

NTP- Pakistan

Specimen Transportation Manual

For TB laboratory Network



**NATIONAL REFERENCE LABORATORY
NATIONAL TUBERCULOSIS CONTROL PROGRAM
GOVERNMENT OF PAKISTAN**



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Scope

The scope of this manual is to provide information on minimum requirements for the quality and quantity of biological specimens sent to a TB laboratory for testing Xpert/MTB rif assay, culture and/or DST and conditions for transportation of specimens to the laboratory, expected reporting time of the test and recording of results received from higher level laboratory in local

1. Definitions and abbreviations

BAL: bronchoalveolar lavage

GA/L: gastric aspirate/lavage

MOTT: mycobacteria other than tuberculosis

NTP: national tuberculosis programme

RR : rifampicin Resistance

TB : tuberculosis

2. Principle

Specimen quality –from the moment of collection to the arrival of specimens at the laboratory where they will be processed – is the responsibility of the setting in which specimens are collected, that is, either the peripheral laboratory where patients were given sputum containers and/or the clinics where sampling/biopsy is performed.

Since laboratory is usually the only place where quality of specimens received can be controlled. Therefore laboratories at all levels must record quality of specimen and monitor quality indicators like. The proportion of saliva in sputum specimens, delay in specimen processing after collection (time delay between specimen collection, shipment, specimen reception and processing), frequency of delay in arrival/processing of specimen. Identified problem should be reported so that corrective action may be taken wherever necessary.

Specimens sent to the laboratory should be of adequate volume, as specified below, accurately labelled for identification, and accompanied by a written laboratory request form according to guideline provided by (NTP/WHO recommendations).

Specimens should be sent to the laboratory **AS SOON AS POSSIBLE AFTER COLLECTION**, in leak proof containers surrounded by absorbent material in a shock-resistant outer package that is properly labelled according to the national and/or international regulations for infectious material.

Since the laboratory is usually the only place where there is quality of specimens received can be controlled, laboratories at all levels must monitor quality indicators, e.g. the proportion of saliva in sputum specimens, frequent late arrival of specimens, and report problems so that corrective action may be taken wherever necessary.

Specimens sent to the laboratory should be of adequate volume, as specified below, accurately labelled for identification, and accompanied by a written laboratory request form according to WHO recommendations.

3. Reason for specimen transportation:

Transportation of specimen to higher level laboratory may be required in circumstances where test is not performed in laboratory receiving request for testing

- Xpert MTB/Rif assay for diagnosis of TB or RR TB
- TB culture for Diagnosis of PTB or extra-pulmonary TB
- TB culture for Treatment monitoring
- TB DST for comprehensive First and second line drug susceptibility testing

4. Type of specimen:

Different biological specimen can be used for diagnosis of TB depending on disease site. Pulmonary TB is the most common type of tuberculosis and sputum is most common respiratory specimen used for diagnosis.

1. Respiratory Specimen:

Sputum: SPUTUM is the most common specimen received for testing.

Gastric lavage: Gastric lavages (GL)/aspirates (GA); sputum specimen swallowed is collected from stomach .It may contain MOTT and are therefore rarely used for adults.

They are mostly indicated for children, who are unable to expectorate absolutely no sputum.

- GA collection are recommended early in the morning on empty stomach.
- GA specimen should be neutralized by adding 100mg of sodium bicarbonate and transport it immediately to the laboratory.

Other respiratory specimens: Includes Bronchial alveolar lavages, secretions, Tran bronchial and other biopsies.

Note: *BIOPSY IN FORMALINE CANNOT BE PROCESSED FOR CULTURE*

2. Extra-pulmonary specimens



The laboratory may receive a variety of specimens for diagnosis of extra-pulmonary TB. Number of MTB in infected specimen is usually very low. These specimen need to be processed with care. These specimens are broadly divided into two groups which are processed in different ways to improve yield of results

- **Aseptically collected Specimen** are usually free from contaminating flora (spinal fluid, pericardial fluid, synovial fluid, ascetic fluid, bone marrow etc.)
- Specimens with resident or contamination flora (e.g. urine)

5. Equipment and materials

- Following supplies will be required for transportation of specimen
- Specimen container
- Cool Transport Boxes
- Frozen Ice packs
- Request form
- Dispatch List
- Marker for labelling
- Masking Tape
- Envelop for request form and dispatch list

Below are examples of recommended sputum containers

	
<p>Sputum container : 50 ml Wide-mouthed, unbreakable, leak-proof, screw-capped made of translucent material</p>	<p>Falcon Tubes are 50ml centrifuge tubes. If specimens are to be cultured using centrifugation, sputa should preferably be collected directly into 50-ml centrifuge tubes to avoid the need for their transfer from one container to another.</p>

6. Sample collection

Sputum

The large majority of specimens received for diagnosis are sputum samples and laboratory technician is responsible for proper specimen collection from patients. Technicians responsible for specimen collection should make all efforts and ensure that good quality specimen is obtained from patient before it is shipped.

