REVIEW ARTICLE

Diagnosis of Childhood Tuberculosis

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Abstract

Tuberculosis (TB) in India accounts for about a quarter of the world's TB cases and children are at high risk of tuberculous infection and TB disease. TB diagnosis in children continues to be a challenge due to difficulty in getting appropriate samples and paucibacillary disease. In recently revised recommendations a major emphasis has been made on microbial diagnosis and more liberal use of molecular diagnostic modalities like cartridge-based nucleic acid amplification test (CBNAAT) and line probe assay. Cartridge based nucleic acid amplification test (CB-NAAT, GeneXpert,) is now considered as a World Health Organization (WHO)-recommended rapid diagnostic (WRD) and is a very simple test ,with a well-established role in the diagnosis of pulmonary tuberculosis (PTB) and extrapulmonary tuberculosis (EPTB) cases in comparison to AFB smear. CBNAAT should be offered upfront whereever TB is suspected. Drug sensitivity tests (DST) should always be attempted in all positive cases. For latent TB infection diagnosis intradermal TST is widely used however interferon- gamma release assays is also an equivalent option.

Keywords: Cartridge-based nucleic acid amplification test, Childhood tuberculosis, Diagnosis.

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Introduction

Tuberculosis (TB) is the ninth leading cause of death worldwide and the leading cause of a single infectious agent, ranking above HIV/AIDS. As per the Global TB report 2017, the estimated incidence of TB in India accounts for about a quarter of the world's TB cases and children are at high risk of tuberculous infection and TB disease.[1] India's Revised National Tuberculosis Control Program (RNTCP) provides guidelines for early case detection, effective management, and prevention of TB and its transmission. Guidelines have recently been revised, and the major emphasis has been made on microbial diagnosis and more liberal use of molecular diagnostic modalities like CBNAAT.

CBNAAT and Line Probe Assay were introduced in 2009 and scaled up from 2012 onwards. In 2017, 7,32,449 patients have been tested using these methods and 38,854 Rifampicin resistant/multidrug resistant (MDR) TB patients have been diagnosed.[2] Being one of the high burden countries for TB, almost 10% of total TB cases in India is Pediatric TB.[3]

Childhood TB Diagnosis: A Challenge

TB diagnosis in children continues to be a challenge due to many reasons, namely:

- Difficulty in getting appropriate samples,
- Paucibacillary disease,
- Relatively high incidence of extrapulmonary TB.

Key risk factors for TB in children (Table 1).

TB Diagnosis

The diagnosis of TB in children usually relies on a combination of clinical features and laboratory tests. TB should be suspected in any case having unremitting symptoms, that persists for more than 2 weeks.

Children having persisting symptoms beyond a period of 14 days, should be evaluated clinically as a case of suspected TB. A careful history and judicious analysis of symptoms and signs are very crucial to identify a presumptive case of TB. Presumptive pediatric TB is one which includes children with persistent fever and/or cough, loss of weight (loss of >5% body weight as compared to highest weight recorded)/no weight gain in last 3 months and/or history of contact with infectious TB cases. Clinical presentations of extrapulmonary TB (EPTB) are mentioned in Table 2.

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Laboratory Diagnosis of Tuberculosis

Direct Evidence

- Microscopy for detection of acid fast bacilli (AFB)
- Culture for isolation of M. Tuberculosis bacilli
- Nucleic acid amplification techniques (NAAT)
- Antigen detection

Indirect Evidence

- *Tuberculin skin test (TST):* Used as a complementary test along with history symptoms, signs and radiology.
- Interferon-gamma release assay (IGRA)
- *Antibody detection tests:* Serological tests for TB are banned by WHO since July 2011 and are not recommended for diagnosing TB.
- *Radiodiagnosis:* Chest X-ray is used as a screening tool. CT scan in very selected cases
- Cytology and histopathology
- *Others:* CBC,ESR,ADA
- *HIV counseling and testing:* All presumptive TB patients should be offered HIV counseling and testing. However, the diagnostic tests for TB should not be delayed.[4]

Specimen for Microbial Diagnosis[5]

Bacteriological diagnosis is a gold standard and is becoming increasingly crucial with the ever increasing burden of MDR/XDR tuberculosis.

Table 1: Risk factors of TB Infection and Disease

For TB infection	*For TB Disease*
Increased exposure • Living in TB endemic communities • Overcrowding • Air pollution including tobacco smoke • Families with HIV	*Young age* • Especially 0–2 years HIV infection Risk of infection and diseases
Source case • Cavitary disease/smear positivity • Cough frequency/cough hygiene • Delay in treatment of adult case	*Other immunosuppression* Severe malnutrition Postmeasles
	Not BCG vaccinated Risk of disseminated disease
Lack of contact screening	Lack of chemoprophylaxis when indicated
Contact with source case • Closeness of contact • Duration of contact	

*Adapted from Basic Traing module. Management of tuberculosis in childrenIAP-RNTCP consensus guidelines 2018–2019

