SPECIMEN COLLECTION & REFERRAL

MODULE 2 (M2)

MODULE CONTENTS

- Introduction to the specimen referral system
- TB-specific considerations and requirements for collection, storage, packaging and transport
- Result delivery & electronic
- Monitoring the specimen referral system

* Refers to Xpert MTB/RIF and Xpert MTB/RIF Ultra

LEARNING OBJECTIVES

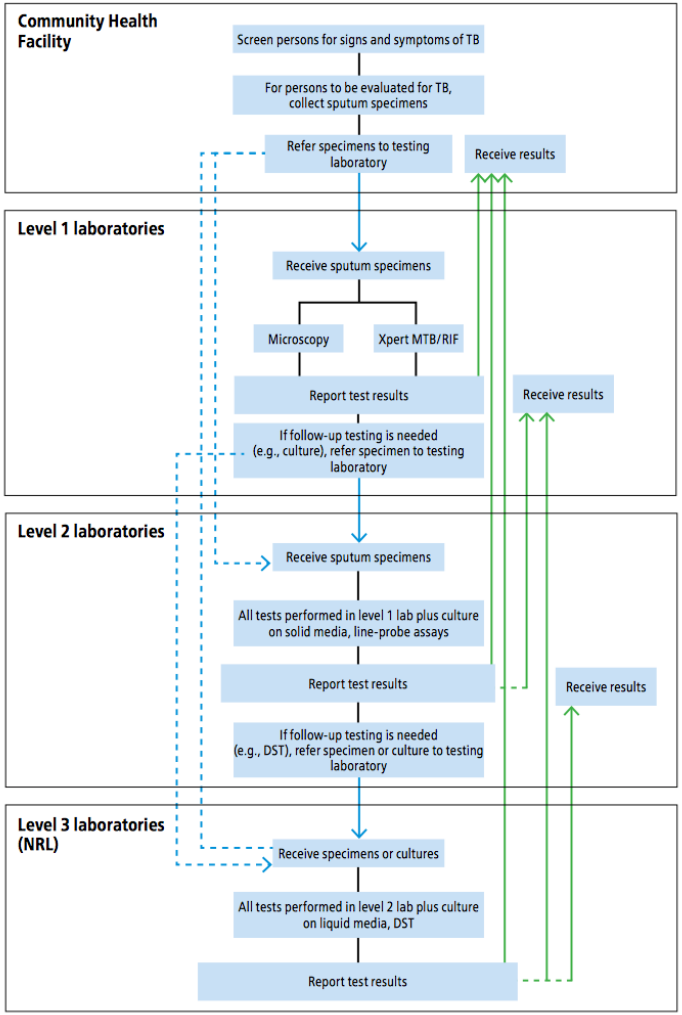
At the end of this module, you will be able to:

- Understand the principles of specimen referral systems
- List the TB-specific considerations and recommendations for specimen collection, storage, packaging and transport
- Describe the specimen collection, storage & transport processes
- Tabulate quality indicators for monitoring the specimen referral system

INTRODUCTION

- A well-designed and well-managed specimen referral system underpins a strong diagnostics network
- In order to refer specimens from health facilities to laboratories reliably, a comprehensive system is needed that not only includes the needed transport mechanisms and equipment to move specimens, but also includes:
  - Logistics
  - Results reporting
  - Trained personnel
  - Data management
  - Monitoring and evaluation
  - Documentation including a policy framework, standard operating procedures, a comprehensive plan with sufficient financing, and proper governance

