

# Table of Contents

[Table of Contents 2](#_gjdgxs)

[Introduction 6](#_30j0zll)

[Training Schedule 6](#_1fob9te)

[Customizing this Training 6](#_3znysh7)

[Training Format 7](#_2et92p0)

[Facilitator Preparation 7](#_tyjcwt)

[Course Introduction 8](#_3dy6vkm)

[Module 1: Introduction to Truenat 9](#_1t3h5sf)

[Target Audience 9](#_4d34og8)

[Learning Objectives 9](#_2s8eyo1)

[Materials 9](#_17dp8vu)

[Advance Preparation 9](#_3rdcrjn)

[Lesson Plans 10](#_26in1rg)

[Introduction 10](#_lnxbz9)

[TB Context 11](#_35nkun2)

[Plenary Session 14](#_1ksv4uv)

[TB Laboratory Tests 14](#_44sinio)

[Placement of Truenat in Diagnostic Networks 16](#_2jxsxqh)

[Diagnostic Accuracy of Truenat 18](#_z337ya)

[Activity: Which Test Should I Use 21](#_36ei31r)

[Summary 22](#_3j2qqm3)

[Module 2: Diagnostic Algorithm and Results Interpretation 26](#_1y810tw)

[Target Audience 26](#_4i7ojhp)

[Learning Objectives 26](#_2xcytpi)

[Materials 26](#_1ci93xb)

[Advance Preparation 26](#_3whwml4)

[Lesson Plans 26](#_2bn6wsx)

[Introduction 27](#_qsh70q)

[WHO Recommendations 28](#_3as4poj)

[Truenat Algorithm 30](#_1pxezwc)

[Activity: Truenat Algorithm 36](#_49x2ik5)

[Patient Flow 36](#_2p2csry)

[Summary 40](#_147n2zr)

[Knowledge Check 41](#_3o7alnk)

[Module 3: Operational Aspects 43](#_23ckvvd)

[Target Audience 43](#_ihv636)

[Learning Objectives 43](#_32hioqz)

[Materials 43](#_1hmsyys)

[Advance Preparation 43](#_41mghml)

[Lesson Plans 44](#_2grqrue)

[Introduction 44](#_vx1227)

[Introduction 45](#_4f1mdlm)

[Equipment and Supplies 46](#_2u6wntf)

[Truenat Test Procedures – Prepare Samples and Extract DNA 50](#_19c6y18)

[Truenat Test Procedures – Run a PCR TB Test 52](#_3tbugp1)

[Truenat Test Procedures – Run a RIF Resistance Test 55](#_28h4qwu)

[Class Activity 56](#_nmf14n)

[Waste Management 56](#_37m2jsg)

[Errors and Troubleshooting 58](#_1mrcu09)

[Class Activity 60](#_46r0co2)

[Infrastructure Requirements 62](#_2lwamvv)

[Class Activity 66](#_111kx3o)

[Recording Testing Activities 67](#_3l18frh)

[Warranty 67](#_206ipza)

[Summary 71](#_4k668n3)

[Knowledge Check 71](#_2zbgiuw)

[Module 4: Order Planning and Quality Assurance (QA) and Control 74](#_1egqt2p)

[Target Audience 74](#_3ygebqi)

[Learning Objectives 74](#_2dlolyb)

[Materials 74](#_sqyw64)

[Advance Preparation 74](#_3cqmetx)

[Lesson Plans 75](#_1rvwp1q)

[Introduction 75](#_4bvk7pj)

[Forecasting and Quantification 76](#_2r0uhxc)

[Activity: Regular Forecasting 79](#_3q5sasy)

[Stock Management 81](#_25b2l0r)

[Quality Assurance and Control 84](#_kgcv8k)

[Monitoring Quality 90](#_34g0dwd)

[Summary 92](#_1jlao46)

[Knowledge Check 93](#_43ky6rz)

[Module 5: Monitoring & Evaluation (M&E) 95](#_2iq8gzs)

[Target Audience 95](#_xvir7l)

[Learning Objectives 95](#_3hv69ve)

[Advance Preparation 95](#_1x0gk37)

[Lesson Plans 96](#_4h042r0)

[Introduction 96](#_2w5ecyt)

[M&E for Truenat 97](#_1baon6m)

[Indicators 98](#_3vac5uf)

[Summary 100](#_2afmg28)

[Knowledge Check 100](#_pkwqa1)

[Module 6: Biosafety and Specimen Collection and Referral 103](#_39kk8xu)

[Target Audience 103](#_1opuj5n)

[Learning Objectives 103](#_48pi1tg)

[Advance Preparation 103](#_2nusc19)

[Lesson Plans 104](#_1302m92)

[Introduction 104](#_3mzq4wv)

[Biosafety Measures and Risk 105](#_2250f4o)

[Assessing Risk 107](#_haapch)

[Laboratory Infrastructure 109](#_1gf8i83)

[Personal Protective Equipment 109](#_2fk6b3p)

[Biosafety Cabinets 112](#_upglbi)

[Generation and Prevention of Aerosols 113](#_3ep43zb)

[Managing Spills 115](#_1tuee74)

[Waste Disposal 116](#_2szc72q)

[Specimen Collection Procedures 118](#_184mhaj)

[Specimen Referral 119](#_3s49zyc)

[Summary 121](#_279ka65)

[Knowledge Check 122](#_meukdy)

# Introduction

The Truenat™ Tests for the Detection of TB and Rifampicin Resistance Central-Level Training was developed to provide users with a toolkit to introduce Truenat to key stakeholders in their country. This multipart training is intended to be delivered at a central or national level, with the following audience; end-users (e.g., lab technicians), lab program managers, and clinicians. Additional hands-on onsite training will be conducted by Molbio, the manufacturer of Truenat, for end-users. This training is divided into six modules:

1. Introduction to Truenat
2. Diagnostic Algorithm and Results Interpretation
3. Operational Aspects
4. Order Planning and Quality Assurance (QA) and Control
5. Monitoring and Evaluation (M&E)
6. Specimen Collection and Referral

This training is based on and intended to complement the [Practical Guide to Implementation of TruenatTM Tests for the Detection of TB and Rifampicin Resistance](http://www.stoptb.org/assets/documents/resources/publications/sd/Truenat_Implementation_Guide.pdf) developed by the United States Agency for International Development (USAID), Stop TB Partnership, and the Global Laboratory Initiative (GLI). The training modules have been endorsed by the GLI.

# Training Schedule

|  | **Estimated Instructional Time** | **Participants** |
| --- | --- | --- |
| Module 1 | 2 hours | End-users; Lab and program managers; clinicians |
| Module 2 | 2 hours | End-users; Lab and program managers; clinicians |
| Module 3 | 9 hours | End-users; Lab and program managers |
| Module 4 | 2 hours | Lab and program managers |
| Module 5 | 1 hour | Lab and program managers |
| Module 6 | 2 hours | End-users; clinicians |

# Customizing this Training

This training is designed to be customized by countries to meet their individual needs. In the PowerPoint slides, text that is highlighted in yellow is intended to be replaced with information pertinent to the country. All other text should also be reviewed by national program managers and may also need to be customized to ensure all content is relevant to their setting.

Countries should also develop a Truenat implementation plan that identifies items such as where Truenat instruments will be placed within the diagnostic network, who will be trained to use Truenat, which forms and reporting tools need to be adapted or developed, and how specimens, patients, and results will flow through the Truenat diagnostic network. For additional considerations on test implementation refer to the [WHO Operational Handbook on Tuberculosis Module 3](https://www.who.int/publications/i/item/9789240030589). This training can be delivered before the implementation plan is developed to prompt conversation and discussion of action items to inform an implementation plan or can be delivered after the implementation plan has been developed. If delivered after implementation plan development, the material in this training should be customized to reflect Plan components. In either circumstance, an implementation plan should be developed and used to sensitize stakeholders in advance of Truenat clinical testing.

The PowerPoint slides use a basic template that was designed to convey information visually and be easy to understand. Countries might consider uploading their logo to the existing template using the Slide Master. They can also change the template to a national one [using these instructions from Microsoft](https://support.microsoft.com/en-us/office/apply-a-template-to-an-existing-presentation-43f7fc75-db26-433b-8248-9fcd0093006b). The slides use the Montserrat font – if this font is not installed on training facilitators’ computers, it can either be downloaded or installed or the font can be changed in the slide master. Similarly, colors can also be changed in the slide master and then using the “reset function” on each slide.

Please note that any customization to the training may require changes to the participant guide, facilitator guide, and PowerPoint slides.

# Training Format

This training is designed to be delivered in person but can be modified to be delivered virtually if preferred. The main changes that need to be considered will be to the activities and discussion questions. Facilitators should consider using features such as breakout rooms, poll questions, and the chat function for virtual training. Participants should be encouraged to participate as much as possible, preferably by coming off mute in a small group, or by actively using the chat if in a large group.

# Facilitator Preparation

Facilitators can use the checklist below to help successfully plan and deliver each training module.

| **SESSION PREPARATION** | |
| --- | --- |
|  | Customize PowerPoint slides, Facilitator Guide, and Participant Guide as needed |
|  | Familiarize yourself with the Facilitator Guide |
|  | Familiarize yourself with the Participant Guide |
|  | Review and test materials requiring technology (website links, video links, etc.) |
|  | Confirm meeting room and technology equipment for the date and time of the session (if needed) |
|  | Print out participant Guides (or for a virtual session, ask participants to print their own Participant Guides) |
| **REGULAR SESSION MATERIALS** | |
| Gather the following materials for each session: | |
|  | Facilitator Guide |
|  | Participant Guide |
|  | Nametags or name tents |
|  | PowerPoint presentation |
|  | Timer (watch, clock, or phone app) used to keep activities within time limits) |
|  | Pens or pencils |
|  | Index cards |
| **DAY OF SESSION** | |
|  | Arrive early |
|  | Arrange tables and chairs in a formation that invites large and small group discussion. |
|  | Test technology (computer, projector, internet) to make sure it is working. |
|  | Write down needed text on a flipchart or whiteboard to prepare for session activities, if needed |
|  | Greet participants |

# Course Introduction

Before beginning the course, the facilitator should provide a basic overview of the training, including what Truenat is and why the training is being delivered, how long the training will last and the schedule/structure of each day, and should explain to participants how to use the participant guide (i.e., that participants should follow along in their participant guide and take notes as they go along). The facilitator should emphasize that this is highly participatory training and facilitate an “ice breaker” for participants to get to know one another. If the training is being delivered virtually, an overview of the technology should also be provided.

# Module 1: Introduction to Truenat

## Target Audience

The target audience for this course is:

* Truenat end-users
* Lab managers
* Program managers
* Clinicians and other healthcare workers

## Learning Objectives

**Terminal Objective**

* At the end of this session, participants should understand the purpose of Truenat and how it is intended to help address TB diagnostic challenges specific to their country.

**Module 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.

## Materials

* Facilitator Guide
* Participant Guide
* Pens/Pencils

## Advance Preparation

* Facilitators will need to customize slides in this module for Patient Flow procedures and the Patient Referral Pathway for their particular country.

## Lesson Plans

### Introduction

| **Introduction**  **Slide: 3**  **Participant Guide Page: 0** |
| --- |
|  |
| **SAY:** Our first topic is an introduction to Truenat. We will talk about challenges with diagnosing TB globally and in our country, the WHO recommendations for TB testing, and then we’ll look at some specific elements of Truenat. |
| **Module 1: Introduction to Truenat**  **Slide: 4**  **Participant Guide Page: 0** |
|  |
| **SAY:** We will look at four topics in this module. The TB context globally and in our country, we’ll hold a plenary session on challenges with diagnosing TB, then we’ll go through the range of lab tests that are available for TB, and then will specifically talk about Truenat, including its placement in diagnostic networks and accuracy, including specificity and sensitivity. |
| **Learning Objectives**  **Slide: 5**  **Participant Guide Page: 0** |
|  |
| **SAY:** By the end of this module, you will 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. |
| **ASK:** What questions do you have before we move into the first lesson for this training? |
| **DO:** Allow participants time to ask questions and respond appropriately. |

### TB Context

| **Global TB Situation: Global Context**  **Slide: 7**  **Participant Guide Page: 0** |
| --- |
|  |
| **SAY:** Let’s take a look at the tuberculosis (TB) situation around the world and in our country. |
| **ASK:** How many people do you think are infected with TB every year? |
| **DO:** Click on the slide for an animation to bring up the content. |
| **ANSWER:** 10 million people fall ill with TB every year. Ensure that participants record this answer in their participant guide. |
| **SAY:** TB is the world’s top infectious disease after COVID-19. One and a half million people die from TB each year. Statistics show that TB is the leading cause of death of people with HIV. It is also a major contributor to antimicrobial resistance. |
| **Global TB Situation: WHO End-TB Strategy**  **Slide: 8**  **Participant Guide Page:** 0 |
|  |
| **SAY:** The World Health Organization (WHO) recommends that rapid TB diagnostics be available to anyone who shows signs or symptoms of TB. Drug susceptibility testing (DST) should be done for all bacteriologically confirmed TB patients. At the very least, all bacteriologically confirmed TB patients should receive DST for rifampin (RIF), and all patients with RIF resistance should receive DST at least for fluoroquinolones. |
| **ASK:** To what extent do you think your country is meeting these goals? |
| **DO:** Allow participants to share their experiences. |
| **Global TB Situation: WHO Guidelines on DST**  **Slide: 9**  **Participant Guide Page: 0** |
|  |
| **SAY:** WHO has updated its guidelines to emphasize the importance of drug susceptibility testing prior to initiating treatment, especially for drugs for which rapid tests are available. The recommended treatments are rapid molecular tests, RIF, FQs, and isoniazid (INH). |
| **DO:** Click on the slide to bring up the discussion question with animation. |
| **ASK:** Does anyone know which medicines have WHO-recommended rapid molecular tests? |
| **DO:** Allow participants time to answer. |
| **DO:** Click on the slide to bring up the answers to this question and read through them. Ensure that participants record the answers in their participant guide. |
| **Country TB Context**  **Slides: 10, 11, and 12**  **Participant Guide Page: 2** |
|  |
| **SAY:** As a country, we face many challenges in ending the TB epidemic. Let’s talk through some basic facts about TB in our country. |
| **DO:** Share the data on the number of TB cases, deaths, pediatric TB cases, and the treatment coverage and undiagnosed cases. Be sure to explain national priorities per the national strategic plan, and provide an overview of the diagnostic network for TB in the country. |