- Specimens should be collected preferably outdoors in open air or in a separate ventilated room.
- If good specimens are to be obtained, patients must be guided properly on how to produce sputum.

Instruction for sputum collection

- Clean mouth with water rinse
- Inhale and exhale for 2-3 time
- Keeping both hands on hips, cough forcibly and collect sputum in the mouth; spit the sputum carefully into a wide-mouthed, unbreakable, leak proof container and close the lid tightly. Avoid spills or soiling the outside the container.
- **Quality of specimen:** Ideally, a sputum specimen should be 2–5ml in volume, although smaller quantities are acceptable if the quality is satisfactory.
- If specimens are to be cultured, sputum specimen should preferably be collected directly into 50-ml centrifuge tubes to avoid the need for their transfer from one container to another.

- In cases where specimen is collected from RR patient for DST (0-month before start of second line treatment) it is strongly recommended to collect two specimens from such patient to ensure that DST_results are made available even if one specimen is contaminated.
- **Labelling of specimen:** Each specimen should be labelled with the name of patient and local lab register number which should match with information on request form.

Other specimen collection:

Beside sputum and urine, mostly other specimen (e.g. gastric aspirates, body fluids, biopsy, needle aspirate) are collected by physicians. Laboratory technician in most cases is responsible for transportation of specimen. Even if no testing is done in local lab. Specimen should be registered in local lab before shipment. See Table-1 for quality of specimen and special instruction and preservative.

Table -1 Specimen Types used for diagnosis of TB

	DISEASE TYPE	SPECIMEN TYPE	RECOMMENDED Quality /Volume	Special Instruction Preservative
1	Pulmonary Specimen	Sputum	2-5ml – Purulent /Muco- purulent	Nil
		Bronchial alveolar lavage (BAL)	20-40ml	NIL
		Bronchial aspirates	2-5ml	NIL
		Trans bronchial Biopsy		taken under sterile condition, should be kept in sterile container with few drops of sterile 0.9% saline to keep tissue wet
		Gastric Aspirate		Neutralize by adding 100mg sodium bicarbonate
2	Extra-pulmonary specimen	CSF	2-3ml	Collect aseptically in sterile container without using any preservative.
		Body Fluids (pleural, pericardial, ascitic, etc)	20-50ml	
		Tissue Biopsy		Add few drops of sterile 0.9% saline to keep tissue wet
		Pus		
		Urine	min. 200ml	single, early-morning, midstream sample, collected in a wide-mouthed sterile vessel

7. Storage/transport of specimen

All specimens should be transported as soon as possible and should be kept in cool temperature/refrigerator in between collection and shipment.

AFB smear microscopy: Laboratories at lower level capable of doing microscopy may also receive request for specimen transportation to higher level laboratories for AFB microscopy.

Most of lower level laboratories are not equipped with specific centrifuge required for TB work. therefore are not capable of processing specimen like body fluids and gastric aspirates for AFB microscopy . These laboratories will be required to ship such specimen for AFB microscopy and/or culture to higher level laboratory. Although maximum of

one week in cold conditions (2-8°C) does not significantly affect the positivity rate of smear microscopy but if AFB culture is also requested specimen should be shipped as soon as possible.

Xpert MTB/Rif assay: Longer transport should not affect Xpert positivity, BUT whenever possible, specimens should be transported and stored at 2-8°C prior to processing which should not exceed maximum of 7 days

Culture and/or DST: Specimens should be transported to the laboratory as soon as possible.. If the transport of specimen is unavoidable for one day, keep specimens cool (refrigerated but not frozen) ; however, additional growth of contaminants may result in an increased contamination rate on culture media. Specimens should reach in culture laboratory within 72 hours and processed for culture within 3 days of collection.