*EXAMPLE OF A SPECIMEN REFERRAL SYSTEM**



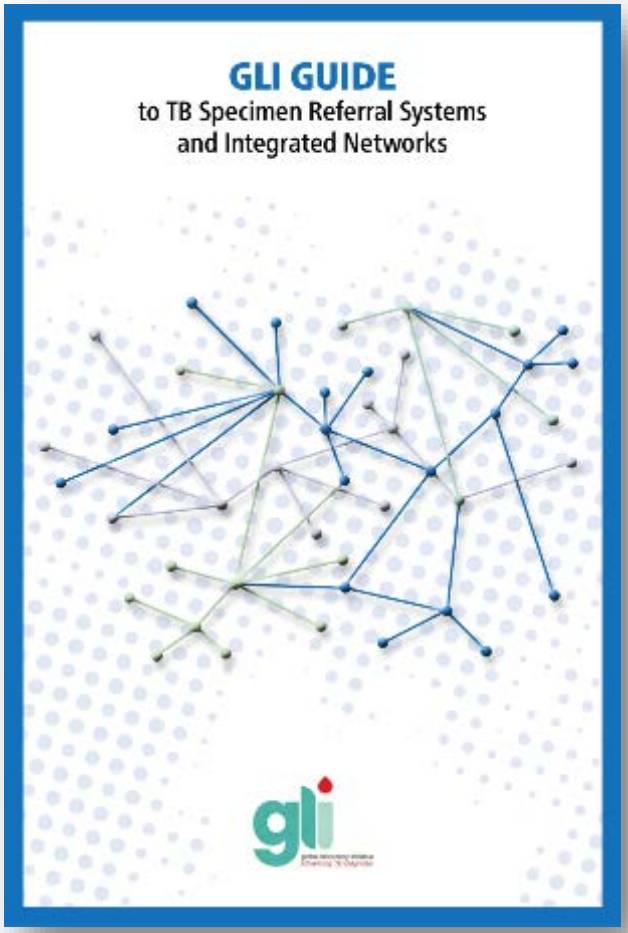
* www.stoptb.org/wg/gli/assets/documents/GLI_Guide_specimens_web_ready.pdf

INTRODUCTION

- The benefit of specimen referral is to relieve patient of the burden (financial, physical, etc.) to travel to the laboratory → **refer the specimen instead of the patient**
- A well-designed specimen referral system can readily support the referral of many types of specimens for many types of tests
- Multiple specimen referral systems can be connected to form an integrated network across a country that meets the needs of various disease programmes
- An integrated network can leverage resources of various disease programmes to improve efficiencies and maximize cost-effectiveness of the network and individual referral systems

INTRODUCTION

- The "GLI Guide to TB Specimen Referral Systems and Integrated Networks"* provides design and technical specifications for the handling and transport of specimens for TB testing and builds a case for integrated approaches to specimen referral across sample types and diseases
- See Programme Module 3: Plan and establish a sample referral network for TB diagnosis



* www.stoptb.org/wg/gli/assets/documents/GLI_Guide_specimens_web_ready.pdf

SPUTUM SPECIMEN COLLECTION

- The most common diagnostic specimen for TB diagnosis is the expectorated sputum specimen:
  - Follow the NTP's guidelines and TB diagnostic algorithm for details on the sample collection for TB diagnosis
  - See Programme Module 1: Implementing TB diagnostics for WHO recommendations on sample collection for TB diagnosis

SPUTUM SPECIMEN COLLECTION: CONTAINER SPECIFICATIONS

To be customized by each country

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

SPUTUM SPECIMEN COLLECTION: SAFETY

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

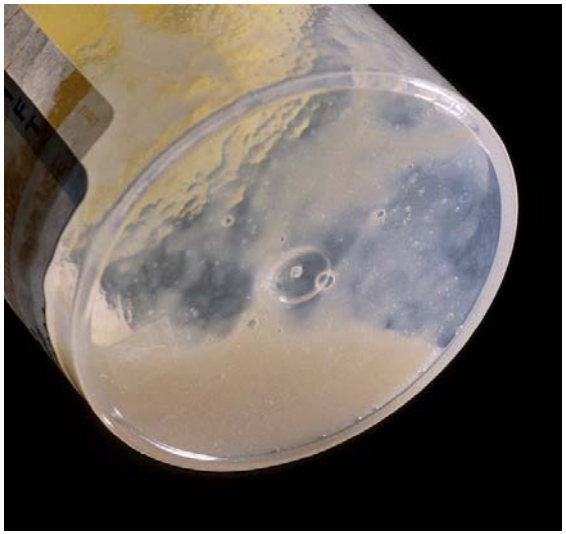
SPUTUM SPECIMEN COLLECTION: PATIENT EDUCATION AND INSTRUCTIONS

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

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

SPUTUM SPECIMEN COLLECTION: GOOD QUALITY

- Obtaining an adequate quantity of good quality sputum is critical to ensuring accurate test results
- For best results, obtain >1ml of purulent/mucoid sputum