### Plenary Session

| **Challenges with Diagnosing TB in *Country***  **Slide: 13**  **Participant Guide Page: 2** |
| --- |
| **DO:** Facilitate a plenary session with key experts in the country to discuss the main challenges with laboratory confirmation of TB. This session should last about one hour, with ample time in the end for Q&A. |
| **ASK:**   1. How would you characterize the level of access to molecular testing in our country? Does this differ in rural vs. urban areas? 2. What are the main challenges with increasing access to molecular testing in our country? 3. How does the country plan to scale up or increase access to molecular testing? 4. Why is it important that more people have access to molecular testing for TB? 5. How does Truenat help us meet these goals? |
| **SAY:** What questions do our participants have for our panelists? |

### TB Laboratory Tests

| **Menu of TB Lab Tests**  **Slide: 15**  **Participant Guide Page: 3** |
| --- |
|  |
| **SAY:** There are many lab tests for the detection of TB and resistance to drugs that treat TB. There is another class of nucleic acid amplification tests that the WHO recommended and categorized as having moderate complexity for the detection of rifampicin and isoniazid resistance. We are going to walk through each one of these tests and discuss their uses, benefits, and limitations. |
| **ASK:** Aside from Truenat, are you familiar with all these tests, or are some of them new? |
| **DO:** Allow participants to share their responses. |

| **Lab Tests for TB**  **Slide: 16-31**  **Participant Guide Page: 4** |
| --- |
|  |
| **DO:** Go over each lab test and talk about its uses, benefits, and limitations. Share the WHO recommendations for each test. Consider asking a different participant to talk through the information on each slide. |
| **Truenat Chips**  **Slide: 32**  **Participant Guide Page: 5** |
|  |
| **SAY:** As you may have been able to tell from the previous slides, there are three different Truenat chips for the three different TB tests. Truenat MTB targets a single copy gene (*nrdB* gene) while Truenat MTBPlus targets a single-copy gene (*nrdZ*) plus a multi-copy gene (*IS6110*, found exclusively in members of the *M.tb* complex). As a result, Truenat MTBPlus is more sensitive than Treunat MTB. |
| **DO:** Using the animation on the slide, show each of the tests and name them. |

### Placement of Truenat in Diagnostic Networks

| **Lab Tests for TB: Placement in the Network**  **Slide: 34**  **Participant Guide Page: 7** |
| --- |
|  |
| **SAY:** This is a visual depiction of where each of the tests we just discussed can be placed in the diagnostic network. Some tests are done at the point of care, some tests are done at peripheral labs, and other more complex tests are typically done at intermediate labs or the central reference laboratory. You can see here that only LF-LAM antigen is a true point-of-care test, but there are three tests that can be done at peripheral labs: microscopy, TB-LAMP, and Xpert MTB RIF/Ultra (hereafter referred to as Xpert). |
| **DO:** Click on the slide for an animation to bring up the discussion question. |
| **ASK:** You may have noticed that Truenat is missing from this diagram. Based on what we talked about in the last lesson, where do you think Truenat should go on here? |
| **DO:** Allow participants to share their responses. Once finished, click on the slide for an animation to bring up the Truenat box. |
| **SAY:** Yes, that is correct (or no, that is not correct). Where a diagnostic test will be placed within your country network will depend on a number of factors such as targets for expanding patient access to rapid testing, projected test volumes, specimen referral networks, and infrastructure requirements. Truenat is designed for use at sites with minimal infrastructure, thereforecan be used at near point of care or in the community, and can also be useful at peripheral laboratories, so you can see here that it is straddling these two levels. Similarly, Xpert MTB/RIF, Xpert Ultra, or Xpert MTB/XDR can be used at peripheral, district, or central laboratories. |
| **Placement of Truenat in Diagnostic Networks**  **Slide: 35**  **Participant Guide Page: 8** |
| Graphical user interface, application, email  Description automatically generated |
| **SAY:** In many countries, Truenat will be placed at peripheral health centers to replace microscopy as the initial diagnostic test for TB. It is possible that an X-ray or digital chest x-ray with computer-aided detection (CAD) or C-reactive protein (in people living with HIV) may be used as a screening tool for confirmatory testing with Truenat in the general population (aged 15 years and older).  Specimen referral networks may be needed to allow for further DST. |
| **ASK: (**If training PMs or clinical staff) What challenges have you faced with the linkage of patients with Rifampicin Resistance results for further DST. What can be done to improve this? |
| **ASK:** Are there any questions before we move on to the next topic? |
| **Positioning vs. Xpert or TB-LAMP**  **Slide: 36**  **Participant Guide Page: 8** |
|  |
| **SAY:** It’s important to note that Truenat is not a replacement for existing Xpert networks. When it comes to positioning Xpert, Truenat or TB LAMP, a country can use more than one test for rapid testing, keeping in mind thatTruenat and TB-LAMP can be placed at lower levels of the network than Xpert.  Three things can happen when placement is at lower levels:   * Patient access to rapid molecular testing for TB can increase * RIF resistance testing can be decentralized * The need for patient travel maybe reduced |

### Diagnostic Accuracy of Truenat

| **Diagnostic accuracy relative to culture, in microscopy center settings**  **Slide: 38**  **Participant Guide Page: 9** |
| --- |
|  |
| **SAY:** This table shows the diagnostic accuracy of each of the Truenat tests (MTB, MTB Plus, and MTB-RIF) relative to culture, in microscopy center settings. |
| **ASK:** Can someone explain to us what sensitivity and specificity mean? |
| **DO:** Allow participants to answer. |
| **SAY:** That’s right, (or, that’s close):sensitivity refers to a test's ability to designate an individual with the disease as positive. A highly sensitive test means that there are few false-negative results, and thus fewer cases of the disease are missed. The specificity of a test is its ability to designate an individual who does not have a disease as negative. A highly specific test means that there are few false-positive results. |
| **ASK:** What do you notice about the specificity and sensitivity of Truenat relative to culture? What stands out to you in this table? |
| **DO:** Allow participants to provide some responses. If not raised by participants, offer the following main points:   * In general, Truenat showed comparable results in terms of diagnostic accuracy relative to culture, which is the gold standard. The validation studies showed high sensitivity and specificity levels compared with culture. * Truenat MTB Plus is a bit more sensitive but slightly less specific, relative to culture than Truenat MTB * Truenat tests have lower sensitivity for patients with smear-negative TB compared to those with smear-positive TB. |
| **Diagnostic Accuracy Relative to Culture, Reference Laboratory Settings**  **Slide: 39**  **Participant Guide Page:10** |
|  |
| **SAY:** This table shows the diagnostic accuracy relative to culture in a reference laboratory setting for Truenat and Xpert tests. |
| **ASK:** What is the main point of the information in this table? |
| **DO:** Allow participants to respond. Emphasize that the main point is that the performance of Truenat MTB, MTB Plus, and MTB-RIF Dx tests were generally comparable to their Xpert counterparts. |
| **Effect of prior treatment on specificity**  **Slide: 40**  **Participant Guide Page:11** |
|  |
| **Sensitivity and Specificity Trade-Offs**  **Slide: 41**  **Participant Guide Page:11** |
|  |
| **SAY:** There is a similar consideration to make when selecting Truenat MTB or MTB Plus. We would have to consider the prevalence of TB, the prevalence of drug-resistant TB, and the prevalence of TB-HIV co-infection. |
| **DO:** Click on the slide for an animation to bring up the discussion question. |
|  |
| **DO:** Allow participants to answer, then move to the next slide. |
| **High HIV Burden Settings**  **Slide: 42**  **Participant Guide Page: 12** |
|  |
| **SAY:** On the reverse side, in a population with a high prevalence of HIV, a more sensitive test like Truenat MTB Plus may be better because of its ability to detect MTBC in smear-negative samples. |
|  |
| **Advantages of Truenat**  **Slide: 43**  **Participant Guide Page: 12** |
|  |
| **SAY:** In addition to a high level of diagnostic accuracy, there are a few other key advantages of Truenat. |
| **DO:** Read through the material on the slide. Click on the slide for an animation to bring up the discussion question, read it, and allow participants to respond. Their answers should speak to the key challenges that were identified at the beginning of this module. |

### Activity: Which Test Should I Use?

| **Activity: Which Test Should I Use?**  **Slides: 44-46**  **Participant Guide Page:13** |
| --- |
|  |
| **SAY:** In your participant guide, starting on page 13, you will find three scenarios. With a partner, you are to read each scenario and determine which test or tests should be used. Be sure to write your rationale. You will have 10 minutes. |
| **DO:** Allow participants to answer the scenario questions in the Participant Guide with their partners. After 10 minutes, call on participants to share their responses. Be sure to correct any incorrect responses. |
| **ANSWERS:**  Scenario 1: According to WHO recommendations, Truenat MTB or MTB Plus testing should be ordered. Xpert or Xpert Ultra could also be correct – choosing between Truenat and Xpert, or other WHO-recommended molecular diagnostic, will depend on national algorithms and which test is more accessible. You might also order a chest X-ray before performing tests to see if there are abnormalities, or a C-reactive protein if the patient is living with HIV  Scenario 2: Truenat MTB, should be ordered and may require a confirmatory test.  Scenario 3: Truenat MTB Plus, should be ordered. |

### Summary

| **Summary**  **Slide: 47**  **Participant Guide Page:15** |
| --- |
|  |
| **SAY:** In this lesson, you learned about the global and country-specific TB situation, different diagnostic TB lab tests, and the diagnostic accuracy of Truenat. The key takeaway from this module is that Truenat is a promising new TB diagnostic tool that is more sensitive and specific than microscopy. Truenat has minimal infrastructure requirements and results are rapidly available. It can also detect RIF resistance within two hours and can be used as an initial drug resistance test. |
| **DO:** Ask participants if they have any questions about today’s lesson. Allow participants to ask questions. Answer any clarifying questions. |
| **SAY:** Thank you for your attention today. Your next session will be on the Truenat diagnostic algorithm and results interpretation. |

Knowledge Check

| **Knowledge Check**  **Slides: 48-51**  **Participant Guide Page: 15** |
| --- |
| **DO:** Explain that you will ask participants three knowledge check questions and may call on participants randomly to provide an answer. (These can also be programmed as poll questions in a virtual training – ensure that all participants respond before proceeding if using the poll feature).  If an answer provided is incorrect, ask if other participants would like to answer. Correct any incorrect answers that are given. If multiple participants get a question wrong, you may need to revisit the topic.  Note that knowledge check questions are not included in participant guides to avoid students seeing them during the lesson and only focusing on those pieces. Encourage participants to write down the answers in their guides in the notes field for future reference. |
|  |
| **Answer:** Answers will vary depending on the country. |
|  |
| **Answer:**   * 2A: AFB-smear microscopy, culture, TB-LAMP, LF-LAM antigen, Xpert, Truenat, Abbott RealTime MTB, Roche Cobas MTB, TB-LAM (in PLWH) * 2B: AFB-smear microscopy, culture * 2C: Phenotypic DST, LPA, Xpert, Truenat (and maybe culture – maybe, provides an isolate for DST), Abbott MTB RIF/INH resistance, FluoroType MTBDR, Roche MTB-RIF/INH * 2D: Phenotypic DST, LPA (and maybe culture – maybe, provides an isolate for DST, Xpert MTB/XDR, Abbott MTB RIF/INH resistance, FluoroType MTBDR, Roche MTB-RIF/INH, Genoscholar PZA-TB II |
|  |
| **Possible Answers:** Cost-effectiveness, patient access, time to result, availability of DNA for other tests, in-built battery, and connectivity, ability to be used in warmer temperatures. |
|  |
| **Answer:** Truenat is generally as accurate as other tests, however, countries and service providers should weigh trade-offs between specificity and sensitivity in deciding which tests to use. |

# Module 2: Diagnostic Algorithm and Results Interpretation

## Target Audience

The target audience for this course is:

* Truenat end-users
* Lab managers
* Program managers
* Clinicians

## Learning Objectives

**Terminal Objective**

* At the end of this session, participants should be able to use the Truenat diagnostic algorithm to guide decisions around TB and drug susceptibility testing.

**Module Objectives**

* By the end of this session, participants should be able to:
  + Implement the WHO recommendations for using Truenat
  + Follow the Truenat diagnostic algorithm and decision tree to use Truenat
  + Understand patient flow within the TB diagnostic network and describe procedures for patient referral

## Materials

* Facilitator Guide
* Participant Guide
* Pens/Pencils

## Advance Preparation

Countries may need to customize the Truenat diagnostic algorithm to reflect the specific context of TB testing in their country. If so, the slides in this module may need to be revised.

