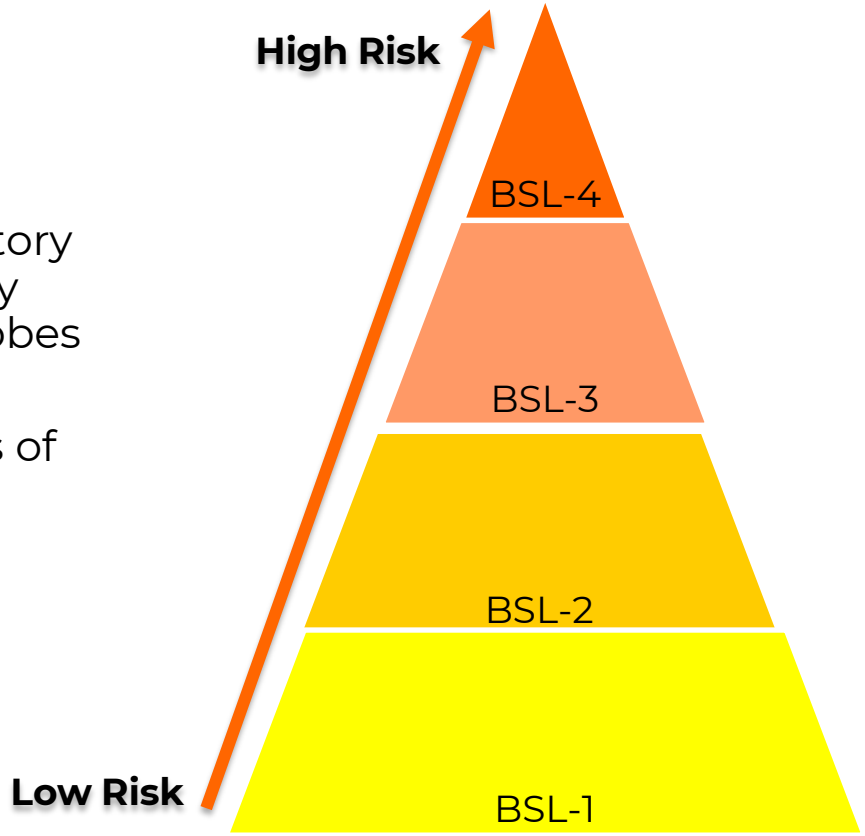
This module will help you:

- Understand and assess the risks
- Laboratory infrastructure, design and layout
- Personal protective equipment
- Biological safety cabinets
- Generation and prevention of aerosols
- Spills
- Waste management

ASSESSING RISK

RISK BIOSAFETY LEVELS

- There are four biosafety levels (BSLs).
- Each level has specific controls (laboratory practices, safety equipment, and facility construction) for containment of microbes and biological agents.
- The primary risks that determine levels of containment are:
  - Infectivity
  - Severity of disease
  - Transmissibility
  - Nature of the work conducted
  - Origin of the microbe or the agent in question
  - Route of exposure



CHARACTERISTICS OF BIOSAFETY LEVELS

Biosafety level		Examples
BSL-4	• Microbes are dangerous and exotic, posing a high risk of aerosol-transmitted infections. • Infections are frequently fatal without treatment or vaccines. • All work with the microbe must be performed within an appropriate Class III Biological Safety Cabinets (BSC), or by wearing a full body protection suit with gloves, mask, and eye protection.	• Ebola • Marburg viruses
BSL-3	• Microbes can be either indigenous or exotic. • They can cause serious or potentially lethal disease through respiratory transmission. • Laboratorians are under medical surveillance and might receive immunizations for microbes they work with. • Access to the laboratory is restricted and controlled at all times.	Tuberculosis
BSL-2	• Microbes pose moderate hazards to laboratorians and the environment. • Microbes are typically indigenous and associated with diseases of varying severity.	• COVID-19 • HIV
BSL-1	• Non-pathogenic organisms or non-aerosol pathogens. • Wear personal protective equipment (PPE), work on open laboratory bench, follow standard microbiological practices.	Non-pathogenic strain of E.coli