Purulent

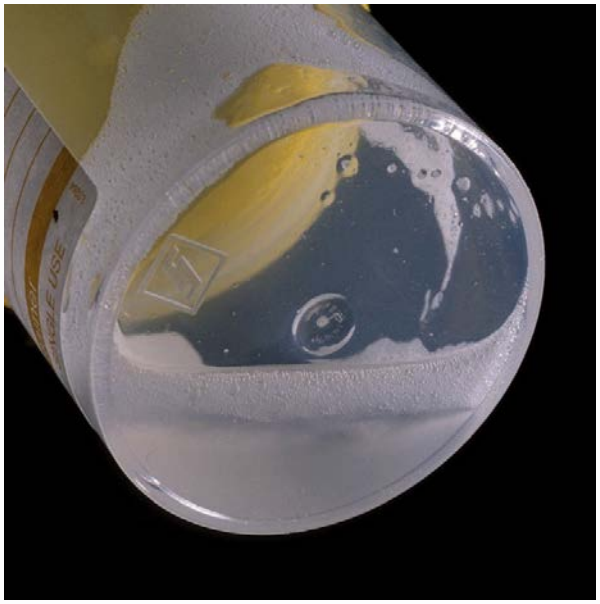
Mucoid

Bloodstained

Photo credit A. van Deun

SPUTUM SPECIMEN COLLECTION: POOR QUALITY

- Poor quality specimens give poor quality results



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

Photo credit A. van Deun

SPUTUM SPECIMEN COLLECTION: LABELLING

- Label with the patient's name, identification number and the date of collection
- Label the outer sides of the container with permanent ink
- Never label the lid
- Complete the Laboratory Request Form. See Technology Module 4: Recording & reporting results
- Once specimens are collected and labelled, they can be packaged and referred to laboratory for testing

SPUTUM SPECIMEN COLLECTION: REQUEST FORMS

To be customized by each country

Request for examination of biological specimen for TB
- MICROSCOPY and XPERT MTB/RIF -

Treatment unit: _____ Date of request: _____

Patient name: _____ Patient/suspect register no.:_____

Age (years): _____ Date of birth: _____ Sex: ☐ Male ☐ Female

Patient address: _____

_____ Telephone: _____

Date sample collected	Specimen type *(Mark ☒/specify)*		Test(s) requested *(Mark ☒)*	
___/___/___	Sputum	Other:	Microscopy	Xpert
___/___/___	Sputum	Other:	Microscopy	Xpert
___/___/___	Sputum	Other:	Microscopy	Xpert

Reason for examination:

☐ Diagnosis. If diagnosis, presumptive RR-TB/MDR-TB: ☐ Yes ☐ No
OR ☐ Follow-up: If follow-up, month(s) of treatment: _____

HIV infection?: ☐ Yes ☐ No ☐ Unknown

Previously treated for TB?: ☐ Yes ☐ No ☐ Unknown

MDR-TB contact?: ☐ Yes ☐ No ☐ Unknown

Requested by (name, qualification, contacts and signature): _____

_____

Add your request form here, and show participants how to correctly complete the form. Does it include:

- Name of the treatment unit
- Date of the request
- Patient's information (that is, name, sex, age, address and the register number of the patient or suspected case)
- Number of specimens and types of specimens sent for testing
- Date the specimens were collected
- Reason for examination (for example, diagnosis or follow-up)
- Signature of the person requesting the examination