## Lesson Plans

### Introduction

| **Introduction**  **Slide: 3**  **Participant Guide Page: 16** |
| --- |
| Graphical user interface, text, application, email  Description automatically generated |
| **SAY:** Our topic today is the diagnostic algorithm for Truenat. We will talk about how to use the algorithm and interpret the results. |
| **Module 2: Truenat Algorithm**  **Slide: 4**  **Participant Guide Page: 16** |
|  |
| **SAY:** We will discuss WHO’s recommendations for utilizing Truenat, the algorithm, interpreting digital results, and how to use patient flow. |
| **Lesson Objectives**  **Slide: 5**  **Participant Guide Page: 16** |
|  |
| **SAY:** By the end of this module, you will be able to:   * Implement 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. |
| **ASK:** What questions do you have before we move into the first lesson for this training? |
| **DO:** Allow participants time to ask questions and respond appropriately. |

### WHO Recommendations

| **WHO Recommendations**  **Slide: 7**  **Participant Guide Page: 16** |
| --- |
|  |
| **SAY:** As we discussed in the last module, WHO has two recommendations related to Truenat testing. Truenat MTB or MTB Plus may now be used as an initial diagnostic test for TB rather than smear microscopy or culture in adults and children with signs and symptoms of pulmonary TB. Truenat MTB-RIF Dx can be used as the initial test for detection of RIF resistance rather than culture and phenotypic DST for persons with a Truenat MTB or MTB Plus positive result. |
| **When to Use Truenat**  **Slide: 8**  **Participant Guide Page: 17** |
| Graphical user interface, text, application, email  Description automatically generated |
| **DO:** Lead participants through a facilitated discussion on this slide. Review the bullets with them and then ask a series of questions to fill in the gaps. Inform participants to write the answer to the discussion questions in the Participant Guide. |
| **SAY:** These recommendations mean that Truenat can be used for all adults and children with signs and symptoms of pulmonary TB. You can use Truenat to detect MTBC in specimens from persons newly presenting with signs and symptoms of pulmonary TB, and to detect RIF resistance in anyone with a positive TB test. Note that even though the kit insert states that Truenat assays can be used to test sputum and non-sputum samples, we do not have sufficient data as yet on the performance of Truenat in detecting EPTB. Therefore, the current WHO recommendation is to use Truenat MTB or MTBPlus on sputum samples.  There are instances when Truenat should not be used. Due to insufficient evidence in this situation, Truenat should not be used for individuals with signs and symptoms of extrapulmonary TB. You should also never use Truenat for treatment monitoring as the presence of dead bacilli may generate a false-positive result. |
| **ASK:** What sample types from children and infants do you think will be compatible with Truenat testing? |
| **ANSWER:** The current recommendation is for sputum specimens based on the extrapolation of data from adults. Although the test is expected to be less sensitive in children, there is no recommendation for its use on non-sputum samples due to a lack of evidence on its accuracy in these sample types. |
| **ASK:** What are the signs and symptoms of pulmonary TB? |
| **ANSWER:** Signs and symptoms of TB include breathing difficulty, chest pain, cough (usually with mucus), coughing up blood, excessive sweating, particularly at night, fatigue, fever, and weight loss. Additionally, a chest X-ray or C-reactive protein may be used as a screening test before using Truenat. |
| **ASK:** Which persons have an elevated risk of RIF-resistant (RR)-TB? (Or, when should you suspect RR-TB?) |
| **ANSWER:**  Persons with an elevated risk of having RR-TB include previously treated patients with a new TB episode or potential relapse, previously treated patients who were non-converters, and previously treated patients who were lost to follow-up. Participants might also include close contacts of RR/MDR-TB patients, patients with treatment failure, and smear-positive patients at the end of the third month of initial treatment. |
| **ASK:** What are the signs and symptoms of extrapulmonary TB? |
| **ANSWER:** Symptoms of extrapulmonary TB are similar to pulmonary TB (including fever, malaise, and weight loss. It is most common among young children, immunocompromised people, and the elderly). For this reason, health care providers may not be able to distinguish between pulmonary and extra-pulmonary TB before ordering a Truenat test. Again, it is not that Truenat does not work to diagnose extrapulmonary TB, but that there is insufficient evidence for WHO to recommend its use in these circumstances. |
| **QUESTION:** As covered in our last module,what tests can you use for TB treatment monitoring? |
| **ANSWER:** Culture, AFB-Smear microscopy |

### Truenat Algorithm

| **Truenat Algorithm**  **Slide: 10**  **Participant Guide Page: 1**9 |
| --- |
|  |
| **SAY:** The Truenat diagnostic algorithm breaks down the testing approach for using Truenat to detect MTBC and RIF resistance. We will break down the algorithm into parts. Molbio has also developed a job aid that can be posted in laboratories for easy reference, and the algorithm is also included in the Truenat Implementation Guide. |
| **Algorithm Part 1: Isolate DNA**  **Slides: 11 – 13**  **Participant Guide Page: 20** |
| **SAY:** When it is decided that a person should be evaluated for pulmonary TB, a sputum sample should be collected from the patient. Programs might consider collecting two specimens upfront – one to test with Truenat, and the other to use for additional testing that may be needed per the algorithm. Once you have the specimen and it is pretreated, the DNA should then be isolated using the Trueprep system. We will talk about how to use the Trueprep and Truelab systems in the next module, but for now, you should just note that you will isolate the DNA. If the DNA is successfully isolated, you are done with the first step. If you get an isolation failure or error, you should repeat the Trueprep procedure, and if the DNA is isolated this time, again you are ready for the next step. |
| **ASK:** According to the algorithm, what should you do if you receive an isolation failure or error twice when trying to isolate the DNA? |
| **DO:** Inform participants that they can reference the algorithm in their participant guide. Ask them to write a response in their own words in the Participant Guide. Call on participants to share their answers. Correct any incorrect answers. |
| **ANSWER:** Conduct additional testing to confirm or exclude TB in accordance with national guidelines or re-evaluate the patient clinically and use clinical judgment for treatment decisions. |
| **ASK:** What are some additional tests you could use if the second attempt is also unsuccessful? |
| **DO:** Call on participants to share their answers. Correct any incorrect answers. |
| **ANSWER:** Additional testing can consist of a repeat test with a new specimen, further DST, a chest X-ray, Xpert or another mWRD, or culture. |
| **Algorithm Part 2: TB Test**  **Slides: 14-17**  **Participant Guide Page: 22** |
|  |
| **SAY:** We are moving to part two of the Truenat algorithm, which is the TB test. Once you have the DNA isolated, use six ul of the DNA eluate for the Truenat TB test. There are three outcomes of the test: MTB not detected, MTB detected and Invalidno result, error or invalid).For Truenat MTBPlus, the result screen will also show the Mtb load as “High” (Ct<20), “Medium” (Ct 20-24), “Low”(Ct 25-29), “Very low” (Ct≥30) for positive samples. |
| **DO:** Go over the procedures for TB results for MTB not detected, MTB detected and TB inconclusive. |
| **ASK:** In your participant guide, list as many re-evaluation options as possible to consider if a TB test result is MTB not detected. |
| **DO:** Allow participants three minutes to write their responses in the Participant Guide. Call on multiple students to answer the question. Correct any incorrect answers. |
| **ANSWER:** Further investigations for TB may include chest X-ray, additional clinical assessments, clinical response following treatment with broad-spectrum antimicrobial agents, additional Truenat TB testing, testing with other WHO-approved rapid diagnostic tests (e.g., Xpert MTB/RIF) or culture. |
| **Algorithm Part 3: RIF Resistance Test**  **Slides: 18-22**  **Participant Guide Page: 25** |
| **SAY: If the result is MTBPlus "MTB detected low or very low" but MTB-RIF indeterminate, it is best to repeat testing on a new sample. Likely the bacillary load is below the limit of detection for the MTB-RIF test (if a repeat is done on the same eluate, it is very likely to have indeterminate again). If a patient is at low risk of MDR-TB, it is best to repeat testing for MTB and MTB/RIF on a new sample to rule out any clerical or technical issues.** |
| **SAY:** The third part of the algorithm is used if the specimen is positive for TB in the second part. In this case, you will:   1. Use 6 ul of the same DNA eluate that was prepared for the TB test. 2. Use the Truelab Real-Time micro PCR Analyzer with the Truenat MTB-RIF Dx test cartridge. 3. If there are delays in conducting the test, initiate treatment using first-line TB drugs in accordance with the national guidelines. If the patient is at high risk for MDR-TB, initiate them on an MDR-TB regimen or treat them in accordance with national guidelines. 4. There are three possible outcomes of the MTB-RIF Dx test:    1. RIF resistance detected    2. RIF resistance not detached    3. RIF indeterminate |
| **DO:** Go over the procedures for each of the three possible outcomes. |

### Activity: Truenat Algorithm

| **Truenat Algorithm**  **Slide: 23**  **Participant Guide Page: 28** |
| --- |
| Text  Description automatically generated |
| **SAY:** You will now get a chance to practice using the Truenat Algorithm. You will be placed in groups of 4-5 members. In your group, you will complete the scenario in your Participant Guide. You have 15 minutes to complete the scenario. |
| **DO:** After 15 minutes, call on participant groups to share their responses. Ask other groups if they chose a different response and share their rationale. Be sure to correct any incorrect responses. |
| **ANSWERS:**   1. Rebeka should be tested for TB. You should collect a sputum sample, transport it to a site with Truenat, and isolate the DNA using the Trueprep system. 2. Repeat the DNA isolation with Trueprep using the same prepared sample and a second Trueprep cartridge. 3. The possible outcomes of the TB test are MTB Detected or MTB not detected or TB Inconclusive (no result, error, or invalid). 4. A RIF-resistance test 5. The patient would be considered low-risk, so you should repeat the Truenat TB test and MTB Rif Dx test on a fresh sample. 6. Assume the test was a false positive and initiate treatment with a first-line regimen in accordance with national guidelines. (If in a country with a high risk of isoniazid resistance, consider additional DST for isoniazid.) |

What methods are available to evaluate drug resistance?

### Patient Flow

| **Patient Flow**  **Slide: 25**  **Participant Guide Page: 29** |
| --- |
| Graphical user interface, application  Description automatically generated |
| **SAY:** Patient flow will vary based on where the Truenat is placed in the diagnostic network. Regardless, it is extremely important that patients are linked to additional testing as needed, treatment, and care. |
| **ASK:** Based on your experience, what are some key considerations in developing a patient referral pathway for Truenat in your country? |
| **DO:** Call on participants to respond to the question.  Answers will vary based on your country. |
| **Questions to Consider About Patient Flow**  **Slide: 26**  **Participant Guide Page: 30** |
| Graphical user interface, text, application, email  Description automatically generated |
| **SAY:** Let’s talk about some key considerations for patient flow for Truenat testing.Respond to the questions in your Participant Guide, starting on page 30. |
| **DO:** Allow participants five minutes to complete the questions in their Participant Guide.  Ask participants to share how the patient flow works in their country. |
| **Example DS-DR-TB Patient Referral Pathway**  **Slide: 27**  **Participant Guide Page: 32** |
|  |
| **SAY:** Let’s take a look at an example patient referral pathway. |
| **DO:** Review the patient referral pathway example slide. |
| **SAY:** Some key facts to remember about the patient referral pathway are:   * The sample flow must follow the patient referral pathway. * It is important that Truenat testing sites ensure that results are transmitted back to the requesting site/community. * If a site cannot initiate DR-TB treatment, end-users/ facility/site staff must ensure Truenat-RR-TB results are transmitted to the DR-TB clinic when patients are referred. * Truenat is portable and can be used at the community level. * Monitor the flow with quality assurance indicators, which will be discussed in a future module. |
| **DO:** Ask participants if they have any questions. Answer any clarifying questions. |

| **Procedures for Patient Transfers and Referrals**  **Slide: 28**  **Participant Guide Page: 33** |
| --- |
| A picture containing text, screenshot, businesscard  Description automatically generated |
| **SAY:** There are procedures that should be followed for patient transfers and referrals.  If TB services are available at the same facility, the patient should be escorted to care and treatment. The care and treatment site should be given the test results.  If TB services are not available at the same facility, follow these steps:   * Provide TB patient with a written referral to the care and treatment facility. * Counsel the patient on the need for immediate treatment. * Call the TB care and treatment facility to alert them of the referral and transmit the test results electronically. * Provide the patient’s name and contact information with the date of the positive test result. * Follow up with patient and treatment facility. |
| **DO:** Ask participants if they have any questions. Answer any clarifying questions. |
| **Digital Results Reporting**  **Slide: 29**  **Participant Guide Page: 34** |
|  |
| **SAY:** Next, we are going to look at some of the features of Truenat related to digital results reporting.  Every Truelab instrument has built-in software for digital results reporting. Third-party connectivity software companies and platforms (SystemOne Aspect and Saavics DataToCare) are currently working to allow for smooth flow of data to referring healthcare workers, Molbio as the Truenat manufacturer, and disease control programs.  Digital results reporting can be used to:   * Send test results to referring healthcare workers * Send information about performance and issues to Molbio (error reads, sample processing information) * Send data to national or regional disease control program servers for surveillance purposes. |
| **DO:** Ask participants if they have any questions. Answer any clarifying questions. |

### Summary

| **Summary**  **Slide: 30**  **Participant Guide Page: 34** |
| --- |
|  |
| **SAY:** We have now come to the end of this session. Let’s recap.   * WHO recommends using Truenat MTB or MTB Plus and MTB-RIF Dx for all adults and children with signs and symptoms of pulmonary TB. * The Truenat algorithm breaks down the testing approach for using Truenat to detect MTBC and RIF resistance. * 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

| **Knowledge Check**  **Slides: 31 – 33**  **Participant Guide Page: 35** |
| --- |
| **DO:** Explain that you will ask participants three knowledge check questions and may call on participants randomly to provide an answer. (These can also be programmed as poll questions in a virtual training – ensure that all participants respond before proceeding if using the poll feature).  If an answer provided is incorrect, ask if other participants would like to answer. Correct any incorrect answers that are given. If multiple participants get a question wrong, you may need to revisit the topic.  Note that knowledge check questions are not included in participant guides to avoid students seeing them during the lesson and only focusing on those pieces. Encourage participants to write down the answers in their guides in the notes field for future reference. |
| Graphical user interface, application  Description automatically generated |
| **ANSWER:** Truenat MTB, MTB Plus, MTB-RIF, and MTB Plus should be used as an initial diagnostic test for TB. MTB-RIF Dx should be used as the additional diagnostic test for RIF resistance. |
| Graphical user interface, application  Description automatically generated |
| **ANSWER:** Isolate DNA, TB test, and RIF resistance test |
| Graphical user interface, text, application  Description automatically generated |
| **ANSWER:**  1. The patient’s specimen should be tested for RIF resistance, 2. The patient should be referred for care and treatment. The facilitator should emphasize country-specific methods for referrals, e.g. ensuring test results are transmitted electronically, counseling, doing a warm-handoff to TB treatment facilities, etc. |

# Module 3: Operational Aspects

## Target Audience

The target audience for this course is:

* Truenat end-users
* Lab managers
* Program managers

## Learning Objectives

**Terminal Objective**

* At the end of this session, participants should understand the operational aspects of Truenat testing, including generally how to use the Truenat equipment.