Table 2: Extrapulmonary sites of TB manifestations

Site of EPTB	*Typical clinical presentation*	*Investigation*
TB lymphadenitis	Asymmetrical, painless, non-tender lymph node enlargement for more than one month +/– discharging sinus Most commonly in neck area	Fine needle aspiration LN biopsy
Pleural TB	Dullness on percussion And reduced breath sounds +/–	Chest pain CXR Pleural tap
TB meningitis	Headache, irritability /abnormal behaviour, lethargic /reduced level of consciousness, convulsions, neck stiffness, bulging fontanel, cranial nerve palsies	Lumbar puncture to obtain CSF Neuroimaging
Miliary TB -	Non-specific, lethargic, fever, wasted	CXR –
Abdominal TB	Painless abdominal swelling with ascites	Ascitic tap Abdominal ultrasound
Spinal TB	Painless deformity of spine—may have lower limb weakness/ paralysis	Lateral X-ray spine Imaging
Pericardial TB	Cardiac failure Distant heart sounds Apex beat difficult to palpate	CXR Ultrasound Echocardiogram Pericardial fluid tap
TB bone and joint (excluding spine)	Painless, non-tender swelling end of long bones with limitation of movement Painless, non-tender unilateral effusion of usually knee or hip	X-ray of affected bone and/or joint Local imaging study Joint tap Synovial biopsy

*Adapted from National Guidelines on Management of Tuberculosis in Children Ministry of Health Division of Leprosy, Tuberculosis and Lung Disease Second Edition August, 2013

Any sample can be used which one can obtain. For pulmonary TB, sputum is the most important sample for laboratory testing and it may be collected as sputum-induced or expectorated, or gastric aspirate (GA), For extrapulmonary TB, it is critical to obtain specimens from the site of disease, and this usually includes collection of tissue (biopsy) and/or body cavity fluids from the suspected disease site. Quality of specimens can often have a big impact on test results, and it is most vital to ensure quality in specimen collection, transport, and processing.

Gastric Aspirates

Young children can not bring out self expectorated sputum hence gastric aspirate is used in young children suspected of having pulmonary TB. During sleep, the mucociliary system of the lung pushes mucus up into the throat which is subsequently swallowed in the stomach. Therefore the first sample collected in the morning gives the highest yield. For this child should have been fasting for at least 4 hours (3 hours for an infant) before the procedure. The gastric aspirate quantity should be minimum 5 mL (ideally at least 5–10 mL) and should be collected into a sterile container. An equal volume of sodium bicarbonate solution is added to the specimen to neutralize the acidic gastric contents to prevent the destruction of tubercle bacilli. The specimen should be transported in a cool box to the laboratory for processing as soon as possible (within 4 hours), otherwise it should be placed in the refrigerator (4–8°C) and stored until transported. The diagnostic yield (positive culture) of a set of three gastric aspirates/ induced sputum is only about 25–50% of children with TB disease, so a negative smear or culture never excludes TB in a child. Table 3 summarizes detection threshold of various diagnostic modalities.

Most young children are not able to expectorate sputum hence alternatively the swallowed sputum is collected from the stomach after a period of fasting (usually 4–6 hours, preferably overnight) as gastric aspirate. An early morning gastric aspirate is a preferred specimen for most young children with presumptive TB. It needs overnight fasting; requires hospitalization and skilled staff. It requires centrifugation. It must be emphasized that gastric aspirates must be collected properly. Increasing evidence now suggests that GA can be collected in ambulatory settings after 4–6 hours of fasting with some compromise of the yield. The overall diagnostic yield of 3 gastric lavages or induced sputum has a wide range from 25% to 30% in a child with active TB disease. However since TB is a paucibacillary disease in children, a negative smear or culture cannot rule out active TB. Table 3 summarizes detection threshold of various diagnostic modalities.

Table 3: Detection threshold of different modalities

Method	*Limit of detection*
Smear	10,000 org/mL
Liquid culture	50–100 org/mL
CBNAAT	100–150 org/mL

Induced Sputum (IS)

Sputum induction is an aerosol-generating procedure, and hence if feasible, this should be performed in an isolation room that has adequate infection control precautions

Since induced sputum provokes cough and carries the risk of infectious aerosol particles, it is mandatory for the procedure to be carried out in an isolation room with appropriate infection control policies

As, GA is invasive, stressful, usually require the admission of children and overnight fasting. IS is less invasive than GA, takes less time and can be performed on outpatients. This technique is preferable to gastric lavage for the diagnosis of pulmonary tuberculosis in both HIV-infected and HIV-uninfected infants and children.[6]

Procedure

- Prenebulization with salbutamol is needed to prevent/restrict bronchoconstriction and wheezing which are likely to be induced by hypertonic sline.
- Administer nebulized hypertonic saline (3% NaCl) for 15 minutes or until 5 mL of the solution have been fully administered. This will cause liquefication of airway secretions and ease out the sputum collection.
- Carry out chest physiotherapy if necessary to mobilize secretions.
- For older children who are able to expectorate, ask them to expectorate sputum coughed up into the sterile jar.
- For children who are unable to expectorate (e.g., young children), carry out either suction of the nasal passages to remove nasal secretions; or nasopharyngeal aspiration to collect a suitable specimen.
- The procedure should be stopped when the patient has produced 1–2 mL of sputum is collected for each specimen, 15 minutes of nebulization completed and/or the patient feels uncomfortable.

A number of samples: Since 2009, the recommended number of samples are 2 (and not three), with one of them being a morning sample. This is because the yield with the third specimen is increased merely by 2–3% and omitting it will help reduce the number of the patient visit, lab workload, patient drop out and will be cost-effective.[7] Presence of at least one acid-fast bacillus in at least one sputum specimen is considered as a positive sample.

