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INFECTIOUS DISEASE DETECTION AND SURVEILLANCE PROJECT (IDDS)

GOOD PRACTICES GUIDE, VOLUME 1: DIAGNOSTIC & SCREENING TOOLS

August 1, 2022

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ABOUT IDDS

The Infectious Disease Detection and Surveillance (IDDS) project is USAID's flagship initiative to strengthen the ability of health systems in sub-Saharan Africa and Asia to quickly detect and stop the spread of infectious diseases and drug-resistant pathogens. A primary focus of the project is to develop the capacity of laboratories and testing facilities in our more than 20 partner countries to provide safe, timely, and accurate diagnostic testing. We also collaborate with partner countries to set up disease surveillance systems that can effectively record cases and quickly analyze and report data—so health officials and other key decision makers have the information they need to take action and help make the world safer for us all.

WHY TB

IDDS prioritizes diagnostic testing for diseases and drug-resistant pathogens that have the potential to spread quickly, devastate public health, disrupt economies, and threaten social and political stability. Tuberculosis (TB) is a major focus for IDDS as it remains the second leading killer among infectious diseases. Globally, TB continues to kill more people each year than HIV and malaria combined, and it is among the top 10 causes of death in Africa and Asia. Drug-resistant forms of TB, which include multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB), are a major public health challenge because they are more deadly, and more difficult and expensive, to diagnose and treat.

See the IDDS TB Fact Sheet for more information about IDDS's work to strengthen the global response to TB.

ABOUT THIS GOOD PRACTICES GUIDE

Timely and accurate diagnoses are essential to guide patient care and to track and prevent the spread of TB and other infectious diseases. The documents in this guide represent standard operating procedures, training materials, and guidelines developed by IDDS and local partners such as national TB program staff, national stakeholders, and domestic and international experts for use in specific countries with World Health Organization (WHO) recommended rapid molecular diagnostic tests and screening tools. We have modified the documents to share best practices across the IDDS consortium. These documents will be made available for implementing partners in other high-burden TB countries.

INTENDED AUDIENCE

The documents included in this guide are intended for national TB programs, TB program implementers, and laboratory technicians.

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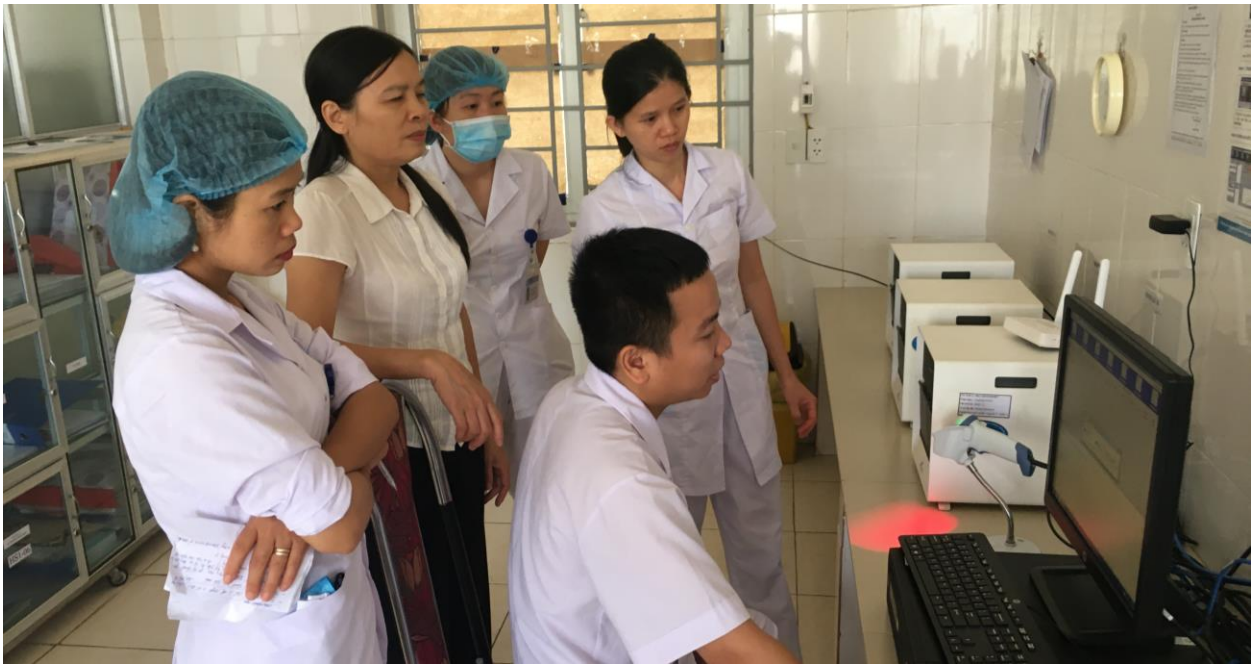
LIST OF ABBREVIATIONS

AMK	Amikacin
AP	Antero-posterior
BSC	Biosafety Cabinet
CAP	Capreomycin
COVID-19	Coronavirus Disease 2019
CR	Central Ray
CSF	Cerebrospinal Fluid
CXR	Chest X-ray
DHIS2	District Health Information Software, Version 2
DNA	Deoxyribonucleic Acid
ECT	Eluate Collection Tube
EID	Early Infant Diagnosis
EQA	External Quality Assessment
ETH	Ethionamide
FFP 1/ NR	Filtering Facepiece 1/ Not Re-useable
FFP 2	Filtering Facepiece 2
FLQ	Fluoroquinolone
IDDS	Infectious Disease Detection and Surveillance
IPC	Infection Protection and Control
IQC	Internal Quality Control
INH	Isoniazid
IR	Image Receptor
KAN	Kanamycin
LAM	Lipoarabinomannan
LFA	Lateral Flow Assay
M&E	Monitoring and Evaluation
MDR	Multidrug Resistant
MoH	Ministry of Health
MTB	*Mycobacterium tuberculosis*
MTBC	*Mycobacterium tuberculosis complex*
NLCDS	National Laboratory Commodity Distribution System
NRL	National Reference Laboratory
OSL	Optically Stimulated Luminescence
PA	Posteroanterior
PCC	Probe Check Control
PCR	Polymerase Chain Reaction
PPE	Personal Protective Equipment
PTB	Pulmonary Tuberculosis
QC	Quality Control
RIF	Rifampicin
RPL	Radio Photoluminescence
SID	Source to Image Receptor Distance
SLID	Second Line Injectable Drug
SOP	Standard Operating Procedure

SPC	Specimen Processing Control
SR	Sample Reagent
SVA	Sample Volume Adequacy
TB	Tuberculosis
TFT	Thin-film Transitor
TLD	Thermo-Luminescent Dosimeter
TWG	Technical Working Group
USAID	United States Agency for International Development
VWS	Ventilated Workstation
WHO	World Health Organization
XDR	Extensively Drug Resistant



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STANDARD OPERATING PROCEDURE FOR THE DIAGNOSIS OF TUBERCULOSIS AND RIFAMPICIN RESISTANCE BY GENEXPERT MTB/RIF[1]

Laboratory services are a key component of national health delivery systems because they provide essential information for disease surveillance, prevention, diagnosis, treatment, and monitoring the effectiveness of treatment.

Good quality laboratory test results must be accurate, clinically relevant, and delivered within an acceptable timeframe, in line with patient requirements. Laboratory test results must also be reproducible and cost effective with a view to benefit the patient and the community at large.

This standard operating procedure (SOP) has been developed to assist medical laboratories in countries in meeting the minimum standards required to produce good quality laboratory results. This document was developed to keep abreast with international best laboratory practices. It is intended to be a practical guide, for use by medical laboratory personnel and other health care workers at all health care levels. The document is generic in nature, and laboratories may need to customize and adapt it to meet the local context. It may be used in conjunction with other available reference materials.

[1] This document was originally developed by the Ministry of Health and Child Care for the Zimbabwe National (TB Program and has been adapted by the Infectious Disease Detection and Surveillance project as a global good. IDDS acknowledges the group of technical experts who developed this manual, whose names are listed below. The Directorate of Laboratory Services and National TB Program deserve special mention for spearheading the development process of the manual.

PURPOSE

This standard operating procedure (SOP) describes the procedure for detecting MTB complex bacteria and their resistance or susceptibility to rifampicin (RIF) using the GeneXpert MTB/RIF Ultra technology in sputum, cerebrospinal fluid (CSF), lymph nodes, tissue biopsies, and fluid aspirates.

1.1 PRINCIPLE

The GeneXpert system integrates and automates sample processing, nucleic acid amplification, and detection of the target sequences in simple or complex samples using real-time polymerase chain reaction (PCR) and melt peak detection. The system consists of an instrument, personal computer, barcode scanner, and preloaded software for running tests on patient samples and viewing the results. The system requires the use of single-use disposable GeneXpert cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the system, see the GeneXpert Dx System Operator Manual or the GeneXpert Infinity System Operator Manual.

PHOTO BY IDDS

GeneXpert MTB/RIF Ultra Assay includes reagents for the detection of MTB and RIF resistance and a sample processing control (SPC) to control for adequate processing of the target bacteria and to monitor for the presence of inhibitors in the PCR reaction and subsequent melt peak detection. The probe check control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The primers in the GeneXpert MTB/RIF Ultra Assay amplify a portion of the rpoB gene containing the 81-base pair "core" region and portions of the multi-copy IS1081 and IS6110 insertion elements target sequences. The melt analysis with four rpoB probes can differentiate between the conserved wild-type sequence and mutations in the core region that are associated with RIF resistance. The two insertion element probes enhance the detection of MTB complex due to the multi-copy insertion element target sequences in most TB strains.

1.2 MATERIALS REQUIRED FOR GENEXPERT TESTING

1.2.1 Equipment

- GeneXpert system

1.2.2 GeneXpert MTB/RIF Assay Kit

- MTB/RIF Ultra cartridges
- Sterile disposable transfer pipettes
- Sample reagent
- Compact disk (assay definition files/instructions, package insert)

1.2.3 Materials Required but not Provided

- Sterile screw-capped specimen collection containers (30–50 ml)
- Disposable powder-free gloves
- Laboratory coats/gowns
- N95 respirators (if performing aerosol-generating activity)
- Plastic bag for waste disposal
- Timer
- Disinfectant solution (70 percent ethanol, 1:10 bleach)
- Labels and indelible labeling marker
- Sterile pipettes for sample processing (optional)
- Centrifuge tubes
- Pasteur pipettes (optional)
- Orange sticks
- Centrifuge
- Vortex mixer (optional)
- Mortar and pestle
- Autoclave

1.2.4 Storage and Handling of Equipment and Materials

- Store the GeneXpert MTB/RIF cartridges and reagents at 2–28° C.
- Do not use reagents or cartridges that have either passed the expiration date or are damaged.
- The cartridge is stable up to seven days after opening the package.

BIOSAFETY

When performed correctly, sputum examination will not place laboratory personnel at increased risk of developing TB.

2.1 RISKS AND TRANSMISSION

TB is an infectious disease. Transmission occurs when small aerosols containing acid-fast bacilli become airborne and are inhaled. When a person coughs, sneezes, sings, or vigorously exhales, they produce aerosols that could be infectious if the person has pulmonary TB.

Properly trained laboratory personnel, when following SOPs and good laboratory practices, have a very low risk of infection in a TB laboratory. However, some activities, such as talking to unprotected pulmonary TB-infected patients and collecting specimens, can carry a greater risk of transmission.

Specimens may contain infectious organisms other than TB. Do not shake or stir samples because aerosols may be generated. Handle all specimens with care.

When working in the laboratory, **DO NOT:**

- Put anything in your mouth (e.g., a pen, your fingers)
- Eat, drink, or smoke
- Pipette by mouth
- Lick labels and envelopes, etc.
- Apply cosmetics or handle contact lenses
- Store food or drinks in the laboratory
- Wear open-toed footwear or bare feet
- Use mobile telephones in the laboratory

2.1.1 Personal Protective Equipment

Laboratory staff must be supplied with personal protective equipment (PPE) that is appropriate for handling TB specimens:

- You must always wear protective clothing in the laboratory.
- You must wear gloves when handling specimens.
- Do not take PPE out of the laboratory.
- Store PPE separately from personal clothing.

2.1.2 Biosafety Requirements for GeneXpert MTB/RIF Ultra Testing

The GeneXpert MTB/RIF Ultra test is a low-risk procedure and requires the same level of precautions as those used for performing direct acid-fast bacilli sputum-smear microscopy.

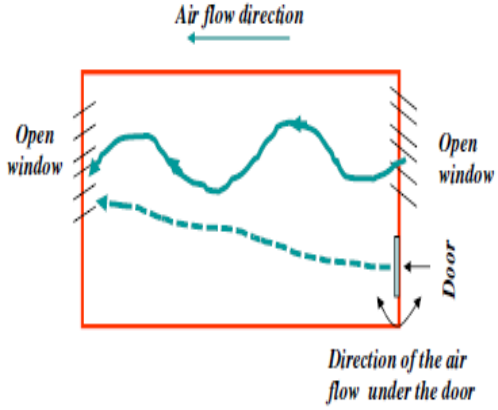
- Work in a well-ventilated area.
- Wear gloves and a laboratory coat when handling samples from patients.
- In settings with a high TB burden, a risk assessment may determine that additional precautions are required in GeneXpert-equipped laboratories; these precautions may include the use of N95 respirators or biosafety cabinets (BSCs).
- Because of the risk of generating aerosols, splitting specimens and handling concentrated specimens must be performed in a certified BSC.

Note: Adding sample reagent to the specimen in a ratio of 2:1 inactivates MTB bacilli in the sample, significantly reducing the biosafety risk to the laboratory staff.

2.2 LABORATORY WORKING AREA

- Use of bench spaces: Specimens are handled (received) and processed in separate designated areas.
- When appropriate microbiological techniques are used, direct smear testing and direct processing of specimens for the GeneXpert MTB/RIF Ultra assay may both be carried out on an open bench in an adequately ventilated area. Avoid processing samples with air flowing from open windows or air conditioners directly toward the working bench.
- When the climate prevents windows from being opened, consideration should be given to using mechanical ventilation systems, such as an exhaust fan.
- If natural or mechanical ventilation is not practical, ventilated workstations (VWS) are an optional solution for managing aerosol containment during direct sputum-smear microscopy or GeneXpert MTB/RIF Ultra testing.
- A BSC is not essential for GeneXpert MTB/RIF Ultra testing on direct specimens (i.e., specimens that do not require splitting or concentration).

Figure 1: Direction of air flow (sample processing area)



- For low-risk procedures, natural ventilation should be sufficient, provided that air flows away from the person and across the work area to the outside.
- Ensure that all windows and doors are open to facilitate unidirectional airflow.

2.3 PERSONAL PROTECTIVE EQUIPMENT USE

2.3.1 Gloves

- Gloves are required for processing specimens for GeneXpert MTB/RIF Ultra testing.
- Use disposable powder-free gloves.
- Wearing gloves may give laboratory staff a false sense of safety.
- Regular and thorough hand washing remains essential.

Figure 2: Hand Washing

- To avoid contamination, remove gloves before using computer terminals or telephones.
- DO NOT reuse gloves.
- DO NOT wear gloves outside the laboratory.
- After removing the gloves, properly wash the hands as clearly illustrated in the diagram.

Figure 3: Misuse of Gloves

- Do not touch telephones, door handles, or water taps with gloved hands.
- Always remove the gloves first before answering telephones and opening doors and water taps.

2.3.2 Laboratory Coats

- Leave coats at the worksite (do not take them out of the laboratory).
- Fasten the laboratory coat when worn.
- Use the appropriate size and type.
- Laundering should be done at least weekly and whenever there is overt contamination.

2.3.3 Masks and Respirators

Figure 4: Surgical Mask

- Surgical masks are *not designed to protect the wearer* against inhaling infectious aerosols.
- They are designed to stop the wearer spreading aerosols.
- N95 respirators are *not required* for performing sputum tests with GeneXpert/RIF Ultra (N95 masks may we worn based on risk assessment).

2.3.4 Disinfectants

- Disinfectants are usually applied to surfaces or inanimate objects.
- Disinfectants recommended for use in TB laboratories that contain phenols, chlorine, or alcohol.
- Fresh household bleach (5 percent sodium hypochlorite) diluted 1:10 with water should be made fresh once every two days because it loses potency over time.

Disinfection methods	Surfaces	Spills	Preparation
Phenol 5%	Yes	Yes	Every 2 days
Alcohol 70% v/v	Yes	No	Weekly
Hypochlorite 0.5%	No	Yes	Daily

- Seventy percent alcohol is a good disinfectant for cleaning bench tops. Formaldehyde is useful in the decontamination of BSCs prior to servicing.
- Disinfectants may be used before autoclaving for pre-decontamination treatment.

2.3.5 Biological Safety Cabinets

A BSC is NOT required for GeneXpert TB testing where there is minimal manipulation of the specimen. Only laboratories performing culture and drug susceptibility testing need a functioning BSC. Only use (if available) a well-functioning and serviced BSC.

2.3.6 Ventilated Workstation

A VWS is a partially enclosed workspace. Air is drawn inward, away from the laboratory worker, and exhausted outside the laboratory. VWS are inexpensive to build and require little maintenance. VWS do not replace careful attention to risk-minimizing laboratory methods.

2.3.7 Safety Procedures during Specimen Handling

Take the following safety precautions before and during laboratory procedures:

- Discard broken containers and leaking specimens and request another specimen.
- Once collected, allow a sputum specimen to stand undisturbed for at least 20 minutes before opening to settle any aerosols.
- Always cover sputum containers with their lids.

- Open sputum containers with care and away from the face. Gently open the sputum container, especially if the lid clicks or snaps on.
- Do not forcefully shake or stir the sputum in the container.

2.3.8 Waste Disposal

- Keep a container with appropriate disinfectant in the working area.
- Place used disposable pipettes in the discard jar.
- Ensure that the container holds enough disinfectant to completely cover the discarded items.
- Soak used disposable pipettes for two hours, after which they can be discarded.
- Keep plastic bins with lids in the immediate proximity of the working area ready to receive discarded materials.
- Line all bins with color-coded biohazardous plastic bin liners in clearly labeled biohazard bins.
- Tie bin liners securely and label clearly as hazardous waste before disposal.
- Discard contaminated fluids into autoclavable containers. Do not pour into drains.
- Remove broken glassware by a brush and dustpan, tongs, or forceps, and decontaminate in an appropriate disinfectant before disposal.

2.4 MANAGING LABORATORY HAZARDS

2.4.1 The Spill Kit

All laboratories handling samples for TB diagnostic testing should have a spill kit containing the following:

Figure 5: Spill Kit

- Instructions (SOPs) for cleaning up spills
- A large biohazard bag (autoclavable)
- Suitable tuberculocidal disinfectants, such as (freshly prepared) 1 percent sodium hypochlorite solutions or phenol-derivatives, stored in opaque bottles
- Laboratory gowns (disposable) and eye goggles
- Box of gloves (different sizes)
- Respirators (N95 or FFP2)
- Paper towels, cotton wool, or absorbent cloths
- Liquid soap and chloramine tablets
- Dustpan
- Sharps container
- "DO NOT ENTER" sign

Check the contents of the spill kit regularly and replenish stock after use or when they have expired.

2.4.2 Spills Procedure

All personnel in the laboratory must be trained in the procedures for handling spills. The actions required depend on where the spill occurs:

- Outside a BSC
- Within a BSC

Spill Outside the BSC (Major Event)

- Immediately vacate and secure the laboratory, and inform the safety officer/laboratory manager.
- Leave the laboratory's ventilation or exhaust systems on, including in the BSC.
- Do not re-enter the room for at least one hour (post **DO NOT ENTER** signs).
- Before re-entering the laboratory, put on clean gloves, a clean coat, and a respirator.
- Cover the spill (or spills) with cloths or absorbent paper and soak the paper with a suitable disinfectant (see above); apply the disinfectant concentrically, from the outer margin toward the center of the spill.
- Allow sufficient time for the disinfectant to act (at least 30–60 minutes) before disposing of any material.
- Collect all containers and clean-up material and place them in the disposal bag provided in the spill kit; tie the disposal bag and place it in a container to be autoclaved (use an appropriate container for sharp objects).
- Change gloves if they have been contaminated; dispose of these along with other infectious waste.
- Clean and disinfect the area of the spill.
- Staff exposed to the spill should be referred for medical advice, and details recorded in the incident logbook.

Spill within the BSC

- Cover the spill area with cloths or absorbent paper, and apply a suitable disinfectant concentrically—that is, from the outer margin toward the center of the spill.
- Any equipment or material that has been splashed must be cleaned (including the interior surfaces and walls of the BSC, or safety buckets).
- Do not use bleach to disinfect metal parts (it is corrosive).
- Allow sufficient time for the disinfectant to act (30–60 minutes) before disposing of any material in the BSC. Place all containers and clean-up material into the disposal bag provided in the spill kit; tie the bag and place it in a container to be autoclaved. All clean-up material, dirty gloves, and cloths should be put into the autoclavable bag while still within the BSC.
- Change gloves if they have been contaminated; dispose of these along with other infectious waste.
- Staff exposed to the spill should be referred for medical advice, and details recorded in the incident logbook.

2.4.3 Waste Management

At the end of each day, seal contaminated material (used sputum containers, pipettes, and cartridges) in an autoclavable bag. Incinerate them as soon as possible. Alternatively, keep the bag in a safe, closed bin or large bucket until it can be taken for incineration. In intermediate or central laboratories where there is an autoclave, infectious waste should be collected in an autoclavable bag and should be autoclaved at 121°C at 15 lbs pressure for 15–30 minutes before incineration.

SPECIMEN COLLECTION AND STORAGE

Note: All specimens must be properly labeled, with a unique identification number. This number must correspond with the one written on the request form and in the laboratory register.

3.1 COLLECTION

Figure 6: Specimen Collection

Suitable Specimen Containers: Use clean, single use disposable, unbreakable transparent plastic containers (50 ml capacity is preferred), that are wide mouthed, screw-capped, and leak-proof.

Never Stand in Front of the Patient During Collection

Instruct the patient to:

1. Relax, take time.
2. Inhale deeply two to three times, breathe out hard each time.
3. Cough deeply from the chest.

Place the open container close to the mouth to collect the sputum. After collection, screw the lid on tightly.

Several attempts may be required to obtain a good quality specimen (see Section 4.1).

***Only one sputum sample with* a minimum of 1 ml is required for GeneXpert MTB/RIF Ultra testing. Do not accept specimens with obvious food particles or other solid particulates.**

3.2 STORAGE

Sputum may be stored at 2–8°C for up to seven days prior to testing.

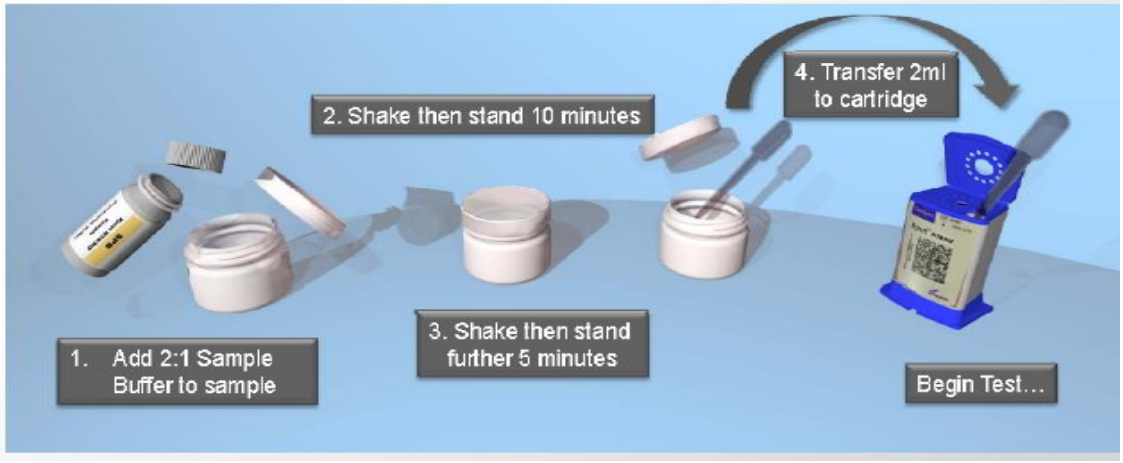
If immediate processing is not possible, the sputum can be stored at a maximum of 35°C for up to three days or at 2–8°C for up to seven days

SAMPLE PREPARATIONS

4.1 SPUTUM

- Carefully unscrew the lid of the sputum container.
- Pour 2 volumes of sample reagent (SR) directly into 1 volume of sputum in the sputum container (1 ml of sputum is the minimum quantity, and 3–4 ml is the optimal quantity required).
- For larger volume specimens (over 4 ml), a portion of SR from a second bottle would be needed, as each bottle contains 8 ml of SR.
- Replace the lid and shake vigorously 10–20 times (one back and forth movement is a single shake), or vortex for at least 10 seconds.
- Incubate at room temperature for 10 minutes.
- After 10 minutes of incubation, shake the specimen vigorously 10–20 times (or vortex for at least 10 seconds).
- After additional 5 minutes of incubation, sample should be perfectly fluid before being tested, with no visible clumps of sputum. If still viscous, wait 5–10 more minutes before inoculating the cartridge with 2–4 ml of the final solution *(see the diagram that follows).* ***Dispense the sample slowly to minimize the risk of aerosol formation***

Figure 7: Sample Processing Procedure for GeneXpert MTB



Note: If using a GeneXpert Dx instrument, start the test as soon as possible and within four hours of adding the SR-treated sample to the cartridge. Once the sample is added to the cartridge, the cartridge should remain at room temperature prior to starting the test within four hours. If using a GeneXpert Infinity system, be sure to start the test and put the cartridge on the conveyor within 30 minutes of adding the SR-treated sample to the cartridge. Remaining shelf life is tracked by the GeneXpert software so that tests are run prior to the four-hour on-board expiration.

4.2 LYMPH NODE BIOPSIES AND OTHER SAMPLES

The WHO issued policy recommendations for the use of GeneXpert MTB/RIF Ultra in the diagnosis of extrapulmonary TB and RIF resistance detection:

- GeneXpert MTB/RIF Ultra should be used in preference to conventional microscopy and culture as the initial diagnostic test in testing CSF specimens from patients presumed to have

TB meningitis *(strong recommendation given the urgency of rapid diagnosis, very low quality of evidence).*

- GeneXpert MTB/RIF Ultra may be used as a replacement test for usual practice, including conventional microscopy, culture, or histopathology, for testing of specific non-respiratory specimens (lymph nodes and other tissues) from patients presumed to have extrapulmonary TB *(conditional recommendation, very low quality of evidence).*

Individuals presumed to have extrapulmonary TB but with a single GeneXpert MTB/RIF Ultra-negative result should undergo further diagnostic testing, and hence processing of tissue samples (lymph nodes and other tissues) for GeneXpert MTB/RIF Ultra should include a decontamination step to enable samples to be concurrently cultured. These recommendations ***do not apply*** to urine or blood, given the lack of data on the utility of GeneXpert MTB/RIF Ultra on these specimens.

Specimens, such as CSF, nodal aspirates, pleural biopsies, stool, organ aspirates, or biopsies, compatible with the clinical presentation should be promptly collected and submitted to the laboratory for the GeneXpert MTB/RIF Ultra assay. As far as feasible, pleural biopsies and not pleural fluid should be submitted for the GeneXpert MTB/RIF Ultra assay for patients presenting with a pleural effusion. All children should have one stool specimen or a naso-pharyngeal aspirate or a naso-gastric aspirate or two sputum specimens if able to produce sputum.