BIOSAFETY MEASURES ACCORDING TO RISK LEVELS

	PPE		BSC	Facility	Lab access
BSL-4	Wearing full body protection and air supplies		• All work performed within an appropriate Class III BSC *or* • Wearing a full body suite and air supplies		• Change clothing before entering • Shower upon exiting • Decontaminate all materials before exiting
BSL-3	Yes + face shield and respirator		• All work done under the Class II BSC • Autoclave or an alternative method of decontamination for proper disposal	• Hands-free sink eyewash near exit • Exhaust air cannot be recirculated	Entrance to the lab is through two sets of self-closing and locking doors
BSL-2	Yes + face shield		Procedures that can cause infection from aerosols or splashes are performed within a Class I BSC	• Self-closing • Sink and eyewash	Access to lab limited to when performing work
BSL-1	Standard microbiological practices are followed		Work can be performed on an open laboratory bench or table		

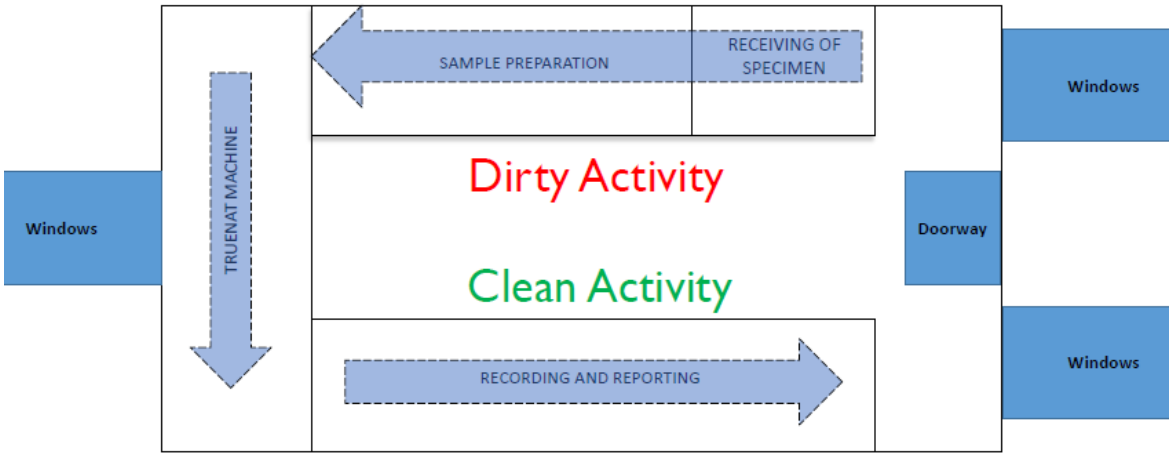
RISK PRECAUTION LEVELS

Risk level of TB	Laboratory activities	Assessment of risk
Low risk	Preparation of specimens for TB microscopy or Xpert MTB/RIF TrueNAT	• Low risk of generating infectious aerosols from specimens • Low concentration of infectious particles
Moderate risk	• Processing and concentration of specimen for Xpert MTB/RIF • Inoculation on primary culture media; splitting specimens; direct DST (e.g., LPA on processed sputum)	• Moderate risk of generating infectious aerosols from specimens • Low to moderate concentration of infectious particles
High risk	• Culture manipulation for identification • DST or LPA on cultured isolates	• High risk of generating infectious aerosols from specimens • High concentration of infectious particles

LABORATORY INFRASTRUCTURE

VENTILATION AND LABORATORY SETUP

- A biosafety cabinet is not essential for Truenat testing on sputum.
- When climate prevents windows from being opened, considerations should be given to using mechanical ventilation systems, e.g., exhaust fans



PERSONAL PROTECTIVE EQUIPMENT

MATRIX OF RECOMMENDED PPE ACCORDING TO ACTIVITY

Activity	Who should wear	Gloves	Disposable lab gown*	Dedicated lab and / or shoe cover	Head cover	Face shield / goggles	Fit-tested N95, N100, P100 respiration**
Specimen Collection (If going with patient to collect)***	Collection unit staff	Double gloves recommended	Recommended		Based on risk assessment	Based on risk assessment	Recommended
Specimen packaging	Collection unit staff	Double gloves recommended	Recommended	Recommended	Based on risk assessment	Based on risk assessment	Recommended
Receiving of Sealed Specimen Package and Accompanying Documents	Laboratory staff	Recommended	Recommended				Based on risk assessment (Face mask may be used as substitute based on risk assessment)
Specimen Transport	Specimen transporter	Recommended					Based on risk assessment (Face mask may be used as substitute based on risk assessment)