Children <5 years of age can not bring out sputum by self expectoration. They tend to swallow their sputum secretions; hence GA or IS are recommended as the preferred approaches to obtain respiratory secretions.[8] One of the South African studies carried out in children <5 years of age hospitalized with clinically suspected pulmonary tuberculosis reported that MTB culture positivity from a single IS sample was similar to that from 3 GA samples collected on consecutive days.[6] A more recent systematic literature review and meta-analysis estimated that 1–3 IS samples may identify 79% (95% confidence interval (CI), 62–92%) of culture-confirmed pediatric pulmonary tuberculosis cases identified through submission of comparator samples, including GA, pleural/lymph node culture, and nasopharyngeal aspirate samples.[9]

Bronchoscopy and bronchoalveolar lavage (BAL) can be used as a technique for specimen collection in very selected cases. It should not be used up front and as a standalone test as the yield is no better than GA. It is available at higher centers only and should be restricted in children with persistent pneumonia/nonresponding pneumonia as a part of work up. In sputum smear-negative and paucibacillary patients with clinico-radiological features of PTB Xpert® MTB/RIF has good sensitivity for diagnosis on BAL fluid. It is useful even when cultures are negative.[10]

Microscopy

Light microscopic examination of a smear using ZN stain is the cheapest, simple, point of care confirmatory test and it provides a direct microbial diagnosis. It is the most widely available test for diagnosing TB in resource-limited settings It is highly specific but sensitivity remains variable (20–80%), and it mainly depends on the bacillary load of the given specimen.[11]

Conventional fluorescence microscopy (CFM) using auramine staining is 10% more sensitive than the Ziehl-Neelsen and takes less time. It has limitations like the high cost of mercury vapor light sources, the need for regular maintenance, and the darkroom requirement.[12] In comparison, WHO recommended Fluorescent light emitting diode (LED) microscopy is cheap, requires less power, and less maintenance has a long half-life, does not require a dark room. It increases sensitivity by 6%. LED microscopy has qualitative, operational, and cost advantages over both conventional fluorescence and Ziehl-Neelsen microscopy with somewhat improved sensitivity. In 2011, WHO recommended that conventional FM needs to be replaced by LED, and LED microscopy can be used as an alternative for conventional ZN microscopy.[13,14]

Optimizing Yield

This can be achieved by various physical and chemical methods. The sensitivity of microscopy as compared to culture can be increased with concentration by centrifugation and/or sedimentation after pretreatment with chemicals such as bleach, NaOH, and NaCl or both, as compared to direct (unconcentrated) smear microscopy. A comprehensive, systematic review of 83 studies have found that concentration resulted in higher sensitivity (15–20% increase) and smear-positivity rate when compared with direct smears.

Remark

It is a standard statement from a guideline and can be quoted verbatim.[15] At concentrations below 1,000 organisms per milliliter of sputum, the chance of observing acid-fast bacilli in a smear is less than 10%,[16,17] whereas culture can detect far lower numbers of acid-fast bacilli (detection limit is about 100 organisms per mL). This implies that culture has a higher sensitivity than microscopy.

Use of Culture Methods to Isolate TB Bacilli

Culture still remains indispensable in a clinical Mycobacteriology Direct microscopic examination of sputum specimens, although specific, has low and variable sensitivity and cannot identify drug-resistant strains. Culture methods of TB diagnosis not only confirm the diagnosis but also provides an opportunity to test material for crucial drug susceptibility testing (DST).

Culture on solid media: Culture on a solid medium such as Lowestein Jenson medium (LJ medium) is most conventional and simple but is insensitive and takes.unacceptably long time, of 4–8 weeks. They also do not give drug sensitivity and currently obsolete due to available newer techniques which use the liquid medium. This includes the BACTEC radiometric assay, speti-check AFB system, and mycobacterial growth indicator tube system (MGIT). These liquid systems are more sensitive, increase the yield by 10% more than solid medium, and are very speedy, give results in 10–15 days only. BACTEC radiometry is a liquid media, which gives results in 9–14 days, but is costly and requires the need for safe disposal of radioactive materials. MGIT culture method is a simple, rapid, and reliable method of Mycobacterial culture without any radioactivity hazard. World Health Organization has recommended liquid culture systems for low and middle-income countries as well.[18]

Mycobacterial Growth Indicator Tube System

MGIT is a system that determines whether or not TB bacteria will grow in the presence of TB drugs. It detects oxygen depletion by MTB using the fluorescent compound. The MGIT consists of liquid broth medium and contains modified Middlebrook 7H9 broth base. The MGIT tube contains an oxygen-quenched fluorochrome, tris 4,7-diphenyl-1, 10-phenanthroline ruthenium chloride pentahydrate, embedded in silicone at the bottom of the tube. During bacterial growth within the tube, free oxygen is used and is replaced with carbon dioxide. The decrease of free oxygen produces a fluorescence within the MGIT tube when visualized under UV light.[19] MGIT is now considered to be the standard method and is widely used. The mean time for MTB detection is 12.9 days with BACTECMGIT 960 and 15 days with BACTEC 460.[18] As per one study, the mean time to the detection of M. tuberculosis complex was 9.9 days with MGIT, 9.7 days with BACTEC, and 20.2 days with solid media.