**Module 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

## Materials

* Facilitator Guide
* Participant Guide
* Pens/Pencils
* Printed notecards for activity

## Advance Preparation

Facilitators should assess whether Truenat equipment is available in the country for practice. If logistics allow, training organizers may consider having a hands-on practice session at a testing site following the delivery of the sections on process flow. An alternative activity is also described in this facilitator’s guide, which will involve printing out notecards for participants to reorder.

Facilitators should work with national program managers to understand how testing activities are currently recorded (i.e. what forms, logs, training materials, registers exist) and how they may need to be modified to incorporate all aspects of Truenat documentation (i.e., commodity management, quality control and assurance, test performance and results, training and competency assessments, supervisory visits). This content in the training may need to be tailored to reflect this information (as noted by the yellow highlight).

## Lesson Plans

### Introduction

| **Introduction**  **Slide: 3**  **Participant Guide Page: 36** |
| --- |
|  |
| **SAY:** In this module, we will learn about the operational aspects of Truenat. We will talk about the different pieces of Truenat equipment, the procedures for conducting TB and RIF-resistance tests, and I will explain the infrastructure requirements for installing Truenat. |
| **Learning Objectives**  **Slide: 4**  **Participant Guide Page: 36** |
|  |
| **SAY:** These are the learning objectives for this module. |
| **DO:** Read through the objectives. Ask if participants have any questions before you get started. |
| **Module 3: Operational Aspects**  **Slide: 5**  **Participant Guide Page:** 36 |
|  |
| **SAY:** There are seven topics in this module. We’ll start with a brief introduction, then will move to equipment and supplies, Truenat test procedures, waste management, errors and troubleshooting, infrastructure requirements, and recording testing activities. |

### Introduction

| **Introduction to Truenat TB PCR Testing**  **Slide: 6**  **Participant Guide Page: 36** |
| --- |
|  |
| **SAY:** Before we get started, let’s talk briefly about how Truenat works. The Truenat TB test is a chip-based real-time polymerase chain reaction (PCR) test for semi-quantitative detection and diagnosis of *Mycobacterium tuberculosis* complex mycobacteria (MTBC) in human sputum samples. |
| **DO:** Click on the slide to bring up the animation one by one and read through each step. |

### Equipment and Supplies

| **Equipment – Trueprep**  **Slide: 8**  **Participant Guide Page: 37** |
| --- |
|  |
| **SAY:** Let’s talk about equipment and supplies, starting with equipment. Truenat really consists of three different machines. The first one is called Trueprep – the formal name is the Truenat AUTO v2 Universal Cartridge Based Sample Prep Device. Trueprep is used for the automated extraction and amplification of DNA, or in other words, to prepare the sample for testing. |
| **Equipment – Truelab**  **Slide: 9**  **Participant Guide Page: 38** |
|  |
| **SAY:** The second piece of equipment is the Truelab Real Time micro-PCR Analyzer, or just Truelab. Truelab is used for performing PCR and it comes in three versions: Uno, Duo, Quattro. Uno runs one test at a time, the Duo version runs two tests at a time, and how many tests do you think Quattro runs at a time? Yes, that’s right, four. |
|  |
| **Different Models of Truenat Analyzers to Match Anticipated Throughput**  **Slide: 10**  **Participant Guide Page: 38** |
|  |
| **SAY:** Let’s look at the throughputs of each version of Truelab. With one Truelab Uno and one Trueprep, you can test between 7-9 specimens during an 8-hour shift or workday under real-world conditions. Under perfect conditions, you would be able to test between 10 and 12 specimens but you should use the real-world conditions for planning purposes. It’s important to note that this combination of devices can be transported in a suitcase, so if you wanted, for example, to conduct Truenat testing in a community and anticipated testing between 7 and 9 specimens in one day, you could choose this combination.  With one Truelab *Duo* and one Trueprep, you can test between 15 and 18 specimens in one workday, and with one Truelab *Quattro*, which would require two Truepreps, you can test between 30-36 specimens.  So, understanding how many specimens you will want to test per day will determine which piece of equipment to procure and where to place it. |
| **Equipment - Truelab Micro-PCR printer**  **Slide: 11**  **Participant Guide Page: 39** |
|  |
| **SAY:** The third piece of Truenat equipment is the Truelab micro-PCR printer, which is a Bluetooth printer that wirelessly prints the results of the PCR tests performed by the Truelab machines. This is an optional piece of equipment – you don’t necessarily need to print the results of the tests, particularly if results are being sent digitally. |
| **Reagents and Consumables**  **Slide: 12**  **Participant Guide Page: 40** |
|  |
| **SAY:** There are three packets that include the reagents and consumables needed to run Truenat. Let’s talk through each of them.  The Truenat Chip pack has three different chips: Truenat MTB, Truenat MTB Plus, and Truenat MTB-RIF Dx.   * + Truenat MTB – for the detection of TB   + Truenat MTB Plus – a more sensitive test for TB detection   + Truenat MTB-RIF Dx – for the detection of RIF resistance |
| **Trueprep AUTO MTB Sample Pretreatment Pack**  **Slide: 13**  **Participant Guide Page: 40** |
|  |
| **SAY:** The first pack is the Trueprep AUTO MTB Sample Pretreatment Pack, which is for pretreating the sample. The packcontains graduated transfer pipettes, lysis buffer bottles, and liquefaction buffer bottles. |
| **Trueprep AUTO v2 Universal Cartridge Based Sample Prep Kit**  **Slide: 14**  **Participant Guide Page: 40** |
|  |
| **SAY:** The Trueprep AUTO v2 Universal Cartridge-Based Sample Prep Kit contains a reagent pack, transfer pipettes, and cartridge pouches that contain a cartridge, and elute collection tube, and a transfer pipette. You can order this pack for 5, 25, or 50 tests. |
| **Truenat Chip Pack**  **Slide: 15**  **Participant Guide Page: 40** |
|  |
| **SAY:** The Truenat Chip Pack (for 5, 20, or 50 tests), contains the chip pouches, a microtube containing freeze-dried PCR reagents, filter barrier pipette tip, and a desiccant pouch. Other material required include the Truelab micro printer, Trupete fixed volume 6µl pipette, Truelab microtube stand, gloves, waste disposal containers and bins.  Remember, Truenat has three types of chips, so three different types of chip packs. The chip packs are MTB, for the detection of TB; MB Plus, a more sensitive test for TB detection; and the MTB-RIF Dx, for the detection of RIF resistance. |
| **SAY:** What questions do you have before we move to the next topic? |
| **DO:** Allow participants time to ask questions and respond appropriately. |

### Truenat Test Procedures – Prepare Samples and Extract DNA

| **Section Introduction: Truenat Test Procedures – Prepare Samples and Extract DNA**  **Slide: 16**  **Participant Guide Page: 41** |
| --- |
|  |
| **DO:** Introduce the topic explaining that you are going to walk participants through the three parts of performing a Truenat test. Note that this training is intended to be followed up with hands-on training by Molbio. Hands-on training should be verified by the trainer and documented, with competency assessments conducted a minimum of once per year. Participants are not expected to be able to use Truenat after this training – this is more of an introduction to running the test. |
| **Video: Prepare Sample and Extract DNA**  **Slide: 17**  **Participant Guide Page: 41** |
|  |
| **DO:** Inform participants that they will watch a short video on preparing samples and extracting DNA. The video is found here: [(85) Truenat- A Point-of-Care Real Time PCR Test for Tuberculosis - YouTube](https://www.youtube.com/watch?v=ydR2I5S2v3c). Start the video at the :05 timestamp and stop the video at 8:41. |
| **Equipment & Supplies for Preparing Samples and Extracting DNA**  **Slide: 18**  **Participant Guide Page: 42** |
|  |
| **SAY:** To prepare the sample and extract DNA, you will use the Trueprep machine.All the supplies needed are included in the sample prep kit, which as we discussed in our last module, contains the reagent pack, transfer pipettes (3ml), and cartridge pouches. The pouches contain a cartridge, elute collection tube (ECT), and a transfer pipette. |
| **Prepare Sample and Extract DNA**  **Slides:19 – 22**  **Participant Guide Page: 42** |
|  |
| **DO:** Explain that there are 14 steps to preparing samples and extracting DNA. Read through each step. Consider asking for a participant to help read through.   1. Make sure you wear the appropriate personal protective equipment, including gloves, for specimen handling and management according to national policies. 2. Collect 2-5ml adult pulmonary sputum sample in sputum cup and label with the time and date of collection and at least two patient identifiers (name and date of birth, for example). 3. Add two drops of liquefaction buffer to the sputum cup. Liquefaction has one main purpose, to decrease viscosity and release the microorganisms from the sputum’s mucin matrix resulting in a homogenous sample. Consequently, you need to be very careful to avoid generating aerosols at this step. 4. Close the cap and swirl gently to mix. 5. Incubate for 10 minutes at room temperature. If after 10 minutes the sample cannot be pipetted, incubate for another five minutes with swirling at two-minute intervals. 6. Label a lysis buffer bottle with the patient ID and transfer 0.5 ml of liquefied sputum sample into the lysis buffer bottle using a 1 ml transfer pipette, being careful not to generate aerosols. Aerosol minimization practices include slowly drawing the sample to be processed into the transfer pipette, careful movement of the transfer pipette to the lysis buffer bottle to minimize the risk of spills or drops onto the work surface, and slow depression of the transfer pipette bulb to release the transferred volume against the interior wall of the bottle to minimize splashing - stopping before bubbles or air are expelled. 7. Add two drops of liquefication buffer into the lysis buffer bottle, swirl gently to mix, and incubate for three to five minutes. Check if contents are fully liquefied, if not, swirl gently and incubate for a further five minutes. Some samples may require up to 15 minutes for complete liquefaction. Do not proceed until the contents are fully liquefied as this is a common source of errors in later steps. 8. NB: Sample pretreatment decontaminates the sample and makes it ready for extraction. The sample in this form is stable for 3 days at up to 40°C and 1 week at 30°C. 9. 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. 10. Transfer the entire contents of the lysis buffer tube to the sample chamber (black cap) of the cartridge using a 3ml transfer pipette and the same aerosol-minimizing technique presented above. 11. Switch “on” the Trueprep Auto v2 device. Press the “eject” button to open and gently pull out the cartridge holder. 12. Place the cartridge in the tray in the orientation and gently push to close the cartridge holder. Press “start.” 13. The device will beep at the end of the DNA extraction process and the cartridge holder will eject automatically. This will take 20 minutes. 14. Gently pull out the cartridge holder, remove the cartridge, and place it on the cartridge stand. NB: do this soon after completion of extraction, otherwise the elute can evaporate which will reduce the final volume. 15. Carefully pierce the elute chamber with the provided elute transfer pipette, and transfer the entire elute into the ETC. Discard the transfer pipette and cartridge according to site-specific waste management policies and procedures. |