The GeneXpert MTB/RIF Ultra assay can be used directly for CSF specimens and homogenized extra-pulmonary samples (lymph node biopsies and other tissues). Whenever possible, specimens should be transported and stored at 2–8°C prior to processing ***(a maximum of seven days).***

4.2.1 Processing of Lymph Nodes and Other Tissues

- Cut the tissue sample into small pieces in a sterile mortar (or homogenizer/tissue grinder) using a clean, sterile pair of forceps or scissors.
- Add approximately 2 ml of sterile phosphate buffer/sterile distilled water.
- Grind tissue/ sterile phosphate buffer solution with a mortar and pestle (or homogenizer/tissue grinder) until a homogeneous suspension is obtained.
- Transfer approximately 0.7 ml of homogenized tissue sample to a sterile conical, screw-capped tube using a transfer pipette.
- **Note:** Avoid transferring any clumps of tissue that have not been properly homogenized.
- Add a double volume of GeneXpert MTB/RIF Ultra SR (1.4 ml) to 0.7 ml of homogenized tissue using a transfer pipette.
- Vigorously shake 10–20 times or vortex for at least 10 seconds.
- Incubate for 10 minutes at room temperature, and again shake the specimen vigorously 10–20 times or vortex for at least 10 seconds.
- Incubate the sample at room temperature for an additional 5 minutes.
- Using a fresh transfer pipette, transfer 2 ml of the processed sample to the GeneXpert MTB/RIF Ultra cartridge.
- Load the cartridge into the GeneXpert MTB/RIF Ultra instrument as per manufacturer's instructions.

4.3 PROCESSING OF CSF AND ASPIRATES

For CSF specimens and aspirates, GeneXpert MTB/RIF Ultra should be preferentially used over culture if the sample volume is low or additional specimens cannot be obtained, to reach a quick diagnosis. If sufficient volume of material is available, concentration methods should be used to increase yield.

The preferred processing method for CSF in GeneXpert MTB/RIF Ultra depends on the volume of sample available for testing.

Note: Blood-stained and xanthochromic CSF samples may cause false negative GeneXpert MTB/RIF Ultra results.

4.3.1 More than 5 ml of CSF

- Transfer all the sample to a conical centrifuge tube and concentrate sample at 3000 g for 15 minutes.
- Carefully pour off the supernatant into another container for biochemistry analysis.
- Re-suspend the deposit to a final volume of 2 ml with GeneXpert MTB/RIF Ultra sample reagent.
- Label a GeneXpert/MTB/RIF Ultra cartridge with the sample ID.
- Using a fresh transfer pipette, transfer 2 ml of the concentrated CSF sample to the GeneXpert MTB/RIF Ultra cartridge.
- Load the cartridge into the GeneXpert MTB/RIF Ultra instrument as per manufacturer's instructions.

4.3.2 Between 1 and 5 ml of CSF (Including Blood-stained/Xanthochromic Samples)

- Add an equal volume of the CSF to the SR.
- Add 2 ml of the sample mixture directly to the GeneXpert MTB/RIF Ultra cartridge.
- Load the cartridge into the GeneXpert MTB/RIF Ultra instrument as per manufacturer's instructions.

4.3.3 Between 0.1 and 1 ml of CSF

- Re-suspend the CSF to a final volume of 2 ml with GeneXpert MTB/RIF Ultra SR.
- Add 2 ml of the sample mixture directly to the GeneXpert MTB/RIF Ultra cartridge.
- Load the cartridge into the GeneXpert MTB/RIF Ultra instrument as per manufacturer's instructions.

4.3.4 Less than 0.1ml of CSF

- Report as insufficient sample for testing in the GeneXpert MTB/RIF Ultra assay.

4.4 PROCESSING OF STOOL

- Store stool specimen 2–8^{0}C up to one week.
- Weigh stool (0.2 to 1.2 g).
- Transfer stool sample into a test tube.
- Add 2 ml of stool processing buffer from the kit provided.
- Add 2 ml SR (GeneXpert MTB/RIF Ultra) + 10 glass beads and snap vortex.
- Incubate 30 minutes at room temperature.
- Pass the sample through the glass wool syringe filter, into a separate container.
- Add 2 ml of this filtrate into GeneXpert MTB/RIF Ultra assay cartridge.
- **Note:** Follow stool kit manufacturer's instructions.

4.5 PROCESSING OF GASTRIC LAVAGE OR GASTRIC WASHINGS

Process the same way as sputum (see Section 4.1).

PROCEDURE FOR TESTING SAMPLES

5.1 STARTING THE GENEXPERT INSTRUMENT

Note: Before starting to process the specimen, check that the GeneXpert machine is functioning, and the modules are available.

- Turn on the computer, and then turn on the GeneXpert diagnostic machine.
- On the Windows desktop, double-click the GeneXpert diagnostic shortcut icon.
- Log on to the GeneXpert diagnostic system software using your username and password.
- Click on "CHECK STATUS" and check whether the modules are available. If not, proceed to "Troubleshooting" in the user's manual.

5.2 PREPARING THE CARTRIDGE

Note: Start the test within 30 minutes of adding the sample to the cartridge.

- Using the sterile transfer pipette provided, aspirate the liquefied sample into the transfer pipette until the meniscus is above the minimum mark (= 2 ml).
- Open the cartridge lid.
- Transfer the sample into the open port of the GeneXpert MTB/RIF Ultra cartridge (see photo).

Figure 8: Xpert MTB / Rif Ultra cartridge open port

Note: It is crucial that no bubbles are created when transferring the specimen into the cartridge because this can lead to an error (no result).

- Dispense slowly to minimize the risk of aerosol formation.
- Close the cartridge lid. Make sure the lid snaps firmly into place.

Note: The remaining liquefied sample may be kept for up to four hours at 2–80 C should repeat testing be required.

5.3 STARTING THE TEST ON THE GENEXPERT MACHINE

In the GeneXpert diagnostic system window, click on "Create Test." The Scan Cartridge Barcode dialog box appears.

Figure 9: Screen shot from GeneXpert diagnostic system window

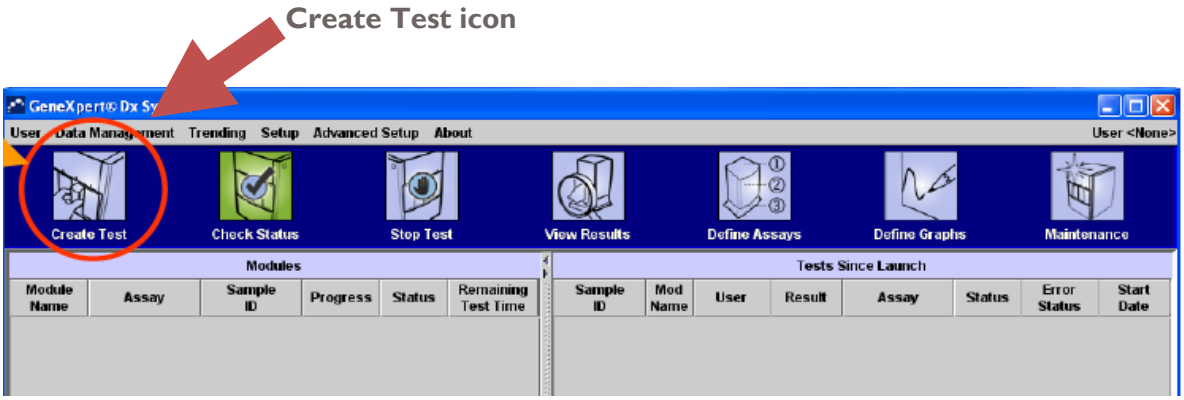
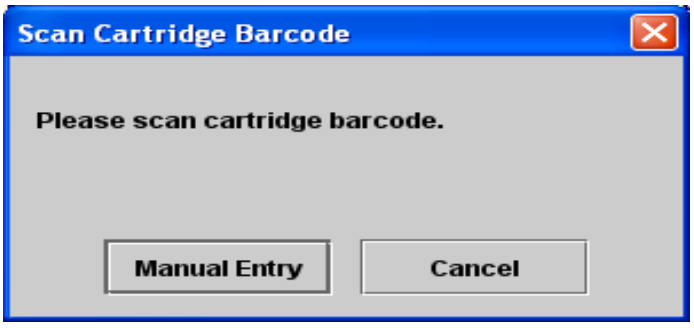
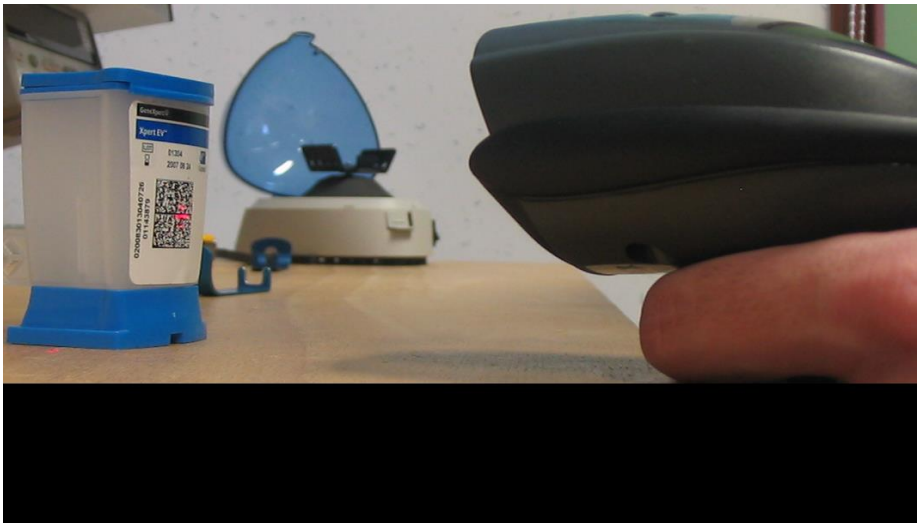


Figure 10: GeneXpert diagnostic system prompt for barcode scanning



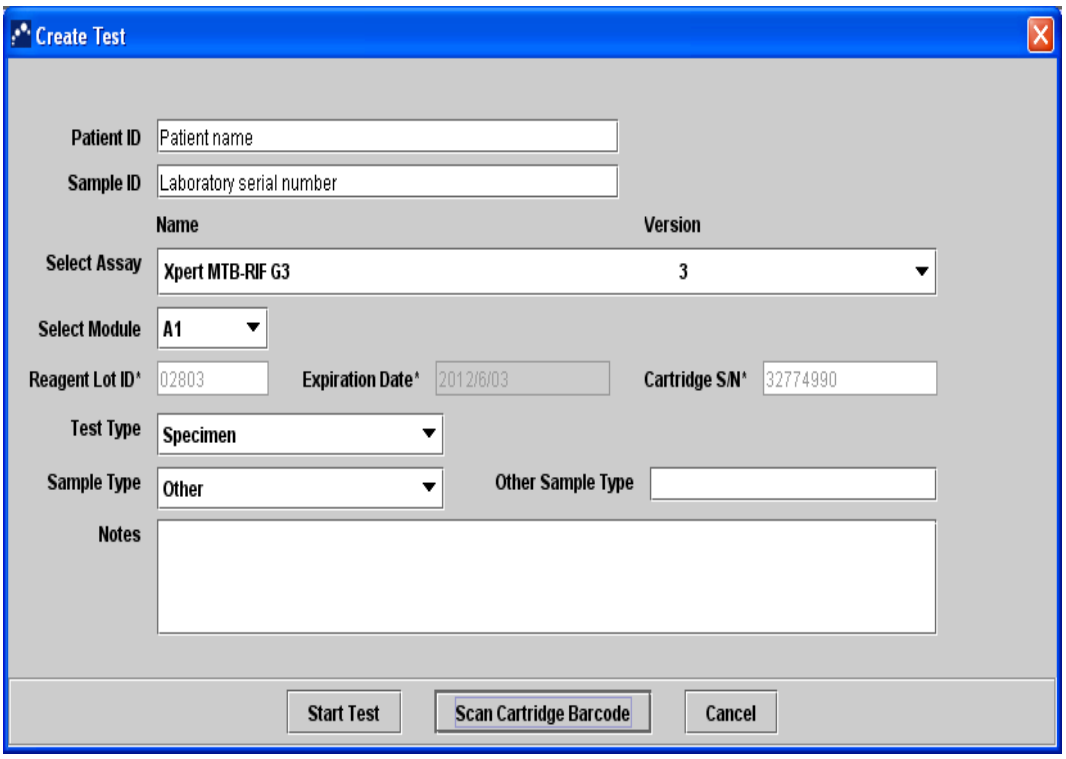
Scan the barcode on the GeneXpert MTB/RIF Ultra cartridge.

Figure 11: Cartridge Barcode Scanning



The Create Test window appears.

Figure 12: Create Test Window



- Using the barcode information, the software automatically fills in the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.
- In the Sample ID box, scan or type the sample ID. Make sure you type the correct sample ID. The sample ID is associated with the test results and is shown in the "View Results" window and all the reports.
- Click on "Start Test."
- In the dialog box that appears, type your password.
- Open the machine module door with the blinking green light and load the cartridge.

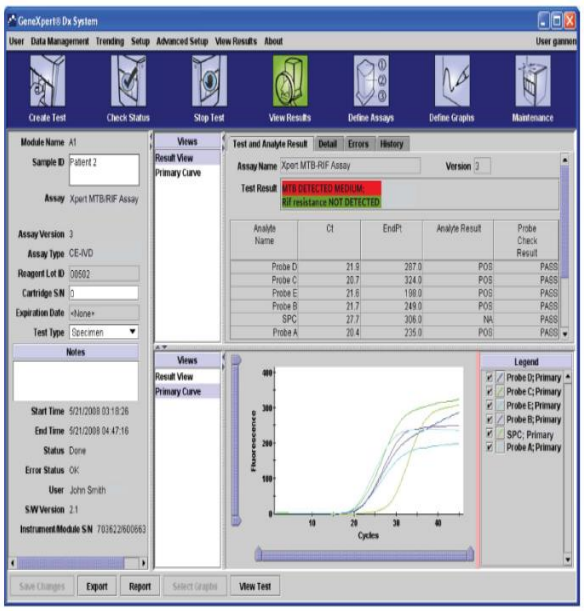
Figure 13: Loading the GeneXpert cartridge

- Close the door.
- The test starts and the green light stops blinking.
- Wait until the system releases the door lock at the end of the run, and then open the module door and remove the cartridge.
- Dispose of used cartridges in the appropriate specimen waste containers according to the waste management policy.

READING, RECORDING, AND REPORTING

6.1 VIEWING RESULTS ON GENEXPERT SOFTWARE (BASIC USER SETTINGS)

Figure 14: Results view from GeneXpert diagnostic software



- In the GeneXpert diagnostic system window, click on "VIEW RESULTS" on the menu bar. The View Results window appears.
- If the software reports "Error," "Invalid," or "No result," repeat the test using the already prepared specimen and a new cartridge.
- Should the test again show "Error," "Invalid," or "No result," proceed to "Troubleshooting" in the user manual to exclude technical problems before requesting a new specimen.

6.2 REPORTING AND INTERPRETATION OF RESULTS

Note: Results must be recorded in the laboratory TB register and laboratory request forms (Annex 1). Use red ink for positive results. Reports must be provided *as soon as possible*.

For MTB	For RIF Resistance
Report as either: • "MTB not detected" • "MTB detected"	Report as either: • "Rif resistance not detected" • "Rif resistance detected" • "Rif resistance indeterminant" • Except for "MTB detected TRACE" when using GeneXpert MTB/RIF Ultra cartridges, in which the RIF sensitivity pattern is not given

Note: "MTB detected, rifampicin resistance indeterminate" means "The concentration of MTB in the sample was very low and resistance could not be determined due to insufficient data collected to interpret resistance-related signals."

In such cases, report "Please submit a new early morning specimen" (for repeat GeneXpert MTB/RIF Ultra) if the system repeatedly did not produce a result and you have excluded or fixed a technical problem (see Annex 4—algorithm for determination of results).

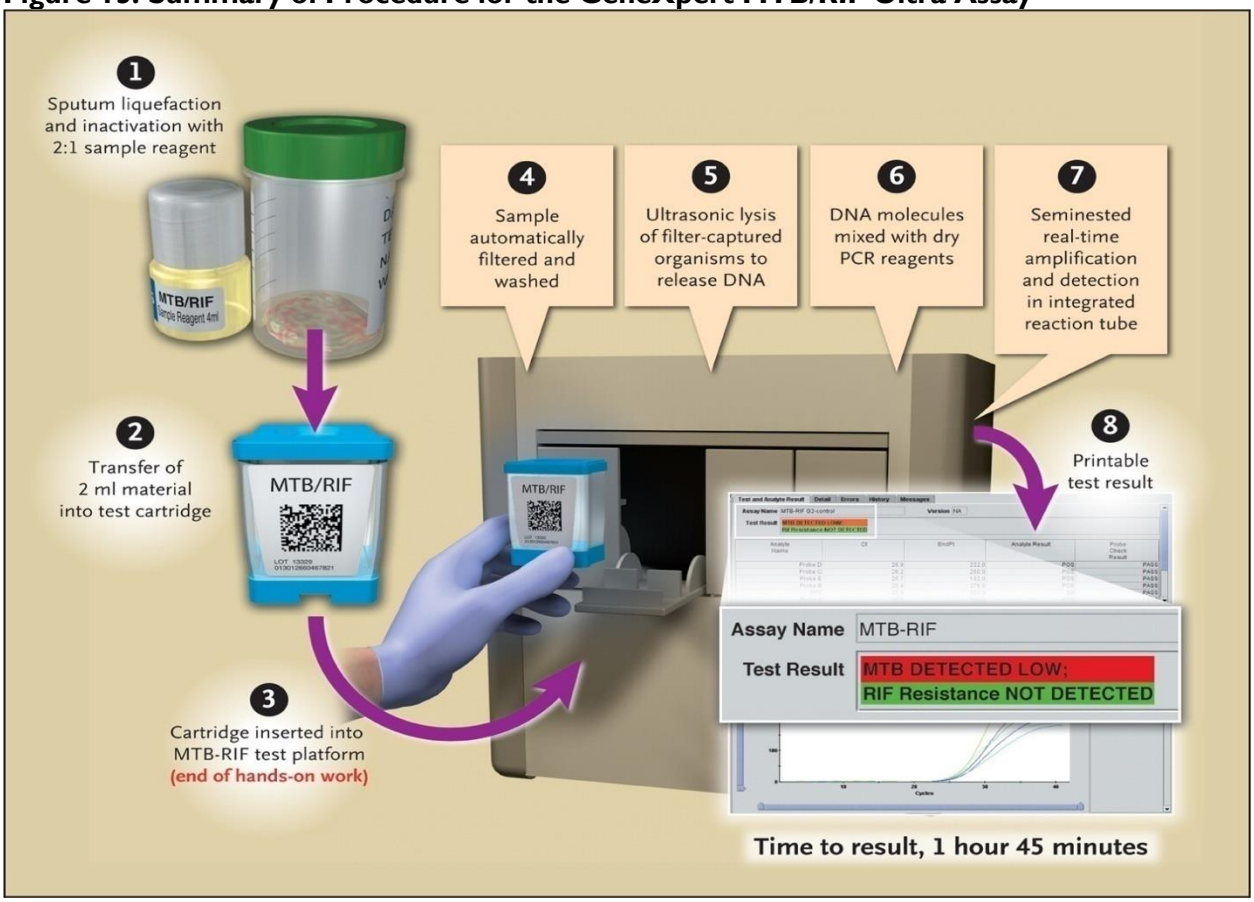
The "MTB detected TRACE" result provides no information on RIF resistance, hence, additional investigations (e.g., culture and phenotypic drug susceptibility testing or molecular testing) are needed to confirm or exclude resistance to RIF.

A GeneXpert MTB/RIF Ultra result of "MTB detected TRACE" indicates that there are very few bacilli in the specimen. Testing of a second sample, which also may contain very few bacilli, maybe, in some cases, generate a result of "MTB not detected."

MTB/RIF Ultra for "MTB detected trace" results:

- In pediatric, people living with HIV and extrapulmonary TB samples, one trace call result is regarded as MTB positive.
- In people with recent history of TB infection or treatment, a trace call result is likely to be a false positive.

Figure 15: Summary of Procedure for the GeneXpert MTB/RIF Ultra Assay



Note: Remember to compile quarterly statistics using the statistics reporting form in Annex 4.

6.3 ELECTRONIC RECORDING AND TRANSMISSION OF TB DATA

- TB recording is done electronically, using appropriate software (e.g., GxAlert).
- GxAlert enables connectivity of all GeneXpert machines in the country.
- Usernames enable one to remotely access information from various machines according user level (e.g., site, district, province and national).
- GxAlert enables real-time access to the web dashboard (e.g., stock management, TB statistics, machine performance including down time, laboratory cadre performance, quality indicators including error rates, quality assessment of machine).
- GX Alert has alerts such as the following:
  - SMS alert when a multidrug-resistant TB case is diagnosed, so the recruitment can be organized faster.
  - Emails sent weekly to laboratory managers showing them error rates compared to those of other nearby GeneXpert laboratories.

6.3.1 Steps to Access the GxAlert Dashboard

- Log in to GxAlert by typing the URL.
- Dialogue box asks for USERNAME.
- Next dialogue box asks for PASSWORD.
- GxAlert dashboard is displayed.
- From the dashboard, you can now select from Devices/Results/Contacts/Modifications/Inventory/Reports.
- Once you select one of the listed options, use the drop-boxes for the time period of interest (e.g., Quarter 1 2017).
- Select GeneXpert MTB/RIF Ultra sites of interest.
- Transfer the displayed data under review to an Excel sheet for analysis.

QUALITY ASSURANCE

7.1 QUALITY CONTROL

7.1.1 Internal Quality Controls

Each test includes a PCC and an SPC.

PCC: Testing of fluorescence readings at different temperatures before the start of thermal cycling is done during this step to evaluate the response of the chemicals contained in a cartridge.

PCC verifies:

- Re-hydration of beads
- Filling of the PCR tube
- Integrity of probes
- Stability of a dye or the reagents/quencher

Results are automatically compared to the pre-established factory settings in the software. A test is stopped if the probe check is not passed.

SPC: Ensures that the sample was correctly processed. It contains non-infectious spores in the form of a dry spore cake that is included in each cartridge to verify adequate processing of MTB.

SPC verifies:

- Conditions for lysis of MTB have occurred if the organisms are present.
- Specimen processing is adequate.

In addition, this control detects specimen-associated inhibition of the real-time PCR reactions and acts as an internal positive control.

The SPC should be positive in an MTB-negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria. The test result will be invalid if the SPC is not detected in an MTB-negative sample.

7.2 EXTERNAL QUALITY ASSESSMENT

This is a process to assess laboratory performance by a higher-level laboratory.

7.2.1 Panel Testing/Proficiency Testing

This is a process in which samples with known results are sent termly (once in four months) to a group of laboratories by reference laboratories for examination. The participants must return their results within a specified time. The results are analyzed and reports with a specific and overall laboratory performance are generated and sent back to the participants.

7.2.2 Onsite Assessment

This occurs when supervisors from a higher-level laboratory periodically visit a lower-level facility to obtain a realistic picture of the conditions and practices in the laboratory. It is also an opportunity to aid with problem areas, including training. Areas assessed for GeneXpert include the following:

- Maintenance of equipment
- Use of SOPs
- Filling in of the laboratory register
- Compilation of statistics
- Stock status of reagents and consumables
- Equipment functionality
- Proficiency testing results/performance

EQUIPMENT MAINTENANCE OVERVIEW

8.1 EQUIPMENT MAINTENANCE

Frequency	Task
Daily	Remove and properly dispose of cartridges Clean and disinfect work area Ensure 10 cm clearance around the instrument Put on dust cover when instrument is not in use
Weekly	Disinfect the interior of the cartridge bay Restart the GeneXpert instrument and computer
Monthly	Disinfect plungers Disinfect the instrument's surfaces Clean the instrument's filter (this applies only to newer model GeneXpert instruments, which have a white cover) Archive and back up test results
Annually or after 2,000 tests per module	Calibrate the module

Refer to the GeneXpert user manual for detailed instructions on maintenance.

8.2 MATERIALS REQUIRED FOR MAINTENANCE

- Sodium hypochlorite solution (bleach with 0.72 percent active chlorine; use within one day after preparation)
- 70 percent ethanol or isopropyl alcohol solution (or equivalent)
- Wipes, tissues, or cotton or polyethylene terephthalate swabs
- Disposable gloves
- Clean water and soap (for washing the filters)
- Replacement filters for the fan (available from Cepheid)

8.3 DISINFECTION PROCEDURES FOR THE WORK AREA, CARTRIDGE BAY, PLUNGER, AND INSTRUMENT SURFACES

1. Dampen the wipe, tissue, or swab with the sodium hypochlorite solution.
2. Wipe the surface or element to be cleaned with the wipe, tissue, or swab.
3. Discard the used wipe, tissue, or swab.
4. Wait 10 minutes.
5. Dampen a new wipe, tissue, or swab with the ethanol or isopropyl alcohol solution.
6. Wipe the surface or element to be cleaned with the wipe, tissue, or swab (ensure residual sodium hypochlorite is removed).
7. Discard the used wipe, tissue, or swab.
8. Repeat Steps 5 through 7.

8.4 EQUIPMENT MAINTENTANCE TASKS

8.4.1 Daily Tasks

- After testing, remove the cartridges from the instrument.
- Dispose of cartridges in the appropriate biohazardous waste container.
- Remove clutter from the work area.
- Disinfect the work area by wiping with sodium hypochlorite followed by 70 percent alcohol (as described above).
- Ensure that there is 10 cm clearance around all sides of the instrument.
- Put on the dust cover when the instrument is not in use, to reduce the amount of dust that may enter the system.

8.4.2 Weekly Task 1: Disinfect the Interior of the Cartridge Bay.

1. Open the module door of the GeneXpert instrument.
2. Clean the surfaces inside the module's cartridge bay.
3. **CAUTION:** Do not touch the slit in the back of the cartridge bay into which the cartridge-reaction tube is inserted.
4. Close the module's door.
5. Repeat Steps 1 through 3 for each module.

8.4.3 Weekly Task 2: Restart the instrument and computer.

Note: This task is necessary only if the instrument and computer are not routinely turned off at the end of each day.

1. On the computer, close the GeneXpert DX software.
2. Turn off the GeneXpert instrument.
3. Turn off the computer.
4. Turn on the GeneXpert instrument.
5. Turn on the computer.
6. Launch the GeneXpert DX software.

