Making the best out of available tools and approaches

Summary guidance for Microbiological and Clinical Diagnosis of pulmonary tuberculosis among Children

Pediatric TB Operational and Sustainability Expertise Exchange (POSEE) Taskforce

June 17, 2021
INTRODUCTION

Of the 10 million people who fall ill with tuberculosis (TB) each year, WHO estimates that 1.2 million (12%) are children under 15 years\(^1\). Of those who become ill, 230,000 children die of TB each year with nearly all deaths occurring among children who did not access treatment\(^2,3\). While nearly 25% of adults with TB remain undiagnosed each year, the case detection gap for children is higher – 56% of all children and 65% of children under five years with TB are “missed” each year (not diagnosed and/or not reported). This youngest age group is at highest risk for rapidly developing severe forms of TB and delays in diagnosis can lead to death. In 2018, heads of state, Ministries of Health, National TB Programs (NTP) and partners committed to the ambitious target of diagnosing and treating more than 3.5 million children with TB by 2022 to reduce mortality and disability due to TB through universal coverage of high-quality TB services, including prevention, diagnosis and treatment support.

Even with recent advances in rapid diagnostics, obtaining a microbiological diagnosis of TB in children remains challenging. Young children often have difficulty producing sputum and may have paucibacillary disease, which can undermine the utility and performance of available laboratory tests for TB diagnosis. Negative laboratory test results cannot reliably exclude TB in this age group. Therefore, clinical diagnosis continues to play an important role in the management of childhood TB. Physical examination, clinical history, contact history, radiography, response to treatment, and other assessments together can lead to a confident, empirical diagnosis of TB in young children and should be paired with available laboratory diagnostic testing to support and confirm a TB diagnosis where feasible. Continued investments to build frontline healthcare worker capacity and confidence to screen and diagnose children with TB clinically must remain a priority for TB programs.

Despite challenges, providers should try whenever possible to obtain a microbiological diagnosis of TB in children as it can simplify the diagnostic pathway, detect rifampicin resistance, allow rapid initiation of appropriate TB treatment, assist the management of complicated cases and build clinicians confidence in their clinical diagnosis. Children with high bacillary loads, who are more likely to have a positive laboratory test, are at greater risk of severe disease and death and stand to benefit most from rapid microbiological diagnosis.

As a time-limited task force of the Child and Adolescent Working Group (hosted by the WHO Global TB Programme), the Pediatric TB Operational and Sustainability Expertise Exchange (POSEE) coordinates and collaborates with key TB stakeholders on the development of tools and documents that can strengthen programs to address TB in children. POSEE developed this information note as an interim resource for TB stakeholders while awaiting new WHO guidelines on the management of TB in children and adolescents, which are expected to be released by the end of 2021.

In this information note, we aim to describe the role, scope and limitations of microbiological diagnosis of pulmonary TB in children in the short- and medium-term. The key guiding principle for this effort is that microbiological diagnostic services must be situated within a comprehensive package of care that provides a pathway to treatment for all children, and importantly, includes robust clinical assessment of all children with presumptive TB.
Specimen collection

The microbiological diagnosis of pulmonary TB usually relies on the examination of sputum. Children are more likely to present with paucibacillary TB, which can result in disproportionately fewer bacilli produced across specimens. Additionally, children, especially young children, often have difficulty producing sputum for testing. In addition to sputum, WHO now recommends collection of gastric aspirates (GA), NPA and stool for Xpert MTB/RIF and NPA for Xpert MTB/RIF Ultra testing. To optimize the performance of microbiological tests for diagnosis of TB in children, it is critical to determine the most appropriate specimen collection methods for the age of the child, the skills of the provider and availability of testing services (on site or by referral).

Sputum induction (SI) may be used in children who are not able to produce quality sputum specimens, yielding induced (as opposed to expectorated) sputum samples. The principle of SI is to help a patient to cough up bronchial secretions by inhaling hypertonic saline. Nasopharyngeal aspiration (NPA) relies on mucus aspiration through the child’s nasopharynx. Induced sputum and NPA are often collected together from young children. Given that Mycobacterium tuberculosis (MTB) can survive the acidic environment of the stomach, GA can be used to collect bronchial secretions that have been swallowed, especially during the night, and are therefore often collected first thing in the morning.