EXERCISE 1: PROPERLY FILLING NTP REQUISITION FORM

Purpose

- The objective of this exercise is to complete a Laboratory Request Form with the information provided

Total time

- 25 minutes

Instructions

- All participants divide into groups (up to 5 groups are permissible)
- Assign each group a number (see above)
- Each group to complete the Laboratory Request Form with the information provided
- Groups to present their completed Laboratory Request Form for discussion

SPUTUM SPECIMEN PACKAGING: TRIPLE-PACKAGING TO ENSURE BIOSAFETY

Primary packaging

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

Secondary packaging

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

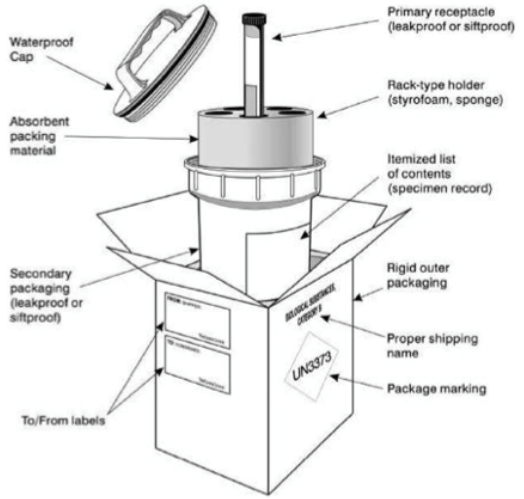


Fig. 3 Example of triple packaging for Category B infectious substances (IATA) from *Guidance on regulations for the transport of infectious substances* 2015. World Health Organization.

UM SPECIMEN PACKAGING: TRIPLE-PACKAGING TO ENSURE BIOSAFETY

Tertiary packaging

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

SPECIMEN TRANSPORT GUIDELINES

- SOPs for occurrence management (i.e. for documenting spills, delays or lost samples), including who to contact in the event of an occurrence should be available to transporters/ couriers
- Transporters or couriers should have access to a spill kit
- Transporters or couriers should be trained on what to do in the case of an accident or spill

SPECIMEN TRANSPORT GUIDELINES

To be customized by each country

- Total number of specimens in a box must correspond to the number of accompanying Laboratory Request Forms
- Identification number on each sputum container must correspond to the number on a request form
- Accompanying forms must contain the required information for each patient
- Documentation should accompany specimens to show Chain of Custody (signed by referring health facility, transporter and laboratory) for tracking
- SOPs should be available for transportation of specimens

HANDOFF TO COURIER OR TRANSPORTER

- Sign the courier logbook/shipping forms to show chain of custody
- Referring facilities should maintain a Sample transportation logbook to keep track of samples that has been sent /referred for testing. This will allow them to keep track of how many samples they should receive results for and monitor if results do not come back
- Hand over properly-packaged specimens to the courier/ transporter – it is not usually their responsibility to package the specimens, only to transport

*EXAMPLE SAMPLE TRANSPORTATION LOGBOOK**

To be completed for samples or results being transported from site to site, kept by laboratory porter or in vehicle								
Date of pick-up (DD-MM-YYYY)	Time of pick-up (HH:MM)	Total number of items transported (samples or results)	Pick-up site	Shipper initials	Date of drop-off (DD-MM-YYYY)	Time of drop-off (HH:MM)	Drop-off site	Recipient initials

* www.stoptb.org/wg/gli/assets/documents/GLI_Guide_specimens_web_ready.pdf

REQUIREMENTS, STORAGE & TRANSPORT OF SPECIMENS: SMEAR MICROSCOPY

- Number of samples: 2
- Minimum volume: 1 ml for direct microscopy; 3–5 ml if samples are to be concentrated
- Storage / shipping conditions: For sputum samples for AFB-smear microscopy, viability is not an issue and storage and transport at ambient temperature (20–30°C) for a total of 1 to 2 days or storage and transport at 2–8°C for a total of up to a week will not significantly affect the positivity

