



Pediatric Tuberculosis

Tuberculosis (TB) is a major cause of childhood morbidity and mortality worldwide. The World Health Organisation (WHO) estimates are that 1 million HIV negative children (<15 years) currently suffer from TB worldwide, and that 239,000 die each year. Children who have TB/HIV co-infection are internationally classified as having died from HIV. A 2016 study estimated that 67 million children have latent TB. In India, pediatric cases represent 6-10% of TB burden of India. About 5.5 lakhs of new cases & 80,000 TB related deaths occur annually in children.

India also has a large burden of multi-drug resistant (MDR) TB and extensively drug resistant (XDR) TB. It is estimated that more than 30,000 children in the world become sick every year with strains of MDR TB. Also, a Revised National Tuberculosis Control Program (RNTCP) survey in India, found that 9% of children with TB were already resistant to rifampicin, before they started treatment. This means that they had become infected with drug resistant (DR) TB.

Progression of Tb Infection to Disease

Unique aspect of TB in children is the imperceptible and often rapid progression of infection to disease. Age <3 years, HIV co-infection & severe malnutrition are the 3 most important risk factors for progression. Risk of developing active disease is 43% below 1yr, 24% below 5 yrs and least in 5-10 year age group. More than 95% of children, who progress to disease, do so within the first 12 months of primary infection.

The most common type of TB disease in children is pulmonary TB but extra pulmonary TB (EPTB) occurs in approximately 20-30% of all cases in children. Infants and young children are at particular risk of developing severe, disseminated and often lethal disease due to immature immunological response. Lymphadenopathy is the most common (67%) extra pulmonary manifestations, followed by central nervous system involvement (13%), pleural (6%), miliary and/or disseminated (5%), and skeletal TB (4%). Disseminated (miliary) disease and TB meningitis are usually found in young (<3 years) and/or HIV infected children. Adolescents are at particular risk of developing adult type disease (i.e. they are often sputum smear positive and highly infectious).

Diagnosis of TB in Children

Diagnosing TB in children is difficult as children are less likely to have obvious symptoms of TB. Hence, it is recommended that evidence in the following categories is collected and carefully considered before a diagnostic decision is made.

Type of evidence	Evidence to be collected
Clinical	Careful history (including TB contacts; symptoms consistent with TB), Physical examination (including growth assessment), HIV testing (in high HIV prevalence areas)
Non-microbiological	Tuberculin skin testing (TST), Other investigations relevant for pulmonary or EPTB (e.g. X-rays)
Microbiological	Bacteriological confirmation whenever possible

Case Finding

Identification of presumptive TB by a symptom based approach is the most important part of case finding.

1. Pediatric Presumptive TB: Refers to persistent fever and/or cough >2 weeks, loss of weight (loss of >5% body weight as compared to highest weight recorded), haemoptysis, and any abnormalities in chest radiography.

2. Presumptive DR TB: Patients who failed treatment with first line drugs, who are contacts of MDR TB or Rifampicin (RIF) resistant TB, previously treated TB and TB patients with HIV co-infection are to be evaluated for DR TB.

All efforts should be made to microbiological confirmation in every presumptive TB case.

Laboratory Diagnosis of Pediatric TB

Smear Microscopy for AFB: Samples such as sputum are more difficult to collect from young children since they are unable to expectorate. Also, even when sputum samples are available either by induction or spontaneous collection method, TB in children is mostly smear negative because pediatric TB disease is paucibacillary in nature. Conventional Ziehl-Neelsen (ZN) stain method and LED based fluorescent microscopy are usually used under RNTCP. However, the sensitivity of smear microscopy in children is less than 15% even with advances in performance of smear microscopy such as concentration of specimens by centrifugation and the use of the relatively newer fluorescence microscopy with auramine-phenol staining. Another significant drawback of AFB smears is that they cannot be used to differentiate Mycobacterium tuberculosis (MTB) from other acid-fast organisms viz. other mycobacterial organisms or Nocardia species. So in children and patients living with HIV (PLWHIV), smear microscopy is now advised only if nucleic acid amplification test (NAAT) is not readily available.

