



Let's Talk **TB:**

A Supplement to GP
CLINICS

Chapter 2: Diagnosis of Tuberculosis: Importance of Appropriate Specimen Collection

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A Good Diagnostic approach for TB requires...

- Collection of the right **clinical specimen**
- Use of the appropriate **laboratory test**
- Clinicians should therefore...
 - Have basic knowledge about the types of specimens collected
 - Be able to provide clear instructions to their patients on how to provide such specimens at the lab or in the clinic

Pulmonary TB

- **Sputum** is the single most important sample for lab testing
- Blood
 - **NO** accepted, valid blood test for TB exists

Blood

- Not ideal for TB testing technologies:
 - Smear microscopy for acid-fast bacilli (**AFB**)
 - Culture and Molecular tests (**e.g. PCR**)
- Blood based antibody detection tests for TB (e.g. IgM/IgG antibodies using ELISA and rapid tests)
 - Strongly discouraged by the **World Health Organization (WHO)**
 - Banned in 2012 by the government of India

Blood Based Tests for latent TB

- Blood based tests for latent TB infection
 - Interferon gamma release assays
 - QuantiFERON TB-Gold/TB Gold by Qiagen

**** These tests have NO VALUE FOR ACTIVE PULMONARY TB DIAGNOSIS and should be avoided!**

Sputum

- All patients (adults, adolescents, and older children who are capable of producing sputum) suspected of having pulmonary TB should have:
 - At least **2 sputum specimens** submitted for microscopic examination
 - OR, a **WHO approved molecular test** (e.g. Xpert MTB/RIF by Cepheid Inc.)

- **2 sputum specimens** can be collected on the same day, a minimum of 1 hour apart
 - Earlier recommendations required the collection of 3 sputum samples
 - Current policy requires 2 specimens for microscopy (provided microscopy is done with quality assurance)

Table 1 – The specimen type is decided by the site of disease or purpose of testing or patient population

Site, purpose, or patient population	Specimen of choice	Comments
Active, pulmonary TB	Sputum (spontaneous or induced)	<ul style="list-style-type: none"> - Sputum must be produced deep from within the lungs - Saliva is not acceptable - At least two sputum samples must be collected - Blood is not acceptable as a sample for active, pulmonary TB - Rarely, bronchoalveolar lavage (BAL) is used to collect lung secretions – this requires expertise and hospital care
Active, extrapulmonary TB		
TB lymphadenitis	Lymph node aspirate or biopsy	<ul style="list-style-type: none"> - Requires needle aspiration and/or excision biopsy - Samples are then sent for smears for AFB, liquid culture, molecular (PCR) tests, and histopathological examination - Histopathology and liquid culture are the most important tests; PCR may help, if positive
Pleural effusion (TB pleuritis)	Pleural fluid and pleural biopsy	<ul style="list-style-type: none"> - Requires pleural tap and/or biopsy - Samples are then sent for pleural fluid analysis, smears for AFB, liquid culture, molecular (PCR) tests, and histopathological examination; pleural fluid adenosine deaminase (ADA) or interferon-gamma is often helpful - Histopathology and liquid culture are the most important tests; PCR may help, if positive
Ascites (abdominal TB)	Ascitic fluid and peritoneal biopsy	<ul style="list-style-type: none"> - Requires ascitic tap and/or biopsy - Samples are then sent for smears for AFB, ascitic fluid analysis, liquid culture, molecular (PCR) tests, and histopathological examination; ascitic fluid ADA or interferon-gamma is often helpful - Histopathology and liquid culture are the most important tests; PCR may help, if positive
TB meningitis	Cerebrospinal fluid (CSF)	<ul style="list-style-type: none"> - Requires spinal tap for CSF collection - Samples are then sent for smears for AFB, CSF analysis, liquid culture, molecular (PCR) tests - Liquid culture of CSF along with CSF analysis is most important; PCR may help, if positive
Bone and joint TB	Bone/synovial tissue via biopsy	<ul style="list-style-type: none"> - Histopathology and liquid culture are the most important tests; PCR may help, if positive
Urinary tract and kidneys TB	Urine and tissue via biopsy	<ul style="list-style-type: none"> - Histopathology and liquid culture are the most important tests; PCR may help, if positive
Genito-urinary tract TB	Tissue via biopsy (e.g., endometrial tissue in women)	<ul style="list-style-type: none"> - Menstrual blood is not ideal; it is important to collect endometrial tissue - Histopathology and liquid culture are the most important tests; PCR may help, if positive
Childhood TB	Sputum in older children; in younger children, gastric aspirates	See Table 2 for additional options and comments
Latent TB infection	Whole blood for interferon-gamma release assays (IGRAs); or Mantoux intra-dermal skin test	<ul style="list-style-type: none"> - IGRAs are only meant for latent TB infection – they cannot separate latent infection from active disease - Mantoux skin test must be correctly performed and read