### Truenat Test Procedures – Run a PCR TB Test

| **Video: Running a PCR TB Test**  **Slide: 24**  **Participant Guide Page: 44** |
| --- |
|  |
| **DO:** Inform participants that they will watch another short video on running a PCR TB test.  The video is found here: [(85) Truenat- A Point-of-Care Real Time PCR Test for Tuberculosis - YouTube](https://www.youtube.com/watch?v=ydR2I5S2v3c). Start the video at the 9:18 timestamp and stop the video at the end. |
| **Equipment and Supplies for Running a PCR TB Test**  **Slide: 25**  **Participant Guide Page: 44** |
|  |
| **DO:** To run the TB test, you will use the Truelab Uno, Duo, or Quattro depending on what you have. For supplies, you’ll need the chip pack, which includes the MTB or MTB Plus Truenat chip and the Truepet 6µl Precision Micropipette. |
| **Process Flow-Running a PCR TB Test**  **Slides: 26 – 30**  **Participant Guide Pages: 45** |
|  |
| **DO:** Explain that you will review 16 steps to running a Truenat PCR TB test. Note that the steps you will review are specific to the testing procedure and may not include all necessary biosafety, quality control and assurance, and waste management information. Indicate that complete testing information will be provided to end-users with the complete Standard Operating Procedure to be used in the country. Read through each illustrative step. Consider asking for a participant to help read through.   1. **Make sure you wear the appropriate personal protective equipment, including gloves, for molecular TB testing according to national policies to minimize test-related contamination.** 2. Switch “on” the Truelab micro-PCR analyzer by pressing the red button in the back right corner for seconds. Wait for 30-50 sections for the “boot-up screen” to appear followed by the “home screen.” 3. Select USER ID and enter your password. Press “Sign in” to log in. 4. Select test profile “MTB.” To confirm selection tap “PROCEED” and enter patient details. 5. Select sample type (sputum). 6. Press “START TEST” on the screen. Chip tray opens. “Please Load Sample” will appear. Be sure not to press “YES” until the chip loading is complete. 7. Inspect a Truenat MTB chip pouch for external integrity (no rips, tears, bloating of the packaging, or other signs of damage). Open the inspected chip pouch,pull out the desiccant pouch and confirm that it is blue. If it is not blue, this suggests there was some humidity during storage which may affect test performance. Use a different chip. 8. Gently take out the chip without touching the white well portion and place it on the chip tray. 9. Open the mastermix tube, discard the stopper and place the tube in the microtube stand. Be sure to check for “white cake” at the bottom of the microtube. Flick the tube (with the stopper on) if you cannot see this “white cake” at the bottom. 10. Next, attach the 6ul micro tip provided in the pouch to the single push pipette. 11. Then transfer 6ul of the elute from ECT into the mastermix tube. You can refrigerate the elute in case the test needs to be repeated or you need to run the Truenat MTB/RIF test. After extraction and amplification steps are complete, store any left-over elute in the ECT tube at -20°C. 12. Allow the mastermix to stand for 30 seconds to get a clear solution. Make sure you do not mix by tapping, shaking, or reverse pipette. Do not discard the pipette tip. 13. Transfer the elute from the mastermix tube to the white reaction well of the chip. Be sure to avoid spillage of the clear solution outside the white reaction well. Then discard the pipette tip and mastermix tube. 14. Click “YES” on the device screen to start the test. It takes 35 minutes for the PCR test to complete. 15. Tap the “Open/Close Tray” button to eject the chip tray and discard the used chip immediately after the reaction. 16. If MTB is detected, test the same elute for RIF resistance using the Truenat MTB RIF Dx chip as a follow-on test. 17. Press “Print” to print the result page using the Truelab micro-PCR print. This is optional. |

### Truenat Test Procedures – Run a RIF Resistance Test

| **Equipment & Supplies for Running a RIF Resistance Test**  **Slide: 32**  **Participant Guide Page: 47** |
| --- |
|  |
| **SAY:** You will use the Truelab Uno, Duo, or Quattro for the RIF resistance test as well, and a RIF chip pack. |
| **Process Flow: Running a RIF-Resistance Test**  **Slide: 33**  **Participant Guide Page: 48** |
|  |
| **SAY:** When 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. When testing for RIF resistance use a Truenat MTB-RIF Dx chip.  To start the RIF resistance test, start from Step 3 in the PCR TB test process and repeat for RIF resistance. This will take an additional 60 minutes.  **SAY**: While the instruments and assay procedures are designed to minimize the risk of contamination by PCR amplification products, **it is essential to follow good laboratory practices** **and ensure careful adherence to procedures to avoid contamination** from previous amplification, positive controls, or specimens. This will be discussed further in Module 5. |

### Class Activity

| **Activity: Let’s Practice**  **Slide: 34**  **Participant Guide Page:** |
| --- |
|  |
| **NOTE:** If Truenat equipment is available at a lab near the training site, training organizers can consider having a hands-on learning session with the equipment. One person can use the equipment while others observe and remind them of the steps in the process flow. |

### Waste Management

| **Waste Management**  **Slide: 36**  **Participant Guide Page: 48** |
| --- |
|  |
| **SAY:** The Truenat tests generate a large amount of plastic waste, and it is important to dispose of or incinerate the plastic based on national guidelines. Some samples and consumables need to be contaminated prior to disposal, so let’s talk about them. |
|  |
| **Waste Disposal**  **Slide: 37**  **Participant Guide Page: 48** |
|  |
| **SAY:** There are specific procedures that must be followed with waste disposal. The items listed on this slide are classified as red biohazardous waste and 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 (2)**  **Slide: 38**  **Participant Guide Page: 48** |
|  |
| **SAY:** PPE made of fiber material or other materials except disposable plastic should be disposed of as “yellow” infectious waste, including   * Face Masks * Gowns * Caps |
| **Waste Disposal (3)**  **Slide: 39**  **Participant Guide Page: 48** |
|  |
| **SAY:** Otheritems are ok to be disposed of as general waste, including   * Cartridge pouches * Chip pouches * Transfer pipette wrappers * Desiccant pouches * Sleeves |

### Errors and Troubleshooting

| **Errors and Troubleshooting**  **Slide: 41**  **Participant Guide Page: 49** |
| --- |
|  |
| **SAY:** There are various errors you may encounter when using Truenat. The machines will alert you if there are hardware malfunctions or errors encountered when performing a test. Truelab automatically records data within the system whenever it encounters an error. If you need to communicate with Molbio to help resolve issues, this can be helpful, so it’s important to generate the log file before you restart a test whenever this happens and it is not a standard error that you can resolve yourself. |
| **DO:** Point out the “Troubleshooting, Alerts, and Errors” job aid to participants and explain that you will be walking through the errors on it. |
| **Trueprep Error Messages**  **Slides: 42 – 48**  **Participant Guide Page: 49** |
|  |
| **DO:** Read through each error and explain how to resolve it. |
| **Truelab Error Messages**  **Slides: 49 and 50**  **Participant Guide Page: 51** |
|  |
| **SAY:** There are additional error messages you might see when using Truelab. There are several types of Error 1 that you will see on this screen. The solution to these is to repeat the run using a fresh chip and reload the elute by pressing the repeat button. You might also see an invalid error message, which means that the Internal control did not amplify in PCR or improper sample extraction. The solution to this is also to rerun the same elute using another chip. |
| **Truelab Alert Messages**  **Slides: 51 – 53**  **Participant Guide Page: 52** |
|  |
| **DO:** Read through each alert and explain how to resolve it. |

### Class Activity

| **Activity: Fix the Error**  **Slide: 54**  **Participant Guide Page: 52** |
| --- |
|  |
| **SAY:** When you are using Truenat, you will sometimes get an error message. It is important to know what the error message means and how to fix it. We’ve gone over several errors that you might see – you can reference the troubleshooting handout to help figure out how to address these errors.  In pairs, you will read through the three scenarios in your Participant Guide, starting on page 52. Read through each scenario and determine the best way to fix the error. Be prepared to share your answers with the class. |
| **DO:** Give participants 8 minutes to work through scenarios and write down their responses. Call on 3 groups to share their answers. Correct any incorrect answers.  Scenario 1: You are preparing a sample and extracting DNA. You switch on the Trueprep AUTO v2 device and pull out the cartridge holder. You place your cartridge in the tray, close the door and receive an E3: Cartridge Clogged error. What does this error mean and what are your next steps?  Answer: E3: Cartridge Clogged error means the specimen used is too thick. Samples need to be liquefied and pipettable. To fix this error, press eject to exit and repeat the extraction in a fresh cartridge.  Scenario 2: You are extracting DNA using the Trueprep AUTO v2 device and receive an error message that says “could not initialize. Please try again.” What do you do?  Answer: This message means the system was unable to establish an internal connection and the test should thermal cycling error attempted again using a new chip and re-loading the elute again. If it happens again, alert Molbio support.  Scenario 3: You are using the Trueprep AUTO v2 device when you receive an alert that says “unable to read chip information.” You know you have completed each step carefully, but continuously get this message. What should you do?  Answer: The user should check if the chip was loaded properly into the tray. Remove the chip and re-select the profile from Status Screen and repeat the steps. If the message reappears, load a new chip and re-load the elute again. |
| **SAY:** What questions do you have about error messages before we move to the next topic? |
| **DO:** Allow participants time to ask questions and respond appropriately. |

### Infrastructure Requirements

| **Power**  **Slide: 56**  **Participant Guide Page: 54** |
| --- |
|  |
| **SAY:** The Truenat system is designed to be operated in peripheral laboratories with minimal infrastructure, but there are key components that need to be considered for site preparation.  For electricity: Truenat is a battery-powered device with in-built batteries that allow for testing without power for up to eight hours. Devices will not even power up if they cannot complete at least one cycle. Batteries are expected to last five years. Solar power or electricity is required to recharge the batteries. Using electric power will allow you to charge the device and run tests at the same time.  Devices are able to operate within the 100-240 voltage range and no additional voltage stabilization is required.  You may also need electrical power may be needed to cool rooms where the temperature exceeds 30C for the proper storage of test chips and refrigerating sputum samples. |
| **Solar Power (Optional)**  **Slide: 57**  **Participant Guide Page: 54** |
|  |
| **SAY:** Again, you can use solar power to charge the Truenat batteries but this is entirely optional.Molbio sells the controller and converters, but the panel, battery, and installation can be locally sourced. |
| **Room Layout**  **Slide: 58**  **Participant Guide Page: 54** |
|  |
| **SAY:** Trueprep and Truelab instruments should be installed on a flat, stable surface. Some key guidelines for the room layout are:   * Devices should be installed away from instruments that cause vibrations or electromagnetic interference. * Devices should be installed 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**  **Slide: 59**  **Participant Guide Page: 54** |
|  |
| **SAY:** This chart shows the ambient temperature of the rooms where the equipment should be installed. Trueprep and Truelab can be stored in rooms that are between 15 and 30 degrees, or 40 degrees for up to 6 months. Relative humidity can be between 10% and 80%. Chips and reagents can also generally be stored at room temperature. |
| **Dust**  **Slide: 60 Participant Guide Page: 55** |
|  |
| **SAY:** Truenat can be operated in dusty environments – but Molbio recommends installing it in a dust-free environment when possible. |
| **Biosafety**  **Slide: 61 Participant Guide Page: 55** |
|  |
| **SAY:** Truenat TB tests require the same biosafety precautions as microscopy, Xpert MTB/RIF, or TB-LAMP. These include operating in a well-ventilated room and use of PPE (lab coats and gloves) per national guidelines. Biosafety cabinets are not needed. You should follow recommendations for low-risk procedures from the *WHO Tuberculosis Laboratory Biosafety Manual*.  You should take standard precautions in handling sputum samples, including:   * Minimize aerosols * Open specimen containers carefully to avoid splatters and spills * Keep specimen containers upright * Decontaminate leaky containers with disinfectant * Wash hands   As discussed in the last module, you should decontaminate samples and consumables prior to disposal by submerging used consumables in freshly prepared 0.5% sodium hypochlorite solution for 30 minutes. This includes TruenatTM chips, microtube, microtube cap, transfer pipette, pipette tips, reagent bottles, etc. |
| **Security**  **Slide: 62 Participant Guide: 55** |
|  |
| **SAY:** All laboratory equipment should be kept in a secure, lockable facility. As previously mentioned, the Truenat equipment can be transported in the portable Truelab® Real Time PCR Workstation Field Case, which should be stored in a secure lockable location when not in use. |
| **Preventive Maintenance**  **Slide: 63**  **Participant Guide Page: 55** |
|  |
| **SAY:** Minimal preventable maintenance is needed for Truelab and Trueprep equipment. Each day, someone should clear and disinfect the work area and discard used chips and cartridges. On a monthly basis, instrument surfaces should be disinfected, Truelab bays should be cleaned, the temperature should be calibrated, and the fixed pipette should be verified. As needed, someone should run a flush protocol for the Trueprep instrument, replace the spillage tray or linear motion group tray, and replace the slider glass. All of these should be tracked in a preventive maintenance log – there is an example of such a log in the Truenat Implementation Guide. |
| **SAY:** What questions do you have about infrastructure requirements before we move to the next topic? |
| **DO:** Allow participants time to ask questions and respond appropriately. |

### Class Activity

| **Activity: Preventive Maintenance**  **Slide: 64**  **Participant Guide Page: 55** |
| --- |
|  |
| **SAY:** When you are using Truenat, you will need to conduct routine and as-needed maintenance.  In pairs, you will define the daily, monthly and as-needed preventive maintenance tasks. |
| **DO:** Working in pairs, have each team define the daily, monthly, and as-needed preventive maintenance tasks. |
| **ANSWER:**  **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 |

### Recording Testing Activities

| **Recording Testing Activities**  **Slide: 66**  **Participant Guide Page: 56** |
| --- |
|  |
| **SAY:** Depending on the current format of the country’s requisition form, it may be necessary to revise accommodate the Truenat TB tests. Similarly, laboratory and clinical registers may need to be modified to record the results of the Truenat TB tests and Truenat MTB-RIF Dx tests. |
| **Recording Testing Activities**  **Slide: 67 Participant Guide Page: 56** |
|  |
| **PLACEHOLDER:** If lab request forms and registers, rejection logs, or other recording forms have already been developed, facilitators should modify this content and walk trainees through them. |