Culture: Culture of mycobacterium is a definitive method to detect bacilli. It is also more sensitive than examination of smear. Another advantage of culture is that it allows specific species identification and testing for recognition of drug susceptibility patterns. Löwenstein-Jensen (LJ) medium is a solid culture medium, while BACTEC-460TB, MGIT-960, BacT Alert, VersaTREK, etc. are automated liquid culture medium. Liquid medium has a yield 10% more than solid media and reduced time to result from weeks to days. However, the turnaround time for results of 2-3 weeks for liquid cultures and even longer for solid cultures, make it of limited use in guiding early therapeutic decision making. The culture system is also prone to contamination. As a consequence, bacteriological confirmation of disease by culture of MTB in children seldom exceeds 30% even when using gastric aspirates, induced sputum, liquid culture media, and molecular diagnostic tools.

Nucleic Acid Amplification Test (NAAT): These are molecular diagnostic tests like polymerase chain reaction (PCR), loop mediated isothermal amplification technology (LAMP), and cartridge based nucleic acid amplification test (CB-NAAT). Line probe assay (LPA) and Xpert MTB/RIF are the two CB-NAATs endorsed by the WHO. LPA detects MTB complex, RIF & Isoniazid (INH) resistance. GeneXpert is the available Xpert MTB/RIF test under RNTCP. It detects tubercle bacilli and RIF resistance from both sputum and specimen from extra pulmonary sites.

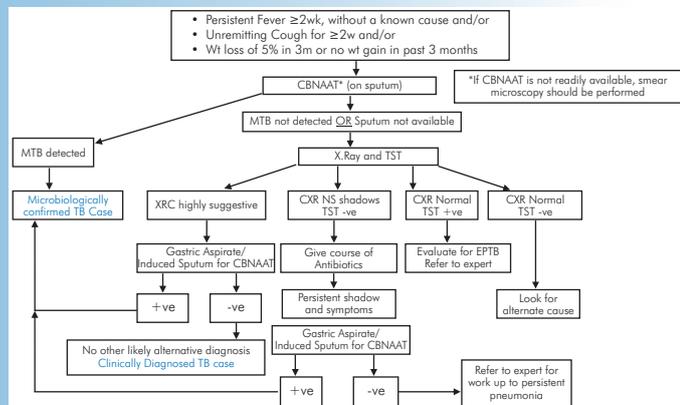
Currently Xpert MTB/RIF is the first line bacteriological test recommended by RNTCP and WHO in suspected DR TB, presumptive TB in children, EPTB and PLWHIV. Although the sensitivity and specificity of NAAT in smear-positive cases exceed 95%, the sensitivity of smear-negative cases varies from 40% to 70%. Thus, discordance between the acid-fast smear result and NAAT requires careful clinical appraisal and judgment.

Interferon Gamma Release Assays (IGRAs): Do not distinguish between active disease and latent TB infection and is used in low prevalence countries to detect latent TB infections. Not recommended in children either by RNTCP or by Indian Academy of Pediatrics (IAP).

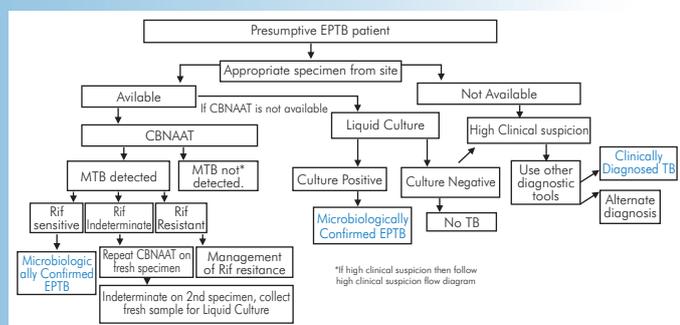
Serologic Tests: Not useful in detection of TB in children and these tests are banned in India.

Diagnosis of Lymph Node TB: Either FNAC or biopsy is needed. Specimen should be sent for cytology (for granuloma) and bacteriological tests like AFB smear, AFB culture and currently GeneXpert is the preferred test. Lymph node TB should not be treated without a tissue or bacteriologic diagnosis. Chest X-ray (CXR) may show findings in 5-40% of cases and tuberculin skin test (TST) positivity in >70% of cases.