Specimens

- Must be collected in sterile, leak-proof, laboratory approved containers
 - Should be labeled on the side with the patient's name and the date of collection
 - Should be accompanied by a carefully completed requisition form providing:
 - The patient's name and age
 - The physicians name and address
 - The date and time of collection
 - Whether the specimen is diagnostic or follow up
 - The specimen type and site
- **specimens for initial diagnosis should be obtained before the initiation of anti-TB therapy

Sputum Collection

- 2 ways of collecting sputum:
 - 1. **Spontaneous expectoration**
 - Patient must cough up a sputum sample from the lung without any assistance
 - 2. **Sputum Induction**
 - Stimulate a deep cough in the patient
 - Ask the patient to inhale nebulised hypertonic saline in a sputum induction chamber to ensure biosafety

Bronchoalveolar Lavage (BAL)

- **Bronchoalveolar Lavage (BAL)** may be necessary to collect samples from the lung
 - Performed in the hospital with the patient sedated
 - **5 ml** portion of the lavage fluid may be submitted for TB testing
 - A post-bronchoscopy sputum specimen should also be collected and submitted

Sputum vs. Saliva

- **Sputum** is a respiratory secretion originating from deep within the lungs
- Unless properly instructed, patients may provide saliva samples instead of sputum
- All patients should be instructed on the difference between sputum and saliva or nasopharyngeal secretions

Important information to provide patients

- 1. The importance of sputum examination for diagnosis or follow-up of pulmonary TB
- 2. TB is curable and if their test is positive, they will receive treatment
- 3. How to open and completely close the screw capped containers without touching the inside
- 4. The need for collecting 1 to 2 teaspoons of real sputum, not saliva

- 5. To wash mouth out with cooled, boiled water to avoid food particles in the specimen
- 6. How to produce good sputum
- 7. If possible, to collect the first specimen in the early morning (since bacillus accumulate in the lungs overnight)
- 8. To expectorate sputum specimens in the open air or in a well ventilated area

- 9. How to avoid contamination of the exterior of the container
- 10. To wash their hands with soap after specimen collection
- 11. How to safely deliver the morning sputum to the laboratory as soon as possible after it is produced
- 12. The need for at least two sputum specimens to facilitate diagnosis

A Good Specimen

- Approximately 5 ml
- All samples should be inspected by clinic staff before sending to lab
- Sputum is usually thick and mucoid and color may vary
 - White, green or bloody
- May be fluid and contain pieces of purulent material

****CLEAR SALIVA OR NASAL DISCHARGE IS NOT A SUITABLE SPECIMEN**



Figure 2 – Saliva is not an acceptable specimen for TB diagnosis

Induced Sputum Samples

- Induced sputum samples are usually watery, but acceptable because they come from lungs
- The accompanied form should state '**induced sputum**'

Specimen Volume

- Specimens should be equal in volume to about two teaspoons of material
- IF specimen is inadequate:
 - the patient must be asked to repeat the procedure until an adequate quantity and quality of the specimen is obtained