REQUIREMENTS, STORAGE & TRANSPORT OF SPECIMENS: XPERT MTB/RIF (ULTRA)

- Number of samples: A single sputum specimen is recommended for Xpert MTB/RIF (Ultra) testing:
  - An additional sputum specimen may be needed in case of an error or invalid Xpert MTB/RIF (Ultra) result
  - An additional sputum is required for re-testing of Xpert MTB/RIF Ultra trace results*
  - Additional sputum specimens may be needed for microscopy, culture and DST, depending on the NTP's guidelines [Xpert MTB/RIF (Ultra) are not recommended for monitoring patient treatment]

* http://www.stoptb.org/wg/gli/assets/documents/GLI_ultra.pdf

REQUIREMENTS, STORAGE & TRANSPORT OF SPECIMENS: XPERT MTB/RIF (ULTRA)

- Minimum volume: 1 ml, 2–4 ml samples are preferred
- Storage / shipping conditions: For sputum samples being transported for Xpert MTB/RIF (Ultra) testing, viability is not an issue, but stability of nucleic acids is a consideration:
  - Whenever possible, samples should be stored at 2-8 °C prior Xpert MTB/RIF (Ultra) testing and during transportation. If necessary, specimens may be stored at ambient temperature (max 35°C) for up to 3 days, then refrigerated at 2-8°C)

REQUIREMENTS, STORAGE & TRANSPORT OF SPECIMENS: NUCLEIC AMPLIFICATION TESTING

- Number of samples: A single sputum sample is required
- Minimum volume: 1 ml, 2–4 ml samples are preferred
- Storage / shipping conditions: For sputum samples being transported for Line Probe Assay (LPA), viability is not an issue, but stability of nucleic acids is a consideration- see Xpert MTB/RIF (Ultra) recommendations
  - Note: some samples may require additional culture and phenotypic DST if the LPA result detects resistance. These samples require more stringent handling (see culture recommendations)

REQUIREMENTS, STORAGE & TRANSPORT OF SPECIMENS: CULTURE

- Minimum volume: 3–5 ml
- The two most important considerations for transporting sputum specimens for culture tests, in addition to biosafety, are:
  - Preserving the viability of the mycobacteria
  - Inhibiting the growth of contaminating flora
- Viability of tubercle bacilli can be maintained for up to 7 days by keeping the specimens at 2–8°C
- Contaminating flora may grow under these conditions and result in increased contamination rates for the culture tests

REQUIREMENTS, STORAGE & TRANSPORT OF SPECIMENS: CULTURE

- For solid culture, if the delay (storage and transport) exceeds 3 days and storage at 2–8°C is not possible, cetyl pyridinium chloride (CPC) may be added to the sputum specimen to inhibit growth of the contaminating flora
- Sputum specimens containing CPC must be stored at ambient temperature (20–30°C) and be delivered to the testing laboratory within 7 days
- CPC recrystallizes at cool temperatures which removes its ability to protect the specimen from contamination
- CPC can't be used in liquid culture systems such as BACTEC MGIT*

* https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5543576/

REQUIREMENTS, STORAGE & TRANSPORT OF SPECIMENS: CULTURE

- Products that can be used instead of CPC to preserve specimens for culture are commercially available (e.g., OMNIgene•SPUTUM from DNA Genotek Ottawa, Canada)
- A recent WHO Technical Expert Group* meeting concluded that there is limited evidence that use of commercial transport products improves test performance compared to untreated specimens transported under ambient conditions for culture

* http://www.who.int/tb/areas-of-work/laboratory/policy_statements/

REQUIREMENTS, STORAGE & TRANSPORT OF SPECIMENS: OTHER THAN SPUTUM

- Little information is available on the stability of tubercle bacilli in specimens other than sputum
- Bronchoalveolar lavage fluid, neutralized gastric aspirates, tissue specimens and cerebrospinal fluid (CSF) should be transported to the testing laboratory immediately, preferably on the same day as collection
- Great care (i.e., storage and transport at the proper temperature, immediate transport to the laboratory, and immediate testing at the laboratory) must be taken to ensure the quality and utility of invasively obtained specimens such as tissue biopsies and CSF. This is due to the difficulty of obtaining repeat specimens for retesting and the urgent nature of testing on such samples