MATRIX OF RECOMMENDED PPE ACCORDING TO ACTIVITY

Activity	Who should wear	Gloves	Disposable lab gown*	Dedicated lab and /or shoe cover	Head cover	Face shield / goggles	Fit-tested N95, N100, P100 respiration**
Unboxing of Specimen Package for Specimen Receiving, Sorting and Verification	Laboratory staff	Double cloves Recommended	Recommended	Recommended	Based on risk assessment	Based on risk assessment (Recommended BSC)	Recommended
Specimen Processing	Laboratory staff	Double cloves Recommended	Recommended	Recommended	Recommended	Based on risk assessment (Recommended BSC)	Recommended
Decontamination of specimen	Laboratory staff	Double cloves Recommended	Recommended	Recommended	Based on risk		Recommended

PROPER ORDER FOR DONNING PPE

1. Laboratory shoes
2. Primary gown: lab coat
3. Secondary gown: disposable
4. Fitted respirators
5. Bouffant cap
6. Inner gloves
    -*Nitrile
7. *Face shield / goggles
8. Outer gloves
    -*Nitrile
9. Shoes cover

* Optional / recommended

PERSONAL PROTECTIVE EQUIPMENT: GLOVES & SHOES

Gloves:

- Disposable, powder-free
- In light of the COVID-19 pandemic, the use of double gloves is *recommended*
- Wearing gloves can give technicians a false sense of security
- Do not reuse or wear gloves outside of the laboratory

Regular and thorough handwashing is most essential

Shoes:

- Shoes must cover the toes, upper part of the feet, and have closure to the back of the heel
- Dedicated laboratory shoes should be available in the facility

PERSONAL PROTECTIVE EQUIPMENT: LABORATORY GOWN & COAT

Laboratory gown:

- Appropriate size
- Buttoned
- Covers the whole arm with elastic cuff
- Length should be up to knee when standing and should cover entire lap when sitting

Laboratory coat:

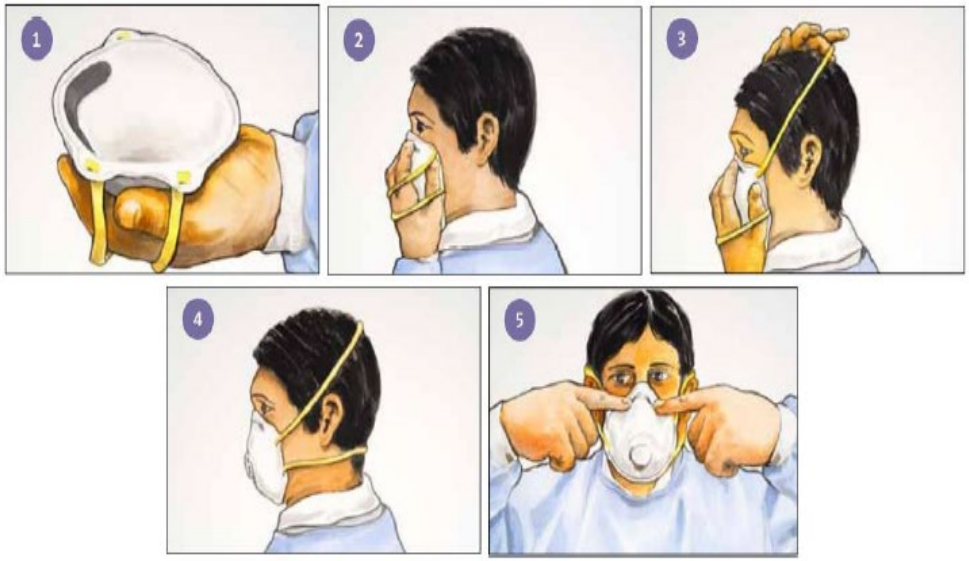
- Leave coats at worksite
- Fasten coat when worn
- Do not wear outside of lab
- Disinfect before laundry

PERSONAL PROTECTIVE EQUIPMENT: RESPIRATORS & MASKS

- Due to the current pandemic, the use of respirators is now recommended.
- Respirators must be fitted to the face (fit-testing)
- Surgical masks do not provide protection to the wearer against inhaling infectious aerosols
- Respirators protect the wearer from inhaling droplet nuclei.
- Masks prevent the spread of microorganisms from the wearer.