8.4.4 Monthly Task 1: Disinfect Plungers

1. Follow guidance in Figure 16

8.4.5 Monthly Task 2: Clean the Instrument Filter

1. Follow guidance in Figures 17 and 18

Figure 16: Steps for disinfection of the GeneXpert plunger

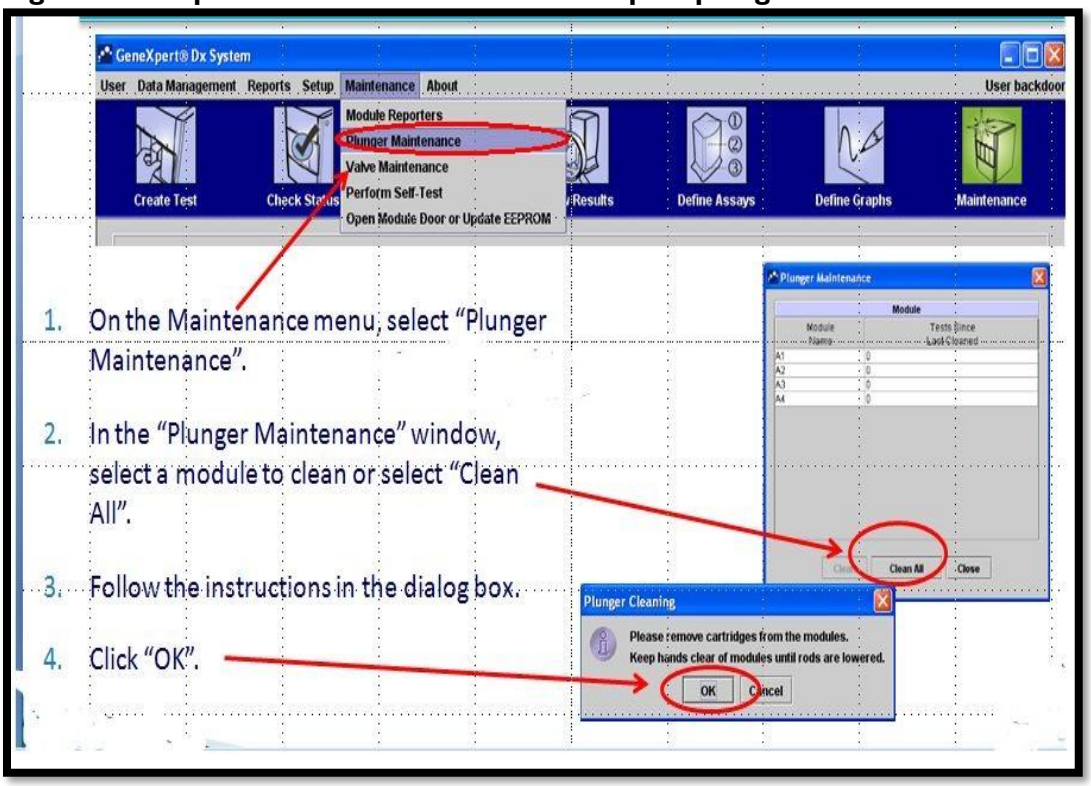


Figure 17: Step 1 for cleaning of the GeneXpert filter

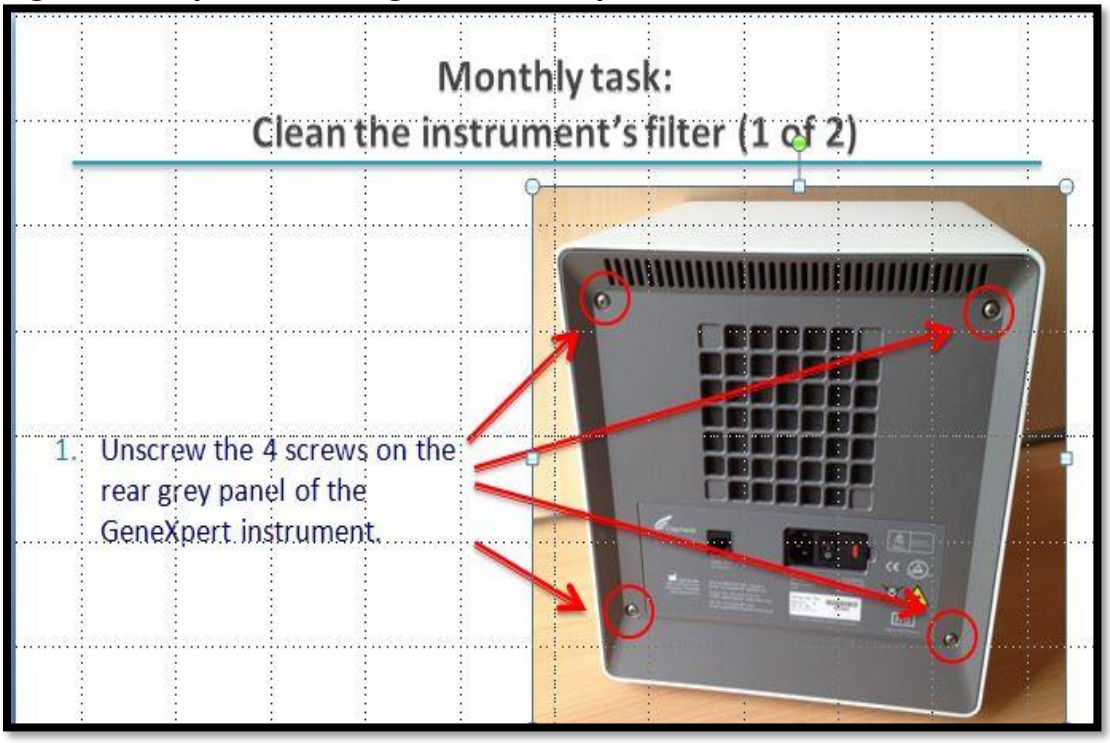
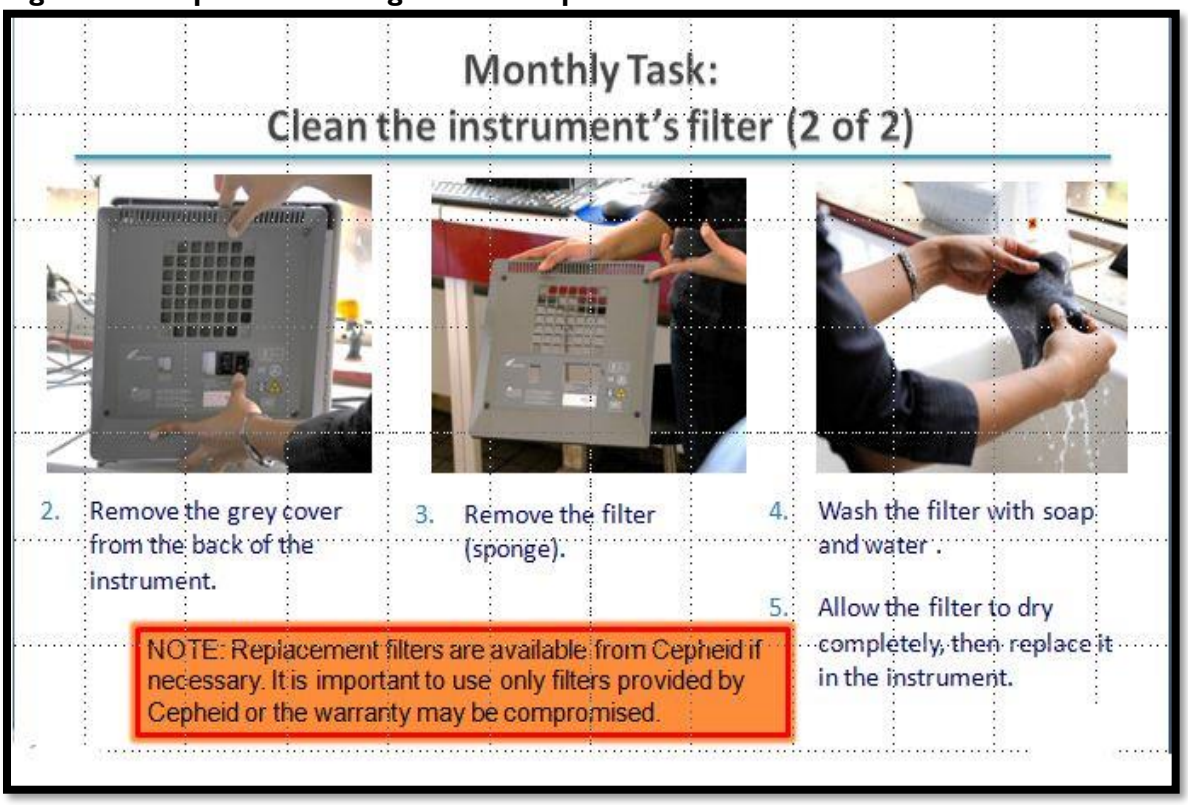


Figure 18: Step 2 for cleaning the GeneXpert filter



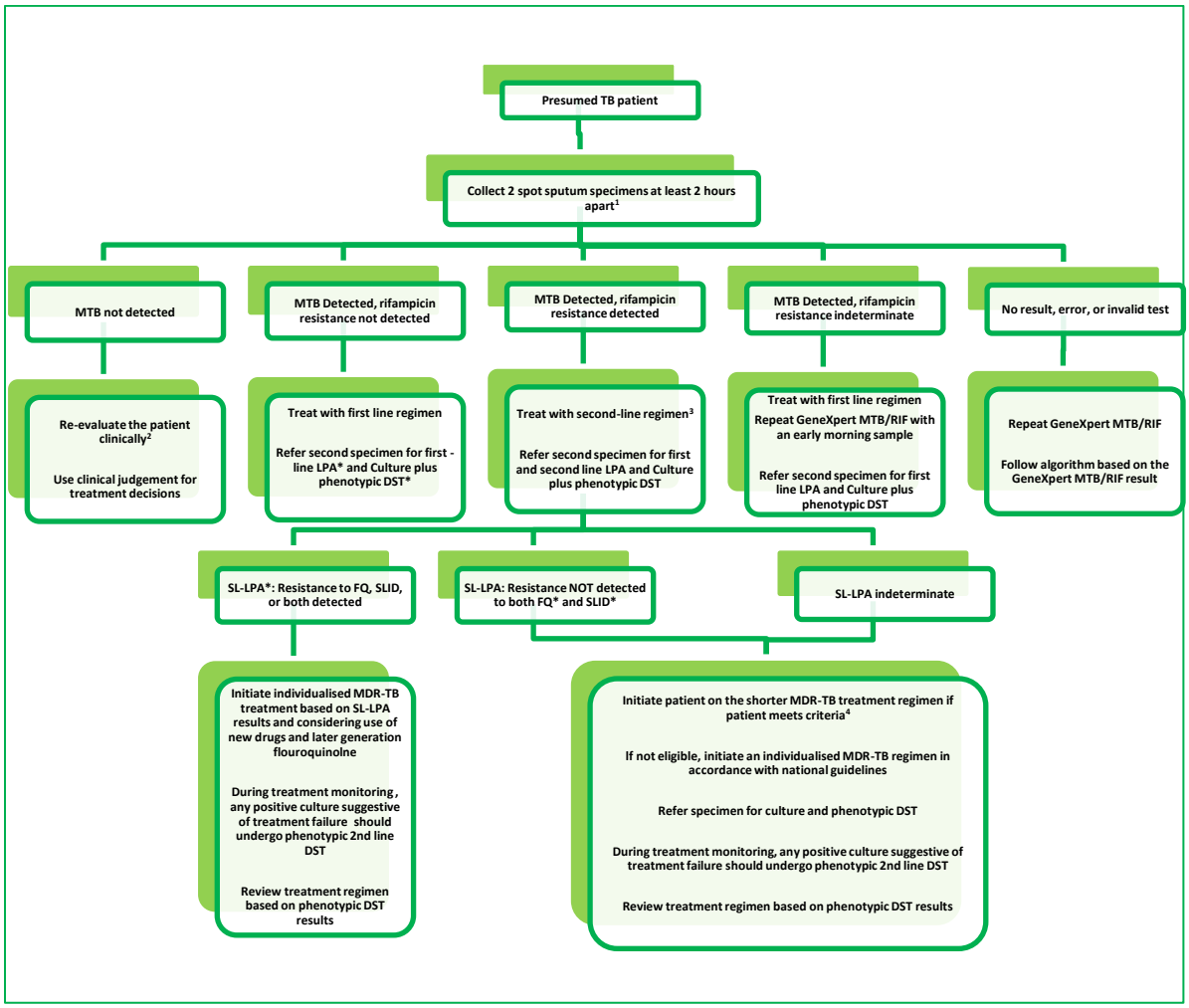
8.4.6 Annual Task: Module Calibration

Calibration should take place each year or after every 2,000 runs on each instrument module (whichever comes first).

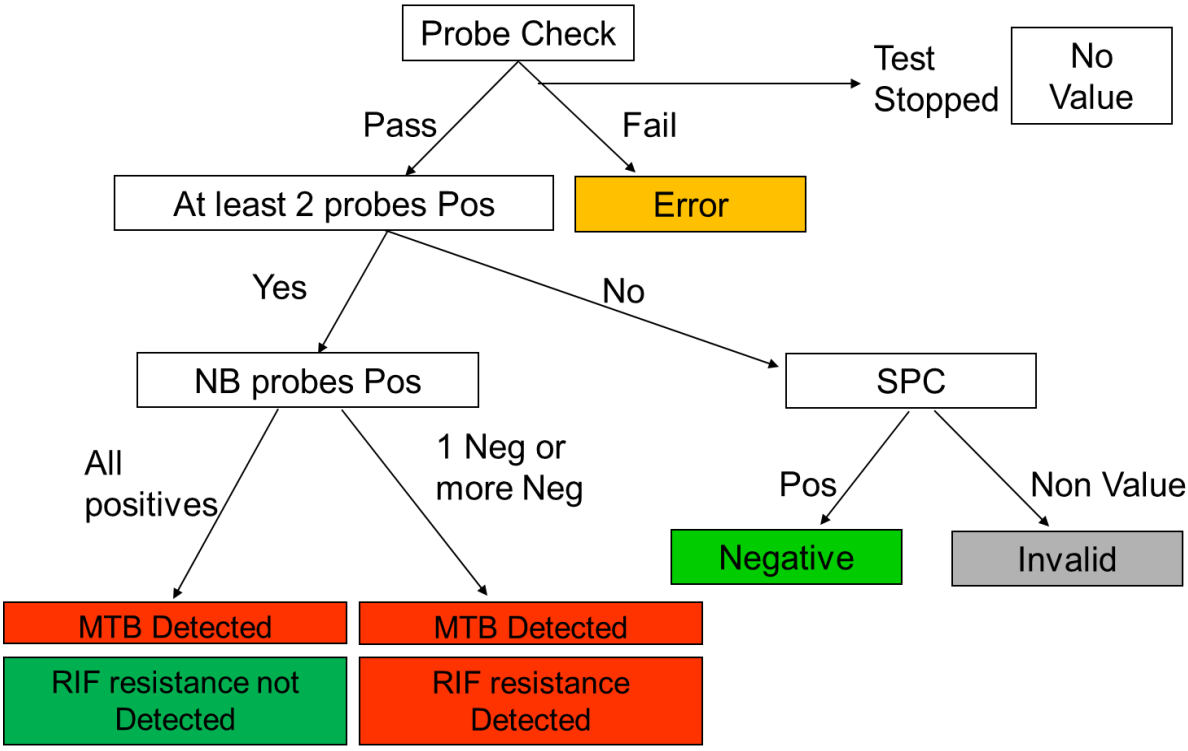
- The date is calculated from the delivery date (or based on the previous calibration date).

ANNEXES

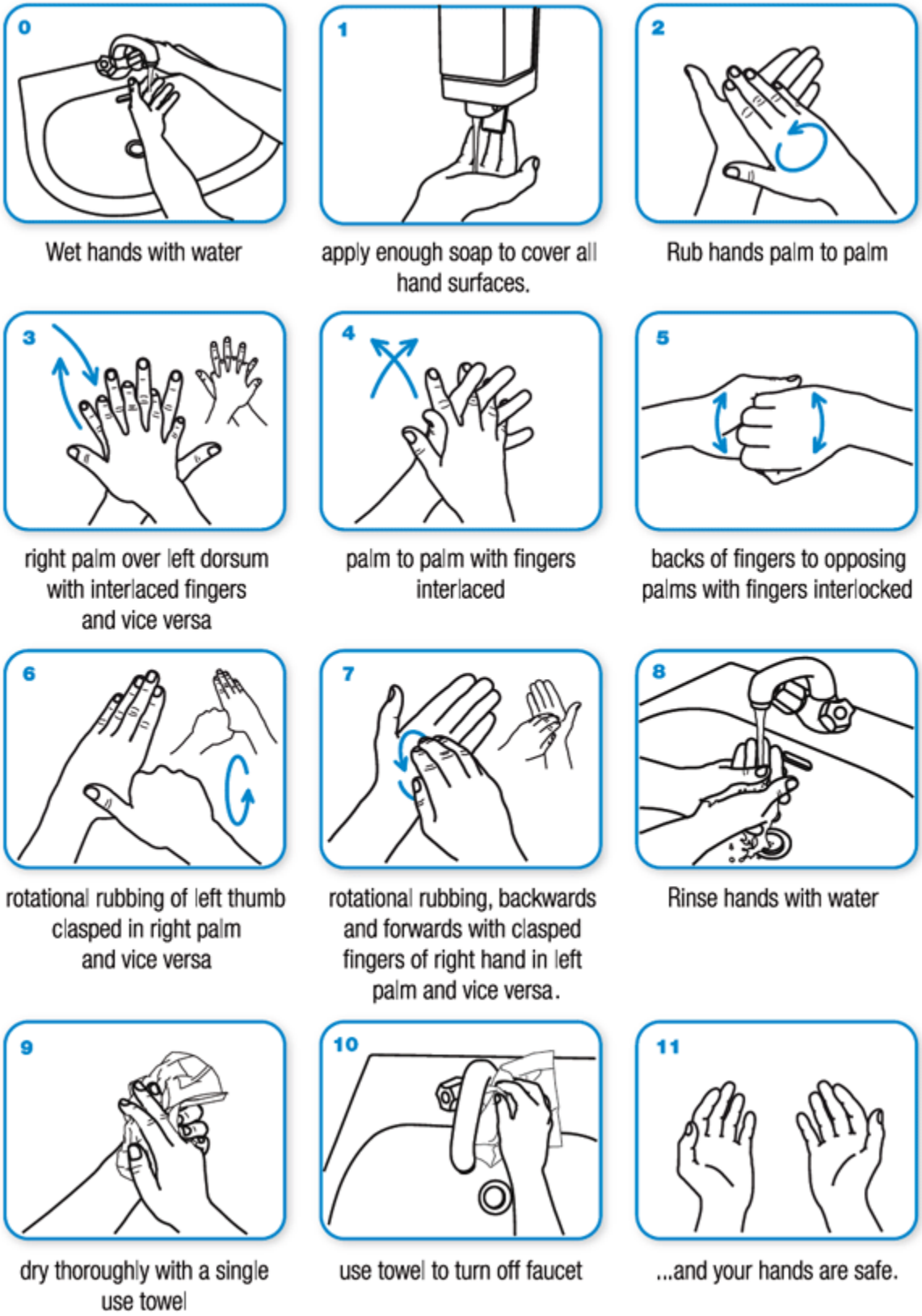
ANNEX 1: TB TESTING ALGORITHM FROM ZIMBABWE, USED IN THE DEVELOPMENT OF THIS MANUAL.



ANNEX 2: ALGORITHM FOR GENEXPERT RESULTS DETERMINATION FROM ZIMBABWE, USED IN THE DEVELOPMENT OF THIS MANUAL



ANNEX 3: HAND WASHING PROCEDURES



ANNEX 4: SAMPLE GENEXPERT MAINTENANCE FORM (FROM ZIMBABWE)

Month:														
	Daily				**Weekly**		**Monthly**							**Annually**
							Archiving data*			Clean GeneXpert instrument**				
Day of the month	Disinfect work surfaces with 1% bleach	Keep module doors vertical after use	Unload and dispose of used cartridges after last run	Ensure 10cm clear around instrument	Reboot Xpert instrument	Reboot software and computer	Archive runs	Delete runs	Save archived data to CD	Disinfect instrument surfaces	Disinfect cartridge bay interior	Disinfect plunger rod	Clean fan filters with soapy water (for GX with white cover)	Calibration annually or every 2000 tests/module ***
1														
2														
3														
4														
5														
6														
7														
8														
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Initial in the appropriate box after completion of the relevant tasks.

* Results archiving to be done monthly, ** cleaning to be done using 1% sodium hypochlorite (bleach) *** calibration to be pre-arranged with supplier

Reviewd by:	Date Reviewed:	Signature :

STANDARD OPERATING PROCEDURE ON DETECTION OF EXTENSIVELY DRUG RESISTANT (XDR) MYCOBACTERIUM TUBERCULOSIS COMPLEX BY XPERT MTB/XDR CARTRIDGE USING THE GENEXPERT TESTING SYSTEM[2]

1.1 PURPOSE

This standard operating procedure (SOP) is written to describe the procedure for detection of extensively drug resistant (XDR) *Mycobacterium tuberculosis* complex (MTBC) using Cepheid's Xpert *Mycobacterium tuberculosis* extensively drug resistant (MTB/XDR) *in vitro* diagnostic test and the GeneXpert (GX) instrument.

PROCEDURES

2.1 PRINCIPLE

The Xpert MTB/XDR assay is an automated in vitro diagnostic test for the detection of XDR-MTB complex Deoxyribonucleic Acid (DNA) and resistance associated mutations. The assay is performed on the Cepheid GX instrument equipped with GX 10 color modules.

The GX instrument integrates and automates specimen processing, nucleic acid amplification, and detection of the target sequences in specimens using nested real-time polymerase chain reaction (PCR) and melt peak detection. GX consists of an instrument, personal computer, barcode scanner, and preloaded software for running tests and viewing the results. The system requires an uninterrupted power supply and the use of single-use disposable GX cartridges that contain target specific PCR reagents and host the PCR process and melt peak detection. Because the GX cartridges are self-contained, risk of cross-contamination between specimens is minimal.

PHOTO BY CEPHEID

The GX MTB/XDR assay cartridge includes reagents for the detection of the XDR-MTB profile and specimen processing control (SPC) to control for adequate processing of the target bacteria and to monitor the presence of inhibitor(s) in the PCR reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability. The sample volume adequacy (SVA) control checks if an adequate volume of specimen is present in the sample chamber before sample processing can start. The GX MTB/XDR assay cartridge contains all reagents except the sample reagent (SR), whereby the user is then required to add the SR to the specimen prior to loading the treated specimen into the cartridge. The test is intended to be run as a reflex test for MTB positive samples.

[2] This document was produced by the IDDS team in Burma with support from the National Tuberculosis Program of Burma.

The results are interpreted by the GX software from measured fluorescent signals and embedded calculation algorithms and are shown in the *View Results* window in tabular and graphic formats. It also reports if the test is invalid, has encountered an error, or produces no result. The GX MTB/XDR test detects XDR MTB with resistance to isoniazid (INH), ethionamide (ETH), fluoroquinolone (FLQs), and second line injectable drugs (SLIDs) directly from unprocessed sputum or from concentrated sediment from sputum in less than 90 minutes.

The GX MTB/XDR assay is intended for use as a reflex test for a specimen (unprocessed sputum, concentrated sputum sediments, or Mycobacteria growth indicator tube culture) that has already been determined to contain MTB. In specimens where MTB is detected, the GX MTB/XDR assay can also detect INH resistance-associated mutations in the *katG*[3] and *fabG1*[4] genes, *oxyR-ahpC* intergenic region[5] and the *inhA* promoter[6]; ETH resistance associated with *inhA* promoter mutations only; FLQ resistance-associated mutations in the *gyrA*[7] and *gyrB*[8] quinolone resistance determining regions; and SLID-associated mutations in the *rrs* gene[9] and the *eis* promoter[10] region. This test is intended as an aid in the diagnosis of XDR tuberculosis (TB) when used in conjunction with clinical and other laboratory findings.

2.2. SPECIMENS

Sputum specimen (Natural/Expectorated or Induced)

- Collect high quality sputum specimens as per the available SOPs. Reject specimens with obvious food particles or other solid particles or particles that are only saliva.
- Proper specimen collection, storage, and transport are essential for correct results.
- Whenever possible, specimens (unprocessed sputum) should be transported and stored at 2–35°C prior to processing (the maximum time for storage and processing is seven days).
- The GX MTB/XDR assay can be used to test leftover SR treated specimens from GX MTB/RIF or GX MTB/RIF Ultra assays. However, in such cases, the volume of the leftover SR treated specimen must be ≥ 2mL and the mix should be stored at 2–8 °C for no longer than four hours or at 35°C for no longer than two and a half hours.

2.3. EQUIPMENT AND MATERIALS

- GX instrument 10 color modules
- GX MTB/XDR cartridges
- Disposable graduated transfer pipettes
- Sterile screw-capped specimen collection containers
- Disposable gloves
- Bio-hazard plastic bag for waste disposal
- Timer

[3] gene encoding the catalase-peroxidase enzyme and associated with INH resistance
[4] gene encoding 3-oxoacyl-ACP-reductase enzyme and associated with INH resistance
[5] intergenic region- associated with INH resistance
[6] associated with INH resistance
[7] gene encoding DNA gyrase subunit A and associated with fluoroquinolone resistance
[8] gene encoding DNA gyrase subunit B and associated with fluoroquinolone resistance
[9] gene encoding 16S rRNA and associated with SLID resistance
[10] associated with SLID resistance

- Indelible labelling marker (permanent marker)
- Sterile pipettes for specimen processing (supplied along with the kit)
- A jar for decontamination of used pipettes
- Rack for placing falcon tubes
- Trays for placing cartridges
- Sterile 50 ml Falcon screw-capped tubes for specimen processing
- N95 Mask
- Absorbent paper
- Vortex mixer
- Thermometer to monitor room temperature (-20 to 50°c)
- Wash bottles
- Towel
- Laboratory coat
- A4 Paper

2.4. REAGENTS AND SOLUTIONS

- Sample reagent (supplied along with the kit)
- 1 percent hypochlorite solution (freshly prepared)
- 5 percent Phenol solution (freshly prepared)

2.5. DETAILED INSTRUCTIONS FOR USE

2.5.1. Start-up of GX instrument

- Perform start-up of the instrument before starting the processing of specimens.
- Turn on the GX instrument, and then turn on the computer.
- On the Windows desktop, double-click the GeneXpert Dx shortcut icon.
- Log on to the GeneXpert Dx System software using your username and password.
- Click on "CHECK STATUS" and check if modules are available and working.
- If modules are not available proceed to the "Troubleshooting" section of the User manual.

2.5.2. Disinfecting the working area

- Disinfect the working area using 1 percent hypochlorite solution.

2.5.3. Labelling

- Remove a cartridge from the package and inspect the cartridge for damage. If damaged, do not use it.
- Label the GX MTB/XDR cartridge with the specimen ID. Do not put the label on the lid of the cartridge—write on the sides of the cartridge or affix an ID label as illustrated in Figure 1.

Figure 1. Write on the side of the cartridge[11]

2.5.4. Preparation of specimens for testing

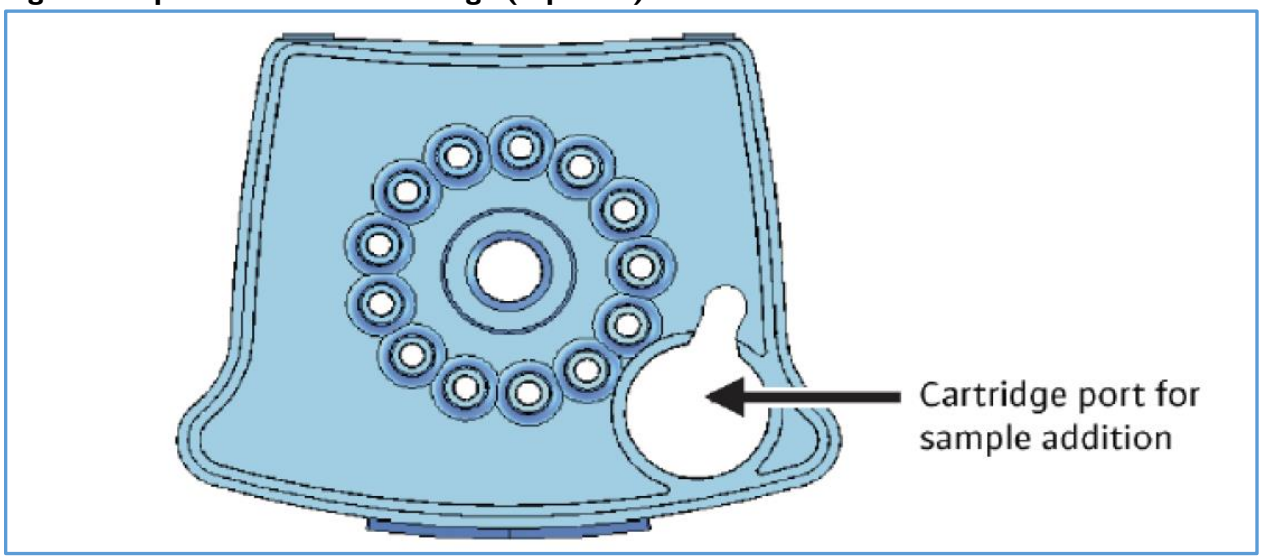
Steps for preparation of sputum specimen:

1. Transfer specimen from a leak-proof sputum collection container to a sterile 50 ml falcon tube.
2. Add Sample Reagent 2:1 (v/v) to specimen and close the lid.
3. Shake vigorously 10 to 20 times or vortex until the specimen is liquefied well (at least 10 seconds).
4. Incubate for 10 minutes at room temperature.
5. Shake the specimen again vigorously 10 to 20 times or vortex until the solution is dissolved well (at least 10 seconds).
6. Incubate for another five minutes at room temperature.
7. If there are still clumps of sputum, shake again vigorously and incubate for another three to five minutes at room temperature.
8. Using the sterile transfer pipette, aspirate the liquefied specimen into the transfer pipette until the meniscus is above the minimum mark (2ml).
9. Open the cartridge lid.
10. Transfer specimen into the open port of the Xpert MTB/RIF cartridge (see Figure 2).
11. Make sure that no bubbles are created when transferring the specimen into the cartridge as this can lead to an error (no result).
12. Dispense slowly to minimize the risk of aerosol formation.
13. Close the cartridge lid.
14. Make sure the lid snaps firmly into place.
15. Keep the remaining liquefied specimen at 2-8°C for repeat testing when required (it can be kept at 2-8°C for a maximum of four hrs.).