SPECIMEN RECEPTION AT LABORATORY: DROP OFF POINT

- Specimens should be received by an individual designated for specimen reception at the laboratory
- Laboratory signs Chain of Custody/shipment form to record receipt of specimens
- Referral lab maintains a logbook where specimen received are recorded
- Laboratory performs quality check of specimens against rejection criteria
- Specimen reception area may also be collection point for results

EXAMPLE OF A TB SPECIMEN REFERRAL REGISTER*

S. No.	Name of patient Address	Age Sex	Medical record No. (MRN)	Lab Serial No.	Type of sample	DD/MM/YY of sample collection Time	DD/MM/YY of sample picked up Time	Shippers name Signature	DD/MM/YY results received from Lab Time	Results	DD/MM/YY results given to the patient/ HCW Time	Sample rejected	Turn-around time (TAT)	Name and signature of technician	Remark

* www.stoptb.org/wg/gli/assets/documents/GLI_Guide_specimens_web_ready.pdf

SAMPLE REJECTION CRITERIA: XPERT MTB/RIF (ULTRA)

To be customized by each country

- No or incomplete Laboratory Request Form
- Specimen leaked in transit
- Important to understand rejection criteria for Xpert MTB/RIF (Ultra):
  - Less than 1ml specimen
  - Particles in specimen
  - Specimen not suitable, i.e. urine, stool, blood
  - ALSO- general sample rejection criteria (see previous)

RESULT DELIVERY & ELECTRONIC REPORTING

- An electronic reporting system is recommended for reporting results to clinicians*
- If a paper-based system in used:

1. Results should follow the same transportation route/ mechanism as specimens (in reverse), if paper result is returned
2. Other supplies may also be collected at the same time and brought to the health facility
3. Results can be collected when specimens are dropped off at the laboratory
4. Laboratory signs Chain of Custody/shipment form to record release of results

* http://www.stoptb.org/wg/gli/assets/documents/gli_connectivity_guide.pdf

RESULT DELIVERY & ELECTRONIC REPORTING

- Connectivity solutions typically comprise:
  1. A connectable diagnostic device that produces electronic data,
  2. A software platform that receives and interprets data,
  3. A means to transmit data from the device to the software platform and to a server
- Systems have been developed by Cepheid, USA (C360), SystemOne (GxAlert™/Aspect™), Savics (DataToCare™) and FIND (Connected Diagnostics Platform)

RESULT DELIVERY & ELECTRONIC REPORTING

- Test results can be sent to the NTPs electronically as real-time data to assist with surveillance
- Electronic reporting can enhance the capacity of NTPs to generate performance indicators and to provide the data needed for several of the top 10 indicators of the End TB Strategy
- Another key feature of electronic systems are to send results automatically to clinicians and automatically into LIMSs and electronic registers

SPECIMEN REFERRAL TO OTHER LABOTATORIES

- If specimens will be referred to a testing laboratory outside of the collection facility, they need to be packaged properly to ensure safe transit and quality control, and documented to track chain of custody:
  - Fill out requisition and shipping forms properly
  - Triple packaging for biosafety reasons
  - Hand-off to couriers or person who will transport specimen to laboratory, if necessary

INDICATORS TO MONITOR SPECIMEN REFERRAL

- Monitoring and evaluation for specimen referral systems should examine the performance of the whole system (high level as well as its operations at a more detailed level)
- Quality indicator monitoring requires:
  - Standardizing registers and forms
  - Quality control practices during specimen collection, packaging and transport
  - Targets should be set for all indicators, and any unexplained change in quality indicators, should be documented and investigated