According to RNTCP all diagnosed cases of TB should be offered HIV testing after counselling.



Diagnostic algorithm of paediatric TB (JACP 2017)



Diagnostic algorithm of EPTB (JACP 2017)

Diagnosis of Drug Resistant (DR) TB

DR TB is a laboratory based diagnosis and is performed in the specimen by either of the following methods:

- 1. Phenotypic** Drug Susceptibility Testing (DST) using solid LJ medium/ liquid culture (MGIT)
- 2. Genotypic** Test by LPA/CBNAAT is faster than phenotypic methods, as these are not growth based tests. Xpert MTB/RIF is recommended for the simultaneous detection of TB and RIF resistance directly from sputum specimens.

DST results by solid LJ medium has a turnaround time of upto 84 days, liquid culture (MGIT) upto 42 days, LPA upto 72 hours and CB-NAAT 2 hours. Under RNTCP, either CB-NAAT or LPA should be used for diagnosis of DR TB. This test can be performed using nasopharyngeal specimens in settings where induced sputum and culture are not practical.

Under the RNTCP, diagnosis of pulmonary TB includes –

1. Collection of atleast 2 sputum samples (spot & early morning) followed by
2. Sputum smear microscopy (both conventional ZN staining / fluorescent staining)
3. Culture (on solid or liquid media using manual or automated machines like BACTEC, MGIT)
4. Conventional PCR based LPA (for MTB complex), and real time PCR based CB-NAAT

If Rifampicin resistance is confirmed by CBNAAT or LPA:

1. Start standardized regimen for MDR TB
2. Perform liquid culture DST at base line to Levofloxacin and Kanamycin, & if facilities are available, perform DST to Moxifloxacin, Capreomycin, Ethambutol, Ethionamide, Linezolid and Pyrazinamide.
3. LPA for detecting INH resistance on sample or culture isolate. If resistance is detected to any second line injectable and/or fluoroquinolones, extended DST is performed for PAS and Clofazimine and treatment modified accordingly.

If Rifampicin sensitive is detected by CB-NAAT among presumptive DR TB cases:

1. Continue treatment with first line drugs
2. Send sample for LPA to detect INH resistance
3. Liquid culture DST for Ethambutol, Pyrazinamide, Kanamycin & Levofloxacin.
4. If resistance is detected to 2nd line injectable and/or fluoroquinolones, perform DST for remaining second line drugs.

Tests Offered at SRL

Test Name	Method	Test Code
Acid Fast Bacilli Culture: MGIT	BACTEC/ MGIT	1464, 1465, 1464EP, 1464BA
Acid Fast Bacilli Smear	Microscopy/Ziehl Neelsen Stain	5320S, 5320U
Acid Fast Bacilli Stain And Culture	Culture-LJ, Fluorescent Stain / Ziehl Neelsen Stain & MGIT 960 + LJ Culture	2419, 1464S, 1464U
Gene Expert (XPRT MTB / RIF)	Real Time PCR on GeneXpert	RD1324
Gene Expert (XPRT MTB / RIF) - Extrapulmonary	Real Time PCR on GeneXpert	RD1324EP
AFB RAPID GENOTYPIC TEST (MDR-TB) (Note: This test is not applicable for MOTT)	Line Probe Assay	9950
AFB SECOND LINE DRUG GENOTYPIC ASSAY (Note: This test is not applicable for MOTT)	Line Probe Assay	9957
Xpert Plus	Semi nested Real time PCR on GeneXpert platform / Acid Fast Bacilli Smear- Microscopy (Ziehl Neelson's Method)/Bactec culture (Fluorescent technology)	9507, 9507EP
Xpert Total (AFB Smear + Genexpert + AFB Culture, Reflex to DST)	Semi nested Real time PCR on GeneXpert platform / Acid Fast Bacilli Smear- Microscopy (Ziehl Neelson's Method)/Bactec culture (Fluorescent technology)	7713, 7713EP
TBferon (M. tuberculosis Interferon Gamma Release Assay (IGRA))	EIA	2405
QUANTIFERON TBferon	EIA	2406

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