[11] Cepheid GXMTB/XDR-10. Package insert. 302-3514, Rev. C, 2021. Cepheid, USA. Retrieved from: https://www.cepheid.com/Package%20Insert%20Files/Xpert%20MTB-XDR%20ENGLISH%20Package%20Insert%20302-3514%20Rev%20C.pdf.

Figure 2. Add processed specimen to the GX MTB/XDR assay cartridge.[12]

Figure 3. Xpert MTB/XDR cartridge (top view)[13]



[12] Cepheid GXMTB/XDR-10. Package insert. 302-3514, Rev. C, 2021. Cepheid, USA. Retrieved from: https://www.cepheid.com/Package%20Insert%20Files/Xpert%20MTB-XDR%20ENGLISH%20Package%20Insert%20302-3514%20Rev%20C.pdf.

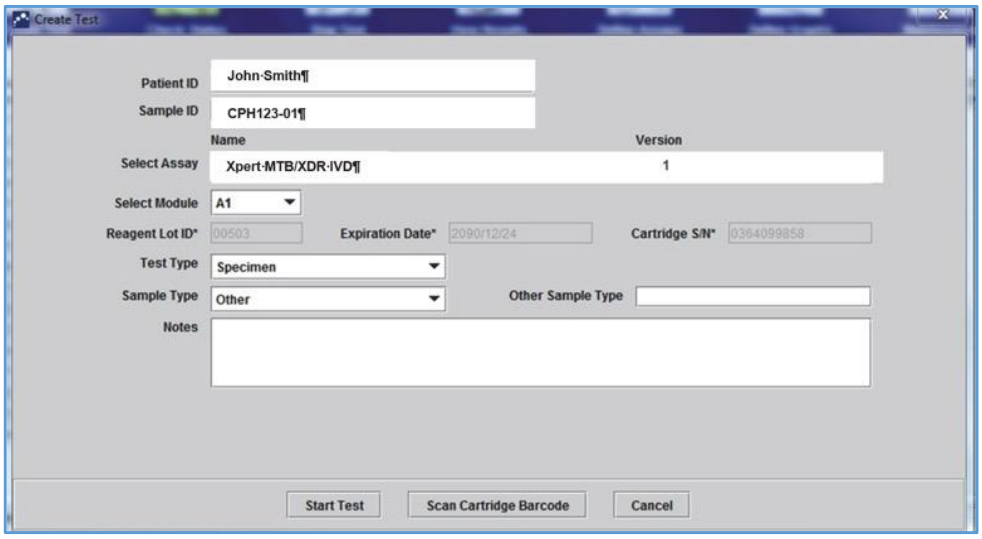
[13] Vanker, N. (2019, July 10). *GX Practical Session 1*. https://media.tghn.org/medialibrary/2020/09/GeneXpert_practical_1_Naadira_Vanker.pdf

2.5.5. Starting the test

Steps for testing:

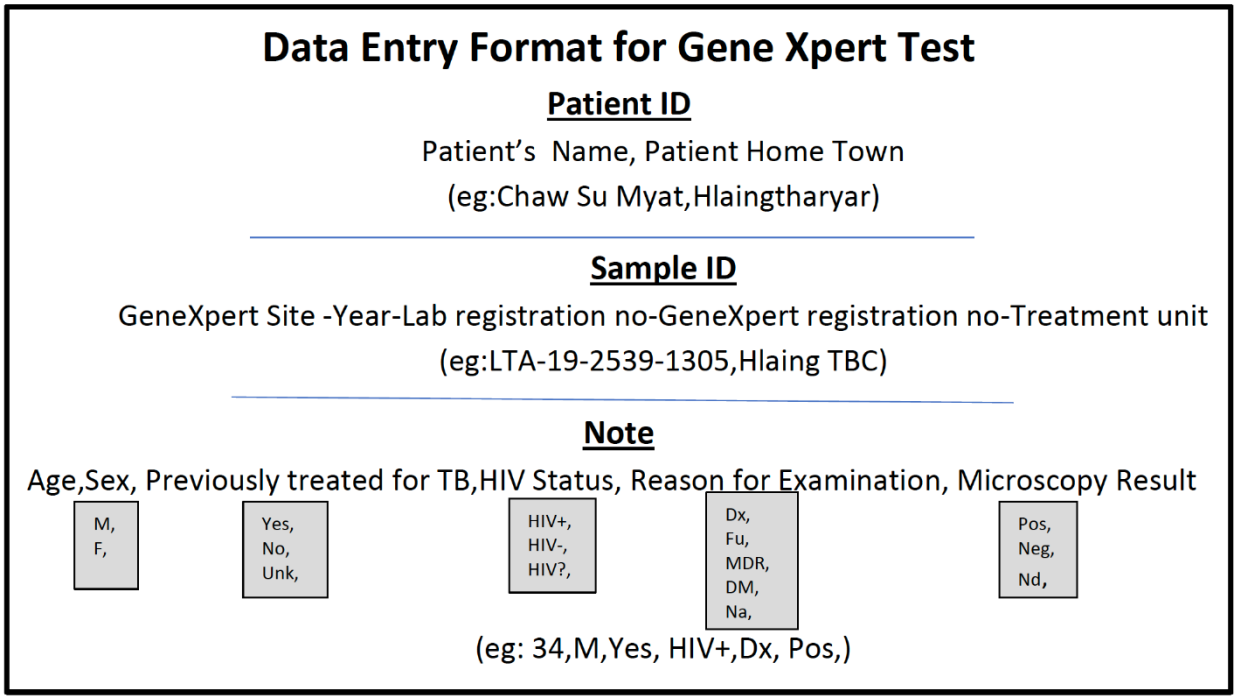
1. Start the test within 30 minutes of adding the specimen to the cartridge.
2. In the GeneXpert Dx System window, click "CREATE TEST." The Scan Cartridge Barcode dialog box appears.
3. Scan the barcode on the GX MTB/XDR cartridge. The Create Test window appears.
4. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.
5. In the Sample ID box, type the specimen laboratory serial number (ID). Make sure you type the correct specimen ID and patient ID and write in the Notes field if needed. The specimen ID is associated with the test results and is shown in the "View Results" window and all the reports.

Figure 4. GX Dx Create Test Window.[14]



[14] Cepheid GXMTB/XDR-10. Package insert. 302-3514, Rev. C, 2021. Cepheid, USA. Retrieved from: https://www.cepheid.com/Package%20Insert%20Files/Xpert%20MTB-XDR%20ENGLISH%20Package%20Insert%20302-3514%20Rev%20C.pdf.

Figure 5. Example of information to be entered in the create test form of the GX DX system.



1. Click "Start Test."
2. In the dialog box that appears, type your username and password.
3. Open the instrument module door with the blinking green light and load the cartridge.
4. Close the door. The test starts and the green light stops blinking. When the test is finished, the green light turns off.
5. Wait until the system releases the door lock at the end of the run, then open the module door and remove the cartridge.

2.5.6. Reading, interpretation, recording and reporting

- In the GeneXpert Dx System window, click "VIEW RESULTS" on the menu bar. The View Results window appears.
- If the software reports "Error," Invalid," or "No result," repeat the test using the already prepared specimen and a new cartridge.
- If the repeated test shows "Error," "Invalid," or "No result" again, proceed according to the troubleshooting manual to exclude technical problems before requesting a new specimen.
- Record the results in the GX register for TB laboratory examination.
- Use red pen to record all "Resistance detected" results.
- Report the results as soon as possible according to the standard process for reporting.

Figure 6. Reading chart by drug class and result call

Drug Class	Result Call
N/A	INVALID/ERROR/NO RESULT MTB DETECTED MTB NOT DETECTED
Isoniazid	Low INH Resistance DETECTED INH Resistance DETECTED INH Resistance NOT DETECTED INH Resistance INDETERMINATE
Fluoroquinolone	Low FLQ Resistance DETECTED FLQ Resistance DETECTED
	FLQ Resistance NOT DETECTED FLQ Resistance INDETERMINATE
Amikacin (AMK)	AMK Resistance DETECTED AMK Resistance NOT DETECTED AMK Resistance INDETERMINATE
Kanamycin (KAN)	KAN Resistance DETECTED KAN Resistance NOT DETECTED KAN Resistance INDETERMINATE
Capreomycin (CAP)	CAP Resistance DETECTED CAP Resistance NOT DETECTED CAP Resistance INDETERMINATE
Ethionamide	ETH Resistance DETECTED ETH Resistance NOT DETECTED

- Report "Please submit a new specimen" if the system repeatedly did not produce a result and you have excluded and/or fixed a technical problem.

2.5.7 Quality Control (QC)

- Maintain the instrument according to the SOP.
- Validate for built-in QCs (SPC, PCC, and SVA) and annual instrument calibration status, and cross-check with patient details using TB-05 test request form prior to reporting.
- Monitor errors and invalid results and investigate promptly.
- Participate in external quality assessment program periodically.

- Only use GX cartridges that are in date and have been stored correctly.

WASTE MANAGEMENT AND OTHER SAFETY PRECAUTIONS

- Dispose of used cartridges in the appropriate specimen waste containers according to your institution's standard practices.
- At the end of each day, the used sputum containers, pipettes, and cartridges must be sealed in a bag and incinerated or autoclaved as soon as possible.
- Keep the waste bag in a safe, closed bin or large bucket until it can be incinerated or autoclaved.
- In intermediate or central laboratories where there is an autoclave, infectious waste should be collected in an autoclavable bag and should be autoclaved before incineration.
- Make sure the tubes are tightly closed before shaking.
- Prepare specimens for testing in a well-ventilated area.
- Clean up spills immediately according to the procedure for management of specimen spills.

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DAVID ROCHKIND / USAID

STANDARD OPERATING PROCEDURES FOR THE DIAGNOSIS OF TUBERCULOSIS AND RIFAMPICIN RESISTANCE BY TRUENAT™ MTB PLUS ASSAY[15]

OBJECTIVES AND SCOPE

The purpose of this manual is to describe the usage of the Truenat™ MTB Plus assay, a chip-based real-time polymerase chain reaction (PCR) test, for the semi-quantitative, detection, and diagnosis of *Mycobacterium tuberculosis* (MTB) complex bacteria in human sputum samples. The manual is to be used at MTB testing sites. The Truenat equipment shall be operated by trained and competent laboratory personnel.

PROCEDURE

1.1 PRINCIPLE

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

1.2 SAMPLE

Sputum samples only

[15] This document was produced by IDDS in collaboration with the Stop TB Partnership and Molbio Diagnostics as part of the USAID supported introducing New Tools Project (iNTP).

EQUIPMENT AND MATERIALS

2.1 EXTRACTION OF DNA

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

2.2 AMPLIFICATION OF PURIFIED DNA

- Truelab Duo Real Time Quantitative micro-PCR analyzer
- Truenat™ MTB Plus micro-PCR chip
- Truelab™ micro-PCR printer
- Truepet Pre-calibrated Single Push Auto ejector fixed volume (6µl) micropipette
- DNase and RNase-free pipette tips with filter barrier

2.3 OTHERS

- Truenat™ Positive Control Kit—Panel I
- Powder-free disposable gloves
- Lab coats
- N95 masks
- Two waste disposal containers, with lids, containing bleach solution
- Timer
- Two waste bags
- Microtube stand cartridge holder
- Two cryovial racks

2.4 REAGENTS AND SOLUTIONS

- Liquefaction buffer
- Lysis buffer
- 0.5 percent bleach and 70 percent alcohol
- Distilled water

2.5 SAMPLE PROCESSING

2.5.1 Specimen Collection

Two spot or early-morning sputum samples are collected from each patient.

2.5.2 Sample Storage and Transportation

Samples collected for testing on the Truenat instruments should be stored in a refrigerator between 2°C to 8°C prior to testing and transportation to the testing laboratory. During transportation cold chain should be maintained (2°C to 8°C) and the samples should be well packaged (triple packaging) in a sample flask/sample transportation container.

2.5.3 Replacement of Trueprep Kit Reagent Pack

Figure 1: Trueprep AUTO cartridge and Trueprep AUTO v2 Reagent Kits

After completion of 50 extractions, the Trueprep AUTO v2 device will prompt the user to change the reagent pack and reset the buffer count.

When prompted to change the reagent pack and reset the buffer count, follow these steps:

1. Press "start" and "eject" simultaneously to reset.
2. Disconnect the used reagent pack by removing the plug-in connector. Take a new reagent pack. Hold the reagent pack's connector and remove the cap.
3. Connect the new reagent pack to the Trueprep Auto v2 device by inserting the plug-in connector into the slot provided (Figure 1).
4. Press the eject button to open the cartridge holder, and gently pull out the door.
5. Insert the reagent card as shown, and gently push to close the cartridge holder (Figure 2).
6. Press start button. It will display "New Reagent Pack Registered" and will eject the reagent reset card.
7. Remove reagent reset card and proceed with further testing.

Figure 2: Inserting the reagent card to the Trueprep AUTO

2.5.4 Sample Processing Procedure

2.5.4.1 Workstation Preparation

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

1. Put on personal protective equipment.
2. Clean the working surfaces with freshly prepared 0.5 percent bleach and then with 70 percent alcohol.
3. Clean the instruments with a paper towel wet with 70 percent alcohol.
4. Empty the two liquid waste containers and fill the two waste containers halfway with 0.5 percent bleach solution.
5. Open a Trueprep AUTO MTB sample pre-treatment kit, which contains a graduated 1 ml transfer pipette, lysis buffer bottle, and liquefaction buffer bottle. Bring all refrigerated samples or reagents to room temperature before use.
6. Arrange the items needed to run a complete batch of two samples:
    a. Liquefaction buffer bottle
    b. Graduated 1 ml and 3 ml transfer pipette
    c. Lysis buffer bottle. Visually check for any damage and that the volume is 2.5 ml. If the volume is less than 2.5ml due to damage, do not use that lysis buffer.
    d. Cartridge pouch and the cartridge holder

e. Result register

7. Ensure that the Trueprep AUTO v2 sample prep device is on.

2.5.4.2 Sample Testing

1. Arrange the cryovials containing the 0.5 ml pipettable sputum in ascending order of sample numbers on the sample rack.
2. Record sample information in the Truenat register as it appears on laboratory request form.
3. Label lysis buffer bottle with corresponding lab number and date of extraction.
4. Place labeled lysis buffer bottle in front of the corresponding sample.
5. Add 2 drops of liquefaction buffer to the sputum container containing the first sample in the batch.
6. Swirl the container to allow the buffer to mix with the sample.
7. Incubate for 10 minutes at room temperature. If the sample is not pipettable after 10 minutes, incubate for another 5 minutes, with swirling at 2-minute intervals.
8. Transfer 0.5 ml of the liquefied sputum sample from the sample container to the corresponding lysis buffer bottle using the 1 ml graduated transfer pipette provided.
9. Dispose the transfer pipette into the container filled with concentrated bleach.
10. Add 2 drops of liquefaction buffer into the lysis buffer bottle.
11. Note: To avoid cross-contamination, DO NOT bring the nozzle of the liquefaction buffer bottle near the sample container.
12. Swirl gently to mix and incubate the lysis buffer bottle at room temperature for 3–5 minutes and observe to ensure that the sample has completely liquified.

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

2.5.5 Nucleic Acid Extraction

1. While incubating the sputum in the lysis buffer, tear open the cartridge pouch. Each pouch contains a cartridge, an eluate collection tube (ECT), and a transfer pipette.
2. Take out the cartridge and place it on the cartridge stand and keep the transfer pipette and the ECT in the pouch for later use.
3. Observe the sample chamber and visually confirm that the reddish internal positive control (IPC) is present. If absent, discard that cartridge and take a different one and report this issue.
4. Label the cartridge pouch with the patient number.
5. Label the cartridge with lab number of the sample and the date of the test.
6. Open the sample chamber of the cartridge by gently pulling the black cap upward.
7. Transfer ALL the contents of the lysis buffer bottle (3 ml) into the sample chamber of the cartridge using the 3 ml transfer pipette provided.

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

8. Dispose the transfer pipette and the used lysis buffer bottle into the waste container filled with concentrated bleach.
9. Recap the cartridge sample chamber with the black cap and put on a fresh pair of gloves.
10. Press the "Eject" button to eject the cartridge holder (Figure 3).
11. Gently pull the cartridge holder and insert the cartridge (Figure 4).

Note: The orientation of the cartridge is to be placed as in Figure 4. Ensure that the site containing the sample is to the right, when looking at the front of the instrument.

12. Gently push to close the cartridge holder. You will hear two clicking sounds when the cartridge is properly loaded in the instrument. **Caution:** Placing the cartridge in the wrong orientation will cause the cartridge holder to remain open, and the cartridge will not be inserted in the device.
13. Press the "Start" button to begin the DNA extraction process.
14. The reagents from the bottles in the back will be automatically added to the cartridge based on the pre-programmed protocol.
15. While the test is in progress, prepare the next sample in the batch (following Steps 1–12).
16. After 18–20 minutes, the device will give a beeping sound, indicating completion of the extraction process with a displayed message.
17. The device will automatically eject the cartridge holder.

Important: To avoid the eluate from being evaporated by heat generated during the extraction process, remove the cartridge as soon as the cartridge holder is ejected.

18. Lift the cartridge up and place it on the cartridge stand.
19. Inspect the tray in the cartridge holder for any spilled liquid. **Note:** In the event of a spill, dispose of the tray in the container filled with concentrated bleach for 30 minutes and spray the cartridge holder with 70 percent isopropyl alcohol. After 5 minutes, place a new tray in the cartridge holder.
20. Take out the ECT tube from the test pouch and label the tube using the sticker provided in the pouch with patient number, age, sex, and date.
21. With the precision and filter barrier pipette tip, pierce the covering of eluate compartment in the cartridge, and aspirate the entire amount of the eluate.
22. Dispense the eluate into the labeled ECT tube and close the ECT tube cap tightly.

Figure 3: Schematic of the Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Device with "EJECT" button

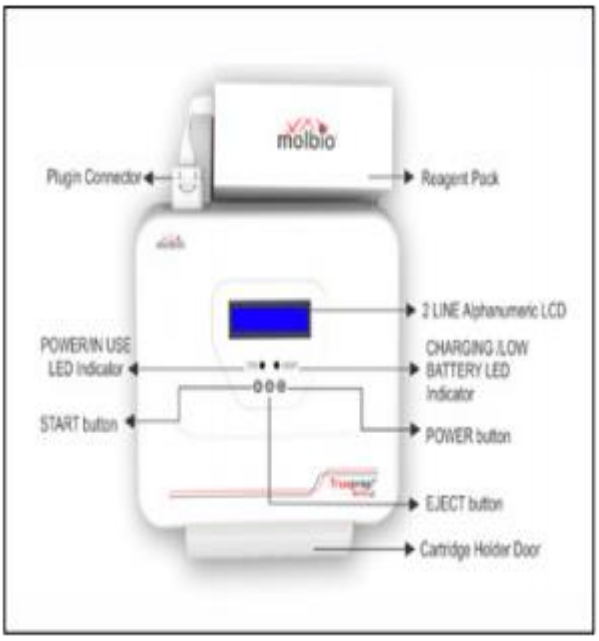

Figure 4: Correct orientation of the Trueprep® AUTO Universal Cartridge Based Sample Prep Kit

23. Put the ECT tube in the labelled ECT holder.
24. If eluate is not to be amplified immediately, store in a refrigerator at 4°C for up to 24 hours or at -20°C for up to 1 year.
25. Dispose the pipette tip and used cartridge into the container filled with concentrated bleach.
26. Dispose used gloves in the dedicated waste container.
27. Load the next sample in the batch into the Trueprep instrument.
28. Transfer the ECT tube containing the eluate to the Truelab micro-PCR analyzer for amplification.

2.5.6 Nucleic Acid Amplification

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

1. Clean the working surfaces with 10 percent bleach, followed by 70 percent alcohol.
2. Clean the instruments with a paper towel wet with 70 percent alcohol.
3. Put on a fresh pair of gloves.
4. Before starting the amplification process for a maximum of two samples, ensure that you have the following arranged on your workstation:
    a. PCR chip set, which contains the chip, micro tube with freeze-dried PCR reagent, micropipette tip, and a blue desiccant
    b. The white, fixed-volume micropipette
    c. The microtube stands to hold the microtubes
    d. ECT tubes containing the extracted DNA
5. Switch on the Truelab Duo device by pressing the red button in the back left corner for 2 seconds. The power/in use indicator will glow green. In 30–50 seconds, the boot screen will appear, followed by the home screen. Ignore the "insert sim" pop-up message.
6. Click on Molbio and select a username from the drop-down menu.
7. Tap on the password text box to pull up the on-screen keyboard.
8. Enter password and press "sign in" to log in the selected user.
9. Inspect the two-chip bay to ensure that there are no used chips in the instrument by clicking on each bay. Select "open/close" to close the bay.
10. Choose either the test bay 1 or 2 and select MTB plus.
11. A pop-up will appear. Confirm selection by pressing "PROCEED."
12. Enter the information required (referred by, patient ID, patient name, age, and gender).
13. Select the sample type "SPUTUM."
14. Press "start test," and the cartridge bay selected will automatically open.

Note: When the "please load sample" prompt appears, DO NOT press "YES" until the chip is loaded.

15. Tear open the chip pouch.
16. Pull out the desiccant pouch and confirm that it is blue.

Note: If the desiccant pouch is white or pink in color, do not use the contents of that pouch. Take another one. (This means that the chip has been exposed to excess moisture.)

17. Pull out the chip enclosed in the chip sleeve.

Note:

    a. NEVER touch the white reagent well.
    b. Minimize the exposure of the chip to light by preparing and running the test immediately after opening.

18. Place the chip on the tray by aligning the registration holes with the tray pins.

Note: The white reaction well should face upward and away from the device.

19. Take out the microtube containing the freeze-dried PCR reagent and remove the lid.
20. Place the microtube containing the freeze-dried PCR reagent in the microtube stand provided.
21. Inspect to be sure that the PCR reagent is at the bottom of the tube.
22. Take the 6 µl precision micropipette and attach the micropipette filter barrier tip enclosed in the chip pouch.
23. Pipette out 6 µl of purified DNA from the ECT and place in the microtube. Confirm visually that the pipetted solution is 6 µl.

Note: DO NOT mix it by tapping, shaking, or by reverse pipetting.

24. Do not dispose the pipette tip. Keep it attached to the fixed volume micropipette but ensure that the tip is retained in the sleeve.
25. Cap the remaining extracted DNA in the ECT tube and move it one step behind.
26. Allow the mixture of eluate and PCR reagent to stand for 30 to 60 seconds to get a clear solution.
27. Pipette out 6 µl of treated DNA from the microtube and put into the reaction well of the chip. **Note:** DO NOT spill eluate on the outsides of the well. Take care not to scratch the internal well surface
28. On the instrument screen and select "YES" when the "please load sample" pops up.
29. Chip tray will close automatically, and reaction will start.
30. Dispose the microtube, microtip, and used gloves into waste container containing concentrated bleach.
31. Truelab will verify chip and begin the test.

Note: If desired, press "PLOT" to view test progress in real time. No user intervention or interpretation is required. Amplification profile is visible in "optical" view.

32. DO NOT touch or shake the instrument while the test is in progress.
33. Follow Steps 9–29 to load the second sample in the batch, but this time select the other test bay.
34. At the end of the run (35 minutes), press "RESULT" to go to the result screen.
35. Possible results:
    a. MTB detected (very low, low, medium, high), Rif resistance detected/not detected/indeterminate
    b. MTB not detected
    c. Invalid
    d. Errors
36. Record the result in the lab register.
37. If result is MTB detected, test same eluate for rifampin resistance using MTB-RIF chip. In this case, select the MTB Rif assay.
38. If the test gives an invalid or error result, record the result and repeat the amplification using the same extracted DNA and a different chip. If a valid result cannot be obtained, run the test with a different sample and eluate.
39. Press "print" to print the results. **Note:** Test results are automatically stored and can be retrieved at any time.
40. Lift the chip from the instrument tray and directly dispose into the waste container filled with concentrated bleach. **Caution:** DO NOT put the chip down on the table or any other place. Do not discard chip anywhere else. The amplicons may contaminate another test and

give a false positive result.

41. Dispose used gloves in the dedicated waste container.
42. Switch off the Truelab analyzer and Trueprep device at the end of the day.
43. Cover each of these instruments with the instrument plastic covers.
44. Clean the surfaces using bleach at the end of the day.

2.6 READING AND INTERPRETATION OF RESULTS

At the end of the test run, the result screen will display "DETECTED" for positive results or "NOT DETECTED" for negative results. The result screen will also display the MTB load as "HIGH," "MEDIUM," "LOW," or "VERY LOW" for positive specimens. The result screen also displays the validity of the test run as "VALID" or "INVALID."

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

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

2.7 STORAGE OF DNA

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

2.8 QUALITY CONTROL

To ensure that the Truelab analyzer is working accurately, positive and negative controls may be run once per month or as needed. The Truenat Positive Control Kit—Panel I containing a positive control and negative control may be used in running these controls; alternatively, phosphate buffered saline may be used as a negative control and a known positive sample (e.g., from culture) as a positive control.

Quality control should be performed if the temperature of the storage area falls outside of 2–30° C.

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

Corrective action: Repeat the control and inform the head of the laboratory.

Documentation: Controls should be recorded in the result register.

WASTE MANAGEMENT AND OTHER SAFETY PRECAUTIONS

- Submerge the used cartridges, replaceable trays, reagent bottles, and other consumables in freshly prepared 0.5 percent bleach for 30 minutes before disposal as per the standard medical waste disposal guidelines.
- Samples and reagents of human origin, as well as contaminated materials, disposables, neutralized acids, and other waste materials, must be discarded after decontamination by immersion in freshly prepared 0.5 percent sodium hypochlorite for 30 minutes (1 volume of 5 percent sodium hypochlorite for 10 volumes of contaminated fluid or water).
- Do not autoclave materials or solutions containing bleach.
- Handle chemicals in accordance with good laboratory practice and dispose of them according to the Biosafety Manual.
- Check all packages before using the kit. Damage to the packaging does not prevent the contents of the kit from being used. However, if the outer packaging is damaged, the user must confirm that individual components of the kit are intact before using them.
- Do not perform the test in the presence of reactive vapors (e.g., from sodium hypochlorite, acids, alkalis, or aldehydes) or dust.
- While retrieving the Truenat™ MTB micro-PCR chip and the DNase and RNase free pipette tip from the pouch, ensure that neither bare hands nor gloves that have been used for previous tests run are used.
- All pipetting steps should be performed with the utmost care and accuracy to prevent cross-contamination between reagents and samples, which may lead to invalid results.

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STANDARD OPERATING PROCEDURE FOR THE DIAGNOSIS OF TUBERCULOSIS BY TB LAM ANTIGEN LATERAL FLOW ASSAY[16]

PURPOSE

The purpose of this standard operating procedure (SOP) is to detail the steps for correctly performing, interpreting, and documenting valid results for tuberculosis (TB) lipoarabinomannan (LAM) antigen (Ag) lateral flow assay (LFA). TB LAM LFA is an immunoassay for the detection of the LAM protein of mycobacteria in human urine as rule-in tool for the diagnosis of active mycobacterium among persons living with HIV as per World Health Organization recommendations.

SCOPE

The procedure applies to all facilities (primary, secondary, tertiary, and quaternary level) performing urine TB LAM Ag LFA for the diagnosis of TB in HIV-infected adults with signs and symptoms of TB or who are seriously ill (World Health Organization stage III/IV) with a CD4 count of <200 cells/ul.

RESPONSIBILITY AND AUTHORIZATION

The persons responsible for performing this test are laboratory personnel and trained non-laboratory personnel (e.g., nurses, HIV testing and service counselors) at a point of care testing site. The person in charge is responsible for ensuring that the SOP is implemented.