In published data by Gaby et al. media concluded that the median time interval was shorter in liquid cultures (MGIT) as compared to solid media (LJ) 9.9 *vs.* 20.2 days.[20]

Due to increased sensitivity, liquid culture media are having higher contamination rates than solid media and NTM are more frequently isolated with liquid media than with solid media.[21] Hence all mycobacterial isolates from solid or liquid cultures must be identified to allow differentiation of the M. tuberculosis complex from NTM. The current guideline recommends that all specimens cultured on liquid media also be inoculated on solid media to ensure purity and sufficient strength for the diagnosis

Cartridge-based Nucleic Acid Amplification Testing (CB NAAT)

Cartridge-based nucleic acid amplification test (CB-NAAT, GeneXpert,) is now considered as a WHO-recommended rapid diagnostic (WRD) test. As per published guidelines by WHO, CBNAAT is the first test of choice for diagnosing all forms of pediatric TB with or without HIV coinfection.[21] It is an automated test, which not only detects MTB but also gives additional information on Rifampicin resistance within a time frame of 2 hours. A variety of respiratory and non-respiratory specimens (BAL, tracheal aspirates, LN biopsy, tissue biopsy, pus, CSF) can be fed into the cartridge for MTB detection. As compared to conventional microscopy, gene expert scores over with a higher sensitivity since it recognizes the DNA of MTB. Samples such as blood, urine, and stool are not suitable for processing via CBNAAT. Since pediatric TB is a paucibacillary disease a negative GeneXpert does not rule out active disease OR other species of mycobacterial causing disease. It is simple, rapid, cost-effective and doesn't require technical expertise and due to use of disposable closed cartridges, it prevents cross contamination.[22] It can be carried out in an automated manner, including bacterial lysis, nucleic acid extraction, and amplification and amplicon detection. CBNAAT is an extremely useful, simple, and reliable test in resource restricted settings where culture and DST are not available.

CBNAAT is used for respiratory specimens such as sputum, bronchial or tracheal aspirates, broncho-alveolar lavage, and gastric lavage. It can also be performed on non-respiratory specimens like tissue biopsy material, lymph node, pus from an abscess, CSF, ascitic and pericardial fluid, pleural fluid.[23] CB-NAAT is not recommended for samples such as stool, urine, and blood.[24] It provides a positive yield of 60–70% in culture positive cases, with a detection threshold of 130–150 cfu/mL.[25] For cultures; the threshold for detection is low (10–100 cfu/mL compared to 130–150 cfu/mL for CB-NAAT). Hence a negative GeneXpert result does not rule out TB, and a child can still have TB with MTB or mycobacteria other than tuberculosis (MOTT) species.

Secondly, this test, being PCR based, amplifies any DNA, whether live or dead bacilli and gives false positive results. So it can give a false positive result and hence a positive GeneXpert, but culture negative results should be well correlated with clinical and treatment history of the patient.[26] GeneXpert also has a higher sensitivity than AFB smear microscopy in respiratory samples. Table 3 summarizes detection threshold of various diagnostic modalities.

In pulmonary TB (PTB), pooled sensitivity of CBNAAT, when testing smear positive and smear negative samples, was 98% (95% CI, 97–99%) and 68% (95% CI, 59–75%), respectively, and a pooled specificity of 98% (95% CI, 97–99%).[27] When used as an initial diagnostic test replacing smear microscopy, Xpert MTB/RIF achieved an overall pooled sensitivity of 88% and a pooled specificity of 99%. When used as an add-on test following a negative smear-microscopy result, Xpert MTB/RIF showed a pooled sensitivity of 68% and a pooled specificity of 99%. For smear-positive culture-positive TB, the pooled sensitivity of Xpert MTB/RIF was 98%, for smear-negative culture-positive TB, the pooled sensitivity was 68%. For people living with HIV, the pooled sensitivity of Xpert MTB/RIF was 79%, for people without HIV infection, the pooled sensitivity was 86%. For the detection of rifampicin resistance, Xpert MTB/RIF achieved a pooled sensitivity of 95% and a pooled specificity of 98%.[28]

Type of test	*Pooled sensitivity*	*Pooled sensitivity*
Initial diagnostic test	88%	99%
Add-on test	68%	99%
Detection of RR	95%	98%

Type of patient	*Sensitivity*	*Specificity*
Smear and culture positive	98% (pooled)	–
Smear-negative and culture positive	68% (pooled)	–
HIV positive	79% (pooled)	–
HIV negative	86% (pooled)	–

In case of extrapulmonary TB, sensitivity is extremely heterogeneous, CBNAAT has proved to have higher sensitivity with lymph node samples (88.3%, CI82–95), other tissue samples (75%) and cerebrospinal fluid (85.7%, CI67–100) as compared to the results of testing pleural fluid (44.4%,CI 21–67) and other

Table 4: Advantages and disadvantages of CBNAAT

Advantages	*Disadvantages*
Can detect TB and rifampin resistance at the same time„	Requires stable uninterruptable electricity (not suitable for regions with power cuts)
Rapid (results in in less than two hours)„	Operating temperature should not exceed 30°C and cartridge must be stored at less than 28°C (in an air-conditioned room)
High sensitivity (88%) and high specificity (99%) when compared to liquid culture in sputum samples„	Cartridges' shelf life must be monitored or are prone to being wasted (MTB/RIF's shelf life is currently 22 months; Ultra, if approved, will likely have a shorter shelf life)
Low biosafety level requirements (compared to culture) and similar to microscopy„	Security measures must be put in place to avoid theft of laptop or desktop computer„
Minimal training of personnel„	Cannot be used to monitor TB treatment„
Can detect both pulmonary TB and EPTB„	The instrument's accuracy needs to be checked (calibrated) every year
Same system can be used for other conditions such as early infant HIV diagnosis and viral load monitoring (pending WHO approval	

serous fluids (50%, CI 19–81).[27] Hence a negative CBNAAT does not rule out TB in all cases. The lower sensitivities in pleural and nonpleural serous fluid reflect low bacillary load in these samples. In comparison to the pleural fluid analysis of pleural biopsies gives a better microbiological diagnosis as well as permits histological evaluation.[29] Major advantages and disadvantages of CBNAAT are described in Table 4.