8. Packaging of specimen for Transportation

The basic packaging system for local surface transport of sputum specimens should be considered as follows:

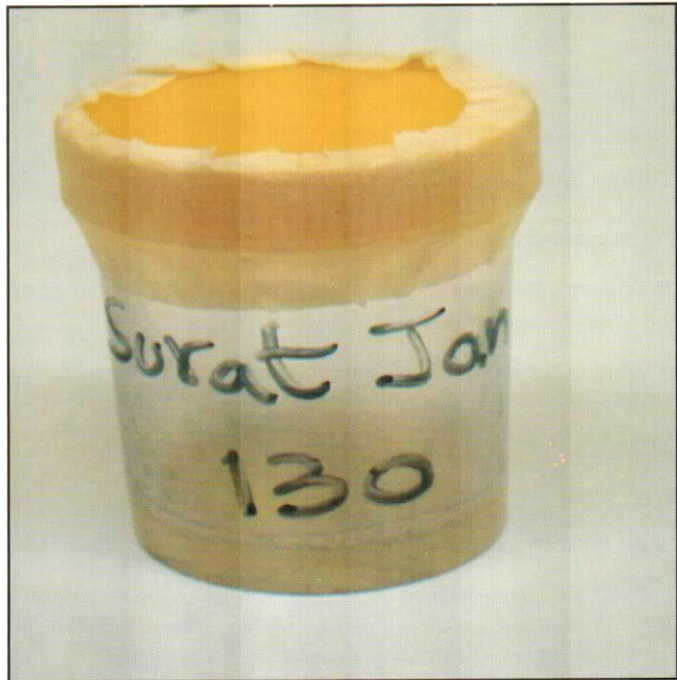
- Primary receptacle – the specimen container
- Secondary packaging – Ziplock vinyl bags (plastic bags)- compatible to the size of specimen container so the vinyl bag could be sealed to avoid leakage and cross contamination.
- Outer packaging – Transport box- Specimen containers packed in vinyl bags are placed in transport box with suitable cushioning material.
- Each transport box should be placed inside with frozen ice packs replenishable for every shipment.
- Outer packaging protects their contents from external influences, such as physical damage, during transit.

Procedure for transportation

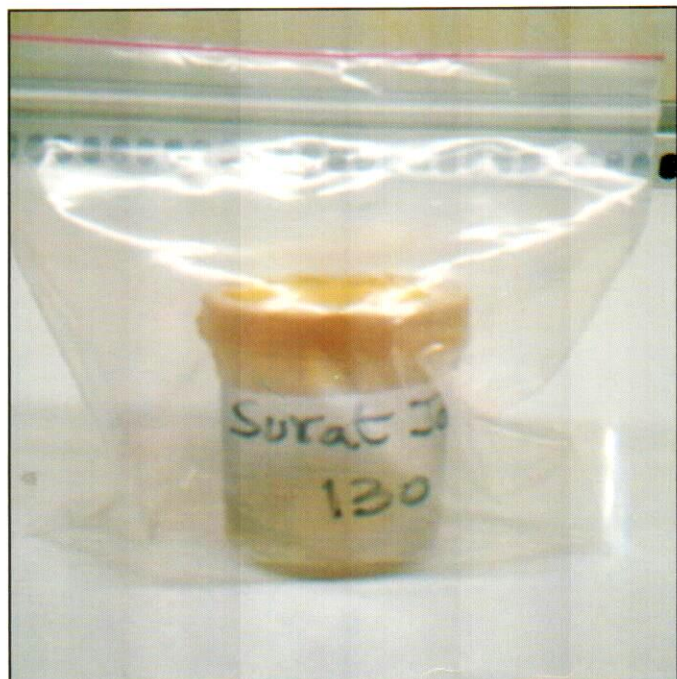
- Match the specimen ID on container with ID registered in request form
- Tightly screw cap specimen container and seal with adhesive paper tape (easily removable)
- Place the specimen container in vinyl bag, seal ziplock.
- Place tissue absorbant in transport box.
- Place frozen ice packs in transport box.
- Place specimens in transport box.
- Seal the lid of transport box with tape to avoid opening during transportation.
- Label the transport box with address of laboratory and Name of person incharge with phone number
- Place dispatch list and request forms in an envelope.
- Paste envelope of dispatch list and request form on one side of transport box.