Sputum containers

- Suitable sputum containers should be:
 - Wide-mouthed
 - Sterile
 - Disposable
 - Translucent
 - Leak-proof
 - Have a screw cap and a space for labeling on the side

Smear and Culture

- If the specimen is for smear and culture, it can be expectorated directly into a sterile 50 ml conical, screw-capped laboratory tube

- Re-usable glass, screw-capped universal containers may be used if the laboratory has a facility for sterilizing and cleaning the vials for re-use
- **All containers must be labeled with the patient's name and the date of specimen collection in indelible ink on the side of the container, not on the cap.

Sputum Samples

- Sputum samples must be collected and transported safely to avoid the risk of infection to clinic and lab staff or other handlers
- Containers must be capped firmly
 - any sputum noticed on the outside of the container must be wiped clean with bleach.
- Specimens should be packed upright in accordance with national requirements for transportation



Source: Madhukar Pai

Figure 1 – Sputum samples are usually thick and mucoid with varying color

Sputum Samples

- Forms must be kept separate from the specimens to avoid contamination
- Specimens should be transported to the laboratory as soon as possible
 - IF there is a delay of > 2hours, they should be refrigerated

Testing for MDR-TB

- The diagnosis of multi-drug resistant TB (MDR-TB) is usually based on molecular tests done on sputum samples
 - Genotype MTBDR plus by Hain Life Science
 - Xpert MTB/RIF by Cepheid
 - Liquid cultures
 - **MGIT** by BD
 - **BacT Alert** by bioMerieux

Specimens for Extra-Pulmonary Active TB

- The most common sites for **Specimens for Extra Pulmonary Active TB (EPTB)** are:
 - Lymph nodes
 - Pleural
 - Abdominal
 - Men-Ingel sites
- *Other sites can include bone and joints, kidneys, genitourinary tract, and pericardial.

EPTB Diagnosis

- **EPTB** cannot be diagnosed with sputum or blood specimens
- It is critical to make an effort to collect tissue and fluids from the site of the disease
- This may require surgical expertise and referral to a center where biopsies can be done safely

Common Diagnostic tests of EPTB samples

- The most common diagnostic tests on EPTB samples are:
 - Smear for acid-fast bacilli (AFB)
 - Liquid culture on fluids or tissue samples
 - Molecular (PCR) tests (e.g. Xpert MTB/RIF® by Cepheid)
 - Histopathological examination of biopsy tissue
 - Adenosine deaminase (ADA) or free interferon-gamma levels in sterile fluids (e.g. pleural, peritoneal and pericardial fluids)

- Smears are often negative in EPTB specimens because of the low numbers of AFB
 - Liquid cultures and histopathology results are therefore CRITICAL
- Molecular/ PCR tests are helpful if positive
 - However, if PCR tests are negative, EPTB cannot be ruled out
 - Molecular tests for EPTB are highly specific, but **sensitivity is not very high**
- Molecular tests for EPTB **should not** be performed on venous blood specimens!
 - They should be used on specimens from the site of the disease.

Specimens for Childhood TB

- Older children may be able to cough up sputum samples, but this is very difficult in young children
 - Young children tend to swallow sputum rather than expectorate them
- In young children (<7-8 years of age), the routine specimens collected are **2 to 3 fasting gastric aspirates** (gastric juice aspirate)
 - Usually cumbersome and requires hospitalization.