Though the Xpert MTB/RIF test provides rapid identification of rifampicin-resistant M. tuberculosis, its results need to be confirmed by culture and drug susceptibility testing (DST) to detect additional drug resistance. Conventional microscopy and culture are also essential for monitoring therapy and for performing DST for anti-TB agents other than rifampicin

Although GeneXpert provides faster detection of RR-MTB, it does not displace the need for conventional microscopy and culture DST, which is pivotal in monitoring treatment and detecting resistance patterns for other drugs as well. (including for isoniazid and second-line anti-TB drugs).[30] Line probe assays (LPAs) are rapid methods, which test for resistance to rifampicin and isoniazid (referred to as the first-line LPAs); a rapid LPA that tests for resistance to fluoroquinolones and injectable anti-TB drugs (referred to as a second-line LPA); and sequencing technologies. WHO first recommended First-line LPAs in 2008; and the second-line LPA in May 2016. Culture-based methods currently remain the reference standard for drug susceptibility testing.

In spite of these very few limitations, CB NAAT offers very good specificity, reasonable sensitivity and has a very short turn- around time and additionally imparts status of rifampicin resistance.[31]

Xpert MTB/RIF Ultra[32]

In smear-negative culture-positive specimens, pediatric specimens, extrapulmonary specimens (notably cerebrospinal fluid) the Ultra cartridge provides better performance for the detection of MTB and is also useful in testing smear-negative culture-positive specimens from HIV-positive individuals as well. In the evaluation of HIV children, with culture positive and smear negative scenarios. Currently WHO recommends that UltraGenexpertcan be used as the initial diagnostic test(like Xpert MTB/RIF) for all adults and children with signs and symptoms of TB and in selected extrapulmonary specimens (CSF, lymph nodes and tissue specimens). The current guidelines laid down by the WHO recommends the use of the ULTRA cartridge for detection of extrapulmonary TB in symptomatic adults and children.

Line Probe Assay

LPAs are WHO-approved tests for rapid detection of drug resistance to the first line (FL-LPA)- and second-line agents (SL-LPA).[33] They can be used for testing of culture isolates (indirect testing), as well as direct testing of acid-fast bacilli (AFB) smear-positive specimens (FL-LPA), and both smear positive and smear negative sputum specimens (SL-LPA).[33,34] LPA is suitable for use at national/central reference laboratories or those with proven capability to conduct molecular testing. LPAs also help differentiate *M. avium*, *M. intracellulare* and *M. kansasii* from MTBC and from other non-tuberculous mycobacteria.[33,34] LPA helps in species differentiation especially MTBC from *M. Intracellulare*, *avium* and *kansasi*, however, this sophisticated method commands appropriate laboratory infrastructure and capacity for processing and manipulating of TB cultures. Adequate and appropriate laboratory infrastructure and equipment must be available which needs facilities for specimen processing for culture and manipulation of cultures with appropriate biological safety cabinets.

FL-LPA showed sensitivity and specificity for the detection of rifampicin resistance of 96.7% and 98.8%, respectively. For INH resistance, it showed a sensitivity and specificity of 90.2% and 99.2%, respectively.[35,36] SL-LPA (Genotype MTBDRsl V1) showed a pooled sensitivity and specificity for the detection of fluoroquinolone resistance by direct testing of 86.2% and 98.6%, respectively, and a pooled sensitivity and specificity for the detection of second-line injectables drugs resistance of 87.0% and 99.5%, respectively.[33]

WHO recommends the use of the SL-LPA for patients with confirmed rifampicin-resistant TB or MDR-TB as the initial test to detect resistance to fluoroquinolones and the second-line injectable drugs, instead of phenotypic culture-based drug-susceptibility testing (DST). However, it will not eliminate the need for phenotypic DST to confirm resistance to other drugs and to monitor the emergence of additional drug resistance during treatment.[34-36] The SL-LPA produces results in just 24–48 hours compared to the 3 months or longer duration currently required.[37] Mean turnaround time of various diagnostic tools are described in Table 5.

TB-LAMP

WHO recommends that loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis.[38] It can also be considered as a follow-on test to microscopy in adults with signs

Table 5: Mean turnaround time

CBNAAT—2 hours
Line Probe Assay (LPA—three days (72 hrs)
Liquid culture and DST (MGIT)—75 days (two and half months)
Solid culture and DST technology—120 days (four months)

and symptoms of pulmonary TB, especially when further testing of sputum smear-negative specimens is necessary.TB-LAMP is a manual assay. It requires less than one hour to perform and can be read with the naked eye under ultraviolet light.

Drug Susceptibility Testing

Drug susceptibility of M. tuberculosis can be determined by means of (a) macroscopic observation of growth in drug-free and drug-containing media; (b) detection or measurement of the metabolic activity or products; (c) lysis with mycobacteriophage; (d) detection of genetic mutations using molecular techniques.

Commercial liquid culture systems and molecular line-probe assays are the gold standard and endorsed by the WHO for rapid detection of MDR TB.

Liquid cultures are superior over conventional LJ cultures (solid media), however sophisticated instrumentation and huge investment for infrastructure are the limitations of its widespread use.

But they carry limitations in terms of technical complexity, cost and the requirement for sophisticated laboratory infrastructure. Use of these techniques is limited in many resource-constrained settings.