Step-1: check if Specimen ID on container match with ID on request form

Step-2: Tightly screw cap specimen container and seal with adhesive paper tape (easily removable)



Step-3 : Place the specimen container in vinyl bag, seal ziplock.



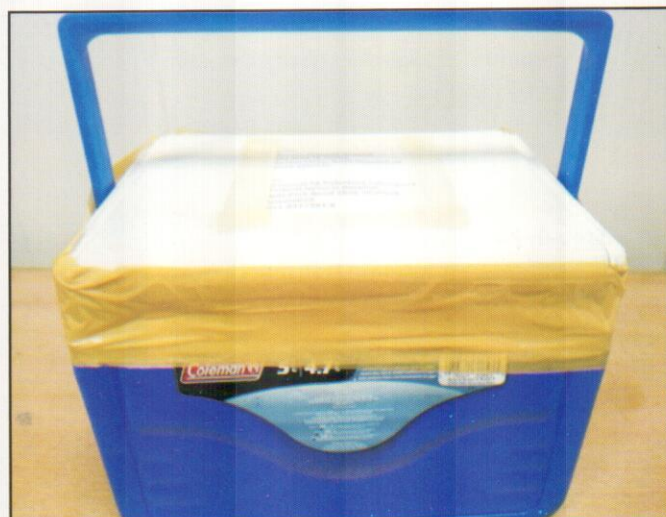
Step-4: Place frozen ice packs in transport box



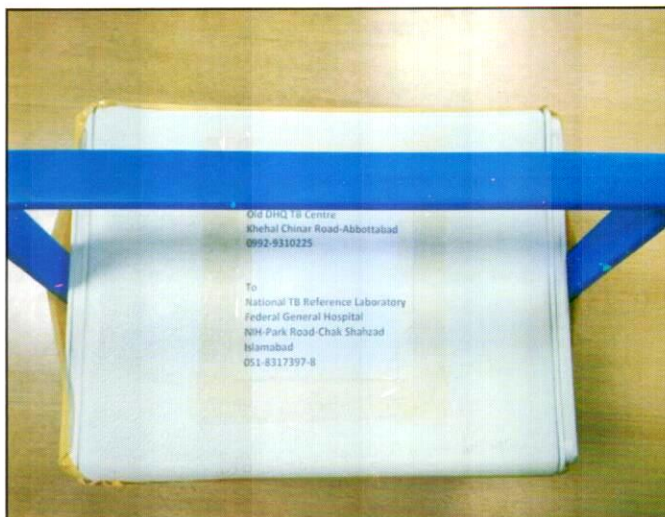
Step-5: Place specimen in Transport Box



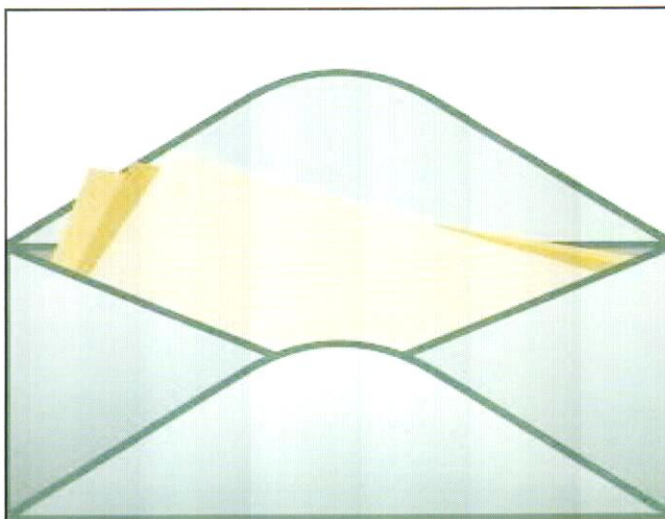
Step-6: Seal the lid of transport box with tape to avoid opening during transportation.



Step-7: Label the transport box with address of laboratory and Name of person incharge with phone number



Step-8: Place dispatch list and request forms in an envelope.



Step-9: Paste envelope of dispatch list and request form on transport box.



9. Reporting time and recording of result

Target reporting time (Turn around Time) of different test is given below Time starts after specimen reaches testing laboratory.

Table: Reporting/turnaround time:

S #	Test	Time
1	AFB smear	01 day
2	Xpert MTB/Rif assay	01 day
3	Culture	08 weeks
4	DST	12 Weeks

Recording of results by sending laboratory

Laboratory results of specimen sent to higher laboratory should be entered in local lab register against lab register number given to specimen on date when specimen was received.