MATERIALS REQUIRED

- TB LAM LFA kit/card
- TB LAM Ag test strip
- TB LAM positive control

Materials required but not provided in the kit:

- Timer
- Gloves
- Micropipette and tips or a disposable pipette that delivers 60 µl
- Sharps discard containers
- Pen and sharp permanent marker
- Biohazard disposable bags

SAFETY, HEALTH, AND ENVIRONMENT

Treat all urine specimens as infectious and follow basic universal precautions. Wear protective clothing (coat/apron and gloves) when handling the specimens. In addition, implement all safety procedures as outlined in the facility's SOPs for biosafety.

[16] This document was prepared by IDDS in collaboration with the National Tuberculosis and Leprosy Programme, without whose participation, this would not have been possible.

PRINCIPLE

Alere Determine TB LAM Ag is an immunochromatographic test for the qualitative detection of LAM Ag of mycobacteria in human urine. The Alere Determine TB LAM Ag is currently (as of February 2022) the only commercially available urinary LAM test that potentially could be used as a rule-in test for TB in patients with advanced HIV-induced immunosuppression and could facilitate the early initiation of anti-TB treatment. Refer to the package insert for detailed principles.

SPECIMEN COLLECTION AND STORAGE

Collect mid-stream urine in a new standard urine collection container. Morning urine is the best sample. TB LAM testing should be done within eight hours of urine collection.

If testing is delayed, the urine samples should be stored at 2-8°C and should be done within a maximum of three days from collection.

REAGENT STORAGE AND PREPARATION

Alere Determine TB LAM Ag test cards must be stored at 2–30°C until the expiration date. Kit components are stable until the expiration date when handled and stored as directed. Do not use kit components beyond the expiration date. Immediately reseal all unused tests in the foil pouch containing the desiccant by pressing the seal from end to end to close. Do not use devices that have become wet or if the packaging has become damaged.

TEST PROCEDURE

- Remove the protective foil cover from the test strip and label the strip with the client identification number.
- Apply 60 µl or two drops of the urine sample to the sample pad.
- Read results between 25 and 35 minutes after sample application.

RESULT INTERPRETATION

LAM ANTIGEN POSITIVE (TWO BARS—CONTROL AND PATIENT BARS)

The test result is positive even if the patient line appears lighter or darker than the control line, **as long as the "Patient" line is equal to or stronger than any of the colored lines in the "Positive" range** on the Reference Scale Card.

NEGATIVE (ONE BAR)

One purple/grey bar appears in the Control window of the strip (labeled "Control"), and no purple/grey bar appears in the Patient window of the strip (labeled "Patient").

INVALID (NO BAR)

If there is no purple/grey bar in the Control window of the strip, even if a purple/grey bar appears in the Patient window of the strip, the result is invalid, and the test should be repeated. If the problem persists, contact your local distributor or Alere Technical Support.

INDEFINITE

One purple/grey bar appears in the Control window of the strip (labeled "Control") with unclear or incomplete purple/grey bar in the Patient window of the strip (labeled "Patient"). For a better clinical decision, the test should be repeated. Alternatively, collect a new urine sample in the following days from the patient and test again. Early morning urine is recommended.

QUALITY CONTROL TESTING

Conduct quality control for the TB LAM test weekly or whenever you open a new kit. This should be done before you test the first specimen to be analyzed during that particular week or using the new kit. If there are no specimens to be tested for TB LAM, quality control may not be evaluated in that week. Record quality control results in the TB LAM result log book.

Follow the following procedure to evaluate TB LAM Ag quality control:

TB LAM AG POSITIVE CONTROL

- Label the test strip as TB LAM positive control.
- Add one drop of the TB LAM Ag positive control on the labeled test strip.

- Read results after 25 minutes.

TB LAM AG NEGATIVE CONTROL

- Label the test strip as TB LAM negative control.
- Add two drops of saline/distilled water.
- Read results after 25 minutes.

If positive control samples have run out, work with nearest lab to prepare in-house controls. Leftover urine samples with TB-LAM 4+ positive result, on the Alere TB-LAM test, can be aliquoted into small cryotubes and frozen (-20o C) for a maximum of three months for use as control samples. Thaw one of the frozen aliquots and conduct quality control for TB LAM test weekly.

REFERENCES

1. Stop TB Partnership – Global Drug Facility: Technical Information Note, Determine™ TB LAM Ag Test.

TB CHEST X-RAY TRAINING CURRICULUM[17] FOR X-RAY TECHNICIANS/RADIOGRAPHERS

BACKGROUND

High-quality radiographic images are essential for the correct interpretation of pulmonary tuberculosis (TB). However, there are several technical challenges involved in the production of high-quality chest X-rays (CXRs), including accurate positioning of the patient, correct understanding of equipment and specifications, reproducibility of images, contrast differences, and uniform optical density of anatomic structures in the radiographs. This curriculum has been developed to build the capacity of radiographers to take quality conventional (analog) and digital CXR images for pulmonary TB (PTB), and to enable correct interpretation by trained medical officers and radiologists. This document is not intended for radiologists and medical officers who specialize in interpreting CXRs for patient care, not *per se* in the production of radiographs.

AIMS AND OBJECTIVES

AIM

To improve the quality of both analogue and digital CXRs taken for PTB diagnosis

OBJECTIVES

- To refresh knowledge and build capacities of radiographers (technologists and technicians) to produce high quality CXR images
- To enable correct interpretation of CXR images by trained medical officers and radiologists
- To refresh the knowledge of radiation hazards and protection against radiation when taking CXR images

ANATOMY OF CHEST

Thorough knowledge of the anatomy of the chest helps a radiographer in producing quality CXR images. The thoracic cage and thoracic cavity are important anatomical structures visible on a CXR radiograph.

THORACIC CAGE

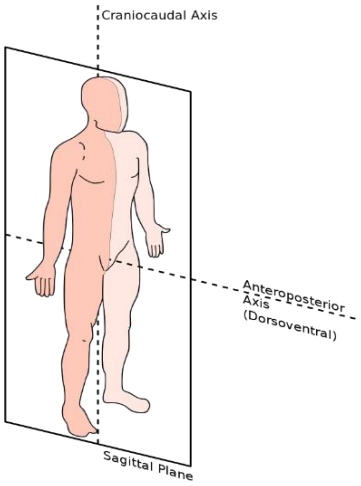
The thoracic cage is bounded:

- Anteriorly, lateral & posteriorly – by 12 pairs of ribs
- Midline anteriorly – by the sternum bone
- Midline posteriorly – by 12 thoracic vertebrae
- Antero-superiorly – by clavicle bones
- Postero-superiorly – by scapular bones

[17] This document was originally produced by IDDS in Burma and is based upon the work and training materials developed by Prof. Khin Hla.

The thoracic cavity is separated by the diaphragm from the abdominal cavity.

Figure 1: The Midsagittal Plane or Median Plane[18] Figure 1 is reproduced by kind permission of Wikimedia commons.



THORACIC CAVITY

Below is detailed information about the thoracic cavity:

- Generally – it is divided into three compartments: The two lateral and one middle compartment. The middle compartment is further divided into the superior, middle, and inferior media sternum.
- The trachea, main bronchus, root of great vessels, and the thymus gland are found in the superior mediastinum and the heart is in the inferior media.
- Two layers of pleurae (known as parietal and visceral) completely cover the lung; they are each filled with a thin film of fluid and mild negative pressure.
- Lungs lie in the thoracic cavity: right lung in the right side of the chest and the left (lung in the left side of the chest. On an X-ray film image, the right lung has three lobes, and the lung has two. Fissures (oblique and horizontal) divide the lung. The horizontal fissure is often seen on a normal frontal view, and the oblique fissure on a normal lateral view.
- Right lung has three lobes (upper, middle, and lower) and is subdivided into ten segments
- Left lung has two lobes (upper and lower) and is subdivided into nine segments.
- On an X-ray film image, the lung can be seen as comprising three zones (upper, middle, and lower zones) according to the ribs:
    - Upper zone = apex up to the second rib zone
    - Middle zone = second to fourth rib zone
    - Lower zone = fourth to sixth rib zone

[18] Edoarado. (2011). *English: Anatomical Sagittal Plane and it's axis*. Own work. https://commons.wikimedia.org/wiki/File:Anatomical_Sagittal_Plane-en.svg

Figure 2: Bony Thorax[19]

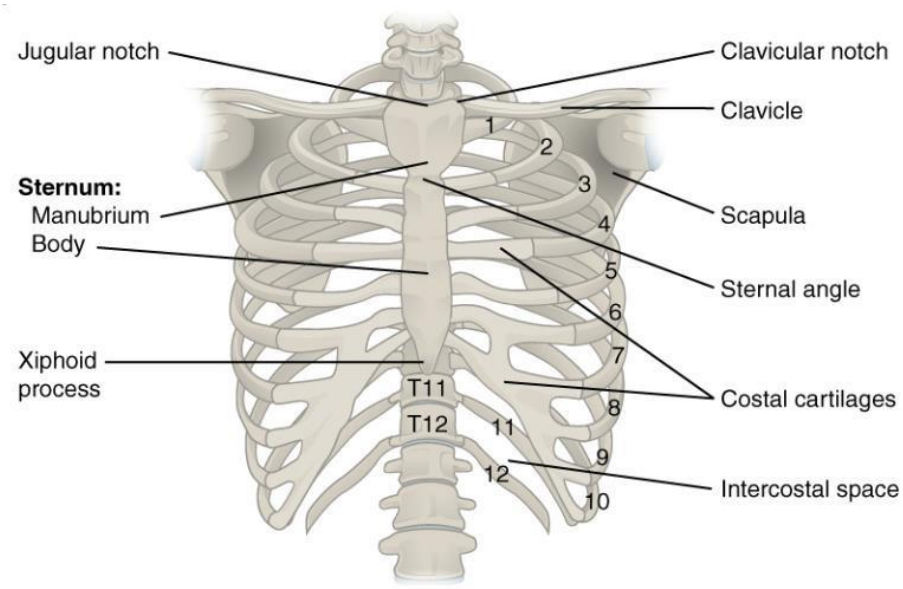
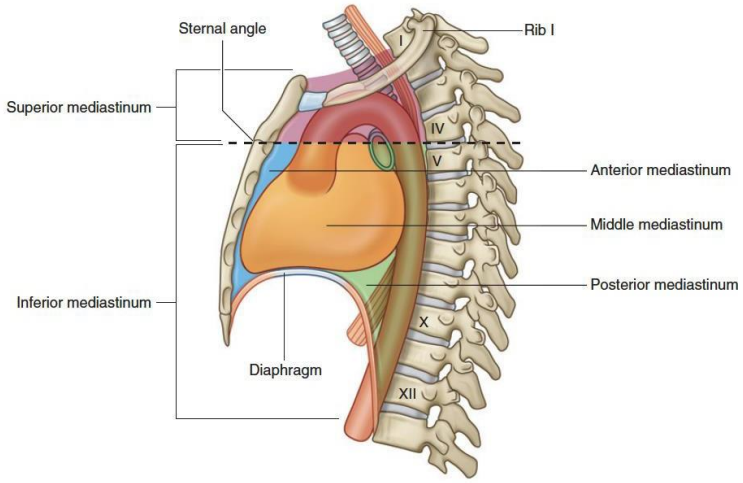


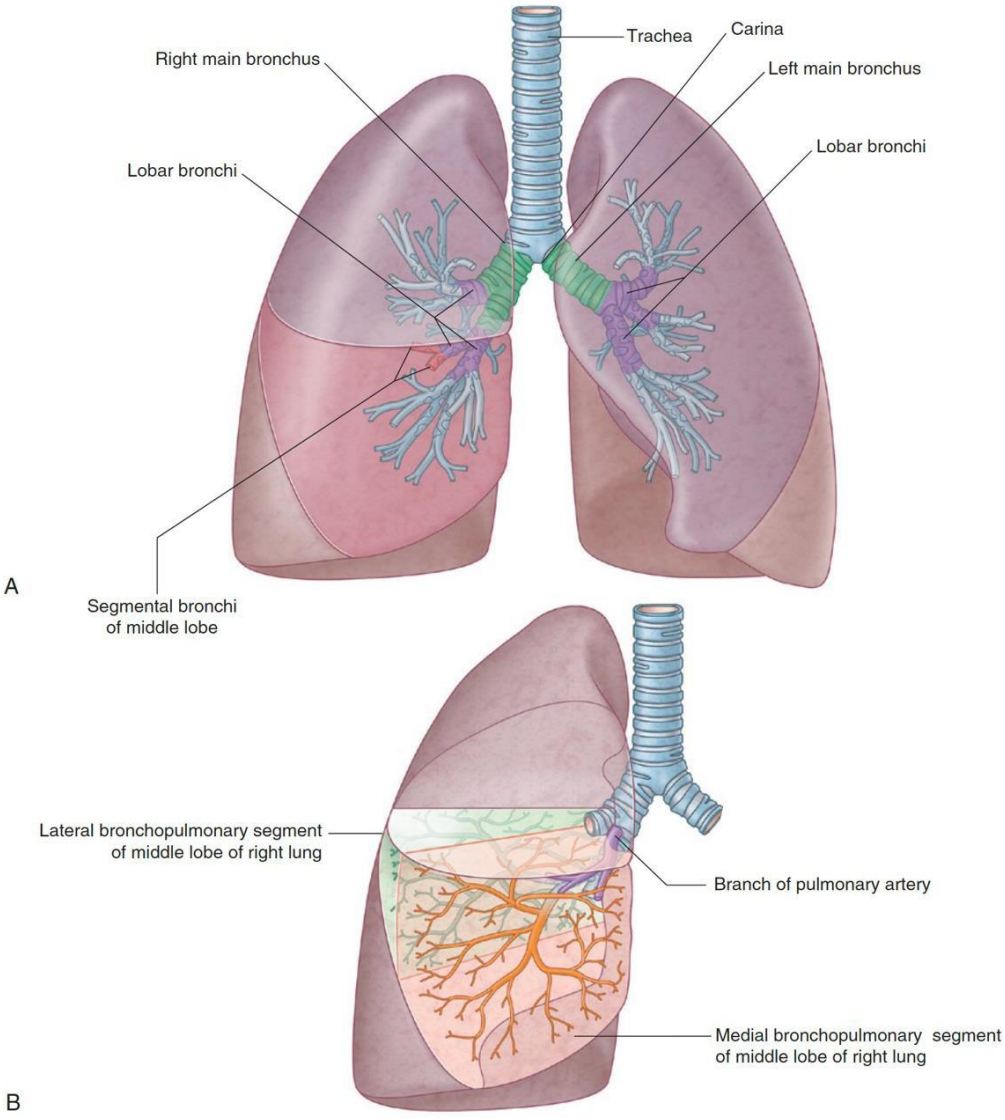
Figure 3: Subdivisions of mediastinum. Topographic Landmarks[20]



[19] Gordon Betts, J., Young, K. A., Wise, J. A., Johnson, E., Poe, B., Kruse, D. H., Korol, O., Johnson, J. E., Womble, M., & DeSaix, P. (2013). The Thoracic Cage. In *Anatomy and Physiology*. OpenStax. https://openstax.org/books/anatomy-and-physiology/pages/7-4-the-thoracic-cage

[20] Drake, R. L., Vogl, A. W., Mitchell, A. W. M. (2020). *Gray's Anatomy for Students* (p.130). USA/Canada: Elsevier Inc.

Figure 4: Pulmonary Diagram A. Bronchial tree B. Bronchopulmonary segments[21]



Schematic representation of segments of the lung from a radiographer's point of view is provided below in figure 7, to help define the segments on the CXR image.

[21] Drake, R. L., Vogl, A. W., Mitchell, A. W. M. (2020). *Gray's Anatomy for Students* (p.175). USA/Canada: Elsevier Inc.

Figure 5: Posterior Ribs in Full Inspiration (K. Hla, personal communication, March 1, 2021)

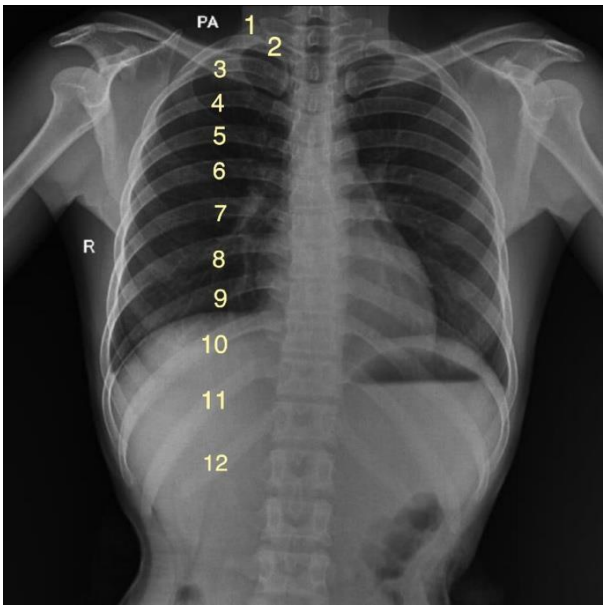


Figure 6: Posterior to Anterior (PA) Chest Radiograph (Adult Male) (K. Hla, personal communication, March 1, 2021)

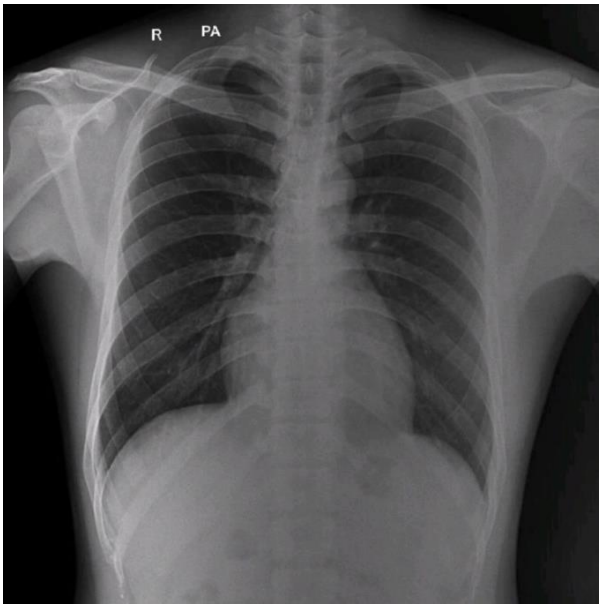
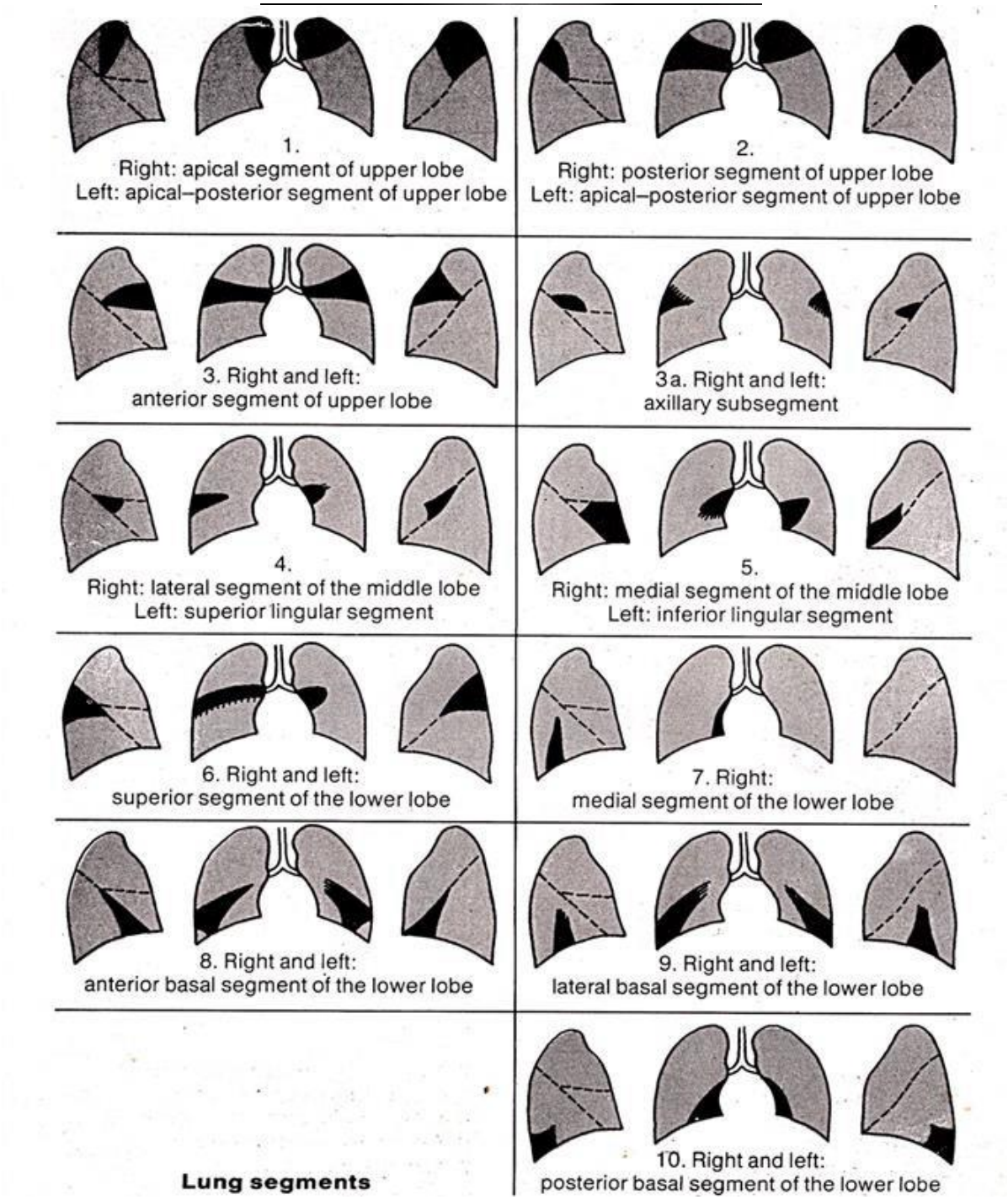


Figure 7: Lung Segments[22]



[22] Moeller, T.B., Reif, E., & Stark, P. (1993). *Pocket Atlas of Radiographic Anatomy* (p.215). Thieme, Germany: Georg Thieme Verlag and Thieme Medical Publishers, Inc.

BASICS OF X-RAYS PRODUCTION AND DETECTION

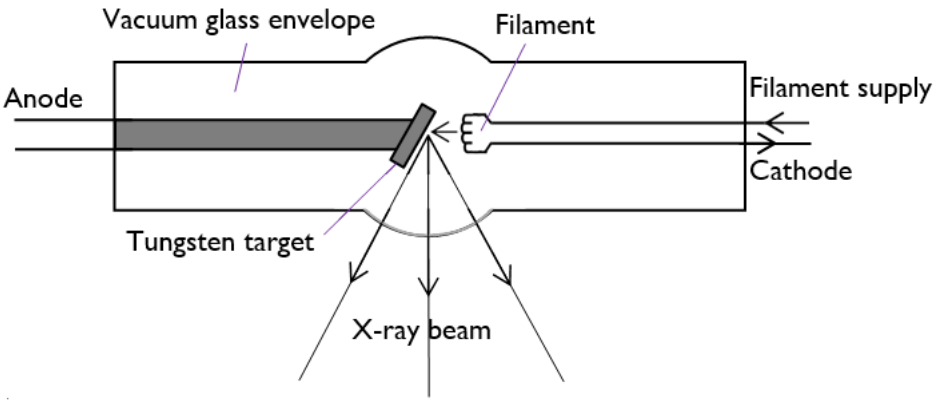
X-rays are used to image the internal anatomical structures of a patient. They depend on an X-ray tube (also called the Coolidge tube) that generates X-rays that pass through a human body and are captured behind on a film that is sensitive to X-rays (conventional CXR) or by a digital detector (digital CXR). Different tissues in the body vary in their absorption of X-rays: dense bone absorbs more radiation, but soft tissue allows more radiation to pass through it. This variance produces contrast within the image to give a two-dimensional representation of the three-dimensional anatomical structures of the human body. As a result, the X-ray image often includes overlapping structures. Thorough knowledge of anatomy is needed to identify an abnormality on an X-ray and understand where it is in the body.

Components involved in X-ray radiography are: (a) X-ray tube as the source of X-rays (b) X-ray film and cassette, and (c) X-ray film processing.

THE X-RAY TUBE

- The X-ray tube is a vacuum glass envelope with a cathode end (filament) and anode end (target) (figure 8).
- The glass envelope is immersed in the transformer oil or insulating oil within the metal case.
- There is an opening (square shape) from which the X-ray beam emerges.
- The collimators (square opening) can easily be adjusted to control the X-ray beam size.

Figure 8: Coolidge Tube[23]



PRODUCTION OF X-RAY BEAM

- An electrical impulse is applied to the filament with a high potential difference between the cathode and anode side resulting in the production of the X-ray beam from the Target.

[23] Hla, K. (2021, August). *Chest X-ray Taking Procedures Training for X-ray technicians/ Radiographer*. On file with author.

- An X-ray beam is an electromagnetic wave that can pass through the object according to the density and thickness of the object.
- The strength (intensity) of the X-ray beam obeys the inverse square law.

A current is passed through the filament and heats it up. As it is heated, the increased energy enables electrons to be released from the filament. The electrons are attracted towards the positively charged anode and hit the tungsten target with a maximum energy determined by the tube potential (voltage). As the electrons bombard the target, they interact and result in the conversion of energy into heat (99 percent) and X-ray photons (1 percent). The X-ray photons are released in a beam with a range of energies and form the basis of X-ray formation.

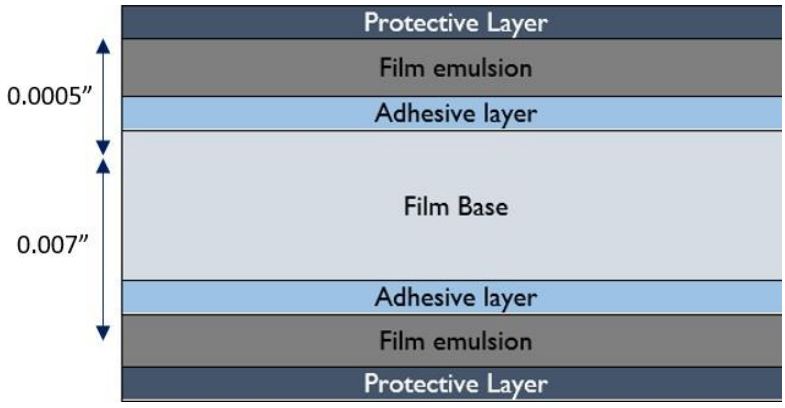
X-RAY FILM AND CASSETTE

Radiographic X-ray film is composed of three elements:

5. A polyester support: solid, sparse, and insensitive to temperature variation
6. One or two emulsions composed of:
7. Silver bromide (AgBr) crystals
8. Gelatin that binds the crystals
9. Achromatic sensitizer making the film only sensitive to a part of the light spectrum
10. A protective layer covering the emulsion

The radiographic film, as shown in Figure 9, is covered with a reinforcing screen (also called an intensifying screen) which aims to convert the X-rays into light photons.

Figure 9: Radiographic Film (K. Hla, personal communication, March 1, 2021)



- The role of the X-ray cassette is to:
  - Protect the film from the light
  - Protect the film from shocks
  - Unify the whole of the radiographic film and the intensifying screen from the couple film/screen
- Under the effect of X-rays, the intensifying screen will emit light of a defined color (blue or green light). The X-ray film will be sensitive to this color.

- The couple film/screen will be locked in the X-ray cassette that will let in the radiation on its front side and stop it through a leaded layer on the back.
- Photographic printing of the film or latent image:
    - Under the effect of light photons, silver bromide crystals will separate into Ag+ and Br- ions.
    - This dissociation of the ions will form the latent image.
    - The latent image is visualized as a radiographic image after the film is suitably processed in the darkroom.