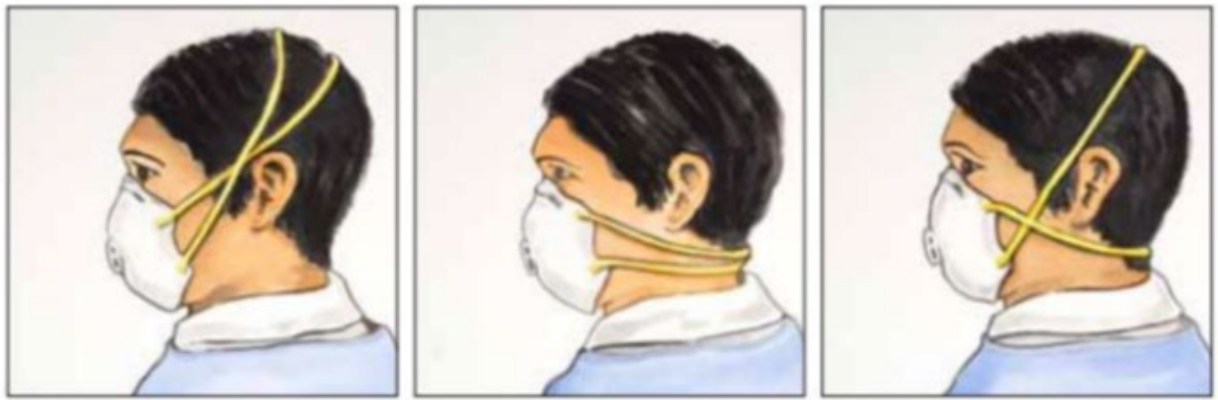
PERSONAL PROTECTIVE EQUIPMENT: RESPIRATORS & MASKS

Properly wearing a respirator



PERSONAL PROTECTIVE EQUIPMENT: RESPIRATORS & MASKS

Common mistakes in wearing respirators



Both straps above the ears

Both straps below the ears

Straps crossed

PERSONAL PROTECTIVE EQUIPMENT: RESPIRATORS & MASKS

Properly removing a respirator

Locate the lower headband strap and pull over the head : release strap tension and hold both straps in one hand

Locate the upper headband strap and pull over the head: release strap tension and hold the strap in one hand

Remove the respirator away from the face and hold both straps in one hand

PERSONAL PROTECTIVE EQUIPMENT: FACE SHIELDS & GOGGLES

Due to the COVID-19 pandemic, the use of face shields and goggles is recommended when there is no BSC in the facility.

BIOSAFETY CABINETS

BIOSAFETY CABINETS

Biosafety cabinets are categorized as Class I, Class II or Class III

- Class II BSCs draw around 70% of purified air from the HEPA filter above the work area and around 30% air through the front grille.
- Class II provides protection for the user, environment and the work area.
- There are four types of Class II: A1, A2, B1 and B2
    - Class II, Type A2 BSCs are recommended for all TB work; however, they are not necessary for Truenat

PERFORMING TESTS WITHOUT BSC

Point of care assays, like Truenat, can be performed on the bench without BSC when local risks dictate that the following conditions are met:

1. Absorbent liner
2. Well-ventilated windows
3. Appropriate PPE
4. Well-trained staff on GMP
5. No rush or increase pressure in TAT
6. Validated infectious process is in place

GENERATION AND PREVENTION OF AEROSOLS

GENERATING AEROSOLS

In the TB laboratory, all aerosols should be considered as potentially infectious. Aerosols are capable of being inhaled and establishing infection. Once they settle onto a surface, they are not re-aerosolised, and are no longer infective. However, they may contaminate specimens, equipment, consumables, and reagents creating a cross-contamination risk.