Use forms in Annex 1, 2 & 3.

10. Quality control

Before specimens can be accepted in the laboratory, the patient information on accompanying dispatch list should be matched carefully with request forms and label on specimen container (sample and request form labelled with the same number). Specimens that cannot be identified exactly should not be processed and sending laboratory should be informed.

Specimens should be examined on receipt, to ensure that they correspond in type, quantity, quality and volume to the appropriate criteria. Any deviations must be documented and noted on the final report since they may affect the results. (Saliva or transport delay)

The transport conditions and duration must be checked. Delays in transportation and/or exposure of specimens to extremes of temperature without protective measures must be documented and noted in the report.

11. Related documents

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Annexure

Annex-1 Request form Xpert MTB/Rif assay

Annex-2 Request and reporting form for TB culture and Drug Susceptibility Test (DST)

Annex-3 Specimen Dispatch list

Annex-4: Strategy for Xpert MTB/Rif assay Testing

REQUEST FORM: AFB MICROSCOPY & XPERT MTB/RIF DIAGNOSTIC ASSAY**Patient identification (ID):**

CNIC # _____

Name of Patient: _____ Age _____ (yrs): Sex: M

/F _____

Address: _____ Contact #: _____

OPD# _____ Ward# _____ Bed# _____ Hospital/Health Facility Name: _____

TB Registration: # _____

DR TB

Registration: _____

Origin of request

Name of Physician: _____ Designation: _____

Contact# (work): _____ Mobile# _____

Patient's Clinical Information:

Disease Type: Pulmonary

Extra Pulmonary (Specify : _____)

Specimen Type: Sputum

Other (Specify _____)

Reason for Testing: Diagnosis

F.U.P. _____ Month

AFB Microscopy Result: **Positive** 3+ 2+ 1+ 1-9AFB Neg **Not Available**

Date tested _____ Lab serial# _____ Lab where tested _____

REASON FOR XPERT MTB/RIF ASSAY TESTING (tick appropriate box)

MDR SUSPECT/ MDR HIGH RISK GROUP	History of previous ATT/	Type of Treatment	Cat-I	Cat-II	Cat I & II	Others
		Treatment outcome	Cured Treatment completed	Defaulted	Treatment failure	Unknown
	Other High Risk Group		MDR contact	Health care worker	Hospitalized	Seriously ill
PRESUMPTIVE TB CASE	HIV positive	Difficult to diagnose PTB case (Adult)	Child hood TB	TB Meningitis	Extra Pulmonary TB	

Date Patient referred /specimen collection: ____/____/20____

LABORATORY REPORT : AFB MICROSCOPY & XPERT MTB/RIF DIAGNOSTIC ASSAY (To be Completed in Laboratory)

Laboratory Name: _____

PIN: _____

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--	--	--	--

CNIC # _____

Name of Patient : _____ (Yrs): _____

Sex: _____

Address: _____ Contact #: _____

Hospital/Health Facility Name: _____ OPD Ward/Bed# _____ TB/DRTB Reg

Date specimen received/Collected: ____/____/20____

AFB MICROSCOPY RESULT

SPECIMEN			AFB Result	AFB Smear Grading (if Positive)			
Type	Appearance	Volume	Positive/Negative	1-9AFB	1+	2+	3+

Technique Used: Hot Ziehl-Neelsen (ZNS) Fluorescence (FM) Direct smear D/S Concentrated smear (Conc. Sm)

Xpert MTB/RIF ASSAY RESULT : _____ Date: ____/____/20____

1.MTB DETECTED High Med Low V. Low

Not detected

2.Rifampicin DETECTED

Resistance

Not detected

Indeterminate

Report prepared by: Name _____

Signature _____

REQUEST FORM FOR TB SMEAR, CULTURE AND DRUG SUSCEPTIBILITY TESTING (DST):-**Patient identification (ID):-**

Patient CNIC:-----

Name of patient: _____ Age _____
(yrs): _____ Sex: _____

Ward/Department: _____ Address: _____ Contact # _____

HIV-status: Pos Neg Unknown

Requested tests: Microscopy Culture DST (specify First Line Second Line)
 Specimen Sputum Sputum in preservative, type Other
 (Specify) _____
 Date of specimen collection: ____/____/20____ Specimen ID number:-