DIGITAL RADIOGRAPHY

Some basics of digital radiography are as follows:

- Digital radiography uses different solid-state detectors (flat panel detectors, charge couple devices) as image receptors (IRs) in place of X-ray films and the cassette used in conventional radiography.
- Components involved in digital radiography are: (i) X-ray generator/tube (same as for the conventional X-ray imaging system described in section 5.1), (ii) digital detector,
- (iii) high-resolution diagnostic display, and (iv) computer system and software.
- The digital detectors are solid-state devices. There are two types of detectors available based on the process of conversion of X-rays to charge:
    - Indirect conversion system or opto-direct system: It uses a scintillator (e.g. Cesium iodide (CsI) or gadolinium oxysulphide (GOS or Gadox) layered on top of an array with light-sensitive photodiodes with thin-film transistors (TFTs). The scintillator converts radiation into light that is detected by the photodiode/TFT array.
    - Direct conversion systems or electro-direct system: It uses a photo conducting layer (amorphous selenium (a-Se), or amorphous silicon (a-Si)), in which the absorbed X-ray energy is directly converted into a charge on top of a TFT array.
- Different types of digital radiography systems are available and are often manufacturer-specific. Thus, detailed orientations to digital radiography should include hands-on trainings facilitated by the manufacturer in collaboration with the national TB program.
- Inter-reader variability of human interpreters of CXRs is substantial and access to trained radiologists is limited in many settings. Computer-aided detection software packages have been developed to automate the interpretation of digital CXR images. They produce a numerical score indicating the likelihood of TB.
- Digital CXR technologies offer several advantages over conventional film-based X-ray technologies: (i) lower operating costs, (ii) improved reproducibility of image quality, (iii) a decreased radiation dose, (iv) computer-aided automation of image interpretation, (v) digital archiving and retrieval facility for images, and (vi) electronic transmission of images, enabling remote monitoring of patients and quality control of images.

Notes: Taking a good quality CXR image: Contributing technical factors

The strength of the X-ray beam depends on the current (in milliampere = mA), duration of current (in seconds= s), and potential difference (in kilo-voltage = kV). Dosage is controlled by two functions: X-ray tube current and exposure time adjustment. Generally, the dosage of the X-ray beam control is referred to as "mAs," which refers to the multiplication of mA and seconds.

Contributing factors for quality X-ray image production depend on specifications for the voltage, current, and time of exposure:

- The voltage (Units of measurement, kV= kilo Voltage)
  - The higher the difference in kV the higher the speed of electron transmission and thus more X-ray penetration; this causes changes in the contrast of the image.
  - 100-120 kV is recommended.
- The current (Units of measurement, mA =milli Ampere)
  - The number of electrons depends on the amount of current applied to the filament and this can change the darkness of the image.

o More than 100mA is recommended.

- Time (units of measurement, s= second)
  - The shorter the time the less movement of a person and therefore a clearer image.
  - Less than 0.05 seconds is recommended.
- Distance between x-ray film and tube focus (SID) and X- ray beam alignment
  - Longer distance improves the image sharpness through geometric means. X-ray beam must be aligned straight with the X-ray film.
  - 140-200 cm is recommended for SID.

Troubleshooting for X-ray image quality:

- Too bright image – most likely causes are:
  - Low current (Low mA)
  - High kV
  - Too short a developing time
  - Developer and Fixer solutions temperature lower than 20° C
  - Low strength of developer solution
- Too dark image – most likely causes are
  - Using too much mA
  - Long exposure time
  - Long developing time
  - Increased strength of developer solution
- Blurring of image
  - Patient cannot hold their breath properly
  - Less than cooperative patient such as children and the elderly
  - Too long exposure time

STEPS IN GENERATING A QUALITY CXR IMAGE FOR PTB

Procedures for (1) patient preparation, (2) identification, (3) patient positioning and techniques for PA projection, (4) patient positioning and techniques for lateral projection, (5) patient positioning and techniques for antero-posterior (AP) projection, and (6) patient positioning and techniques for generating quality CXR images for children are detailed below.

PATIENT PREPARATION

Patient preparation for CXR includes the removal of all opaque objects from the chest and neck regions, including clothes with buttons, snaps, hooks, jewelry, or any objects that would be visualized on the radiograph as a shadow (radiopaque artifact). To ensure that all opaque objects are removed from the chest region, ask the patient to remove all clothing and jewelry (necklaces, or other objects) around the neck and chest area. The patient then puts on a hospital gown, which usually has an opening in the back.

Long hair braided or tied together in bunches with rubber bands or other fasteners may cause suspicious shadows on the radiograph if left superimposing the chest area.

In hospitalized patients, oxygen lines or electrocardiogram monitor leads should be moved carefully to the side of the chest if possible. All radiopaque objects should be moved carefully from the radiographic field of interest to prevent them from interfering with the quality of the diagnostic image.

IDENTIFICATION OF PATIENT

When identifying a patient, take the following steps:

- Check the CXR request form for patient information – (name, age, sex, name of the requesting health facility, date of examination).
- Obtain menstrual history information for females of childbearing age. If the patient could be pregnant, take necessary radiation safety precautions.
- Make an identification mark on the X-ray film to positively identify the patient. This is essential to avoid misidentification by the radiographer. These markings are done prior to preparing the X-ray equipment. At a minimum, the marking should indicate the orientation of the film as left or right (L/R), date of radiography (date), name of the X-ray facility and hospital, and X- ray registration number.

PATIENT POSITIONING AND TECHNIQUE FOR PA PROJECTION: CHEST

The PA chest view examines the lungs, bony thoracic cavity, mediastinum, and great vessels. Position the patient for PA projection:

- Patient is erect, feet spread slightly apart, weight equally distributed on both feet
- Chin raised, resting against the IR
- Hands on lower hips, palms out, elbows partially flexed
- Shoulders rotated forward against the IR to allow the scapulae to move laterally clear of lung fields and shoulders depressed downward to move clavicles below the apices

Position the instrument for an ideal image:

- Align midsagittal plane with the Central Ray (CR) and with the midline of the IR with equal margins between lateral thorax and sides of the IR.
- Ensure no rotation of thorax by placing the midcoronal plane parallel to the IR.
- Raise or lower the CR and IR as needed to the level of T7 for an average patient. (Top of the IR is approximately 1.5 to 2 inches [4 to 5 cm] above the shoulders for most patients)

Make sure the shielding is place correctly:

- Shield radiosensitive tissues outside the region of interest such as testes, uterus, and ovaries.

Position the Central Ray for optimum imaging:

- CR perpendicular to the IR and centered to midsagittal plane at the level of T7 (7 to 8 inches [18 to 20 cm] below vertebra prominent, or the inferior angle of the scapula)
- IR centered to the CR

Follow the recommended Collimation:

- Collimate on four sides to the area of the lung fields. (Top border of the illuminated field should be to the level of vertebra prominent, and the lateral border should be to the outer skin margins)

Please give the following instructions to the patient for respiration:

- Breathing instructions are very important in chest radiography because any chest or lung movement that occurs during the exposure results in "blurring" of the radiographic image. Chest radiographs must be taken on full inspiration to show the lungs as they appear fully expanded.
- Exposure is made at the end of the second full inspiration. More air can be inhaled without too much strain on the second breath compared with the first. Therefore, the patient should be asked to hold the second full inspiration rather than the first. However, the full inspiration should not be forced to the point of strain that causes unsteadiness; this should be explained to the patient as they are being positioned and before the exposure starts.
- For hypersthene and broad-chested patients, place 14 × 17 inch (35 × 43 cm) IR in a crosswise position.

Check the following technical Factors for X-ray equipment operation:

- Minimum SID—72 inches (183 cm) mean distance between the X-ray tube and cassette
- IR size—35 × 43 cm (14 ×17 inches), lengthwise or crosswise, size of the cassette
- X-ray grid
  - Used according to the radiology department protocol
- Voltage for Analog and digital systems—110 to 125 kV range

Remember the following factors for a Standard CXR (PA):

- Included are both lungs from apices to costo-phrenic angles and the air-filled trachea from T1 down
- Hilum region markings, heart, great vessels, and bony thorax are demonstrated

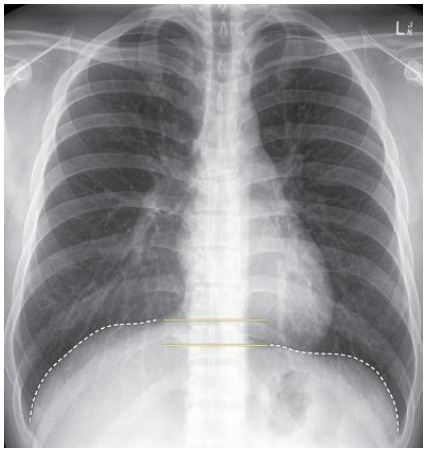
Evaluate the PA image:

- Chin sufficiently elevated to prevent superimposing apices
- Sufficient forward shoulder rotation to prevent superimposition of scapulae over the lung fields
- Larger breast shadows (if present) primarily lateral to lung fields
- No rotation: Both sternoclavicular joints the same distance from the centerline of the spine
- Distance from the lateral rib margins to the vertebral column the same on each side from the upper to lower rib cage
- Collimation margins near equal on top and bottom with the center of collimation field (considered as equivalent to CR) to T 7 region on most patients
- Full inspiration with no motion
- Visualizes a minimum of 10 posterior ribs above the diaphragm (11 on many patients)
- Note: Scoliosis and kyphosis may cause asymmetry of sternoclavicular joints and rib cage margins, as evidenced by Rt. to Lt. spinal curvature

Review the quality of the exposure:

- No motion evident by sharp outlines of the rib margins, diaphragm, and heart borders as well as sharp lung markings in the hilar region and throughout the lungs
- Sufficient long-scale contrast for visualization of fine vascular markings within the lungs
- Faint outlines of at least the mid-thoracic and upper thoracic vertebrae and posterior ribs visible through the heart and mediastinal structures

Figure 10: PA Projection Chest[24]



PATIENT POSITIONING AND TECHNIQUE FOR LATERAL PROJECTION

Lateral chest view examines the lungs, bony thoracic cavity, mediastinum, and great

vessels. Position the patient for lateral projection:

- Standing upright

[24] Gaillard, F. (n.d.). *Normal frontal chest x-ray*. Radiopaedia. https://radiopaedia.org/cases/normal-frontal-chest-x-ray?lang=us

- The left side of the thorax adjacent to the IR
- Both arms raised above the head, preventing superimposition over the chest
  - Arms can be placed on the head or holding onto handles, if available
- Chin raised out of the image field
- The midsagittal plane must be perpendicular to the divergent beam:
  - using the paravertebral gutter technique + right side rotated 5-10° anterior

Position the part lateral projection:

- Centering point
  - The level of the 7th thoracic vertebra, approximately seven cm below the jugular notch of the sternum
  - The CR is angled to be perpendicular to the long axis of the patient's sternum generally resulting in a caudal angle

Position the shielding

- Shield radiosensitive tissues outside the region of interest such as testes, uterus, and ovaries.

Follow the recommended Collimation

- Superiorly five cm above the shoulder joint to allow proper visualization of the upper airways
- Inferior of the inferior border of the 12th rib
- Anterior posterior to the level of the acromioclavicular joints

Instruct the patient on optimum respiration:

- Suspended inspiration: The patient should be asked to take a deep breath and hold the breath while the exposure is taken

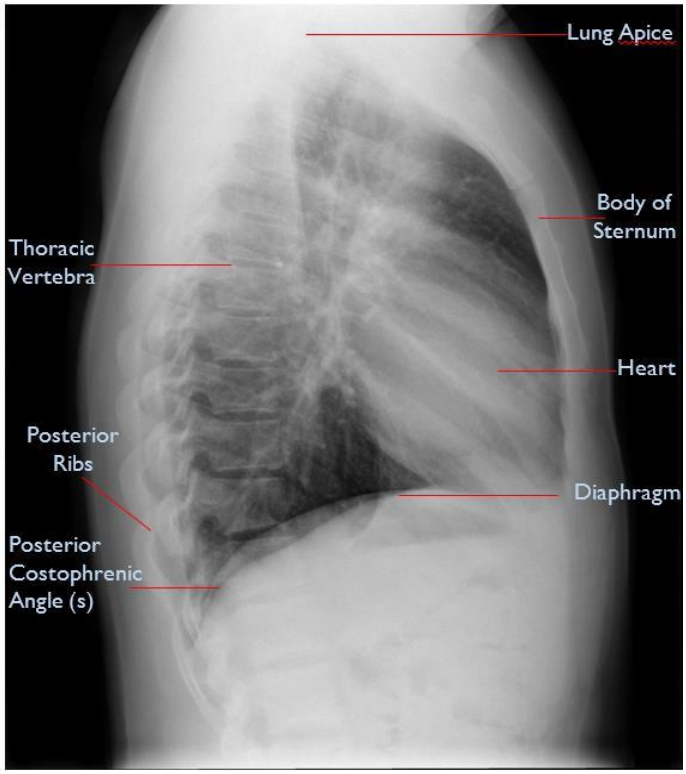
Remember to review the following technical factors for X-ray equipment operation

- Orientation
  - portrait or landscape
- SID
  - 180 cm mean distance between the X-ray tube and cassette
- IR size
  - 35 cm x43 cm or 43cm x 35 cm lengthwise or crosswise, size of the cassette

Check the X-ray Grid

- Exposure
  - 100-110 kVp
  - 8-12 mAs

Figure 11: Lateral Projection[25]



PATIENT POSITIONING AND TECHNIQUE FOR AP PROJECTION

The AP view examines the lungs, bony thoracic cavity, mediastinum, and great vessels.

Position the patient for an anteroposterior image:

- The patient is upright as possible with their back against the IR.
- The chin is raised to be out of the image field.
- If possible, the hands are placed by the patients' side.
- Shoulders are depressed to move the clavicles below the lung apices.

Position the part for an anterioposterior image:

- Centering point
  - The level of the 7th thoracic vertebra, approximately seven cm below the jugular notch
  - The CR is angled to be perpendicular to the long axis of the patient's sternum generally resulting in a caudal angle

Position the shielding for an anteroposterior image:

- Shield radiosensitive tissues outside the region of interest such as testes, uterus, and ovaries

[25] Case courtesy of Assoc Prof Frank Gaillard, rID: 8090, Radiopaedia.org. https://radiopaedia.org/cases/normal-frontal-chest-x-ray?lang=gb

Follow the recommended Collimation:

- Superiorly five cm above the shoulder joint to allow proper visualization of the upper airways
- Inferior to the inferior border of the 12th rib
- Lateral to the level of the acromioclavicular joints

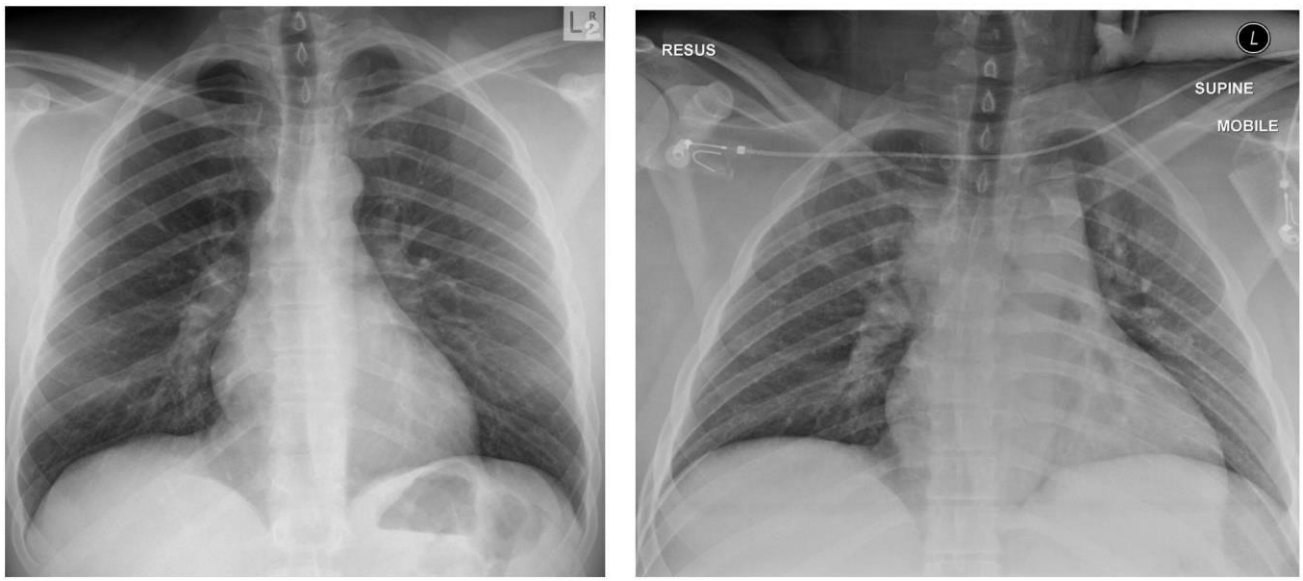
Instruct the patient on optimal respiration:

- Suspended inspiration: The patient should be asked to take a deep breath and hold the breath while the exposure is taken

Review the technical factors for X-ray equipment operation:

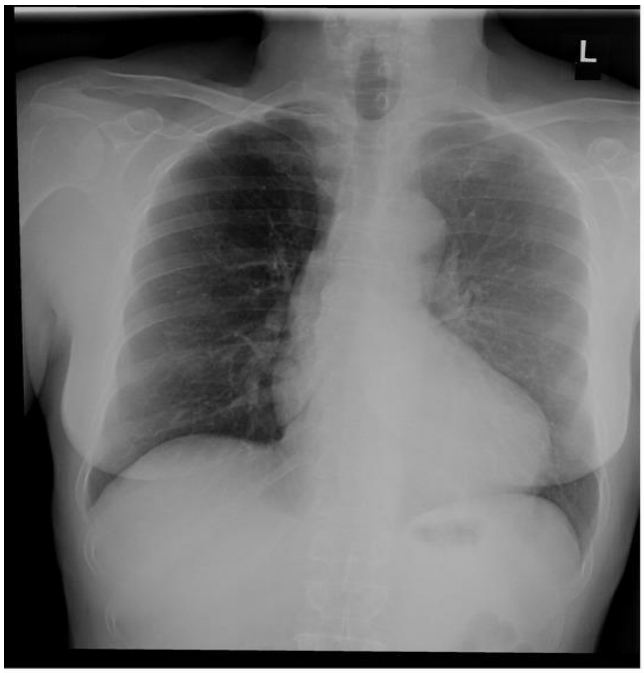
- Orientation
  - Portrait
- Detector size
  - 35 cm x 43 cm or 43cm x 35 cm
- Exposure
  - ○ 100-110 kVp
  - 4-8 mAs
- SID
  - 180 cm
- X-ray Grid
  - Yes

Figure 12: PA vs. AP chest projection on the same patient[26]



[26] Kuok, Y.-J. (n.d.). *Differences in PA versus AP projection on a chest radiograph*. Radiopaedia. https://doi.org/10.53347/rID-17910

Figure 13: Grid Artifacts[27]



PATIENT POSITIONING AND TECHNIQUE FOR CHILDREN (AP/SUPINE)

In pediatric imaging, the PA erect is performed on older children (teenage years) and is not advised for younger children due to their attention span. The AP erect is ideal for cooperative younger children (three to seven years old). The anteroposterior supine (AP supine) CXR is beneficial for imaging unconscious or uncooperative patients. This view is preferred in infant and neonate imaging.

As radiation protection is necessary for pediatric patients, it is essential to image the chest properly and avoid unnecessary repeats. If the pediatric patient can only manage a supine view, this is more ideal than performing a poor erect view.

Family members may assist in distracting or holding the child. It is important to give the parents a focused task, particularly when they are feeling anxious for their child.

Position the patient:

- Patient is supine
- The detector is placed underneath the patient, or the patient is placed on top of the detector
- Head is straight and chin ideally out of the field of view
- Arms are placed by the patients' side outside of the field of view or above the head; either method is equally effective
- Centering point:
    - The level of the 7th thoracic vertebra; on or above the level of the nipple
    - For lordotic patients, the centering point can be used at the 10 degrees caudal angle.

[27] Murphy, A. (n.d.). *Grid cut off—Upside down grid*. Radiopaedia. https://doi.org/10.53347/rID-62516

Place the shielding correctly:

- Shield radiosensitive tissues outside the region of interest such as testes, uterus, and ovaries

Instruct the patient of the proper respiration

- Suspended inspiration
  - Observe breathing by watching the patient's chest

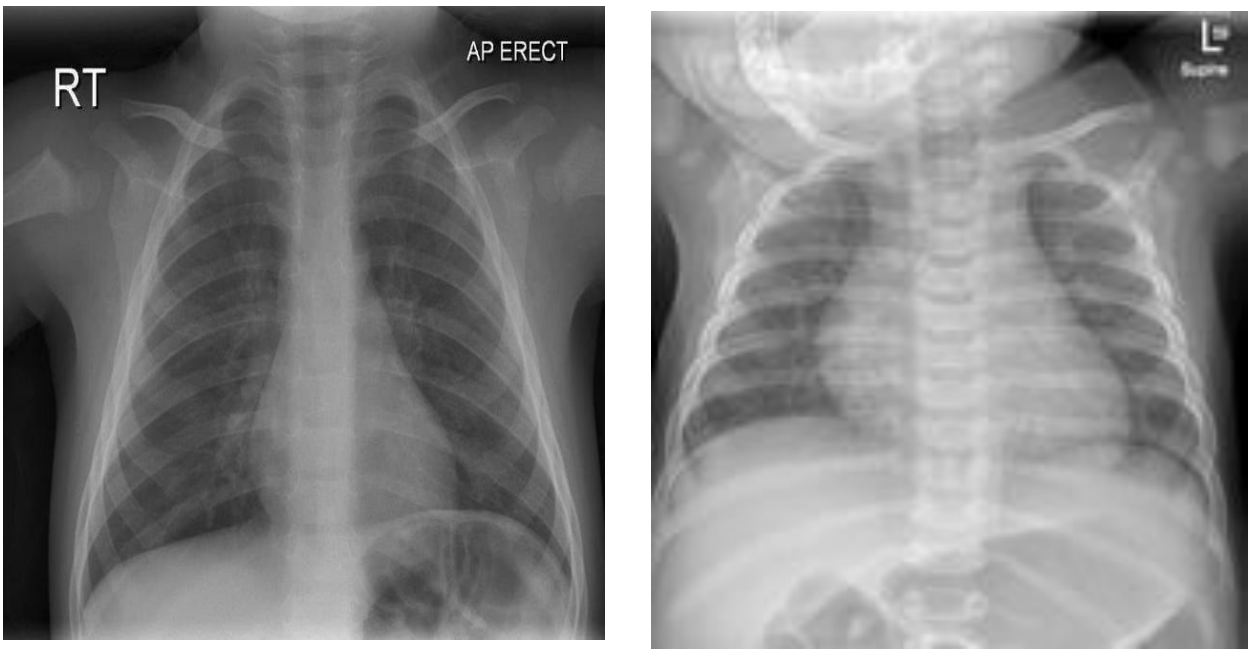
Following the recommended Collimation

- Superior to the 3rd cervical vertebrae
- Inferior to the thoracolumbar junction
- It is advised not to collimate too tightly at the apices as breathing may cause the apices to move superiorly

Review the following technical factors for X-ray equipment operation

- Orientation
  - Portrait
- Detector size
  - 24 cm x 30 cm or 35 cm x 43 cm depending on the patient's size
- Exposure
  - 55-65 kVp
  - 1-2 mAs
- SID
  - 110 cm
- X-ray Grid
  - No

Figure 14: Pediatrics Chest X-ray (AP Erect/ Supine)[28]



RADIOGRAPHIC FILM PROCESSING

Correct processing of radiographs is a key factor in good radiography. Correct processing is not an expensive procedure. However, an understanding of the basic fundamentals and needs is required to avoid unnecessary handling that might destroy the detail of a radiograph. The first consideration will be the X-ray film and its processing.

The latent image produced when a radiographic film is exposed to a beam of X-ray can be visualized and examined only after the film has been suitably processed in the darkroom.

The exposed film is removed from the cassette in a safely lighted dark room and placed in a stainless-steel processing frame. It is then immersed in a tank of the developer which completes the reduction of the exposed grains of silver halide and makes the image visible.

After a specified time, the film is taken out of the developer, rinsed in water, and then immersed in the fixer bath. This solution removes the undeveloped emulsion. The image can be inspected in white light. After 10 minutes in the fixer bath, the film is washed again in running water for half an hour to remove the processing chemicals, and then hung up to dry.

CORRECT MANAGEMENT OF X-RAY FILM

Unexposed X-ray film should be stored in a cool, dry place protected from radiation and in an upright position in cassettes in the darkroom. To load the film in the cassettes, be sure to correctly position the film and only touch the corners; the central portion should never be touched. Once loaded, the cassett should be closed and locked.

[28] Murphy, A. (2021, August 2). *Pediatric chest (supine view)*. Radiopaedia. https://doi.org/10.53347/rID-64922

When you are ready to use the film, remove it from the cassette in a darkened processing room with only a safe light.

Great care must be taken in removing the X-ray film from the cassette to prevent damage to the intensifying screens. The workers' fingers should not touch the screens of the cassette. Rubbing the film across the end of the cassette must be avoided to prevent black pressure scratches on the developed radiograph.

Developing X-ray film:

- The processing solutions should be stirred before processing the film. The X-ray film should be placed in the developing solution and agitated briefly to remove any air bubbles.
- It should be left in the developer for five minutes at a temperature of 20° C. If the temperature varies from 20° C the developing time should be varied accordingly. Below 16° C, developing chemicals are quite sluggish casing under development and inadequate fixation.
- The developing chemicals reduce the exposed silver halides in the emulsion to metallic Ag+, which is black. An increase in the intensity of light from the intensifying screens causes more of the silver halides to turn to metallic Ag+, thus producing various shades of gray and black on the developed radiograph.
- Two forms of developing chemicals are used, one a liquid and the other a powder. Liquid chemicals are more convenient; the powder variety may disseminate powder dust throughout the room.
- The developing solution should be tightly covered when not in use to reduce oxidation. The solution should be discarded and replaced after three months of use because oxidation and accumulation of gelatin sludge and other impurities will cause poor development. A more practical method of determining the strength of the developing solution is by looking at the color of the solution (first it turns yellow, then brown). When it turns brown, indicating exhaustion, it should be replaced.
- The X-ray film should be quickly removed from the developer and, in one motion, placed into the post-developer water rinse. The developing solution should not be allowed to drip from the film back into the developer tank. The developing solution on the film will be nearly exhausted and a certain amount of developer should be removed each time to reduce the level so that developer replenisher solution may be added periodically. This keeps the developer at the proper level and the correct chemical strength.

Reloading the cassette:

- During the time that the X-ray film is in the developer, the cassettes should be reloaded as previously described.

Post-development rinse:

- The post-development rinse, which ordinarily takes 30 seconds, should be circulating clean water. The rinsing process can be shortened by continually agitating the film. After the rinse is completed, the film should be drained to prevent excess dilution of the fixer.

Clearing and fixing the X-ray film:

- After its removal from the rinse, the X-ray film should be placed in the fixing solution and agitated for 15 seconds. This helps prevent streaking and staining of the finished radiograph and hastens the fixation process.
- The temperature range for fixation should be the same as that for development, with 20° C being optimal. The developing and fixing solutions should be at the same temperatures to avoid unevenness of development and reticulation of the final radiograph.

Final washing of the radiograph:

- Adequate washing prevents discoloration, and it should be performed in running water at 20° C. Washing for 20 minutes is adequate.

Drying the radiograph:

- The radiograph may be dried in the open air or in an automatically heated, circulating air dryer. Fresh fixer solution causes faster drying of the film. If the temperature of the fixer is above 24° C, the drying time will be markedly slower due to swelling of the emulsion.

Storing radiographs:

- Processed radiographs should have the corners cut off and be placed in a properly labeled envelope. The envelope should be stored in an upright position in a storage bin. A numbering system should be used so that radiographs can be easily found.

Hanging X-ray film:

- X-ray film should be grasped only at the corners and inserted into the clips of the film hangers and locked in place. The upper corner of the film should be grasped and attached to the top clips. The film is now ready to be placed in the developing tank.
- The clips of the film hanger should be cleaned periodically to prevent an accumulation of chemicals that may run down on the film during processing and causing streaks.

Safe-light efficiency:

- Safe lights should be placed so that the work of the darkroom can be done without fumbling. Where the dry and wet benches are separate, a small direct wall light should be provided for each.