SI, NPA and GA are procedures that require specific training of health workers. SI also requires equipment for the nebulization of the hypertonic solution and a mucus aspirator to retrieve the specimen in young children. SI should be performed in a properly ventilated area and requires specific infection control and biosafety measures to reduce the risk of MTB transmission that could result from aerosolization during the procedure. Due to the potential risk of bronchospasm caused by inhalation of the hypertonic solution, SI is contraindicated in children with respiratory distress. Mild nose bleeding resulting from the mucus extraction and vomiting are the most common adverse events reported with IS followed by wheezing and transient hypoxia in less than 2% of children.

NPA requires minimal infection control and biosafety measures compared to SI, does not require nebulization and can be easily done in an outpatient setting under observed biosafety and infection control conditions to reduce aerosol-based infection risk. Although there is no standardized procedure, NPA still requires an aspirator, which can be battery operated, and mucus extraction material, for example, a mucus trap and catheter. Data on feasibility and patient safety for NPA are expected by the end of the 2021 from the UNITAID TB-Speed funded project.

GA does not require specific equipment but does require consumables (e.g., syringes and nasogastric-tubes). Similar to NPA, it requires minimal infection control and biosafety measures and can be implemented in inpatient or outpatient settings. However, GA requires insertion of a nasogastric tube and is more invasive, less well tolerated by children and often not accepted by parents and caregivers. Furthermore, to ensure a good quality of the sample, GA should be done early in the morning, after overnight fasting, in the supine position or after more than one hour in the supine position if done in an outpatient setting.

Despite SI and GA having been recommended for several years as sample collection procedures for TB diagnosis in children, they are not routinely implemented in most TB programs due to the operational challenges described above.

The use of Xpert MTB/RIF testing on stool specimens is based on the same concept of retrieving MTB from swallowed respiratory samples during GA, since MTB travels through the gastrointestinal tract and is excreted in stool. The collection of stool is simple and not invasive. In very sick hospitalized children, a rectal swab may be used to collect the specimen. The main challenge with collection of stool occurs in outpatient settings, where it is often difficult to obtain a specimen on demand. This
may result in the parents or caregivers needing to collect a sample at home and return to the facility for specimen submission. These additional steps may raise operational challenges and increase the risk of dropout during the diagnostic pathway.

However, both NPA and stool specimen collection offer the advantage of being minimally invasive or not invasive at all, meaning that they can be implemented in outpatient settings, with limited infrastructure and equipment, minimum infection control and biosafety measures, and no need for the child to fast before sample collection. Given the majority of sick children seek care at primary or secondary health care levels, these methods offer the most children with presumptive TB in high burden and resource-limited countries the opportunity for microbiological testing.

Lastly, recent studies have demonstrated that TB detection is substantially improved when molecular WHO-recommended diagnostics (mWRD) are used to test more than one specimen per pediatric patient or with the combination of different samples8–10.

Microbiological tests

Nucleic acid amplification tests (NAAT) that detect MTB and genes associated with resistance to rifampicin (RIF) such as the Xpert MTB/RIF and Xpert MTB/RIF Ultra (Cepheid, USA), as well as the Truenat MTB, Truenat MTB Plus and Truenat MTB RIF Dx (Molbio, India) assays, are major laboratory testing breakthroughs, bringing TB diagnosis closer to the patient. Additionally, some of these methods can achieve performance similar to mycobacterial culture.

In 2013, WHO recommended that Xpert MTB/RIF replace smear microscopy in all children, especially children living with HIV or with presumptive multidrug-resistant TB5. In their most recent guidelines, WHO strongly recommended the use of Xpert MTB/RIF in children with signs and symptoms of pulmonary TB as the initial diagnostic test for TB and detection of rifampicin resistance in sputum, gastric aspirate, nasopharyngeal aspirate and stool9. WHO recommends also the use of Xpert MTB/RIF from extra-pulmonary samples based on clinical presentation (lymph node fine needle aspiration biopsy (FNAB), pleural fluid, cerebrospinal fluid (CSF) and tissue samples). As for adults, the urine LAM antigen point of care test (Alere Determine Urine-LAM Ag test, Alere Inc, Waltham, MA, USA) is recommended to assist the diagnosis of active TB in HIV-positive children with presumptive TB or advanced HIV disease or who are seriously ill or irrespective of TB suggestive signs if they have CD4 count < 200cells/mm³ (inpatients) or CD4< 100cells/mm³ (outpatients)11.