Hence several noncommercial cultures and drug susceptibility testing (DST) methods have been developed. They are as follows:

- *Microscopic observation drug susceptibility testing (MODS)*: MODS can be performed as a direct or an indirect test, by observing microcolony growth and typical cord formation of *M. tuberculosis* in sealed microtitre plates containing liquid culture medium, through an inverted microscope. MODS is highly sensitive (pooled estimate, 98%; 95% CI, 95–99%) and specific (pooled estimate, 99%; 95% CI, 96–100%) for the detection of rifampicin resistance and slightly less so for isoniazid (pooled sensitivity, 91%; 95% CI, 87–95%). In comparison with the conventional indirect proportion DST method on LJ medium, MODS requires additional staff skills, an additional inverted microscope and additional consumables that may be difficult to obtain. In a smear-negative specimen, conventional liquid culture is required before using MODS and hence its utility in paucibacillary diseases is likely to be low.
- *Nitrate reductase assay (NRA)*: It is liquid or solid medium based method relying on the ability of MTBC to reduce nitrate to nitrite which can be detected using Griess reagent. All MTBC cannot reduce nitrates, and hence negative results may require further testing. NRA done on smear-positive samples provides detection of MDR in just 6–9 days. It can be applied as a direct or indirect method. Its sensitivity and specificity for INH is 97% and 99%, respectively and for rifampicin 97% and 100% respectively. Smear-negative samples require a further test with solid medium and turn around time for MDR TB detection is 7–11 weeks.[39]
- *Thin-layer agar (TLA):* It is a direct method on solid culture, based on inoculation of specimens into drug-free and drug-containing media, followed by microscopic examination of early growth. The growth results are available in seven days with DST results within 10–15 days. It is not as sensitive as liquid media but specificity for INH and rifampicin resistance has been reported to be 100 %.
- *Colorimetric redox indicator (CRI) methods*: It is an indirect method based on the reduction of a colored indicator added to liquid culture medium on a microtitre plate after exposure of Mycobacterium tuberculosis strains to anti-TB drugs *in vitro*. Different indicators which are commonly used include tetrazolium salts and resazurin. Its sensitivity and specificity is nearly 96–98% for INH and rifampicin.
- *Phage-based assays*: Assays in which bacteriophages are used to infect and detect the presence of viable *M. tuberculosis* in clinical specimens and culture isolates. Turnaround time is only 2 days false overdiagnosis of RIF resistance is quite common.

Considerable evidence is available for the use of CRI methods, MODS, and NRA, in reference laboratories. They are used under the clearly defined program and operational conditions and strict laboratory protocols, and as an interim solution while capacity for genotypic or automated liquid culture and DST is being developed.[40,41] Summary of WHO recommended diagnostic tools are given in Table 6.

Indirect methods for TB diagnosis

Tuberculin Skin Test (TST) or Mantoux Test (MT)[42]

It is an intradermal immunological test carried out by injecting tuberculoproteln, i.e., purified protein derivative (PPD) which elicits the delayed type of hypersensitivity response till date different tuberculin proteins have been used as under:

- *Old tuberculi*: not now used
- PPD S is used in the developed world like the US. 5TU PPD-S has shown the best discriminatory power between infected and noninfected cases.

Table 6: Summary of WHO recommended diagnostic tools[41]

1. *LED microscopy*: Can be used at all laboratory levels as replacement of conventional fluorochrome and light microscopy.
2. *Automated real-time nucleic acid amplification*: Xpert MTB/RIF system: For rapid detection of pulmonary and extrapulmonary TB and for detection of rifampicin resistance.
3. *Commercial liquid culture and DST systems*: For use at central/regional reference laboratory level, as current reference standard.
4. *Rapid speciation strip technology*: Speciation is necessary to differentiate M. tuberculosis complex and other mycobacteria grown in cultures. This test can detect a TB-specific antigen (MPB64) from positive liquid or solid cultures to confirm the presence of organisms belonging to M. tuberculosis complex.
5. *Commercial molecular line probe assays for 1st-line anti-TB drugs*: for rapid detection of rifampicin (alone or with isoniazid) resistance. Suitable for use on smear-positive specimens or culture isolates.
6. *Selected noncommercial DST methods: (MODS, NRA, CRI)*: For conditional use at central/ reference laboratory level for detection of rifampicin resistance only. MODS and NRA suitable for use on smear-positive specimens or culture isolates, CRI suitable for use on culture isolates only.

- Rest of the world uses PPD RT 23 currently and 5 TU of PPD-S is equivalent to 2 TURT 23 TWEEN 80.
- Hence PPD RT23 with Tween 80-2TU, which is equivalent to 5 TU PPD (plain) is the recommended strength currently.

» The test result is read as mm of induration in the transverse diameter of the forearm.

» Induration of >10 mm after 48–72 hours is considered as a positive test. If the patient reports beyond 72 hours but within 7 days, a positive test should be considered as positive but the negative test is to be repeated on another forearm. If the patient reports after 7 days for TST reading, the test should be repeated on another forearm.

» A positive TST indicates that a person is or was infected with M. tuberculosis but does not necessarily indicate the presence of TB disease. At the same time, negative TST does not rule out TB. It is a test that measures immune response, not the presence/absence of bacteria. It may, therefore, be used as an adjunct in diagnosing TB in children with signs and symptoms of TB and in conjunction with other diagnostic tests. The TST can also be used to screen children exposed to TB (such as household contact with TB)

» In children with HIV and other immunocompromised states, induration of >5 mm is considered as a positive TST.