High-risk procedures and practices that may increase the potential of creating aerosols (which then become droplet nuclei) include

- Mechanical (vortexing, centrifugation, shaking)
- Pouring/tipping
- Pipetting

MINIMIZING AEROSOL FORMATION

Allow enough contact time of liquefaction buffer to sample, including adequate standing time.

This ensures:

1. Adequate liquefaction of samples will enable complete exposure of TB bacilli to lysis buffer
2. Pipettable consistency of the sample to prevent forceful pipetting
3. Settlement of aerosols possibly produced during swirling/gentle mixing

Updated Guidelines on Handling of Specimens for TB Testing in the Presence of Coronavirus Disease (COVID-19) Community Transmission

MINIMIZING AEROSOL FORMATION

1. Do not forcibly expel air from a pipette when aspirating the liquefied and lysed sputum sample

2. Place the pipette against the inner wall of the lysis buffer bottle when dispensing liquified sputum sample

3. Do not forcibly expel lysis buffer sample while dispensing into the cartridge

MANAGING SPILLS

MANAGING AND RESPONDING TO SPILLS

A spill outside of a BSC is a major incident and places staff at greatest risk

Spills usually involve liquids and aerosols of infectious droplet nuclei are generated

Even in the best laboratories, infectious spills do occur. Spill management procedures and training are a fundamental requirement for working safely in a TB laboratory. Management must ensure procedures are in place, reviewed regularly and that staff are trained in the use of the spills kit. Conducting an effective debrief and implementation of corrective actions will reduce the likelihood of future spills.

MANAGING AND RESPONDING TO SPILLS

Always keep two spill response kits in lockable containers in the laboratory.

Each kit will include a contents list detailing each item, the quantity, and expiry dates for stock solutions should be placed on the container lid and checked quarterly by staff

Recommended spill kit contents:

- Laboratory gowns (disposable) and goggles
- Box of gloves (different sizes) and respirators (N95/FFP2)
- Paper towel, cotton wool or absorbent cloth
- Soap and chloramine tablets
- Dustpan and sharp container

COMMONLY USED DISINFECTANTS FOR SPILLS

Disinfectant	Advantages	Disadvantages
Sodium hypochlorite • Should be used at a concentration of 10% in water • Solutions should be prepared daily • Chlorine residue can be removed with alcohol.	• Broad spectrum of antimicrobial activity • Readily available • Inexpensive • Property to decontaminate and degrade DNA	• Corrosive to metals • Inactivated by organic matter • Discolors fabrics • Unstable
Alcohol	• Does not leave residue on treated items • Can be used in BSC and bench top	• Volatile • Flammable • Does NOT decontaminate and remove DNA
Phenolic compounds • Should be used at a concentration of 10% in water	• Used for decontaminating equipment and single-use items prior to disposal	• Inhalation and dermal exposure irritates skin • Ingestion is considered toxic • Because of its toxicity and odor, phenol derivatives are generally used in place of phenol • Does NOT decontaminate and remove DNA

WASTE DISPOSAL

WASTE DISPOSAL

TB medical waste is category B and requires an autoclave or incinerate.

Be sure to follow country policies and guidelines.

Waste Type	Classification	Colour Coding	Description & Disposal Method
Infectious	Hazardous	YELLOW	Infectious waste which requires disposal by incineration.
Infectious	Hazardous	ORANGE	Infectious waste which may be treated to render safe prior to disposal or alternatively it can be incinerated.
Cytotoxic / Cytostatic	Hazardous	PURPLE	Waste consisting of, or contaminated with, cytotoxic and/or cytostatic products which requires disposal by incineration.
Offensive	Non-Hazardous	YELLOW & BLACK	Non-infectious, offensive/hygiene waste which may be recycled, incinerated or deep landfilled.
Anatomical	Hazardous	RED	Anatomical waste which requires disposal by incineration.
Medicinal	Non-Hazardous	BLUE	Waste medicines, out of date medicines, denatured drugs, which requires disposal by incineration.
Dental	Hazardous	WHITE	Dental amalgam & mercury including spent and out of date capsules, excess mixed amalgam & contents of amalgam separators which requires disposal by recovery or recycling.
Domestic	Non-Hazardous	BLACK	This waste should not contain any infectious materials, sharps or medicinal products, and requires disposal by landfill.