In a systematic review of Xpert MTB/RIF testing on stool specimens, the pooled sensitivity and specificity were 67% (52-79) and 99% (98-99), respectively against culture (from a respiratory specimen)12,13. There was high heterogeneity between the studies, mainly explained by differences in the amount of stool tested and stool processing methods. Stool includes more PCR inhibitors compared to sputum, which can result in a higher rate of invalid results and its potential for greater solid mass within the sample may lead to an increase in error rates.

Several methods have been developed to process stool specimens prior to testing. Historically, these methods involved multiple steps, including centrifugation, and required a more advanced laboratory setting with skilled laboratory staff. Such processing methods represent a major challenge to decentralization of stool testing. However, three simplified, centrifuge-free stool processing methods have now been developed to address the need for decentralized stool testing and are currently under evaluation. The Stool Processing Kit (SPK) by FIND, the Simple One Step (SOS) method by the KNCV Tuberculosis Foundation and the Optimised Sucrose Flotation (OSF) method by the Unitaid-funded TB SPEED project are all under evaluation in two multicentre head-to-head studies. The results from these studies are expected to be reported by the end of 2021 (TB-Speed, KNCV Tuberculosis Foundation and FIND).
In addition to the stool processing method, the choice of mWRD test requires consideration. The next-generation Xpert MTB/RIF assay, Xpert MTB/RIF Ultra (Ultra), has a limit of detection (LOD) of 16 colony forming units (CFU)/mL compared to 114 CFU/mL achieved by Xpert MTB/RIF. This lower LOD is similar to the detection level of culture and should improve the diagnosis of paucibacillary disease in children. Initial studies report Ultra sensitivities between 64% and 75% for sputum, 46% for NPA, and high specificity (97% to 100%) compared to culture. Using composite reference standard including microbiologically and clinically diagnosed TB cases, the sensitivity of Ultra for sputum ranges between 22 and 25% with a specificity between 98 and 100%. Initial reports on stool specimens show an increase in sensitivity from 37.9% with Xpert MTB/RIF to 58.6% with Ultra. However, the WHO 2020 guidelines include a strong recommendation on the use of Xpert Ultra in children as initial diagnostic test for TB and detection of RIF resistance in sputum or nasopharyngeal aspirate due to a lack of data on other sample types (e.g. stool). This indication is expected to be extended to other samples in the revised guidelines by the end of 2021.

**Clinical Diagnosis**

Regardless of the type of specimen collected and testing method, only a small proportion of children with TB disease currently have a microbiological diagnosis (less than 40%) and a negative test result does not exclude TB, particularly in young children. Microbiological diagnosis is dependent on disease stage, age, and setting. For example, children with more advanced disease admitted in referral centres are more likely to have a microbiological diagnosis than children seeking care at primary health centres. Even in settings where a full array of microbiological diagnosis services is available, providers will continue to rely on clinical judgement. Clinical screening and decision-making algorithms remain a critical component for diagnosis and treatment of TB in children and clinical diagnosis will remain the pathway to treatment for the majority of children with TB.

**Overview of clinical diagnosis**

Clinical TB diagnosis in children includes history and presentation. One of the most important aspects of clinical history is exposure to another person in the household or family with TB. For many children who are identified as part of a contact investigation, this history is already known. However, for children evaluated outside of a contact investigation, detailed exposure history should be elicited; if an index TB case is identified then a source case investigation can be conducted to identify any other adults and children who may have been exposed. Children living with HIV (CLHIV) and those with severe acute malnutrition (SAM*) are particularly vulnerable to rapidly developing, severe forms of TB and death, and these comorbidities should be assessed as part of the clinical history.

The most common signs and symptoms of TB in children include persistent cough, fever (which may be accompanied by night sweats), failure to thrive or weight loss and unusual fatigue or lethargy, and decreased playfulness. Due to immune suppression, CLHIV and children with SAM are less likely to present with well-characterised symptoms and are at higher risk of having a missed diagnosis. Young children with pulmonary TB may present with more acute symptoms and present with pneumonia/severe pneumonia; young children may also present with minimal signs, (e.g., wheeze due

---

* Defined by WHO in children below the age of 5 years as either a weight for height Z-score <-3 SD, a mid upper arm circumference (MUAC) <115 mm, or bilateral pitting edema
to enlarged intrathoracic lymph nodes) or enlarged matted lymph nodes (more commonly in cervical or supraclavicular regions\textsuperscript{17–21}.