### Warranty

| **Warranty Conditions**  **Slide: 69**  **Participant Guide Page: 56** |
| --- |
|  |
| **SAY:**   * 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 to repair or free of charge substitution/replacement of spare parts 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 on-site (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**  **Slide: 70**  **Participant Guide Page: 57** |
|  |
| **SAY:** 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 an unauthorized person &/or with spare parts which are not originals. * Alterations 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 is very important to stress as many instruments may be transferred at some point.]**   + 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**  **Slide: 71**  **Participant Guide Page: 57** |
| Graphical user interface, text  Description automatically generated |
| **SAY:** Check for the following parameters with respect to the location of installation:   1. The workstation should be positioned on the workplace/table/workbench in an upright position on a 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 that may cause vibrations or electromagnetic interference. 4. Installation site should be free from any atmosphere of potentially explosive liquids, vapors, and gas. 5. Room temperature should be between 15ºC and 40ºC. 6. Relative humidity (RH) should be between 10% - 80% (non-condensing). 7. Power Supply minimum requirement is 100 to 240V/5Amps AC for all the devices as AC to DC adapters which are provided along with the device for charging an Inbuilt Battery can function. 8. Check for Earthing Voltage which should be less than 5V 9. Dimensions of our Devices are as Follows:    * Trueprep Autov2: 215 mm x 235 mm x 115 mm    * Truelab Quattro: 400 mm x 242 mm x 159 mm    * Truelab Duo: 240 mm x 242 mm x 159 mm    * Truelab UnoDX: 248 mm x 185 mm x 112 mm 10. Table space requirement should be after taking other accessories like MicroTube stand, Cartridge stand, Thermal Printer (small Size) and MicroPipette (6uL) Stand also into consideration. |
| **Other Warranty Information**  **Slide: 72**  **Participant Guide Page: 58** |
| Text  Description automatically generated |
| **SAY:**  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 the 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 are 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

| **Summary**  **Slide: 73**  **Participant Guide Page: 58** |
| --- |
|  |
| **SAY:** Let’s review what we learned in this module.  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 micro PCR printer   Procedures for operating Truenat are summarized in an easy-to-follow job aid.  Truenat equipment requires minimal infrastructure and minimal preventive maintenance. |
| **ASK:** Does anyone have any questions before the quick knowledge check? |
| **DO:** Answer any clarifying questions from participants. |

### Knowledge Check

| **Knowledge Check**  **Slides: 74 – 76**  **Participant Guide Page: 59** |
| --- |
| **DO:** Explain that you will ask participants three knowledge check questions and may call on participants randomly to provide an answer. (These can also be programmed as poll questions in a virtual training – ensure that all participants respond before proceeding if using the poll feature).  If an answer provided is incorrect, ask if other participants would like to answer. Correct any incorrect answers that are given. If multiple participants get a question wrong, you may need to revisit the topic.  Note that knowledge check questions are not included in participant guides to avoid students seeing them during the lesson and only focusing on those pieces. Encourage participants to write down the answers in their guides in the notes field for future reference. |
|  |
| **ANSWER:**   1. 3 – Trueprep, Truelab, and Truelab printer 2. 3 – Sample pretreatment pack, cartridge-based sample prep kit, chip pack |
|  |
| **ANSWER:**   1. Uno, Duo, and Quattro |
|  |
| **ANSWER:**   * Disinfect instrument surfaces * Clean Truelab bays * Temperature calibration * Verification of the fixed 6µl pipette |

# Module 4: Order Planning and Quality Assurance (QA) and Control

## Target Audience

The target audience for this course is:

* Lab managers
* Program managers

## Learning Objectives

**Terminal Objective**

* At the end of this session, participants should have a foundation in how to ensure the quality of Truenat testing at their sites.

**Module 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

## Materials

* Facilitator Guide
* Participant Guide
* Pens/Pencils

## Advance Preparation

Facilitators should ensure they understand the activity on stock management in advance of delivering the lesson.

## Lesson Plans

### Introduction

| **Introduction**  **Slide: 3**  **Participant Guide Page: 60** |
| --- |
| Graphical user interface, text, application  Description automatically generated |
| **SAY:** In this lesson, we will talk about how to forecast and plan supply orders, and how to develop and follow quality assurance procedures at test sites. |
| **Learning Objectives**  **Slide: 4**  **Participant Guide Page: 60** |
|  |
| **SAY:** By the end of this module, you will be able to:   * Explain how to forecast Truenat supplies. * List the key elements of good stock management.   Identify some quality assurance procedures for Truenat testing. |
| **ASK:** What questions do you have before we move into this training? |
| **DO:** Allow participants time to ask questions and respond appropriately. |
|  |
| **Module 4: Order Planning and Quality Assurance and Control**  **Slide: 5**  **Participant Guide Page: 60** |
|  |
| **SAY:** We will look at four topics in this module: forecasting and quantification, quality assurance and control and monitoring quality. |

### Forecasting and Quantification

| **Reagents and Consumables**  **Slide: 7**  **Participant Guide Page: 60** |
| --- |
| A picture containing diagram  Description automatically generated |
| **SAY:** Remember there are three packets that include the reagents and consumables that are needed to run Truenat: Trueprep AUTO MTB Smample Pre-treatment Pack, Trueprep AUTO v2 Universal Cartridge Based Sample Prep Kit and the Truenat Chip Pack.  Do not forget the Truenat Chip pack has three different chips: Truenat MTB-for the detection of TB, Truenat MTB Plus- a more sensitive test for TB detection, and Truenat MTB-RIF Dx-for the detection of RIF resistance. |
|  |
| **Ordering Reagents and Consumables**  **Slide: 8**  **Participant Guide Page: 60** |
| Graphical user interface, text, application, email  Description automatically generated |
| **DO:** Review the type of questions that participants should consider when ordering reagents and consumables. |
| **Pricing**  **Slide: 9**  **Participant Guide Page: 61** |
| Table  Description automatically generated |
| **SAY:** The Global Drug Facility (GDF) Diagnostics Catalog outlines the pricing of equipment, reagents and service packages through Stop TB Partnership’s GDF. |
| **DO:** If working online, open the link to the [GDF Diagnostics Catalog](http://stoptb.org/assets/documents/gdf/drugsupply/GDFDiagnosticsCatalog.pdf) and explore with participants the items on there. |
| **Storage Conditions and Shelf-Life of Consumables**  **Slide: 10**  **Participant Guide Page: 61** |
|  |
| **SAY:** The shelf life of reagents and their required storage conditions must be taken into consideration.  The recommended storage conditions for the Truenat TB chips is 2°C–30°C and for the Sample Pre-treatment Pack and Prep Kit is 2°C–40°C.  The shelf-life of all reagents is 2 years under recommended storage conditions. Truenat TB chips can be stored for up one month at a temperature under 45°C and up to 6 months at a temperature under 40°C, if conditions do not allow for storage under 30°C. |
| **Quantities for an Initial Order of Reagents**  **Slide: 11**  **Participant Guide Page: 62** |
| Graphical user interface, table  Description automatically generated |
| **SAY:** This table shows the number of reagents to order for one year of testing based on planned average number of tests per day in 260 working days. |
| **DO:** Review the overall chart with participants. Consider having a participant go through the slide. |
| **Order Quantities**  **Slide: 12**  **Participant Guide Page: 63** |
|  |
| **SAY:** The number of MTB-RIF Dx kits to order should depend on the anticipated proportion of people tested that will be MTB positive, and therefore in need of a test for rifampicin resistance.  The anticipated number of tests needed should include the number of repeat rifampicin resistance tests that will be required given the need to confirm rifampicin-resistant results among patients in whom the result is unexpected and for tests that give errors or indeterminate results.  In the previous table, an estimate of 20% is used. Tests are increased by 5% to account for potential wastage, and the resulting number of tests is rounded up to the nearest kit (50 tests per kit). Note that other kit sizes are available (kits with 5 or 20 tests). |
| **Data Needed for Regular Forecasting**  **Slide: 13**  **Participant Guide Page: 63** |
| Calendar  Description automatically generated |
| **SAY:** There is certain data you need for regular forecasting. |
| **DO:**  Review the data to collect with participants.  Share with participants that stock currently available is the physical count. The amount of stock currently needed by the laboratory to be able to continue testing for one quarter, plus buffer considering what the lab already has currently available (F). |

### Activity: Regular Forecasting

| **Regular Forecasting Activity**  **Slide: 14 Participant Guide Page: 64** |
| --- |
|  |
| **SAY:** In groups of two, you are going to practice calculating supply requirements by completing the Quarterly Supply Requirement table in your Participant Guide. Be prepared to share your findings in a whole group discussion. |
| **DO:** Before participants start, share with them how to calculate how much to order. Have them refer to their participant guide for the formula. The calculations are:   * + Calculating how much of a supply to order:     - a = quarterly number of tests performed (e.g, 210 tests)     - b = number of months (e.g, 3 months)     - c = average usage per month (a ÷ b) (e.g, 210 ÷ 3 = 70 tests per month)     - d = lead time (e.g, 4 months)     - e = stock in-hand (e.g, 140 cartridges)     - f = recommended buffer stock (2 months average usage = 140 tests)   + Minimum quantity to order for a four-month lead time: (c × d) – e + f = (70 × 4) – 80+140 = 340 cartridges.   Give participants 15 minutes to complete the table. Help pairs as they go through the calculations with their partner. Use Module 3 Technical Modules for Xpert Stop TB Partnership – Global laboratory Initiative (GLI) for guidance.  After 10 minutes, call on participants to share their calculations. Correct any incorrect answers and allow other participants to explain their answer if it is different. |
| **ANSWERS:**   | **Quarterly Supply Requirements for Truenat Testing** | | | | | | | | | | | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | | **Laboratory:** Regional Reference Laboratory | | | | | | | | | | | **Region:** Western Region | | | Supply Quarter 3 | | | | | | | | **District:** Urban | | | **Year:** 2021 | | | | | | | | Total MTB tests performed in previous quarter, including failed tests (A): 630 | | | | | | | | | | | **Item** | **Quantity Needed per Test (B)** | **Stock for one month (C) = (A/3) \* B** | | **Buffer stock requirement**  **(2 month average usage)** | **Stock for quarter with 1 month buffer (D)= C\*5** | **Stock on hand (E)** | **Calculated request (F) = D-E** | **Order unit (G)** | **Actual order (H) = F/G round up** | | Trueprep® AUTO MTB Sample Pre-treatment Pack | 1 | 210 | | 420 | 1,050 | 450 | 600 | 1 pack | 600 packs | | Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Kit (for 25 tests) | 1 | 210 | | 420 | 1,050 | 420 | 630 | 25 tests | 26 kits | | Truenat MTB Chip Pack | 1 | 210 | | 420 | 1,050 | 300 | 750 | 1 pack | 750 packs | |
| **SAY:** What questions do you have before we move into the next slide? |
| **DO:** Allow participants time to ask questions and respond appropriately. |

### Stock Management

| **Stock Management**  **Slide: 16 Participant Guide Page: 66** |
| --- |
| Timeline  Description automatically generated |
| **SAY:** The purpose of determining how much supplies to order is because it helps avoid stock-outs and expiring of cartridges. A designated person is needed for supply management.  There are six key components to maintaining an adequate stock of Truenat supplies. The components are as follows:   1. Do a monthly “stock count.” 2. Maintain inventory records. 3. Determine how much to order. 4. Place orders in accordance with local ordering practices. 5. Inspect and verify supplies received. 6. Ensure proper storage of stock. |
| **Stock Log**  **Slide: 17**  **Participant Guide Page: 67** |
| Chart, diagram  Description automatically generated |
| **ASK:** What fields should be included in a stock log for Truenat? |
| **DO:** Lead a whole group discussion and help the participants come up with the different content in the surrounding circles. Once the discussion reaches a logical stopping point, show the rest of the content on the slide. |
| **ANSWER:**   * 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 |
| **Temperature and Shelf Life**  **Slide: 18**  **Participant Guide Page: 67** |
| Graphical user interface, text, application, email  Description automatically generated |
| **NOTE:** Storage information on this slide has been discussed previously. This slide is a recap. The key point on this slide is the note on GDF-negotiated minimum shelf life at time of readiness for delivery is 19 months. |
| **SAY:** The recommended storage conditions for the Sample Pre-treatment Pack and Prep Kit is 2°C–40°C. Shelf life of reagents under recommended storage conditions is 2 years from the date it was manufactured. GDF-negotiated minimum shelf life at time of readiness for deliver is 19 months.  If conditions do not allow for storage under 30°C – Truenat TB chips can be stored for up to 6 months under 40°C. |

| **Storage and Expiry**  **Slide: 19**  **Participant Guide Page: 68** |
| --- |
| Graphical user interface, text, application  Description automatically generated |
| **SAY:** When storing equipment, organize any existing and new shipments by the expiry date. This is so supplies with the soonest expiry date will be used first. |