For digital X-ray machine:

- Patient preparation, positioning, and technical factors are all the same as for analog X-ray.
- A control unit is used containing a computer that can easily control the brightness and contrast of the image.
- Film processing is performed by the printer which is connected to the computer-controlled unit.

RADIATION PROTECTION

Patients should be protected from unnecessary radiation for all diagnostic radiographic examinations, especially for CXRs because these are the most common radiographic examinations.

Although the chest radiographic examination is often considered the simplest of all radiographic procedures, it is also the examination with the highest number of repeats in many radiology departments. Therefore, unnecessary radiation exposure from repeat exposures should be minimized by taking extra care in positioning, CR centering, and selecting correct exposure factors if automatic exposure control systems are not used. Reduce patient dose as much as by using close collimation and protective shielding.

Careful collimation is important in chest radiography. Restricting the primary X-ray beam by collimation not only reduces patient dose by reducing the volume of tissue irradiated but also improves image quality by reducing scatter radiation.

In addition to careful collimation, a lead shield should be used to protect the abdominal area below the lungs. This shielding is especially important for children, pregnant women, and all individuals of childbearing age. A minimal rule is that shielding should be used on all patients of reproductive age.

However, many departments have a general policy of shielding for all patients undergoing chest radiography.

To protect the gonads from scatter and secondary radiation from the cassette or IR holder device and the wall behind it, a freestanding shield or a wraparound shield also should be placed over the radiosensitive structures outside the anatomy of interest between the patient and the IR.

For patient safety, the following three points must be checked before starting any examination:

- Identity
- Known or potential pregnancy
- Closure of the radiology room

For patient comfort, provide reasonable accommodations such as:

- Suitable adjustments to the X-ray room and procedures for handicapped patients
- A changing room for changing of clothing and putting on a hospital gown

Take the following hygiene precautions:

- Ensure disinfection between each patient of all equipment used (radio tube, control console, potter, sensor, handles) for patients with TB and presumptive TB (if possible, examine them after all other patients).
- Ensure sufficient air exchange and control airflow direction by using natural and mechanical ventilation systems.
- Wear a mask type Filtering Facepiece (FFP)1 or at least FFP2 and provide the patient with a surgical type face mask.

Figure 15a: Filtering Facepiece 1 mask (Dust mask 3M 9310 FFP1/NR) (K. Hla, personal communication, March 1, 2021)

Figure 15b: Filtering Facepiece 2 mask (3M™ Aura™ Particulate Respirator 9320+) (K. Hla, personal communication, March 1, 2021)

To protect the public and the staff, take the following precautions:

- Stand behind a lead screen or wear a lead apron.
- Stand as far as possible from the X-ray source (as a reminder, the radioactivity decreases by the inverse of the square of the distance: at 1m the dose is divided by two, at 2m by four, etc.).
- If there is a need for exposure, try to do it for the least amount of time.

To protect the patient:

- Lock the examination room to prevent someone from entering during the imaging procedure.
- If the X-ray is taken in a patient ward, make sure other people stay away as far as possible from the X-ray machine.
- In the case of CXR in children, often a parent is necessary for the proper conduct of the examination.
- For elderly patients make sure to explain very clearly about the examination.
- Principle of Justification / Optimization / Limitation
- Justification: Radiation can only be undertaken if it is justified by the benefits, it provides
- Optimization: Principal low as reasonably achievable: reduces the dose as low as it is acceptable to read the radiograph properly
- Limitation: respect the dose limitations implemented by the health system
- For thorax imaging, use a lead loin cloth or a gonad cache to limit the exposure of the gonads.

PERSONAL MONITORING DEVICES

Many instruments are used for individual monitoring of radiation exposure. Aims of personal monitoring include:

- Monitor and control the individual dose.
- Report and investigate over-exposure and recommend necessary remedial measures, if needed.
- Maintain life-time cumulative dose record.

The **film badge** is used to measure the individual dose from:

- X-rays
- Beta particle
- Gamma radiation
- Thermal neutrons

A **thermo-luminescent dosimeter (TLD) badge** can be used instead of a film badge:

- It is based on the phenomenon of thermos-luminescence, the emission of light when certain materials are heated after radiation exposure.
- It is used to measure individual dose from X-rays, beta and gamma radiation.

An **optically stimulated luminescence (OSL) dosimeter** can be used instead of a film or thermo-luminescent dosimeter badge:

- In comparison with TLDs, luminescence is produced by a light beam in OSLs, rather than by heat.
- The trapped electrons are subsequently freed by stimulation with light. After that, the detector releases the stored energy in the form of light. The light output measured with photomultipliers is a measure unit for the dose.
- It is designed to provide X, gamma, beta and neutron radiation monitoring using OSL technology that is a method that has established itself in the whole-body dosimetry.

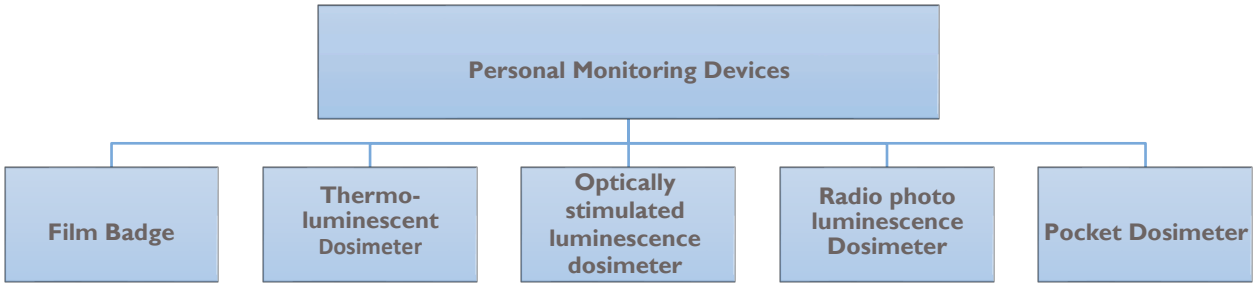
Or, a **radio photoluminescence (RPL) dosimeter** can be used instead of a film or thermo-luminescent dosimeter badge.

- It was developed for use in high temperature conditions such as nuclear emergencies because TLDs are not suitable for use in high temperature environments because of the large fading of the main dosimetry peak at high temperature.
- It consists of a glass plate doped with Ag+ atoms. When exposed to ionizing X, γ and β radiations, electrons are trapped in an excited state by these atoms. The electrons trapped in Ag+ atoms are excited and produced a characteristic orange luminescence by de-excitation. The measurement of this "luminescence" provides the exposure received by the glass plate.

A **Pocket Dosimeter** is used to provide an immediate reading of individual exposure to x-rays and gamma rays. Typical industrial radiography pocket dosimeters have a full-scale reading of 200 milliroentgens. It can be reusable but the limited range, inability to provide a permanent record, and the potential for discharging and reading loss due to dropping or bumping are the disadvantages. There are two common types:

- The **Direct Read Pocket Dosimeter** is the size of a fountain pen and comprises a small ionization chamber with a central wire anode inside and metal coated quartz fiber is attached to this anode. By pointing the instrument at a light source, the position of the fiber due to electrostatic repulsion between the anode and quartz fiber caused by ionization may be observed through a system of built-in lenses. The fiber is viewed on a translucent scale which is graduated in units of exposure.
- In **Digital Electronic Dosimeter**, the collected charge is discharged to trigger an electronic counter and displays the accumulated exposure and dose rate in digital form when a predetermined exposure has been reached.

Figure 16: Types of personal monitoring devices (K. Hla, personal communication, March 1, 2021)



RADIATION HAZARD

Ionizing radiation can cause tissue damage. Tissue damage occurs through the change in chemical properties of molecules in the tissue following radiation exposure. The major contributor to damage from radiation is through radiation changing a water molecule into a new form called a "free radical." Free radicals are chemically highly active and as such can have reactions with genetic molecules of the cell (i.e., the Deoxyribonucleic Acid [DNA]). This can cause damage to the DNA most of which is readily repaired by the cell. If it is not, it can result in cell death. Alternatively, if the DNA damage is repaired erroneously, it can result in an alteration of the genetic encoding leading to hereditary changes or cancer induction.

DETERMINISTIC EFFECTS (CELL DEATH)

Deterministic effects are usually divided into tissue-specific/local changes and whole-body effects. Examples of tissues that are known to demonstrate deterministic effects from radiation exposure are:

- The lens of the eye
  - Detectable opacities
  - Cataract formation
- Skin
  - Skin reddening (erythema)
  - Hair loss (depilation)
  - Skin cell death with scarring (necrosis)
- Reproductive organs
  - Infertility

Whole-body radiation damage (only occurs in extremely high radiation exposures beyond those produced by any diagnostic imaging system):

- Bone marrow damage/reduction of blood cell production
- Gastrointestinal mucosa lining loss
- Central nervous system tissue damage

STOCHASTIC EFFECTS (GENETIC CHANGES AND CANCER)

Radiation exposure can cause damage in germ cells that ultimately can result in mutations in the exposed person's fetus if she is pregnant. However, such mutations are not radiation-specific; the radiation only produces DNA sequencing errors that might have occurred naturally. Therefore, instead

of producing unique mutations, damage from radiation exposure only results in a higher frequency of normal/spontaneous mutations. This means radiation does not cause the production of monsters as seen in the movies. Besides, many animal and human studies have shown that the adverse effects from radiation exposure are negligible in subsequent generations.

Specifically, there is no direct evidence at any radiation dose that exposure of parents leads to excess genetic disease in their offspring. Although radiation exposure can cause mutations in children if the reproductive cells of the parent are exposed, the child does not carry any adverse genetic trait produced by the radiation exposure that can be passed on to their offspring. Still, it is very important to know if patients might be pregnant before taking an X-ray. The exposure of a fetus to radiation is a major concern for radiation effects that may occur in a child if the child is exposed before birth.

QUALITY ASSURANCE (QA)

QA is the summation of internal quality control (IQC), external quality assessment (EQA), and quality improvement. IQC measures are performed routinely whenever a radiographer produces images. EQA is supervisory support from a higher-level radiology department (regional/state/district) to a lower-level radiology department (township/peripheral). Quality improvement is the response of the system over a time, based on IQC and EQA.

Radiographers should take these IQC measures:

- Daily cleaning of the department
- Weekly cleaning of the film cassette
- Monthly cleaning of the lead apron, gonad shield, etc.
- Ensuring calibration of the X-ray unit, annually
- Immediately reporting any fault detected in the X-ray unit or control panel to the appropriate engineers
- Obtaining and annually renewing radiation safety certificates from the authorized department

TB supervisors from the higher level should undertake EQA visits to the radiology section at the lower level. During these visits, the assessor should:

- Assess overall performance of the radiographer for TB CXR for IQC measures
- Randomly select radiography images (three to five) and assess their quality
- Interview radiographer to note achievements and challenges in quality and workload
- Compile and share assessment report with radiologists/trained medical officer that outlines corrective and improvement measures

MOST COMMON RADIOLOGICAL FEATURES SEEN IN PTB

Below are the most common radiological features seen in PTB:

1. Consolidation shadows with air bronchogram (unilateral or bilateral) in Tuberculous pneumonia (Figure 18)
2. Cavity formation mostly thin wall cavity (anywhere in the lungs but common in the upper lobes) (Figure 19)
3. Homogeneous opacity in the lung field may be lower or up to the whole hemi-thorax usually with

4. 'meniscus Sign' (Pleural Effusion) (Figure 20)
5. Hyper translucency of the chest with absence of lung markings pneumothorax (Figure 21)
6. Multiple small size rounded shadows (pin head size) in both lungs in miliary TB (Figure 22)
7. Lung Abscess with or without air fluid level (Figure 23)
8. Gas detected under the diaphragm (Figure 24)
9. Enlarged Hilar lymph nodes-Primary Complex (Figure 25)

Figure 17: Consolidation shadows with air bronchogram (unilateral or bilateral) in Tuberculous pneumonia (K. Hla, personal communication, March 1, 2021)

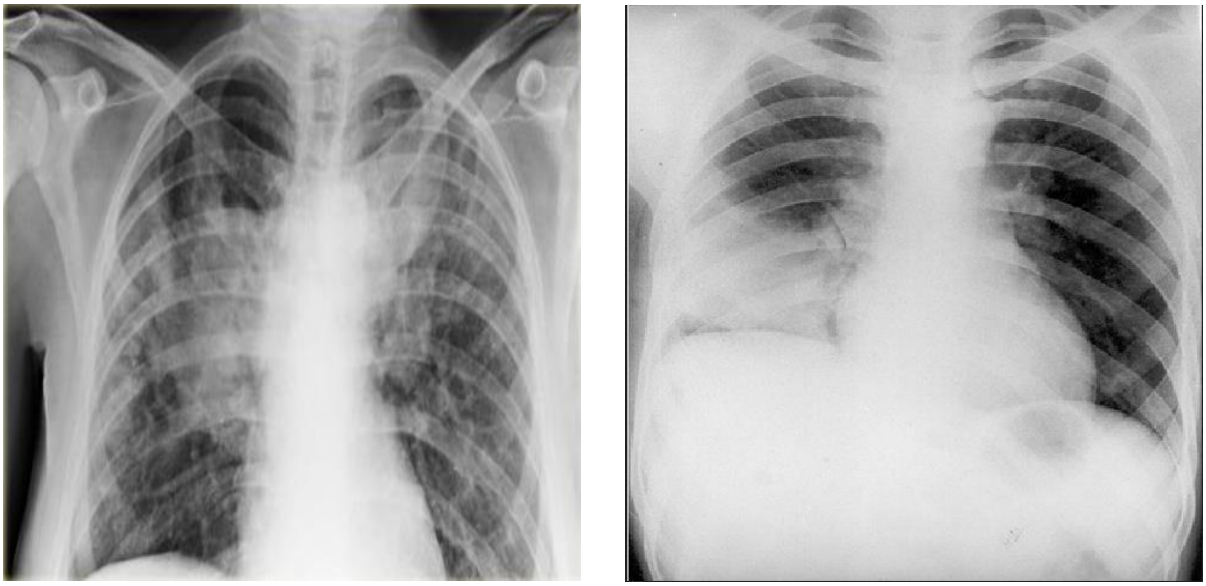


Figure 18: Cavity formation mostly in the thin wall cavity (anywhere in the lungs but common in the upper lobes) (K. Hla, personal communication, March 1, 2021)

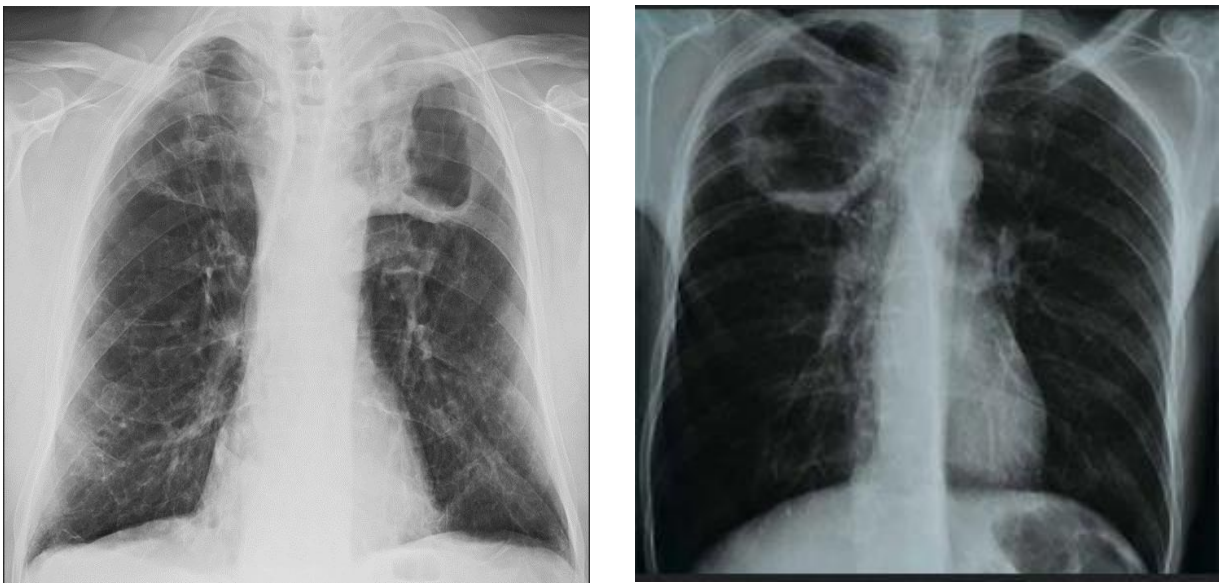


Figure 19: Homogeneous opacity in the lung field may be lower or up to the whole hemi- thorax usually with 'meniscus Sign' (Pleural Effusion) (K. Hla, personal communication, March 1, 2021)

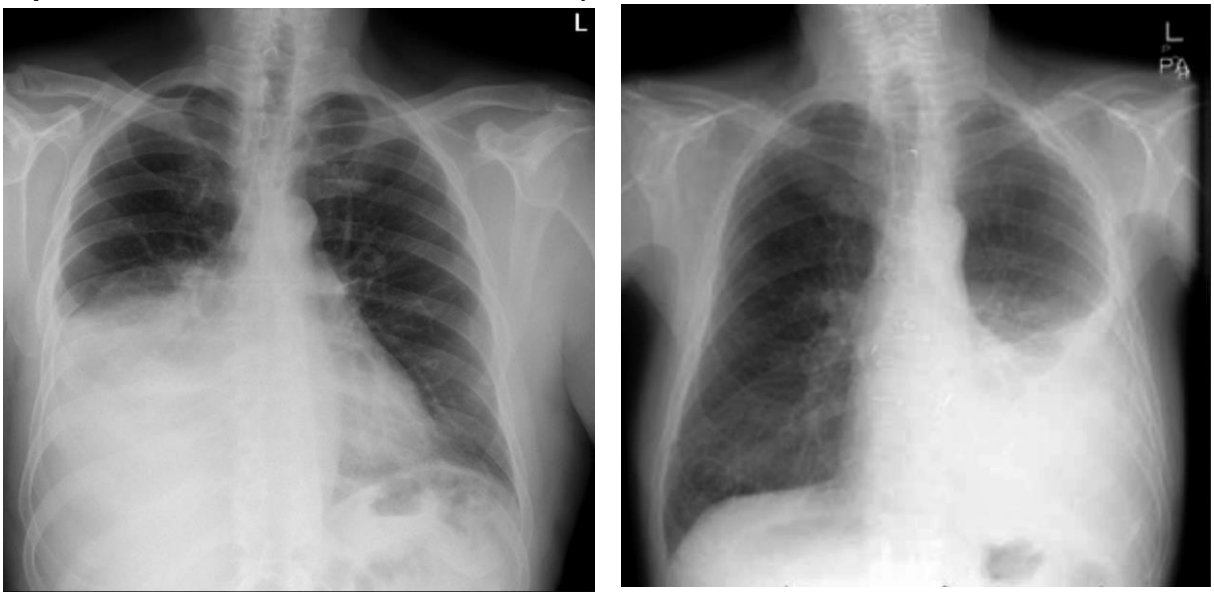


Figure 20: Hyper translucency of the chest with absence of lung markings pneumothorax (K. Hla, personal communication, March 1, 2021)

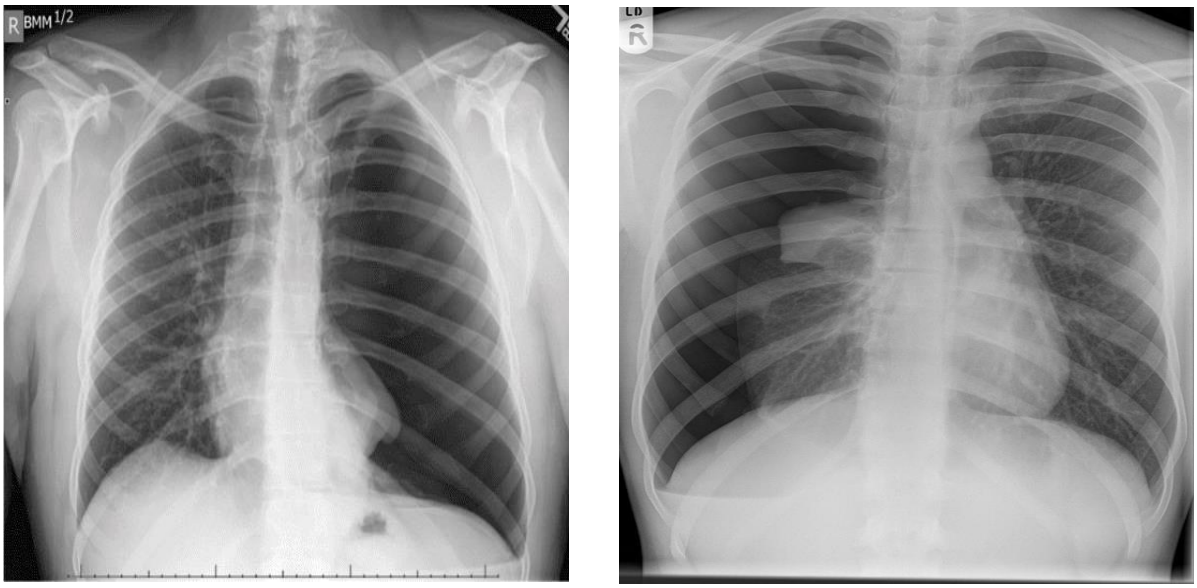


Figure 21: Multiple small size rounded shadows (pin head size) in both lungs in miliary TB (K. Hla, personal communication, March 1, 2021)

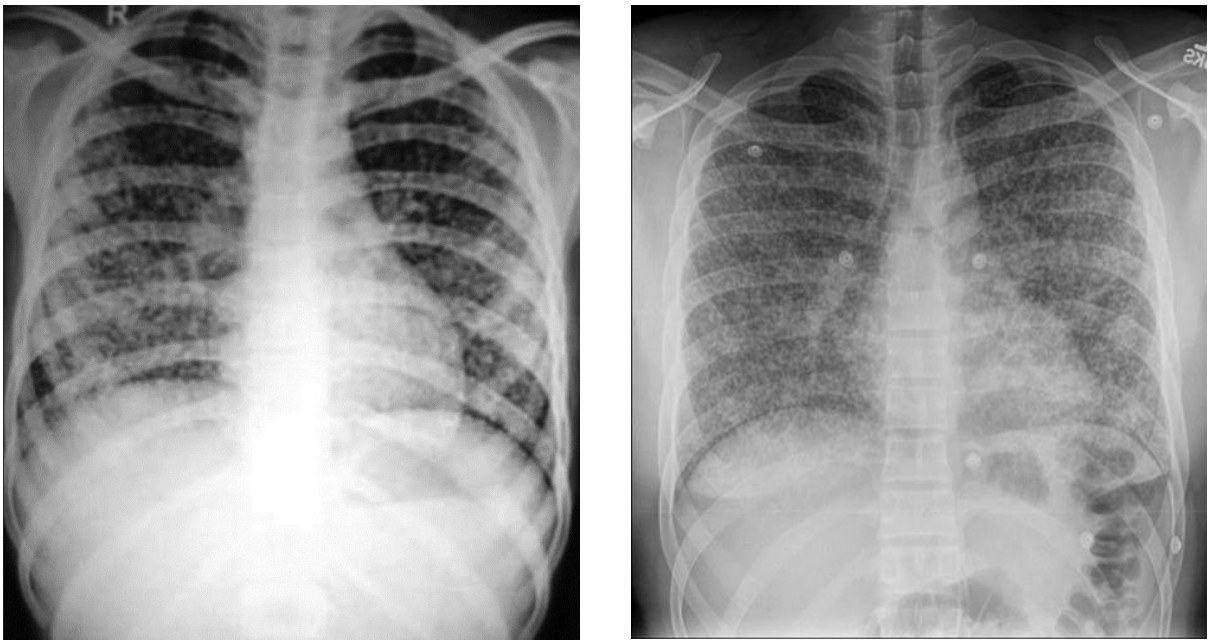


Figure 22: Lung Abscess with or without air fluid level (K. Hla, personal communication, March 1, 2021)

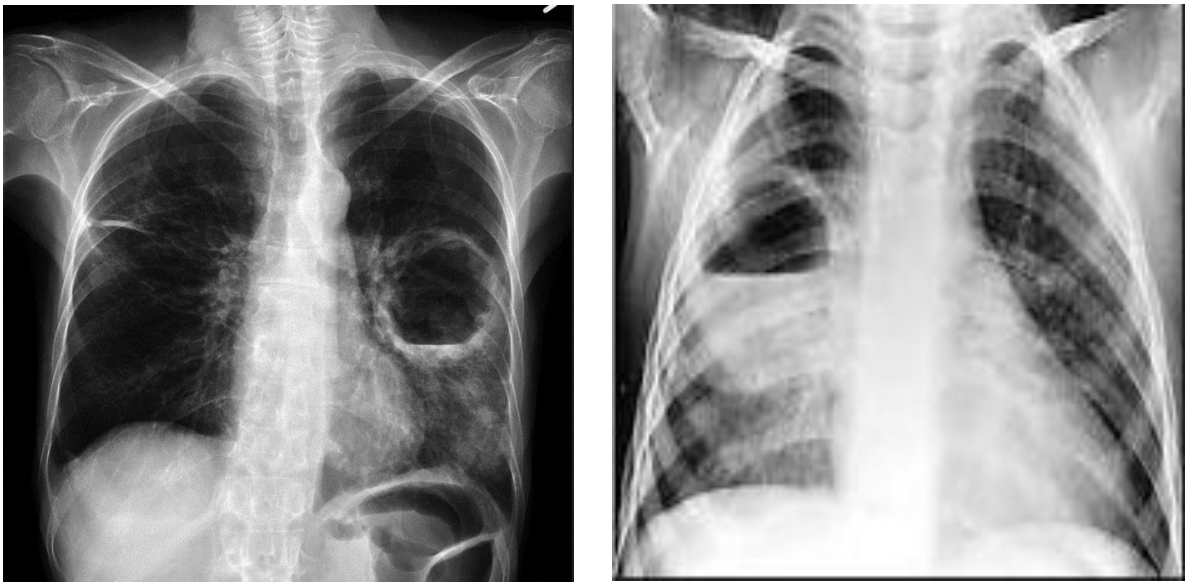


Figure 23: Gas detected under the diaphragm (K. Hla, personal communication, March 1, 2021)

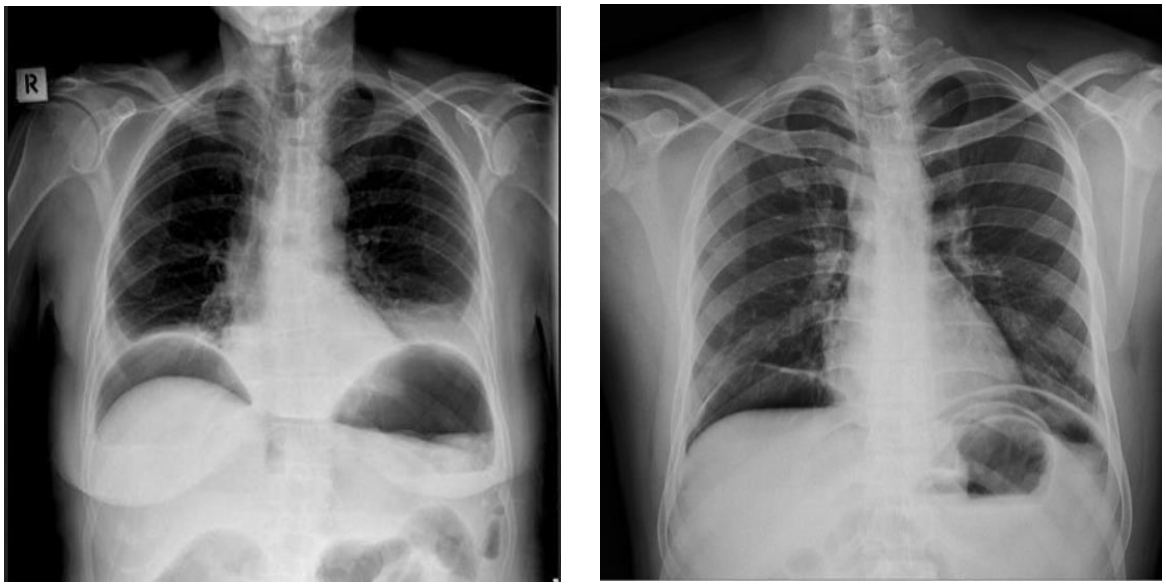
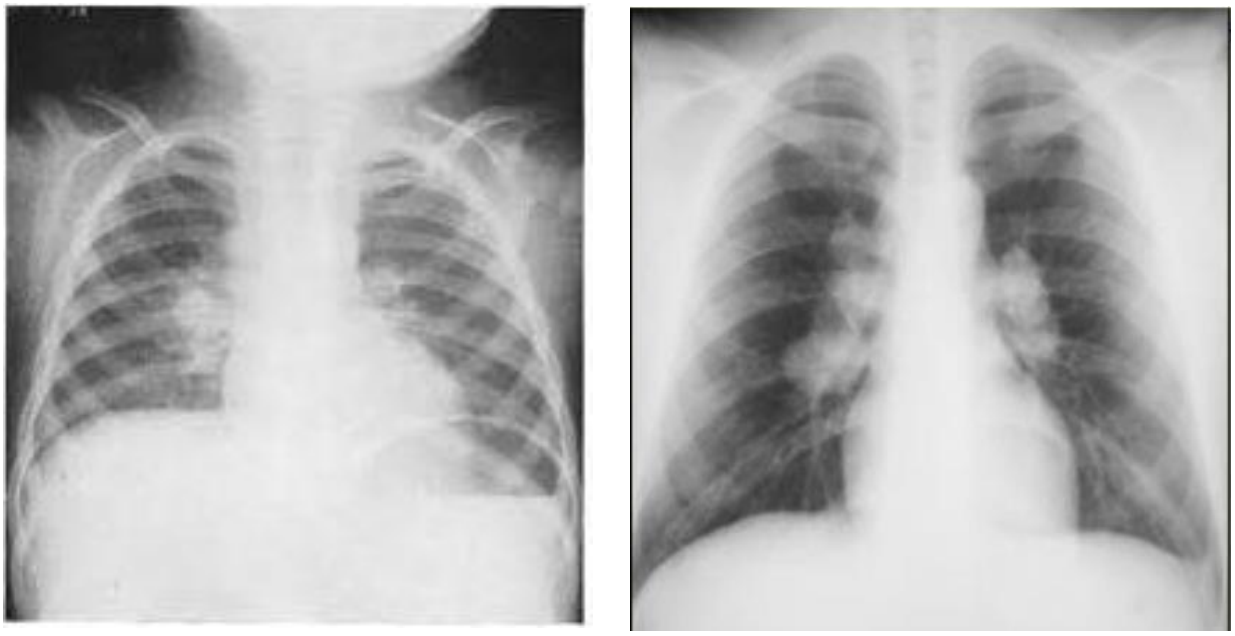


Figure 24: Enlarged Hilar lymph nodes-Primary Complex (K. Hla, personal communication, March 1, 2021)



VALUE OF CXR IN TB

The following list outlines the value of CXR in TB diagnosis:

- CXR has historically been one of the primary tools for the detection of TB, especially PTB. It forms an essential tool for countries to achieve the goals of the END TB strategy.
- It has higher sensitivity for PTB than screening for TB symptoms. However, it suffers from lower specificity.
- An abnormal CXR is an indication for full diagnostic evaluation. Bacteriologically confirmed diagnosis of TB is always preferred.
- Being a multi-disease diagnosis platform, CXR is most easily accessible and repeatable even in resource-limited settings.
- CXR remains an important tool for diagnosis of childhood TB in combination with history, evidence of TB infection, and microbiological testing.
- CXR as a triage tool improves the efficiency of using the Xpert Mycobacterium tuberculosis/resistance to rifampicin (MTB/RIF) assay.
- CXR can assist in the diagnosis of TB among people living with HIV/AIDS.
- CXR helps rule out active TB before treating latent TB infections.
- CXR is an essential tool for TB disease prevalence surveys.
- Apart from lung pathology, CXR can serve as a differential diagnostic tool to get information about the heart and great vessels; mediastinal pathology; tumors of various origin in the thorax; detect gas under the diaphragm; air in soft tissues of the chest wall; and mediastinum (surgical emphysema).