SAFE MANAGEMENT OF TB-CONTAMINATED MEDICAL WASTE

- Manage like any other regulated medical waste.
- Use typical engineering and administrative controls, safe work practices, and PPE to prevent exposure to the waste streams (or types of wastes);
    - Including any contaminants in the materials they manage
    - Measures can help protect workers from sharps and other items that can cause injuries or exposures to infectious materials.
- For regulated medical waste information, consult the regulated medical waste information in CDC's Guidelines for Environmental Infection Control in Health-Care Facilities. This document provides information on management of waste streams from hospitals and other healthcare facilities.

LABORATORY WASTE MANAGEMENT & DISPOSAL

- Where decontamination cannot be performed in the laboratory area or onsite:
    - Package the contaminated waste in an approved (sealed and leak-proof) manner for transfer to another facility with decontamination capacity.
- Handle waste from testing (suspected or confirmed) TB patients as all other biohazardous waste in the laboratory.
- Completely soak waste materials in a 1:10 dilution of household bleach
- Disposal should be carried out following existing local and national regulations on management of healthcare waste

All materials used should be considered contaminated!

MENTAL CHECKLIST FOR BIOSAFETY AND RISK MANAGEMENT:

- ✓ Correct PPE
- ✓ Adequate collection supplies
- ✓ Collect the appropriate specimen
- ✓ Use appropriate collection material/equipment
- ✓ Practice IPC during/after collection
- ✓ Label specimen
- ✓ Decide where to test
- ✓ In house or refer? Referral contact and process
- ✓ Adequate material for storage
- ✓ Adequate material for packaging and shipping
- ✓ Labels and documentation
- ✓ Appropriate handling of medical waste

USEFUL BIOSAFETY RESOURCES

1. **WHO Tuberculosis Laboratory Biosafety Manual**
Available at: https://www.who.int/publications/i/item/9789241504638

2. WHO Laboratory Biosafety Manual, 4th Edition
Available at: https://www.who.int/publications/i/item/9789240011311

3. **CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th Edition**
Available at: https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf

SPECIMEN COLLECTION

SPUTUM SPECIMEN COLLECTION: CONTAINER SPECIFICATIONS

To be customized by each country

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

- Insert a picture of container used by the country

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!

SPUTUM SPECIMEN COLLECTION: PATIENT EDUCATION AND INSTRUCTIONS

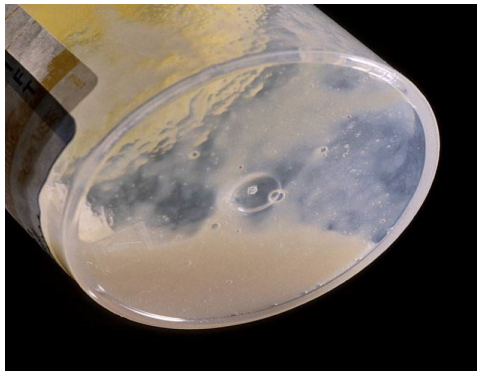
- Saliva or nasal secretions are unsatisfactory
- Specimens should not contain food or other particles
- Patients should be instructed to take the following steps to produce the best specimen:

    1. Wash your mouth with clean water to remove food and other particles
    2. Inhale deeply 2–3 times and breathe out strongly each time
    3. Cough deeply from your chest to produce sputum
    4. Place the open container close to your mouth to collect the specimen; do not get sputum on the outside of the container
    5. Wash your hands after collecting the sample