### Quality Assurance and Control

| **QA and Control**  **Slide: 21**  **Participant Guide Page: 68** |
| --- |
| Diagram  Description automatically generated |
| **ASK:** What types of quality control processes do you currently institute in your lab that would be relevant to Truenat? |
| **DO:** Call on participants to share their experiences about QA programs and external quality control processes in their labs. |
| **Roles of Ensuring Quality**  **Slide: 22**  **Participant Guide Page: 69** |
| **SAY:** Lab managers are responsible for overseeing QA activities at large facilities. The Health Facility Quality Committee (HFQC) may provide oversight and coordination for QA activities at larger facilities. |
| **Quality Assurance Program**  **Slide: 23**  **Participant Guide Page: 69** |
| Diagram  Description automatically generated |
| **SAY:** Many components go into creating a quality assurance program. For Truenat having standardized documents, use of good molecular biology practices, competency assessment internal quality controls, EQAs, on-site supervision and continuous quality improvement processes are essential elements of a quality assurance system for any rapid TB diagnostic test. |
| **DO:** Inform participants that Standardized documents include identification, tailoring and ensuring use. |
| **Good Molecular Biology Practices**  **Slide: 24**  **Participant Guide Page: 70** |
| Graphical user interface, text, application, email  Description automatically generated |
| **SAY:** The Truenat TB test procedures require multiple hands-on steps as well as precision micro-pipetting. Laboratory technicians should be properly trained on all procedures and in good molecular biology practices. |

| **Competency Assessment**  **Slide: 25**  **Participant Guide Page: 70** |
| --- |
| Graphical user interface, application, email  Description automatically generated |
| **SAY:** Competency assessments of a lab technician should be performed after training and periodically (annually). After competency assessments of laboratory techs, there should be assessments of the knowledge and skills for performing each of the tasks involved in a diagnostic test. |
| **Standardized Documents (SOPs)**  **Slide: 26**  **Participant Guide Page: 71** |
| Graphical user interface, text, application, email  Description automatically generated |
| **SAY:** Standardized Documents should be provided as reference materials for lab technicians. The following job aids are available in Annex 11 of the Truenat Implementation guide:   * Truenat PCR Process Flow: Sample to Result * Troubleshooting, Alerts, and Errors * Do’s and Don’ts |

| **Internal Quality Control (IQC)**  **Slide: 27**  **Participant Guide Page: 71** |
| --- |
|  |
| **SAY:** Truenat TB assays incorporate internal quality controls through positive and negative controls that follow the same processes as the specimen. |
| **External Quality Assessment (EQA)**  **Slide: 28**  **Participant Guide Page: 72** |
|  |
| **SAY:** External Quality Assessments or EQAs for Truenat are not yet available, but it can be modeled after the proficiency testing programme used for the Xpert MTB/RIF test.  It is recommended to test 10-15 specimens per week to maintain proficiency of staff. |

| **Initial Calibration/Verification Panels**  **Slide: 29**  **Participant Guide Page: 72** |
| --- |
| A picture containing timeline  Description automatically generated |
| **SAY:** New lot testing, positive and negative controls and maintaining QC records are the components that involve initial calibration and verification panels.  New lot testing (lot-to-lot verification) control:  Positive and negative controls are used for new batches of reagents.  Maintaining and monitoring QC records of lot testing should be reviewed by testing site manager and retention on site for a period according to local or national policy. |
| **Regular Maintenance**  **Slide: 30**  **Participant Guide Page: 73** |
|  |
| **SAY:** Although, Truelab and Trueprep instruments require minimal preventive maintenance as per the manufacturer, there are some tasks that should be done monthly.  **SAY**: The Truenat system is a closed amplification system (i.e., the amplified product is sealed in the chip) and an enzyme system is incorporated in the reaction mix to prevent previously amplified material from getting re-amplified. Nonetheless, swab testing of surfaces and instruments is recommended monthly and negative control tests using Trueprep AUTO lysis buffer reagent and sterile PBS should be run monthly or when contamination is suspected (e.g., unusually high proportion of specimens with ‘MTB detected’). Note that if the benches are made of “sub-optimal” material (e.g. wood), you may likely get false positives, in that case then, consider only swab testing instruments. |
| **DO:** Review the maintenance chart with participants. |
|  |
| **Warranty and Repair**  **Slide: 31**  **Participant Guide Page: 74** |
| Graphical user interface, text, application, email  Description automatically generated |
| **SAY:** Molbio offers one-to-five-year extended warranties per year through GDF.  A 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   For the repair and replacement of parts remember that:   * If a machine is not repairable at the site, a stand-by machine will be provided. * If the machine needs to be replaced free replacement will be provided at Molbio’s cost. |

### Monitoring Quality

| **General Laboratory Performance Indicators**  **Slide: 33**  **Participant Guide Page: 75** |
| --- |
| Table  Description automatically generated |
| **SAY:** On this slide, you will see general lab performance indicators and their targets. |
| **DO:** Review what is on the slide with participants. |
| **ASK:** Which of these indicators do you already track for other tests? |
| **DO:** Call on participants to share their experiences. |
| **Performance Indicators for Truenat TB Test**  **Slides: 34 and 35**  **Participant Guide Page: 75** |
| Table  Description automatically generated with low confidence |
| **DO:** Review what is on the slide with participants. |
| **SAY:** The performance indicators for Truenat TB tests are modeled after GLI-recommended performance indicators for Xpert MTB/RIF testing that should be monitored monthly by each testing site. Some targets are setting-specific. Labs should monitor indicators and establish local targets and ranges. Deviations from expected value should be investigated. |
| **Performance Indicators for Monitoring Patient and Sample Flow**  **Slide: 36**  **Participant Guide Page: 76** |
| Graphical user interface, text, application  Description automatically generated with medium confidence |
| **SAY:** On this slide, you will see performance indicators for monitoring patient and sample flows. |
| **DO:** Review what is on the slide with participants. |
| **ASK:** What targets would you set for these, based on your experience? |
| **DO:** Call on participants to share their experiences. |

### Summary

| **Summary**  **Slide: 37**  **Participant Guide Page: 77** |
| --- |
| Text  Description automatically generated with medium confidence |
| **SAY:** It is important to monitor inventory, ensure supplies have not expired and forecasting future needs.  There are procedures and programs that allow for oversight and coordination of QA activities.  Performance indicators help track and monitor each testing site to ensure quality tasks are carried out appropriately. |

### Knowledge Check

| **Knowledge Check**  **Participant Guide Page: 78**  **Slides: 39 – 41** |
| --- |
| **DO:** Explain that you will ask participants three knowledge check questions and may call on participants randomly to provide an answer. (These can also be programmed as poll questions in a virtual training – ensure that all participants respond before proceeding if using the poll feature).  If an answer provided is incorrect, ask if other participants would like to answer. Correct any incorrect answers that are given. If multiple participants get a question wrong, you may need to revisit the topic.  Note that knowledge check questions are not included in the participant guides to avoid students seeing them during the lesson and only focusing on those pieces. Encourage participants to write down the answers in their guides in the notes field for future reference. |
|  |
| **ANSWER:** 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. More information is needed about the proportion of the 210 tests that were positive. |
| Graphical user interface, text, application  Description automatically generated |
| **ANSWER:**   * Do a monthly “stock count” * Maintain inventory records * Determine how much to order * Place orders in accordance with local ordering practices * Inspect and verify supplies received * Ensure proper storage of stock * Keep a stock log * Organize existing and new shipments by the expiry date |
| Graphical user interface, text, application  Description automatically generated |
| **ANSWER:**   * Standardized documents * Use of good molecular biology practices * Competency assessment internal quality controls * External quality assessment (EQA) * On-site supervision * Continuous quality improvement processes |

# Module 5: Monitoring & Evaluation (M&E)

The monitoring and evaluation lesson provides participants with the knowledge of establishing a monitoring and evaluation plan for Truenat.

## Target Audience

The target audience for this course is:

* Lab managers
* Program managers

## Learning Objectives

**Terminal Objective**

* At the end of this session, participants should be able to describe how to establish a monitoring and evaluation plan for Truenat.

**Module Objectives**

* By the end of this module, the participant will be able to:
  + Outline a general approach to monitoring and evaluating the impact of Truenat on TB-related targets and goals.

## Advance Preparation

Facilitators may need to tailor the content at the end of this module to reflect any changes to M&E processes and systems that have already been developed. This section is indicated in yellow highlight.

## Lesson Plans

### Introduction

| **Introduction**  **Slide: 3**  **Participant Guide Page: 79** |
| --- |
|  |
| **SAY:** In this module, we will talk about M&E aspects of Truenat. The focus will be on indicators and how to establish or revise an M&E plan that will allow you as a country to assess the impact of Truenat on meeting targets and goals. |
| **Module Learning Objectives**  **Slide: 4**  **Participant Guide Page: 79** |
|  |
| **SAY:** The learning objective for this module is that by the end of this training topic, you will be able to outline a general approach to monitoring and evaluating the impact of Truenat on TB-related targets and goals. |
| **Introduction**  **Slide: 5**  **Participant Guide Page: 79** |
|  |
| **SAY:** This is a short module, with just two topics. First we’ll talk in general about M&E For Truenat, then we’ll walk through what indicators may be available to track. |

### M&E for Truenat

| **Inroduction**  **Slide: 7**  **Participant Guide Page: 79** |
| --- |
|  |
| **SAY:** The main idea behind developing an M&E system for Truenat is the idea that integrating Truenat into a diagnostic network should help a country meet its existing targets for case detection, bacteriological confirmation, drug resistance testing, or other TB-related indicators. If adding Truenat does not help achieve targets, then either the targets need to be revised or Truenat is not the right solution. |
| **ASK:** What targets will Truenat help achieve in *country*? (Answers will vary but should be tied to the discussion in Module 1 about priority diagnostic challenges.) |
|  |
| **QA vs. M&E**  **Slide: 8**  **Participant Guide Page: 79** |
|  |
| **SAY:** Before we move on, I just wanted to make a quick note about the difference between the QA indicators that we talked about in the last module versus the indicators we are about to talk about for M&E. QA indicators should be used to monitor the performance of the instruments and the technicians using the instruments to make sure everything is working properly. Impact indicators described in this lesson should be used to monitor and evaluate progress towards achieving 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**  **Slide: 10**  **Participant Guide Page: 80** |
| --- |
|  |
| **SAY:** WHO has indicators on laboratory strengthening that you are probably already familiar with. Let’s go over them. |
| **DO:** Go through each possible impact indicator. |
| **ASK:** Which of these indicators are already being measured in your country? (Answers will depend on the country.) |
| **DO:** Ask for a volunteer to provide an answer and have participants note correct answers in their participant guide.  **REMIND**: The impact indicators should be in line with the national data collection tools to capture it routinely. |
| **Other Possible Impact Indicators**  **Slides: 11 and 12**  **Participant Guide Page: 82** |
|  |
| **SAY:** There are other impact indicators that also may be relevant. |
| **DO:** Go through each possible impact indicator. |
| **Diagnostics Connectivity**  **Slides: 13 and 14**  **Participant Guide Page: 83** |
|  |
| **SAY:** To help monitor the indicators that are chosen more easily, you might consider using the connectivity features of Truenat. Software can rapidly and automatically calculate many of the key performance indicators and facilitate the M&E process.  Third-party connectivity software platform (Aspect and DataToCare) companies are currently working to allow for smooth flow of data to these platforms​.  Digital results reporting can be used to send data to national servers for M&E and for surveillance purposes​. |

### Summary

| **Summary**  **Slide: 16**  **Participant Guide Page: 84** |
| --- |
|  |
| **SAY:** The main point of this module was to emphasize that progress toward achieving WHO and other impact indicators should be measured to assess the impact of Truenat. |
| **ASK:** Does anyone have any questions about this lesson? |
| **DO:** Answer any clarifying questions from participants. |

### Knowledge Check

| **Knowledge Check**  **Slide: 17 and 18**  **Participant Guide Page: 84** |
| --- |
| **DO:** Explain that you will ask participants three knowledge check questions and may call on participants randomly to provide an answer. (These can also be programmed as poll questions in a virtual training – ensure that all participants respond before proceeding if using the poll feature).  If an answer provided in incorrect, ask if other participants would like to answer. Correct any incorrect answers that are given. If multiple participants get a question wrong, you may need to revisit the topic.  Note that knowledge check questions are not included in participant guides to avoid students seeing them during the lesson and only focusing on those pieces. Encourage participants to write down the answers in their guides in the notes field for future reference. |
|  |
| **SAY:** Open your participant guide to page 85. Read and answer the knowledge check question. Be ready to share your answer. |
| **DO:** Allow participants five minutes to respond to the knowledge check question. Inform participants to write down their answers in their participant guide. Allow a participant to share their response. |
| **ANSWERS:**   1. 100% 2. 100% 3. 100% 4. 80% 5. 80%, Relapse: 90% |
| **Knowledge Check** |
|  |
| **DO:** Read question aloud and allow participants time to respond in their participant guide. Depending on the size of the group, consider calling on all participants to share their answers. |
| **ANSWER:** Answers will vary. |

# Module 6: Biosafety and Specimen Collection and Referral

This lesson introduces the proper procedures for specimen collection and referral.

## Target Audience

The target audience for this course is:

* Lab technicians
* Lab clinicians

## Learning Objectives

**Terminal Objective**

* At the end of this session, participants should be able to explain the processes for specimen collection, pretreatment, and referral for Truenat.

**Module Objectives**

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

* Demonstrate good biosafety practices and risks when performing Truenat testing
* Collect and pretreat a sputum sample
* Describe storage requirements for collected specimens
* Understand the process for specimen referral

**Materials**

* Facilitator Guide
* Participant Guide
* Pens/Pencils
* Index cards

## Advance Preparation

* Facilitators will need to understand if the national program has already made decisions about the number of specimens that should be collected, or about referral processes more generally. If so, the content of this module should be tailored to reflect these decisions.