Table 2 – Specimen collection methods for childhood TB¹²

Specimen collection method	Problems/Benefits	Potential clinical application
Sputum	Not feasible in very young children; Assistance and supervision may improve the quality of the specimen	Routine sample to be collected in children >7yrs of age (all children who can produce a good quality specimen)
Induced sputum	Increased yield compared to gastric aspirate; No age restriction; Specialized technique, which requires nebulization and suction facilities; Use outside hospital setting not studied; Potential transmission risk	To be considered in the hospital setting on an in- or out-patient basis
Gastric aspirate	Difficult and invasive procedure; Not easily performed on an outpatient basis; Requires prolonged fasting; Sample collection advised on 3 consecutive days	Routine sample to be collected in hospitalized patients who cannot produce a good quality sputum specimen
Nasopharyngeal aspiration	Less invasive than gastric aspirate; No fasting required; Comparable yield to gastric aspirate	To be considered in primary health care clinics or on an outpatient basis
String test	Less invasive than gastric aspirate; Tolerated well in children >4 years; Bacteriologic yield and feasibility requires further investigation	Potential to become the routine sample collected in children who can swallow the capsule, but cannot produce a good quality sputum specimen
Bronchoalveolar lavage	Extremely invasive	Only for use in patients who are intubated or who require diagnostic bronchoscopy
Urine/Stool	Not invasive; Excretion of <i>M. tuberculosis</i> well documented	To be considered with novel sensitive bacteriologic or antigen-based tests
Blood/Bone marrow	Good sample sources to consider in the case of probable disseminated TB	To be considered for the confirmation of probable disseminated TB in hospitalized patients
Cerebrospinal fluid (CSF)	Fairly invasive; bacteriologic yield low	To be considered if signs of tuberculous meningitis
Fine needle aspiration biopsy (FNAB)	Minimally invasive using a fine 23G needle; excellent bacteriologic yield,	Procedure of choice in children with superficial lymphadenopathy; minimal side-effects

Adapted from: Marais BJ, Pai M. Specimen collection methods in the diagnosis of childhood tuberculosis. *Indian J Med Microbiol* 2006;24:249-251.¹²

Basic Guidelines for collecting Gastric Aspirates

- 1) Specimens are collected after the child has fasted for 8-10 hours
 - preferably, while the child is still in bed
- 2) Specimens are usually collected daily for 3 days

- There is no adequate “Gold Standard Test” for childhood TB
 - Diagnosis requires multiple tests
- Smears for AFB are often negative because of the low numbers of AFB in childhood TB.
- Liquid culture and molecular tests may be most helpful
 - Along with signs, symptoms, chest radiology, evidence of TB infection (e.g. positive Mantoux skin test), and history of contact with active TB.

Specimens for Latent TB Infection (LTBI)

- The goal of testing for latent TB infection is to identify individuals (e.g. close contacts of active TB cases) who are at increased risk for the development of active TB
 - Therefore, would benefit from treatment of latent TB infection
 - e.g. Isoniazid for 6-9 months, after active TB is ruled out
- Only those who would benefit from treatment should be tested, so a decision to test presupposes a decision to treat if the test is positive

Identification of LTBI

- There are two accepted tests for identification of LTBI:
 - The tuberculin skin test (TST)
 - The interferon gamma release assay (IGRA)

- As with the TST, IGRAs are surrogate markers of *Mycobacterium tuberculosis* infection and indicate a cellular immune response to *M. tuberculosis*
 - Both tests provide indirect evidence that the patient has been sensitized to *Mycobacterium tuberculosis* in the past
- **Neither** test proves that the patient has current active TB disease, and **SHOULD NOT** be used to diagnose active TB

- IGRAs require blood samples
- TST is an intra-dermal skin test (Mantoux technique)

IGRAs

- For IGRAs such as QuantiFERON-TB Gold[®], blood must be collected in special antigen-coated tubes and shaken after blood collection to ensure that blood comes into contact with TB-specific antigens
 - Blood tubes are incubated overnight
 - Supernatants are then assayed via ELISA for interferon-gamma levels
 - **It is important to strictly follow manufacturers' recommendations on IGRAs.
 - Delays in incubating the blood can cause loss in sensitivity and increase the rate of indeterminate results