» If TST is positive one should rule out active disease and search for a contact. There are many false-negatives and positives (Table 7) in Tuberculin test and one should be very careful in its interpretation.

Interferon-gamma Release Assays (IGRA)

They are *in vitro* tests to detect the gamma interferon (IFN-γ) released by activated T lymphocytes.[43]

Table 7: TST: False-positive and -negative

False-negative	*False-positive*
Incorrect administration or interpretation of test	Incorrect interpretation of test
HIV infection	BCG vaccination
Improper storage of tuberculin	Infection with non-tuberculous mycobacteria
Viral infections (e.g., measles, varicella)	
Vaccinated with live viral vaccines (within 6 weeks)	
Malnutrition	
Bacterial infections (e.g. typhoid, leprosy, pertussis)	
Immunosuppressive medications (e.g. corticosteroids)	
Neonatal patient	
Primary immunodeficiencies	
Diseases of lymphoid tissue (e.g. Hodgkin disease, lymphoma, leukemia, sarcoidosis)	
Low protein states	
Severe TB	

National Tuberculosis Programs on the Management of Tuberculosis in Children. 2nd edition. Geneva: World Health Organization; 2014

These assays use antigens specific for M. tuberculosis, such as early secretory antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), which are two low-molecular-mass secretory proteins encoded by genes located within the region of difference I (RD 1) of the M. tuberculosis genome.[44] IGRAs are more specific than the TST because contain only two or three antigens compared with the hundreds of antigens in tuberculin used in the TST, and they do not become positive because of previous BCG vaccination or infection with most nontuberculous mycobacteria as does the TST. Therefore, the IGRAs are especially advantageous for children who have or may have received a BCG vaccine.

Two types of tests are available:

- Quantiferon TB Gold in tube
- ELIS SPOT-TB.

Quantiferon TB Gold in Tube

It is an enzyme-linked immunosorbent assay (ELISA) that measures the production of interferon gamma (IFN-γ) by T-cells after sensitization with M. tuberculosis antigens using whole blood.[45] QuantiFERON-TB Gold In-Tube is one of only two commercially available IGRAs that were reviewed and recommended by the WHO in its 2018 guidelines. WHO 2018 guideline supports IGRA testing globally for at-risk populations—removing its 2011 guidance against interferon-gamma release assays (IGRAs) in low and middle-income countries.[46]

Advantages and Limitations

- Single patient visit
- Unaffected by prior BCG vaccination
- 95% test sensitivity
- Highest specificity of any test for TB infection
- Less reliable below 4 years
- Similar to MT—cannot distinguish between infection and disease.

QuantiFERON-TB Gold Plus (QFT-Plus)

QFT-Plus employs innovative CD8 T cell technology, providing a more complete picture of a patient's immune response to TB.

Clinical implications of TST and IGRA

Latent tuberculosis infection can only be diagnosed by a positive TST or a positive IGRA result. Latent (asymptomatic) tuberculosis is defined as infection by *Mycobacterium tuberculosis* in a person with a positive tuberculin skin test (TST) or positive IGRA without any physical evidence of disease, a normal chest X-ray or an X-ray with a healed lesion. Nearly 10% of persons with latent TB will develop active tuberculosis, emphasizing the importance of identifying and treating the enormous reservoir of asymptomatically infected people.[47] The sensitivity of the IGRAs, especially for children less than 2 years of age is less who have a much higher risk of rapidly progressing to severe TB disease soon after infection. IGRAs do lack sensitivity for children with advanced tuberculosis disease, even in the absence of an underlying cause such as HIV infection.[48] Thus IGRA lacks sensitivity just like TST.

Radiology in TB Diagnosis

Chest X-ray

Diagnosis of tuberculosis based only on X-ray is now described as 'clinically diagnosed tuberculosis. Some of the X-ray findings are useful to clinch the diagnosis (Figs 1 to 3). The most common picture in young children are persistent opacification (not responding to antibiotics) in lung together with enlarged hilar or subcarinal lymph glands, others are collapse-consolidation, a miliary pattern of opacification and cavitary lesions. Adolescent patients with TB will have radiographic changes similar to adult patients which includes large pleural effusions , apical infiltrates with cavity formation and bronchogenic spread of TB. However, adolescents may also develop primary disease with hilar adenopathy and collapse lesions. The specificity of X-ray as a diagnostic tool increases with positive TB symptoms and positive TST.

CT Chest

Indicated only in selected cases as a work-up for pyrexia of unknown origin, persistent lung opacity, nonresolving pneumonia, mediastinal or hilar mass. CT guided lymph node biopsy is increasingly used with the availability of this kind of facilities.

CT/MRI Brain

Basal meningitis, hydrocephalus, tuberculoma, infarcts.

Hematology Findings

- CBC ESR is nonspecific and will never help in TB diagnosis. High total WBC count with lymphocytic predominance is a common association.
- ESR is usually remained high but has many variables and is highly nonspecific
- *Adenosine deaminase (ADA):* ADA is an enzyme produced by Lymphocytes in the presence of intracellular tubercle bacilli. The reported cut-off value for ADA varies and is quite different. It has a different variable for different body fluids. It has a sensitivity of 90% and a specificity of 89% in adults but in children around 65–70%. It is not at all recommended for TB diagnosis at least in children.[49]
- *HIV testing:* Routine HIV testing should be offered to all patients, including children, with presumptive and diagnosed TB.