## Lesson Plans

### Introduction

| **Introduction**  **Slide: 3**  **Participant Guide Page: 85** |
| --- |
|  |
| **SAY:** Our final module introduces biosafety and the procedures for specimen collection and referral. |
| **Course Outline**  **Slide: 4**  **Participant Guide Page: 85** |
|  |
| **SAY:** We will look at three topics– biosafety, specimen collection and specimen referral |

| **Learning Objectives**  **Slide: 5**  **Participant Guide Page: 85** |
| --- |
|  |
| **SAY:** By the end of this module, you should be able to:   * Demonstrate good biosafety practice when using Truenat * Collect and pretreat a sputum sample for Truenat testing (note that we are assuming that all training participants are familiar with the basic principles of collecting sputum samples) * Describe storage requirements for collected specimens * Describe packaging of specimens for transportation * Understand the process for specimen referral |
| **ASK:** What questions do you have before we move into the first lesson for this training? |
| **DO:** Allow participants time to ask questions and respond appropriately. |

### Biosafety Measures and Risk

| **General Principles of Biosafety**  **Slide: 7**  **Participant Guide Page: 85** |
| --- |
| Graphical user interface, text, letter, email  Description automatically generated |
| **SAY:** Biosafety has three key important components needed to handle TB bacilli safely: Primary safe working practices to minimize generation of infectious aerosols, Secondary infrastructure and layout support for primary activities and Tertiary buildings to contain the laboratory and its activities. |
| **Importance of Biosafety**  **Slide: 8**  **Participant Guide Page: 85** |
| Graphical user interface, text, application, email  Description automatically generated |
| **SAY:** The importance of biosafety is to reduce the risk of infection or injury to you, coworkers, and the community. The main procedural risk in a TB laboratory is generation of aerosols. |
| **Standard Microbiological Procedures**  **Slide: 9**  **Participant Guide Page: 85** |
| Graphical user interface  Description automatically generated with medium confidence |
| **SAY:** The standard microbiological procedures are not eating, drinking, or applying cosmetics in the laboratory. This also includes washing hands after working with infectious materials and before leaving the laboratory and routinely decontaminating work surfaces in the laboratory. |
| **Addressing Biosafety**  **Slide: 10**  **Participant Guide Page: 85** |
| Graphical user interface, text, letter  Description automatically generated |
| **SAY:** This section of the module will help you understand the following: a) The risks in a TB lab, b) Laboratory infrastructure, design and layout, c) Personal protective equipment, d) Biological safety cabinets, e) Generation and prevention of aerosols, f) Spills and g) Waste management. |

### Assessing Risk

| **Risk Biosafety Levels**  **Slide: 12**  **Participant Guide Page: 86** |
| --- |
| Chart  Description automatically generated |
| **SAY:** We are going to discuss the four biosafety levels (BSLs 1,2, 3 and 4) as well the risks that determine the levels of containment. Each level has a specific control (laboratory practices, safety equipment, and construction) for containment of microbes and biological agents. |
| **Characteristics of Biosafety Levels**  **Slide: 13**  **Participant Guide Page: 86** |
|  |
| **SAY:** We will discuss the characteristics of the biosafety levels (BLS-1, BLS-2, BLS 3, and BLS-4) and give examples of the microbes**.** |
| **DO:** Read off each biosafety levels and examples of each. |
|  |
| **Biosafety Measures According to Risk Levels**  **Slide: 14**  **Participant Guide Page: 86** |
|  |
| **SAY:** We will walk through each BSL to discuss biosafety measures according to risk levels. |
| **DO:** Provide an overview of biosafety measures at each risk level. |
| **Risk Precaution Levels**  **Slide: 15**  **Participant Guide Page: 86** |
| Table  Description automatically generated |
| **SAY:** We will discuss the risk precaution levels of TB with the laboratory activities and assessment of risk**.** |
| **DO:** Discuss the laboratory activities and risk at each level. |

### Laboratory Infrastructure

| **Ventilation and Laboratory Setup**  **Slide: 17**  **Participant Guide Page: 87** |
| --- |
| Word  Description automatically generated |
| **SAY:** It is important to note that a biosafety cabinet is not essential for Truenat testing. You only need a safe, well-ventilated laboratory setup. Where windows cannot be opened, consider using mechanical ventilation systems, such as exhaust fans. |

### Personal Protective Equipment

| **Matrix of Recommended PPE According to Activity**  **Slide: 19 and 20**  **Participant Guide Page: 88** |
| --- |
| Table  Description automatically generated  Table  Description automatically generated |
| **SAY:** We will walk through the matrix of the recommended PPE according to activity. |
| **DO:** Highlight the PPE needed for each activity. |
| **Proper Order for Donning PPE**  **Slide: 21**  **Participant Guide Page: 89** |
|  |
| **SAY:** On this slide, you will see a list of proper order for donning PPE. |
| **PPE: Gloves and Shoes**  **Slide: 22**  **Participant Guide Page: 89** |
| Graphical user interface, text  Description automatically generated |
| **DO:** Read through the uses for gloves and shoes. |
|  |
| **PPE: Laboratory Coat and Gown**  **Slide: 23**  **Participant Guide Page: 89** |
| Graphical user interface  Description automatically generated |
| **DO:** Read through the uses for gowns and coats. |
| **PPE: Respirators and Masks**  **Slide: 24 – 27**  **Participant Guide Page: 89** |
| Graphical user interface, text  Description automatically generatedGraphical user interface  Description automatically generated  Graphical user interface  Description automatically generated with low confidenceTimeline  Description automatically generated |
| **SAY:** These slides describe the respirators and masks, and how to properly wear the respirators as well as properly removing |
| **DO:** Discuss common mistakes in wearing respirators and how to properly wear one. |
|  |
| **PPE: Face Shields and Goggles**  **Slide: 28**  **Participant Guide Page: 89** |
| Graphical user interface, text  Description automatically generated |
| **SAY:** Face shields and goggles should be used in facilities where there is no BSC due to the COVID-19 pandemic. |

### Biosafety Cabinets

| **Biosafety Cabinets**  **Slide: 30**  **Participant Guide Page: 89** |
| --- |
| Graphical user interface, text, application, email  Description automatically generated |
| **SAY:** Biosafety cabinets are categorized as Class 1, II or Class III. Class 1I provides protection for the user, environment and work area. There four types of Class I1:A1, A2, B1, and B2. Class 11 type A2 BSC are recommended for all TB work but are not necessary for Truenat testing. |
| **Performing Tests without BSC**  **Slide: 31**  **Participant Guide Page: 90** |
|  |
| **SAY:** This slide discusses the essential minimum biosafety measures for a TB laboratory without BSC.  These conditions are:   1. Adequate ventilation and directional airflow are required 2. Bench spaces should be separate from areas where samples are received and from administrative areas eg paperwork, and telephones 3. Appropriate PPE and well-trained staff on GLP 4. Benchtops should be impervious to water, and resistant to the chemicals and disinfectants used in the laboratory. 5. Storage space must be adequate to hold supplies for immediate use and prevent clutter on bench tops 6. Minimizing the generation of aerosols by using good microbiological techniques 7. Appropriate handling of leaking sample containers (e.g., decontaminate container before processing, or discard and request a fresh sample) |

### Generation and Prevention of Aerosols

| **Generating Aerosols**  **Slide: 33**  **Participant Guide Page: 90** |
| --- |
| Graphical user interface, text, application, email  Description automatically generated |
| **SAY:** In the TB laboratory, highly infectious aerosols can be generated. High-risk procedures and practices that may increase the potential of creating aerosols include mechanical (vortexing, centrifugation, shaking), pouring /tipping, and pipetting. |
| **Minimizing Aerosol Formation**  **Slide: 34**  **Participant Guide Page: 91** |
| Graphical user interface, text, application, email  Description automatically generated |
| **SAY**: To minimize aerosol formation, always allow enough contact time of liquefication buffer to sample, including adequate standing time. |
|  |
| **Minimizing Aerosol Formation**  **Slide: 35**  **Participant Guide Page: 91** |
| Text  Description automatically generated |
| **SAY:** Minimize aerosol formation during pipetting by not forcibly expelling air from the pipette when aspirating the liquified and lysed sputum and place the pipette against the inner wall of the lysis buffer bottle when dispensing liquified sputum sample. Do not forcibly expel the lysis buffer sample while dispensing it into the cartridge. |

### Managing Spills

| **Managing and Responding to Spills**  **Slide: 37 and 38**  **Participant Guide Page: 92** |
| --- |
| Graphical user interface, text, email  Description automatically generated  Graphical user interface, text  Description automatically generated |
| **SAY:** Spills usually involve liquids, and aerosols of infectious droplet nuclei can be generated. Spills outside of a BSC are a major incident and places staff at the greatest risk. Spill management procedures and training are important requirements for working safely in a TB laboratory. The staff should be trained in the use of spill kits. Always keep two spill kits with the recommended contents.Let us list the contents of the spill kit. |
| **Commonly Used Disinfectants for Spills**  **Slide: 39**  **Participant Guide Page: 92** |
| Table  Description automatically generated |
| **SAY:** This slide shows the commonly used disinfectants for spills and their advantages and disadvantages. |
| **DO:** Discuss each disinfectant and when its use would be appropriate. |

### Waste Disposal

| **Waste Disposal**  **Slide: 41**  **Participant Guide Page: 93** |
| --- |
| Graphical user interface, text, application  Description automatically generated |
| **SAY:** TB medical waste is category B and requires an autoclave or incineration disposal mechanism. Be sure to follow country policies and guidelines on waste disposal. |
| **Safe Management of TB-Contaminated Medical Waste**  **Slide: 42**  **Participant Guide Page: 93** |
| Graphical user interface, text, letter  Description automatically generated |
| **SAY:** Manage all TB contaminated medical waste safely, like any other regulated medical waste. For regulated medical waste, use reference information from CDC’s Guidelines for Environment Infection Control in Health Care Facilities. |
| **Laboratory Waste Management and Disposal**  **Slide: 43**  **Participant Guide Page: 93** |
| Graphical user interface, text, letter, email  Description automatically generated |
| **SAY:** For laboratory waste management and disposal, remember that all waste materials generated in the laboratory are regarded as highly infectious and should be decontaminated by soaking in a 1:10 dilution of household bleach or autoclaving and disposal by incineration. Always follow existing local and national regulation on management of health care waste. |
| **Mental Checklist for Biosafety and Risk Management**  **Slide: 44**  **Participant Guide Page: 93** |
| Graphical user interface, text, application  Description automatically generated |
| **SAY:** We will walk through the mental checklist for biosafety and risk management. |
| **DO:** Read off the points on the checklist |
| **Useful Biosafety Resources**  **Slide: 45**  **Participant Guide Page: 94** |
| Graphical user interface, text, application  Description automatically generated |
| **SAY:** The slide shows a list of useful biosafety reference materials, which you may want to refer back to. |

### Specimen Collection Procedures

| **Specimen Collection Slides 47 – 52**  **Participant Guide Page : 94** |
| --- |
|  |
| **SAY:** There are four key points to note about collecting specimens for Truenat.   * Induced or expectorated sputum samples may be used. Remember that there is insufficient evidence on using specimens other than sputum for Truenat testing so this is not recommended. * Spot and morning sputum samples can be collected from each patient. * The algorithm describes the collection of one initial specimen to be used for Truenat TB testing and the collection of additional specimens as needed. If two specimens are to be collected, they can be spot specimens, or a spot and morning specimen, to use for repeat testing or further DST, for smear microscopy or for culture as a baseline for treatment monitoring as needed. * Sputum samples with contaminants such as betel nut, khat, tobacco, or food particles should be rejected. |
| **Packaging and Storing the Specimen**  **Slides: 53 and 54**  **Participant Guide Page: 95** |
|  |
| **SAY:** Truenat is meant to be used at facilities without uninterrupted power supplies,so their ability to store samples for more than a few hours is limited. Ideally, specimens should be stored in a fridge between 2 and 8 degrees and transported to the testing lab. A sample should always be packaged in a sample flask or transportation box. |

### Specimen Referral

| **Lab Forms Slide: 57**  **Participant Guide Page: 97** |
| --- |
| Graphical user interface, text  Description automatically generated |
| **PLACEHOLDER:** Lab forms are country-specific. Edit this section to include the specific forms used for your country. Be sure to update the corresponding PowerPoint and Participant Guide with the updated information. |
|  |
| **Integrated Specimen Referral Systems Slide: 58**  **Participant Guide Page: 97** |
| Graphical user interface, text, application, email  Description automatically generated |
| **SAY:** Depending on where Truenat is placed in the diagnostic network, a specimen referral system may need to be developed for Truenat and incorporated into a more extensive diagnostic network specimen referrals system. GLI’s [Guide to TB Specimen Referral Systems and Integrated Networks](http://stoptb.org/wg/gli/assets/documents/GLI_Guide_specimens_web_ready.pdf) provides guidance on establishing integrated solutions for specimen referral. |
| **Specimen Referral - Results Reporting**  **Slide: 59**  **Participant Guide Page: 98** |
| Graphical user interface, text, application, email  Description automatically generated |
| **DO:** Go over specimen referral guidelines for transportation and results reporting. |
| **Questions to Consider about Sample Flow**  **Slide: 60**  **Participant Guide Page: 98** |
| Graphical user interface, text, application, email  Description automatically generated |
| **DO:** Note that some of these questions were previously explored in a previous module. Ask for a volunteer to recount the responses that were developed last time and respond to the new ones. |
| **Specimen Referral System-Example**  **Slide: 61**  **Participant Guide Page: 100** |
|  |
| **DO:** Go over the specimen referral system example. |

### Summary

| **Summary**  **Slide: 62**  **Participant Guide Page: 101** |
| --- |
|  |
| **SAY:** In this module, you learned about the importance of biosafety when using Truenat and how to collect a quality specimen and pretreat it for Truenat testing. You also learned that specimen referral networks may need to be established or adapted for Truenat testing. It is vital that Truenat testing sites ensure that results are transmitted back to the requesting site, health care worker, or community – digitally where feasible. |
| **ASK:** Does anyone have any questions about this lesson? |
| **DO:** Answer any clarifying questions from participants. |

### Knowledge Check

| **Knowledge Check**  **Slides: 63 – 65**  **Participant Guide Page: 102** |
| --- |
| **DO:** Explain that you will ask participants three knowledge check questions and may call on participants randomly to provide an answer. (These can also be programmed as poll questions in a virtual training – ensure that all participants respond before proceeding if using the poll feature).  If an answer provided in incorrect, ask if other participants would like to answer. Correct any incorrect answers that are given. If multiple participants get a question wrong, you may need to revisit the topic.  Note that knowledge check questions are not included in participant guides to avoid students seeing them during the lesson and only focusing on those pieces. Encourage participants to write down the answers in their guides in the notes field for future reference. |
|  |
| **ANSWER:**   1. Sputum 2. Sputum samples with contaminants such as betel nut, khat, tobacco, or food particles should be rejected. |
| Graphical user interface, text, application, email  Description automatically generated |
| **ANSWER:** Specimens should be stored in a refrigerator or coolbox until they are pretreated. After pretreatment, specimens should be stored at -20° C. |
| Text  Description automatically generated |
| **ANSWER:** Answers will vary depending on the country. Samples that test positive for RIF resistance may need to be referred to for culture/DST. The patient should be referred for treatment (if not available at the site where he or she was tested), and their results should be sent (preferably digitally) to the site where he or she will go. |