CONCLUSION

CXR remains an important rapid tool for TB detection. Although CXRs are not the gold standard for confirming a diagnosis of TB, they have high sensitivity for detecting PTB-related abnormalities in the lungs (opacities, scars, pleural effusion, etc.,). It is a valuable tool for the END TB strategy. However, an accurate CXR-based diagnosis of TB needs high-quality interpretable X-ray image generation and consistent and correct interpretation. Although radiologists and medical officers are frequently trained on how to correctly interpret CXR images, radiographers as a cadre do not often undergo periodic orientation/refresher courses under national TB control programs. This training curriculum is expected to build capacity for radiographers for quality X-ray image generation, and thus help radiologists and trained medical officers to correctly diagnose and treat TB.

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GLOSSARY

Anode: An electrode through which the conventional current enters into a polarized electrical device such as an X-ray tube. It is the positive terminal of the X-ray tube.

Bucky: A device that is a part of the X-ray equipment unit which contains the moving grid system for reducing unnecessary scattered radiation. It is the abbreviation for the Potter-Bucky moving grid system.

Cathode: An electrode through which the conventional current leaves a polarized electrical device such as an X-ray tube. It is the negative terminal of the X-ray tube.

Collimation: The method, in radiology, of restricting and confining the X-ray beam to a given area. Beam collimators (that are used to achieve collimation) are devices used in the X-ray tube housing, along with an arrangement of mirrors and lights, in such a way that the light and X-ray fields match each other. They are made of lead shutters that completely absorb the photons, and thus reduce the patient dose as well as focus the radiation according to the area of interest.

Full inspiration: Inspiration in which the lungs are filled with air as completely as possible.

Image Receptor (IR): An IR is a device that changes an X-ray beam into a visible image. An IR may be a radiographic film and cassette, a phosphorescent screen (used in fluoroscopy or computed radiography), or a special flat panel detector placed in a table or a bucky (used in direct digital radiography).

Inverse Square Law: The law states that "the strength (intensity) of the X-ray beam is inversely proportional to the square of the distance." It describes the principle of radiation dose reduction as the distance from the source increases. It pertains to radiation safety and allows users to create safe distances from the source of radiation, specific times for exposure to radiation, or amounts of radiation to be used.

Intensifying screen: Thin sheets of radiation sensitive fluorescent materials placed inside a cassette on either side of the exposed X-ray film. They are used to allow low-intensity X-rays to convert incident X- ray photons to light photons for sufficient intensity to provide a viewable image.

Latent Image: Under the effect of light photons, silver bromide (AgBr) crystals will separate into silver (Ag+) and bromide (Br-) ions. This dissociation of the ions will form the latent image. The subsequent film developing process converts the invisible latent image into a visible radiographic image.

Medical officers: Medical professionals who oversee health care at various levels of health care delivery and are trained to interpret the X-ray images for diagnosis and patient care.

The midsagittal plane or median plane: It divides the body into two parts. It vertically splits any object into two relatively equal halves – left and right. In humans, each of the two bisected divisions includes half of the head, thorax, abdomen, and genitals; one arm; and one leg.

Posteroanterior (PA) view/projection: PA chest view of patient that examines the lungs, bony thoracic cavity, mediastinum, and great vessels.

Radiograph: A rapid image produced on a photosensitive film by X-rays, gamma rays, or similar radiation, and typically used in medical examinations.

Radiographers: The job title of a health care professional (variously called X-ray technicians/technologists) who specialize in generating radiographic images including X-ray images.

Radiologists: The job title of medical professionals who specializes in interpreting radiographic images including X-ray images for diagnosis and patient care.

SID: X-ray source/tube to image receptor distance.

X-ray Grid: A device for absorbing unnecessary scattered radiation. It is of the same size as the X-ray film cassette and contains very narrow lead strips. The minimum required specification for the fixed grid for a chest X-ray is 34 lead strip lines per cm and an 8:1 grid ratio.

X-rays: Penetrating form of high-energy electromagnetic radiations. The wavelength of X-rays ranges between 10 picometers to 10 nanometers, corresponding to frequencies in the range of 30 petahertz to 30 exahertz (30x1015 Hz to 30x1018 Hz). X-ray wavelengths are shorter than those of ultraviolet rays and typically longer than those of gamma rays.

TRUENAT TRAINING CURRICULUM

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

Materials are available in English and French. Any errors in translation are the sole responsibility of IDDS.

TRUENAT TRAINING MODULES | STOP TB PARTNERSHIP

Truenat Training Modules Overview	English (only)	
Module 1: Introduction to Truenat	English	French
Module 2: Diagnostic Algorithm and Results Interpretation	English	French
Module 3: Operational Aspects	English	French
Module 4: Order Planning and Quality Assurance (QA)	English	French
Module 5: Monitoring & Evaluation	English	French
Module 6: Biosafety and Specimen Collection and Referral	English	French
Facilitator's Guide: Truenat Tests for the Detection of TB and Rifampicin Resistance Central-Level Training	English	French
Participant Guide: Truenat Tests for the Detection of TB and Rifampicin Resistance Central-Level Training	English	French

X-RAY VIDEOS

These videos were developed and filmed by IDDS in Burma to share best practices and information with radiologists in the country. The original videos are in Burmese and for wider audiences, IDDS produced alternate versions with English and French voiceovers. Any errors in the script or content are solely the responsibility of IDDS.

The first video explains the equipment to prepare before taking a chest X-ray. The second explains steps for taking a chest X-ray in a mobile setting, using the portable Amadeo P-100/20HB instrument.

Checklist for Equipment Review before Taking a Chest X-ray	Burmese	English	French
CXR Taking Procedures for TB Diagnosis in Mobile Settings	Burmese	English	French

GENEXPERT VIDEOS

This set of videos, developed and produced by IDDS in Burma, demonstrates good practices in the use and care of the GeneXpert instrument. The videos are intended as refresher and reminder sessions for regular users of the instrument. The first video provides a demonstration for performing Xpert MTB/RIF testing, using the GeneXpert system. The second video shows a step-by-step procedure for removing a stuck cartridge inside a GeneXpert machine. The third video provides a demonstration for performing GeneXpert machine calibration, using Xpert Check. The final video provides a demonstration for performing the GeneXpert maintenance procedure.

The original videos featured a Burmese voiceover. IDDS produced English-language versions with English-language voiceover. Any errors in translation or content are solely the responsibility of IDDS.

GeneXpert MTB/RIF Testing Procedure	Burmese	English
GeneXpert Stuck Cartridge Procedure	Burmese	English
GeneXpert Machine Calibration	Burmese	English
GeneXpert Machine Maintenance	Burmese	English

GENEXPERT MULTIPLEXING GUIDE[29]

INTRODUCTION

OBJECTIVES

Overall Objective

This document aims to inform policy makers, program managers, care providers and other in-country stakeholders on the implementation of GeneXpert devices multiplexing.

Specific Objectives

- To define norms and standards for GeneXpert multiplexing
- To provide guidance on quality management systems for GeneXpert multiplexing assays
- To provide structure for monitoring and evaluation (M&E) of multiplexing activities
- To provide guidance on supply chain management of multiplexing activities
- To define the leadership and governance framework for multiplexing

GUIDING PRINCIPLES

- Integration of services
- Cost sharing across programs
- Implementation of quality systems in testing
- Patient-centered approach

ETHICS

Multiplexing will ensure that patients can access testing services at no cost at each level of the health delivery system while receiving their results within clinically acceptable turnaround times.

INTENDED AUDIENCE

This guide is intended for use by health care workers, clinicians and laboratorians, policy makers, implementing partners, and other stakeholders.

NORMS AND STANDARDS

OPERATIONAL REQUIREMENTS FOR GENEXPERT MULTIPLEXING

[29] The original version of this GeneXpert Multiplexing Guide was developed by Zimbabwe Ministry of Health and Child Care with technical and financial assistance from IDDS. This was an important achievement, because Zimbabwe was among the first countries to pilot the integrated testing for TB, HIV (viral load and EID) on the GeneXpert platform. Special thanks are due to the leadership of the AIDS and TB Unit: Dr. Owen Mugurungi, Dr. Charles Sandy, and Director of Laboratory Services, Mr. Arnold Mukaratirwa. The IDDS team appreciates the leadership of this writing team for their invaluable contributions.

The following prerequisites must be met before multiplexing:

1. GeneXpert must be the main machine to be multiplexed with TB remaining the priority test.
2. Biosafety requirements for additional testing must be in place.
3. Infrastructure must provide adequate storage space for extra cartridges at the recommendedtemperature of 2–28°C.
4. Facility layout must provide an operating temperature between 15°C and 30°C.
5. A reliable procurement process must allow for regular supply of consumables with sufficient shelf life.
6. Health care workers, including clinicians, must be trained on the utility of the GeneXpert machines, patients to be targeted, and interpretation of results.
7. Financing must be sustainable to support service and maintenance, continued training, and procurement of reagents and consumables.
8. Coordination mechanisms in country and analysis of diagnostics and treatments must guide implementation of the multiplexing process.
9. A quality assurance system must be implemented throughout the network.
10. An approved mechanism for biohazard disposal must be in place for both TB and HIV tests.

REQUIREMENTS FOR LABORATORY GENEXPERT MULTIPLEXING

The laboratory must have adequate staff with minimum qualifications and capabilities to run additional tests on the GeneXpert machines.

1. Standard operating procedures (SOPs) on the use and maintenance of GeneXpert machines must be in place.
2. Personnel must be trained on the SOPs and must be assessed for competency before startingthe new tests and at least annually thereafter.
3. The GeneXpert testing method must be verified before use.
4. A service contract for GeneXpert machines must be in place.
5. A risk assessment must be done, and additional biosafety measures must be put in place to ensure a safe working environment.
6. Remote monitoring systems, such as GxAlert, must be connected to the GeneXpert machine tofacilitate data transmission to national levels.
7. The testing laboratory must be registered for an external quality assessment scheme for the multiplexed tests.
8. A laboratory information management system must be in use to capture all the testing data, andtechnical support must be in place for the laboratory information management system.

INFRASTRUCTURE REQUIREMENTS FOR GENEXPERT TESTING

The following infrastructural requirements must be in place before GeneXpert multiplexing:

1. The electricity supply must be stable in the facilities in which the test will be implemented, andmeasures must be in place to ensure uninterrupted supply (an uninterrupted power supply unit may be used with additional batteries, a generator, or solar panels).
2. Premises must be secure to prevent theft of the GeneXpert machine and computer/laptop.
3. Adequate storage space must be provided for the cartridges, which must be stored at the recommended temperature range (2–28°C).

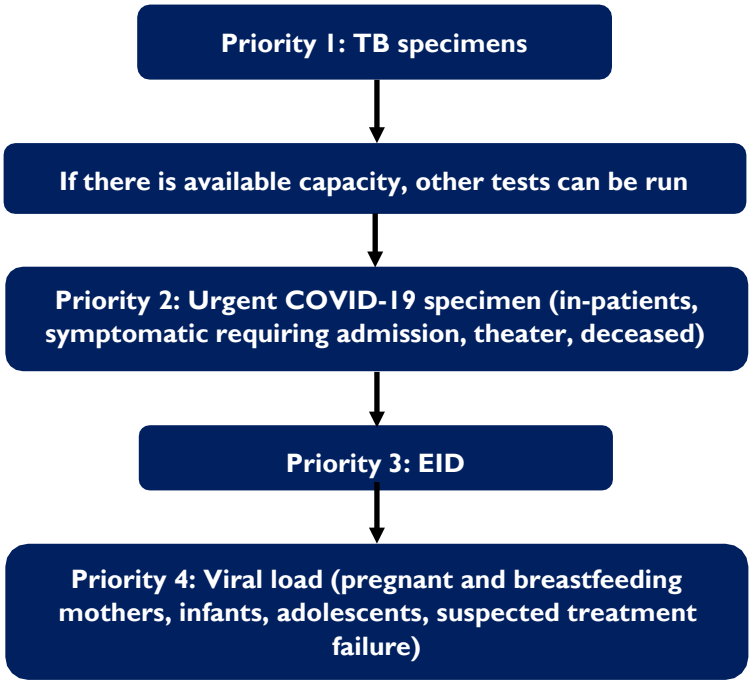
4. Appropriate measures must be taken to prevent ambient temperatures from rising above 30°Cor falling below 15°C in the room in which the machine will be installed (ventilation or air conditioning may be necessary).
5. Adequate space to place the machine must be provided, and this must be free from dust and away from direct sunlight.

ALGORITHM AND SOPS

GeneXpert provides a testing platform for different diseases and gives the country additional testing capacity during outbreaks; however, redeploying machines must be done in consultation with the MoH. GeneXpert is the first choice for TB testing. Therefore, the following steps must be taken before multiplexing:

1. Determine the TB capacity use as follows:
2. Calculate the number of TB tests performed in the last six months as (22 working days X six months X 12 tests per day).
3. Establish reserve capacity.
4. If the GeneXpert machine or facility has a use rate of 80 percent or above, then that machine is not a candidate for multiplexing.
5. Determine the annual demand of each test to be multiplexed for the site (e.g., in collaboration with the HIV/prevention of mother-to-child transmission focal persons, establish the expected EID tests annually to set the demand for EID, and determine the targeted population for viral load).
6. Share this demand with the TB laboratory coordinator for consideration during the annual quantification processes.
7. Multiplexing must prioritize TB. The rest of the tests should be prioritized in this order:
8. Coronavirus Disease 2019 (COVID-19) testing (in-patients, symptomatic patients requiring admission, theater cases, and deceased)
9. EID testing
10. Targeted viral load (defined as pregnant and breastfeeding mothers, infants, adolescents, clients suspected of clinical failure)
11. Human papillomavirus nucleic acid amplification test screening
12. Multiplexing for COVID-19 and viral load should be done for the above-mentioned priority groups only. All other patients should be referred to conventional facilities.
13. Re-evaluate the capacity use annually for presentation at the national laboratory quantification committee. Each submission should clearly state the earmarked capacity for the intended tests following the above priority order.
14. A site-level sensitization meeting must be conducted to align everyone on the availability of thetest menu on site. A site should put in place agreed measures to encourage the multiplexing capacity.
15. A laboratory report should be generated and presented to the district health executive/provincial health executive monthly.

Figure 1: Sample Prioritization Matrix and Flow Chart



PERSONNEL REQUIREMENTS AND TRAINING FOR MULTIPLEXING

1. Staff who operate the machine and perform the assay must be trained in testing for all/new tests. A competency assessment must be done after training and at least annually thereafter.
2. Training should focus on specimen analysis, data reporting, preventive maintenance of equipment, and waste management.
3. Clinical staff members must be sensitized on GeneXpert, including available tests and appropriate specimens. Clinicians and medical associations must be included in GeneXpert stakeholder meetings and trainings.

SPECIMEN STORAGE—PRE- AND POST-TESTING

		Pre-Analysis		Post-Analysis	
Specimen	**Test**	**Temperature**	**Period**	**Temperature**	**Period**
Sputum	TB analysis	Room temp	0–48 hours	2–8°C	7 days
Sputum	TB analysis	2–8°C	3–10 days	2–8°C	7 days
Stool	TB analysis	2–8°C	3–10 days	2–8°C	7 days
Plasma	Viral load	15–30°C	24 hours	2–8°C	7 days
Plasma	Viral load	-18–-70°C	6 weeks	2–8°C	7 days
Dried blood spot	Viral load/EID	Room temp	6 weeks	2–8°C	7 days
Dried blood spot	Viral load/EID	-70–20°C	5 years	-70°–20°C	5 years
Viral transport media swabs	COVID-19	2–8°C	7 days	2–8°C	7 days
Viral transport media swabs	COVID-19	-70–20°C	5 years	-70–20°C	5 years

EQUIPMENT

Verification

Conduct method verification for every new test to be added according to the verification protocol.

Repair, Service, and Maintenance

Costs must be shared across programs with tests being multiplexed on the GeneXpert machine through a surcharge. Cost sharing is provided under the Service Level Agreement between the MoH and Cepheidthrough the AccessCare Program. Each cartridge has a surcharge for servicing and repairs, and the more tests that are conducted on the GeneXpert machine, the lower the surcharge.

WASTE MANAGEMENT

All GeneXpert Cartridges must be disposed as per the manufacturer's recommendation, as follows:

- EID/viral load/SARS-CoV-2 cartridges must be incinerated at temperatures above 1,000°C; therefore, they must be separated from the rest of waste material and sent to facilities where these services are available.
- All other contaminated waste, including TB cartridges/sputum containers, must be incinerated/burned before disposal.

LABORATORY SAFETY

All procedures must be performed based on results of a risk assessment, with appropriate safety measures in place.

QUALITY ASSURANCE AND MANAGEMENT

QUALITY MANAGEMENT SYSTEM

To continuously provide quality service to clients, all testing facilities must implement a quality management system that meets the requirements of ISO 15189:2012.

Quality Assurance for Multiplexing Testing

MoH ensures that multiplexing laboratories are running smoothly, safely, and effectively in all three quality assurance phases—namely, pre-analytical, analytical, and post-analytical.

External Quality Assessment

This is a process to assess laboratory performance by a higher-level laboratory. External quality assessment shall comprise the following:

Panel Testing/Proficiency Testing

This is a process whereby samples with known results are sent on a regular basis to a group of laboratories by reference laboratories for examination. The participant laboratories must return their results within a specified time, and results are then analyzed. Performance reports are issued with specific and overall laboratory performance.

Onsite Training and Supportive Supervision

This is when supervisors from a higher-level facility periodically visit a lower-level facility to obtain a realistic picture of the conditions and practices in the laboratory. It is also an opportunity to provide assistance with challenging areas, including training.

Areas assessed for multiplexing testing include:

- Equipment maintenance
- Use of SOPs
- Documentation
- Compilation of statistics
- Stock status of reagent and consumables
- Verification
- Lot-to-lot comparison
- Equipment functionality
- Review of proficiency testing results

POST MARKET SURVEILLANCE OF COMMODITIES OF MULTIPLEXING

1. The National Reference Laboratories (NRLs) are responsible for the post-marketing surveillance of multiplexing commodities. Health facilities also play a critical role in identifying non-conformities, reporting them to NRLs, and assisting NRLs in carrying out detailedinvestigations when necessary.
2. The roles and responsibilities of end users include:
3. Identifying poor quality commodities and non-conformities with products procured or supplied
4. Documenting complaints
5. Verifying complaints coming from other health facilities and the national level when requested by NRLs
6. Cooperating in any post-market investigations
7. The roles and responsibilities of NRLs include:
8. Regularly carrying out post-market surveillance activities on multiplexing commodities
9. Receiving and documenting all complaints from testing personnel
10. Preparing and maintaining lot-to-lot verification testing panels
11. Conducting investigations of complaints and giving appropriate feedback to all stakeholders
12. Taking corrective and preventive actions on identified non-conformities
13. Notifying authorities, the World Health Organization, and manufacturers of verified kit defects

MONITORING AND EVALUATION

DOCUMENTATION AND MONITORING AND EVALUATION

1. Service data must be documented using both manual paper-based records (standard registers) and electronic connectivity systems, such as GxAlert, SENAITE, DistrictHealth Information Software, version 2 (DHIS2).
2. Service data must be manually documented in different standard registers for therespective

test.

3. Multiplexing must be monitored by tracking proportions of the available machine capacity thatare taken by the multiplexed tests.
4. Program-defined quality indicators per test type must be monitored at the site level and reported to district, provincial, and national levels.
5. Evaluations must be based on data collected in manual registers or electronic systems.

QUALITY ASSURANCE INDICATORS

1. Quality indicators must be subject to routine monitoring of test volumes, number of positive and negative results, and number of failed tests, stratified by type of test performed.
2. Data must be reviewed in real time using available diagnostic connectivity systems, such as GxAlert, SENAITE, and DHIS2. Diagnostic connectivity systems allow for real-time monitoring to detected issues early andallow for rapid response.

DATA MANAGEMENT AND INTEGRATION

1. Data must be consolidated, analyzed, used, and reported at site, district, provincial, and nationallevels.
2. Users of multiplexed machines must have access to GxAlert site-level data for real-time monitoring.
3. Selected individuals at district, provincial, and national levels must have access to GxAlert datafor their level of clearance.
4. All patient-level data must be treated with confidentiality.

ROLES AND RESPONSIBILITIES FOR INFORMATION FLOW

1. Staff at site, district, provincial, and national levels must be trained in the available electronic monitoring systems (GxAlert, SENAITE, DHIS2).
2. The head of the testing site is responsible for M&E of performance of multiplexing using proportional capacity utilization, and reports to the site TB focal person.
3. The head of the testing site is also responsible for M&E of the performance of program-defined indicators and timely reporting to the district laboratory head.
4. The district laboratory head is responsible for M&E of site performance using program-defined indicators and timely providing the consolidated district report to the provincial scientist.
5. The provincial scientist is responsible for M&E of site and performance using program-defined indicators and timely providing the consolidated provincial report to the national TB laboratories coordinator.

GXALERT OPERATION

1. GxAlert connectivity software must be installed and used to monitor and report on all tests done and inventory movements in real time.
2. Trainings for users and super users of the electronic monitoring systems must be done at thesite, district, provincial, and national levels.
3. GxAlert data must be interfaced with other electronic connectivity systems, such as DHIS2 and SENAITE.

SUPPLY CHAIN MANAGEMENT

FORECASTING, QUANTIFICATION, PROCUREMENT, AND SUPPLY PLANNING

Forecasting, quantification, procurement, and supply planning must be coordinated centrally by the Laboratory Logistics Unit using approved processes and methodologies.

ORDERING OF SUPPLIES

1. The National Laboratory Commodity Distribution System (NLCDS) tools must be used for reporting and ordering.
2. Reports must include service statistics, equipment functionality, and stock status.
3. The GxAlert inventory control module must be used to monitor stocks at different levels as a supplement to the NLCDS.

STORAGE OF SUPPLIES

1. At the national level, supplies must be stored at the existing central warehouse, as determinedby the MoH Logistics Unit.
2. Site-level storage must be done according to manufacturer's requirements and in accordance with national standards or guidelines.

DISTRIBUTION

1. Supplies should be distributed via existing distribution systems such as the NLCDS.

STOCK MAINTENANCE

1. Inventory should be managed with existing tools, such as the minimum and maximum forced ordering pull system, to always maintain adequate stock levels.

PROGRAMMATIC MANAGEMENT

LEADERSHIP AND GOVERNANCE

The Directorates of Laboratory Services and AIDS and TB programs provides the overall leadershipand governance on the implementation of the multiplexing guideline, with input from the laboratory services technical working group (TWG) comprising representatives from the following committees:

- TB diagnostic network TWG
- Viral load TWG
- EID TWG
- COVID-19 laboratory pillar
- Integrated specimen transport TWG
- Procurement and supply management
- HIV/TB TWG

REGULATORY APPROVAL AND VALIDATION

Multiplexing is guided by existing regulatory and validation frameworks according to the laboratory policy and strategic plan.

SITE SELECTION

Site selection is guided by epidemiological data and trends, existing capacity (machines, staffing, reagents, andconsumables), and other emerging factors.

INTEGRATED SPECIMEN TRANSPORT SYSTEMS

Sample transportation is guided by the existing MoH integrated transport system.

INTEGRATED SUPPORT, SUPERVISION, AND MONITORING SYSTEMS

Progress on the implementation of multiplexing should be assessed through integrated site support and supervision. Recommendations of support and supervisory visits should be shared and discussed with the laboratory services.

SENSITIZATION AND TRAINING FOR HEALTH CARE WORKERS

Joint trainings and sensitization meetings for multiplexing are to be conducted for all stakeholders.

About IDDS

Established in May 2018, USAID's Infectious Disease Detection and Surveillance (IDDS) project is a five-year, $120 million initiative that operates in more than 20 countries in sub-Saharan Africa and Asia where there are significant gaps in health systems' ability to detect, track, and rapidly respond to infectious diseases and drug-resistant infections that pose a major threat to public health and global health security.

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This publication was produced with the support of the United States Agency for International Development (USAID), Global Health under the terms of the Infectious Disease Detection and Surveillance contract GS00Q14OADU119. Views expressed are not necessarily those of USAID or the United States government.