Investigations Relevant for Suspected Extrapulmonary TB

Extrapulmonary TB will be suspected from the clinical picture and confirmed by imaging, histology or other special investigations.

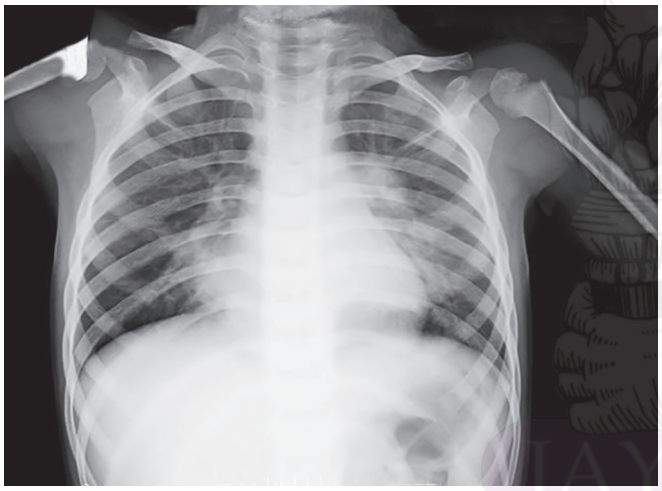


Fig. 1: Left hilar

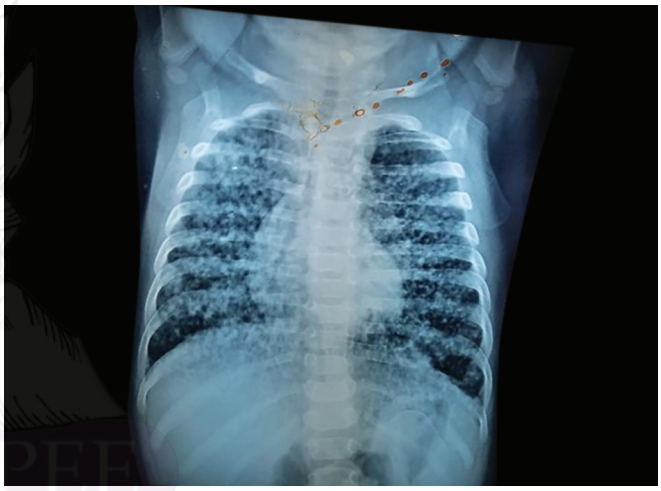
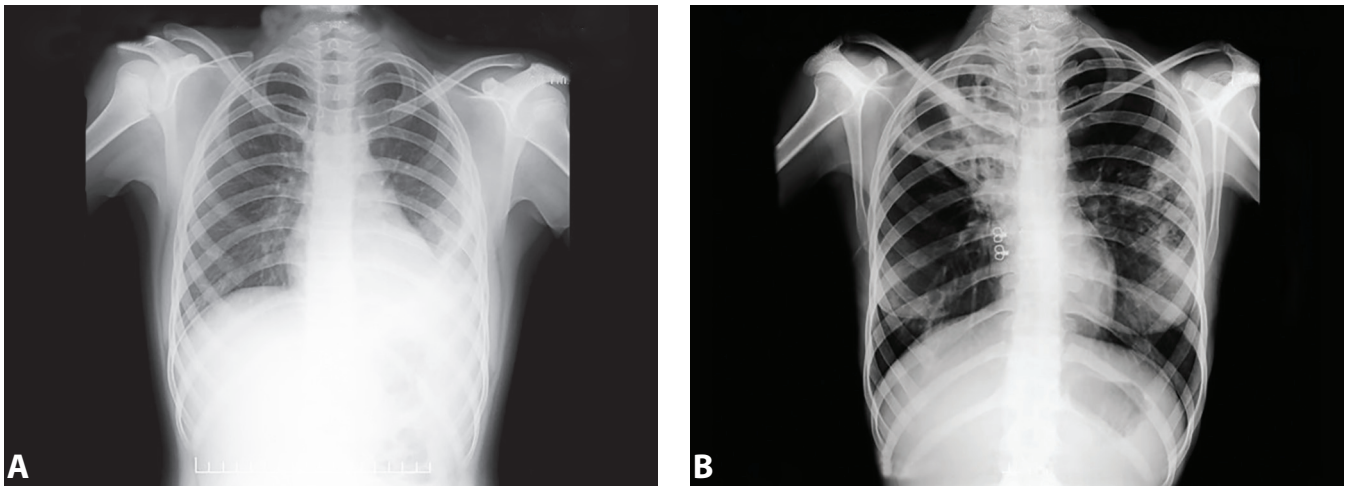


Fig. 2: Miliary TB



Figs 3A and B: TST: False-positive and -negative

This includes some of the specialized tests like:

- Computerized tomography
- Endoscopy with biopsy
- Cytology and histopathology—the presence of granuloma, caseation are highly suggestive of TB

Conclusion

Diagnosis of childhood TB includes pertinent symptoms,presence of genuine source contact collection of at least 2 sputum samples (spot and early morning), followed by sputum smear microscopy (both conventional Ziehl-Neelson staining/fluorescent staining), Real-time PCR based CB-NAAT ,culture (on solid or liquid media using manual or automated machines like BacTAlert, MGIT)and conventional PCR based line probe assay (LPA for Mycobacterium Tuberculosis complex). For latent TB infection diagnosis, intradermal TST is widely used; however, interferon-gamma release assays is also an equivalent option. CBNAAT should be offered upfront where ever TB is suspected. DST should always be attempted in all positive cases.

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