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

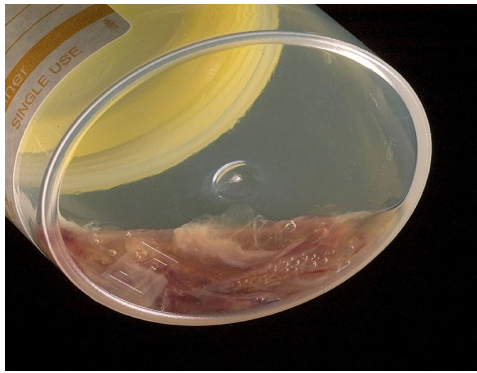
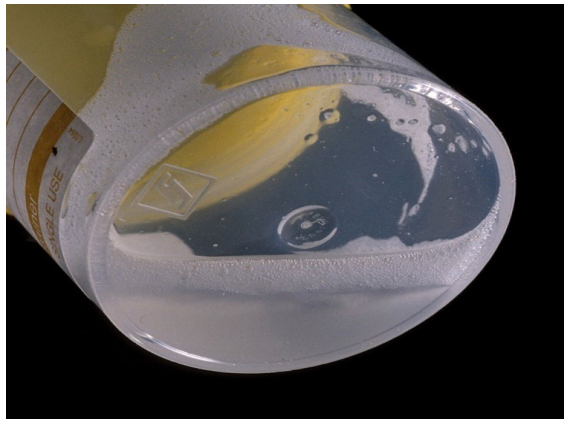


Photo credit A. van Deun

SPUTUM SPECIMEN COLLECTION: POOR QUALITY

- Poor quality specimens will give poor quality results



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

Photo credit A. van Deun

SPUTUM SPECIMEN COLLECTION: LABELLING

- Label the container with the patient's name, identification number, and date of collection
- 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, they can be packaged and sent to the Truenat testing site.

SPUTUM SPECIMEN PACKAGING: TRIPLE-PACKAGING TO ENSURE BIOSAFETY

Primary packaging

- Wrap the leak-proof container in cotton wool or paper towels in a sufficient quantity to absorb the entire contents in case of leaks

Secondary packaging

- Place the wrapped container inside a secondary container, such as a self-sealing plastic bag or another container
- Place secondary container in a rack to prevent leakage

Tertiary packaging

- Place the secondary container and its contents in an approved safety cooler box or another appropriate container in an upright position
- Place a biohazard sign – with markings and labelling appropriate for the specimen category – on the tertiary container

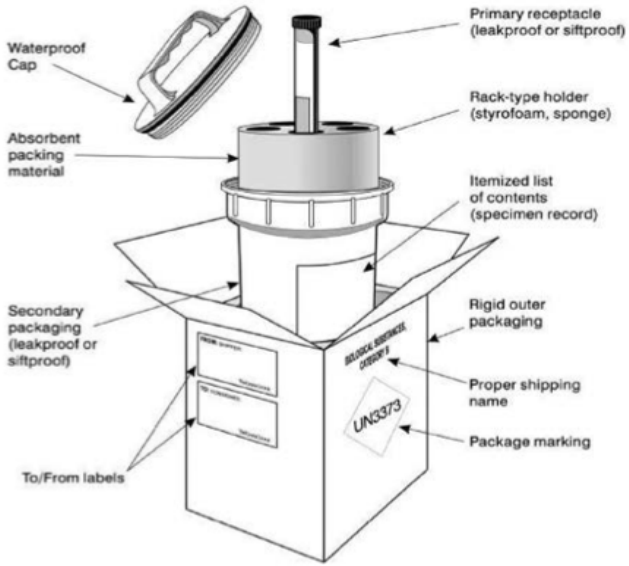


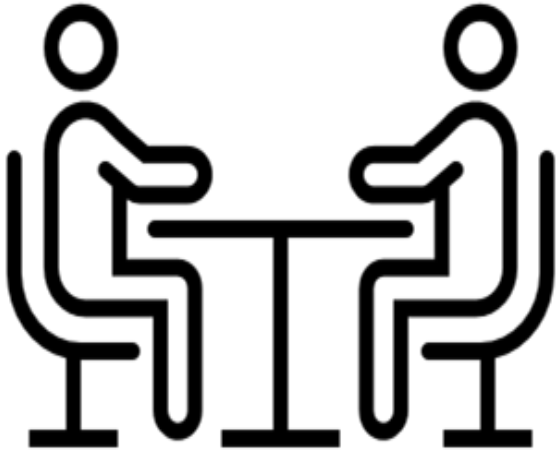
Fig. 3 Example of triple packaging for Category B infectious substances (IATA) from *Guidance on regulations for the transport of infectious substances* 2015. World Health Organization.