TST

- TST should be performed using the Mantoux technique
 - Intradermal injection of tuberculin material (**0.1 ml of purified protein derivative - 2TU of PPD RT23**) on the inner surface of the forearm
 - A clear, raised wheal of **6-10 mm** diameter should appear when the PPD is slowly injected into the skin
 - The results should be read **48-72 hours** after administration, by a trained professional
 - Transverse induration should be measured in mm
 - ***Redness (erythema) is not measured**
 - An induration of **10 mm or more** is usually considered **positive for TB infection**

Common Specimen Related Errors

- The most common error in the Indian context is use of blood (instead of sputum) as the specimen for active pulmonary and extra-pulmonary TB diagnosis
- Indian labs not only perform blood tests like serology for TB, they also perform PCR tests on blood samples
- **Exception:** The use of blood culture or PCR for the diagnosis of disseminated TB in children or immune-suppressed persons
- More recent use of IGRAs (e.g. TB Gold) for active TB is another cause for concern that will need to be addressed by clinicians and laboratory professionals in India

Box 1. Frequently asked questions about the Indian governmental ban on TB serological tests, published in several Indian newspapers in December 2012 in India



Let Us Stop Malpractices in TB Diagnosis



Inaccurate Serological Blood Tests for Diagnosis of TB banned by the Government of India in Public Interest



MINISTRY OF HEALTH AND FAMILY WELFARE
(Department of Health and Family Welfare)
NOTIFICATION
New Delhi, the 7th June, 2012

G.S.R. 432(E).— Whereas the Central Government is satisfied that the use of the serodiagnostic test kits for diagnosis of tuberculosis are giving inconsistent and imprecise results leading to wrong diagnosis and their use is likely to involve risk to human beings and whereas safer alternatives are available:

And whereas the Central Government is satisfied that it is necessary and expedient to prohibit the manufacture, sale, distribution and use of the said test kits in public interest;

Now, therefore, in exercise of the powers conferred by Section 26A of the Drugs and Cosmetics Act, 1940 (23 of 1940), the Central Government hereby prohibit the manufacture for sale, distribution and use of the following test kits with immediate effect.

“Serodiagnostic test kits for diagnosis of tuberculosis”

Frequently asked questions on the notification

Q. What is the reason behind the ban?

ANS: There is proven scientific evidence that serodiagnostic tests for TB provide inconsistent and imprecise results despite high claims of its accuracy

**No More Deaths From TB
Together We Can Make India TB Free**

**Free Diagnosis and Treatment for TB is Available
For More Details Please Contact Concerned District TB Officer**

Q. What is the consequence of inconsistent and imprecise results?

ANS: The dependence on such unreliable tests can be harmful as many patients will end up undergoing TB treatment without any need for it as they are wrongly diagnosed as TB. At the same time, the test also misses many TB patients thus denying treatment at the right time. Such patients will continue to suffer and even spread the infection to other healthy individuals.

Q: What is meant by “serodiagnostic test kits” for tuberculosis?

ANS: Serodiagnostic tests for tuberculosis are tests that detect the antibody response to tuberculosis causing bacteria in blood samples of suspected tuberculosis patients.

Q. Is the ban applicable to Indian as well as imported TB serodiagnostic kits?

ANS: Yes, the ban is applicable to all kits manufactured in India as well as all types of imported kits.

Q. How can TB be detected if all blood tests have been banned? Are there any alternative tests available?

ANS: Government of India has approved the following tests for diagnosis of TB:

- Sputum examination under microscope
- Culture tests
- Newer molecular tests.

Q. What are Interferon-gamma release assays (IGRAs)?

ANS: IGRAs are laboratory blood test that measure the cell-mediated immune response of TB in infected individuals.

Q. In which situation should IGRAs not be used?

ANS: IGRAs blood tests have limited use as they cannot differentiate between active pulmonary TB disease and latent TB infection. Hence IGRAs should not be used as stand alone tests to detect active TB disease.

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Ministry of Health and Family Welfare, Government of India

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