Reason for Request for testing at the reference laboratory:-

Diagnosis: (If yes specify) PTB MDR EPTB

Follow-up: (If yes specify) PTB DR TB TB/DRTB Register #: _____ Months ____ of Rx after

Rx

Origin of request:

Name Physician: _____ Name Health Facility:- _____ District:-

Disease type:-

Site: Pulmonary

Extra-pulmonary

(specify): _____

History of ATT	Never Treated	Previously treated					On treatment
Treatment Category	Month/Y ear treatment started	Treatment outcome					On treatment
		Cured	Completed	default	Failure	Other (specify)	
Cat.1							
Cat.2							
Cat.4 (SLD)							
Other							
Other Risk Factor	MDR Contact	HCW			Hospitalized/Seriously Ill		Any other specify

Test	Date Exam	Lab Name & S#	Latest Laboratory Test Results			
AFB Microscopy			Pos ----- Neg	Positive Smear Grading:		
Xpert MTB/RIF Assay			1. MTB Detected Not detected	2. Rifampicin Resistance Detected Not detected		

				IND
AFB Culture			Pos ----- Neg Cont	MTBC NTM
DST			Resistant: _ _ _ _ _ _	Susceptible _ _ _ _ _
Date Sp. collected: / / / Date Sp. dispatched: / / / Sp. Dispatched by (Name): _____ Contact #: _____				
REFERENCE LABORATORY RESULTS : (To be completed in Laboratory)				

Date Specimen collected	Date specimen received	Lab Serial #	Type of Specimen	Visual Appearance	Quantity (ml)

Date reported:- ____/____/20____

SMEAR MICROSCOPIC RESULT			XPRT MTB/RIF ASSAY RESULT		
Lab. S.#	POS/ NEG	Grading if Positive	Lab S. #	MTB	Rifampicin Resistance
				Detected	Detected
				Not detected	Not detected IND

Hot Ziehl-Neelsen (ZN) Fluorescent (FM) Direct smear (Dsm) Concentrated smear (Conc.Sm)

CULTURE RESULT				Date reported : ____/____/20____
Laboratory S.No	Media Used (LJ/MGIT)	Culture Growth (Pos/Neg/Contaminated)	Culture ID MTBC/NTM	Culture Grading if Positive (1-9 Col/1+/2+/3+)

M. TUBERCULOSIS DST RESULT										Lab. S. #:	Date reported ____/____/20____
Drug Conc (µg/ml)	Tech Used	S	I	R	E	Z	K	A	C	O	Remarks
Solid	4.0	0.2	40.0	2.0	--	30.0	30.0	40.0	4.0		
MGIT	1.0	0.1	1.0	5.0	100	2.5	1.0	2.5	2.0		
DST Result											
Legend		S-Streptomycin, I-Isoniazid, R-Rifampicin, E-Ethambutol, Z-Pyrazinamide, K-Kanamycin, A-Amikacin, C-Capreomycin, O-Ofloxacin									
		S = susceptible; R = resistant; C = contaminated; ND = Not done									

Date: ____/____/20____ Report prepared by:-NameSignature

Annex-3

Dispatch list for specimen shipment						
Name Health Facility:						
S #	Patient Name	Reference #	CNIC #	Local Lab Specimen ID	No. of specimen	Date specimen collected
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
Name & signature of shipper:						
Shipment Date:						

Strategy for Xpert MTB/Rif assay Testing :

The Xpert MTB/RIF assay is the only fully automated cartridge based real-time DNA based test which can detect both TB and resistance to rifampicin in less than two hours,

WHO policy recommendation:

WHO endorsed use of Xpert MTB/RIF assay in 2010, Policy recommendations on the Xpert MTB/RIF assay® (Xpert MTB/RIF) were issued by WHO early in 2011, and updated in 2013. Following are Key recommendation by WHO .

Xpert MTB/RIF for the diagnosis of pulmonary TB and rifampicin resistance

- Xpert MTB/RIF should be used as an initial diagnostic test in individuals (adults and children) suspected of MDR or HIV-associated TB
- Xpert MTB/RIF may be used as an initial diagnostic test in individuals (adults and children) presumed to have TB (conditional recommendation based on resource implication)
- Xpert MTB/RIF may be used as a follow-on test to microscopy in adults presumed to have TB but not at risk of MDR-TB or HIV associated TB (conditional recommendation based on resource implication)

Xpert MTB/RIF for the diagnosis of extra-pulmonary TB and rifampicin resistance

- Xpert MTB/RIF should be used in preference to conventional microscopy and culture as the initial diagnostic test in testing cerebrospinal fluid specimens from patients presumed to have TB meningitis
- Xpert MTB/RIF may be used as a replacement test for usual practice (including conventional microscopy, culture, and/or histopathology) for testing of specific non-respiratory specimens (lymph nodes and other tissues) from patients presumed to have extrapulmonary TB (conditional recommendation).