Packaging and Storing the Specimen

- Specimens should be stored in a refrigerator or a cool box between 2°C to 8°C and transported to the Truenat testing site.
- Specimens should be well parceled in a specimen flask or transportation box
- During transportation, specimens should be kept between 2°C to 8°C in ice packs.
- Once specimens reach Truenat sites, specimens needs to be brought to room temperature before processing with Trueprep® AUTO MTB sample pre-treatment pack.

Class Activity

- Pair Off
- Place the index cards in order based on the steps in the specimen collection process.
- One observe and assist



SPECIMEN REFERRAL

Lab Forms

- National programs should tailor this content to show what the actual lab forms look like and walk through how they should be filled in.

Integrated Specimen Referral Systems

- A specimen referral system may need to be developed for Truenat.
- Truenat testing should be incorporated into larger diagnostic network specimen referral system.
- GLI's Guide to TB Specimen Referral Systems and Integrated Networks provides guidance on establishing integrated solutions for specimen referral.

Results Reporting

- Electronic reporting of digital results should be used where available followed by paper reporting.
- Truenat testing sites must ensure that results are transmitted back to requesting facility.

Questions to Consider about Sample Flow

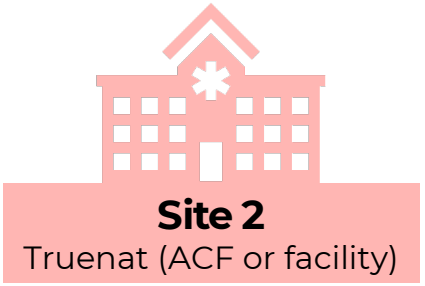
- How are patients referred for TB screening and testing?
- Are patients referred from the community to the facility for TB Testing?
- Are specimens collected at the community level and transported to the facility for testing or are they testing at the community level and TB patients referred to the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

Specimen Referral System-Example

Site 1
No Truenat/ACF

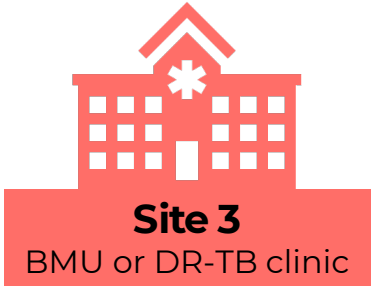
- Screen the patient
- Send specimens for testing (Site 2)

Initiate treatment



Site 2
Truenat (ACF or facility)

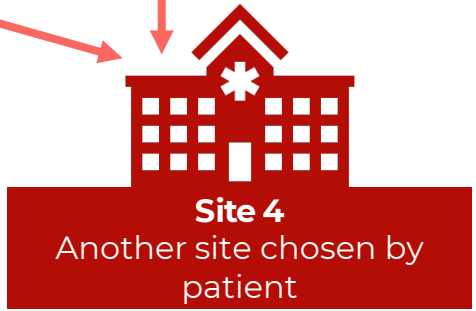
- Detect TB
- Request 2nd specimen to NTRL for culture / DST
- Electronic transmission of results to either Site 1, 3, or 4 *register result



Site 3
BMU or DR-TB clinic

- Receive electronic Truenat results
- Initiate treatment or
- Refer patient to (site 4) another facility for Rx

If RR-TB or DS-TB patient cannot be treated at site 1:
Electronically send or print results and attach to referral form with patient to where patient has been referred (TB BMU or DR-TB clinic).



Site 4
Another site chosen by patient

SUMMARY

Biosafety

- Biosafety is the safe handling and containment of infectious microorganisms and hazardous biological materials.
- The main procedural risk in a TB laboratory is generation of aerosols.
- Use of engineering controls, PPE, and administrative controls are important components of TB laboratory biosafety.

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.

Knowledge Check – Question 1

What equipment is needed for specimen collection?

Knowledge Check – Question 2

At what temperature / where should specimens be stored before being pre-treated?

At what temperature should specimens be stored after being pre-treated?

Knowledge Check – Question 3

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?

THANK YOU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Stop TB Partnership