QUALITY INDICATOR MONITORING

Adapt according to NTP guidelines in your country

Indicators that should be monitored monthly by the referring facility
Number of specimens referred for testing
Proportion of referred specimens for which a result was returned
Proportion of referred specimens for which a result was received within the target turnaround time
Proportion of specimens which were picked up by the transport service within the target turnaround time

Indicators that should be monitored monthly by the receiving (referral) laboratory
Number of referred specimens tested at the referral laboratory
Proportion of shipments that arrive at the referral laboratory within the specified transport time
Proportion of test results that were picked up by the transport service or transmitted electronically within the specified turnaround time after generation of the test result
Proportion of specimens that were rejected because of factors related to inadequate or improper transport, packaging or documentation (disaggregated by referring site)

QUALITY INDICATOR MONITORING

Adapt according to NTP guidelines in your country

Indicators that should be monitored monthly by the courier as part of their service agreement
Number of shipments and number of specimens transported
Proportion of shipments that are delivered within the specified transport time
Proportion of shipments that were lost or damaged (disaggregated by route or district)

Indicators that should be monitored annually at the regional or national level by the TWG or MoH
Number of specimen collection sites participating in the specimen referral system
Unit costs such as cost per specimen or result transported per facility or per month
Annual review of consolidated indicators for each region and for the country

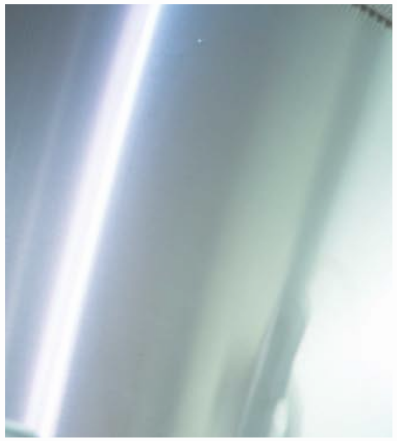
DISCUSSION QUESTIONS

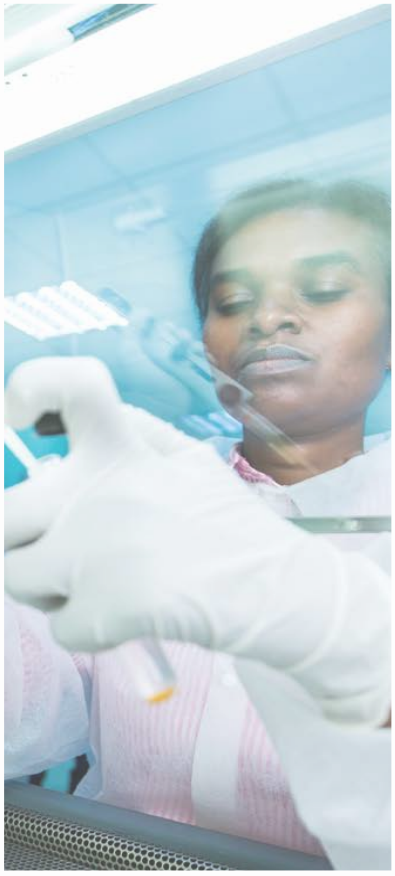
- Describe the process of sputum collection
- Describe the process for triple packaging a specimen
- What are the specimen requirements for Xpert MTB/RIF (Ultra) testing?
- What are the storage & transport requirements for Xpert MTB/RIF (Ultra) samples?
- List two quality indicators that should be collected by the referring facility

KEY MESSAGES

- A well-designed and well-managed specimen referral system underpins a strong diagnostics network
- Proper collection, storage and transport procedures are need to be followed to ensure specimen quality and biosafety
- All specimen referrals must be tracked
- Results should follow the same transport pathway/mechanism as specimens, but in reverse
- Electronic reporting of results should be prioritised
- Targets should be set for all indicators collected to monitor sample referral. Any unexplained change in quality indicators, should be documented and investigated

global laboratory initiative
advancing TB diagnosis





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THANK YOU