National Policy Recommendation:

NTP Pakistan keeping in view WHO recommendation, current accessibility to testing, existing infrastructure and available resources has formulated following strategies for diagnoses of DRTB and TB using Xpert MTB/Rif assay .

I. Xpert MTB/RIF assay testing for Diagnosis of DRTB :

It is recommended to use Xpert MTB/RIF assay to test all individual at risk of MDR. Following three groups of TB patient and individual presumed are included in this group.

1. *ALL RETREATMENT TB CASES :*

All TB cases (Both AFB sm+ve and negative) with history of previous ATT should be tested using Xpert MTB/Rif assay at zero month. This includes all retreatment cases

- Treatment Failure Cat-I (F-1)
- Treatment Failure Cat-II (F-2)
- Relapse after Cat-I (R-1)
- Relapse after Cat-II (R-2)
- Treatment after loss to follow up cat-1(D-1)
- Treatment after loss to follow up cat-II (D-2)
- Other Retreatment

2. *SYMPTOMATIC CONTACTS OF DRTB PATIENT:*

- All house hold and workplace symptomatic contact of DRTB patients should be screened for RRTB .

Keeping in view limited access to Xpert testing facilities it is recommended that specimen from all presumptive TB cases at risk of DRTB should be tested for AFB smear and same specimen then referred for Xpert MTB/RIF assay irrespective of Smear results to xpert testing facility .

3. *TB PATIENTS UNDER TREATMENT WHO FAIL TO CONVERT AT THE END OF INTENSIVE PHASE:*

Keeping in view low prevalence of Drug resistance' in New cases (no history of previous treatment) and resource implication of testing all presumptive/ TB cases, NTP recommends limited use of Xpert MTB/RIF assay testing for following group

- AFB smear +ve patient on Cat-1 who fail to convert end of 2 month
- AFB smear +ve patient on Cat- II who fail to convert end of 3 month (if not tested at Zero month)
- AFB smear Negative Patient who is reported AFB smear positive end of intensive phase

Retreatment cases tested with Xpert at zero month and reported as "RR not detected" but fail to convert on first line treatment should be tested using conventional phenotypic DST (few of drug resistant rifampicin mutation are not detected by Xpert/MTB Rif assay)

II. **Xpert MTB/RIF assay testing for Diagnosis of TB :**

1. *Individual with Presumptive TB NOT AT RISK OF DRTB*

Keeping in view current situation of available resources and limited accessibility it is recommend to use of Xpert MT/Rif testing for Individual who although are not at risk of DRTB but early diagnosis of TB and screening of RRTB is critically important for clinical management and infection control perspective .

- Children under 15years of age
- HIV positive
- Other immune-compromised (Diabetic, on immunosuppressive or chemotherapy)
- Injecting drug users
- Contact of TB
- Health Care workers including laboratory workers
- Hospitalized
- Seriously Ill
- Prisoners

At health facilities where Xpert testing is not available on site, Specimen should be processed for AFB smear and same specimen then referred for Xpert MTB/RIF assay irrespective of Smear results.

2. *Individual with presumptive EPTB*

It is recommended that Xpert MTB/RIF should be used

- As the initial diagnostic test in testing cerebrospinal fluid specimens from patients presumed to have TB meningitis.
- Xpert MTB/RIF should be used for bacteriological diagnosis of specific non-respiratory specimens (lymph nodes and other tissues) in setting where services are available and hospital Dots linkages are established.

3. *Individual having AFB same negative (Clinically diagnosed PTB)*

Xpert MTB/RIF assay as follow-on test to AFB smear for bacteriological diagnosis of all AFB smear negative /clinically diagnosed cases will improve quality of diagnosis and proportion of bacteriological positive Tb cases .However keeping in view resource implication it is not yet recommended to implement this strategy in all setting. Programme may formulate or revise policies for this group based on resources



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