**Overview of radiological diagnosis**

Chest X-ray (CXR) is an important tool for the diagnosis of childhood TB. Although the spectrum of radiological abnormalities found in children with TB can be broad and non-specific, certain radiological patterns are highly suggestive of TB such as perihilar and mediastinal adenopathy\textsuperscript{22,23}. Routine use of CXR is limited by numerous practical challenges including poor access to services, variable image quality and lack of reading skills to interpret CXR in children\textsuperscript{24,25}. A simplified approach to CXR reading based on the detection of six common features is currently being evaluated for accuracy and added value in the TB-Speed Decentralization study\textsuperscript{†}.

Computer aided detection (CAD) technology, which meets WHO-recommended sensitivity and specificity thresholds for screening in adults, could improve access to CXR\textsuperscript{26}. Several providers of CAD software have developed versions that are certified for use in children as young as four years of age. More evidence in the younger age group is needed. CXR digitization using digital radiography (DR) plates on analogue X-ray machines is feasible in most district hospitals, and is an essential system strengthening intervention that would expand access to this critical tool in the diagnostic pathway of childhood TB.

The use of abdominal ultrasound to detect enlarged abdominal lymph nodes in CLHIV has been evaluated in a number of studies. Delivered as a point-of-care tool, this modality may have value in combination with other elements of clinical diagnosis\textsuperscript{27,28}. As children with SAM are also immune-deficient, point-of-care ultrasound (POCUS) could be particularly valuable for timely diagnosis in low resource settings. POCUS is currently under evaluation as a TB screening tool by the Desmond Tutu TB Centre.

**Diagnostic algorithms for tuberculosis in children and adolescents**

Numerous diagnostic scoring systems and algorithms have been developed and implemented in high burden settings, and their use is now familiar to decision makers and healthcare workers. Generally, the goal of these systems and algorithms is to standardize and improve access to paediatric TB diagnosis to enable decentralized, timely treatment initiation. It is important that these systems are developed using recommended methods for diagnostic prediction models and scores and should be evaluated and validated for use in children. Although the evidence base for scoring systems and algorithms has improved over time, there is no single preferred scoring system that has been recommended for widespread use. The WHO is currently reviewing the use of treatment decision algorithms for paediatric diagnosis, and updated guidance is expected at the end of 2021.

Urgent investment is needed to improve access to quality, context-specific clinical diagnosis of TB in children. Algorithms, scoring system, and other approaches can play a role in standardizing application of common criteria for rapid diagnosis and treatment initiation. Critical areas for investment include training as well as clinical mentoring and supportive supervision to build provider capacity and confidence. Integrated approaches emphasizing long-term engagement and sustainability can empower healthcare professionals at peripheral health care facilities to make timely and appropriate TB treatment decisions in children. Expanded access to radiography and CAD should also be prioritised and has health system benefits beyond the diagnosis of TB. The limitations of microbiological testing, and the variability of clinical and radiological diagnosis highlight the need for a comprehensive package approach to ensuring a successful pathway to treatment for children with TB.

\textsuperscript{*} https://clinicaltrials.gov/ct2/show/NCT04038632
Which Xpert cartridge should be used for TB diagnosis in children, Ultra or MTB/RIF?

Providers may use the cartridges available in their setting for respiratory samples. Where Ultra is available, there may be a higher yield of detection due to increased sensitivity. Ultra is also currently being evaluated on GA and stool specimens in many research projects; the current recommendation is to use Xpert MTB/RIF on GA, NPA and stool specimens and Ultra on NPA, but this may change in the near future as WHO is evaluating the evidence use of the Ultra cartridge with GA and stool specimens as part of the upcoming pediatric TB guideline revisions.

How many samples should be tested to confirm microbiological diagnosis of TB in children?

One respiratory (including stool specimen) is sufficient to confirm TB diagnosis in children. However, a negative test does not rule out TB, and clinicians must often rely on clinical judgement to make a treatment decision. This is especially true when the likelihood of having TB is high due to severity of disease, documented exposure or a high prevalence setting. However, where possible, two samples are better than one. And two samples from different specimens is best. The decision to retrieve two samples from separate specimens must be balanced with the concern of subjecting children to invasive procedures; if one sample plus a thorough clinical examination favor TB diagnosis, it likely is not necessary to repeat the microbiological test.

Is the performance of the microbiological diagnostic methods the same in all children?

The likelihood of microbiological diagnosis depends on the clinical spectrum of the disease in children which varies with the age. For example, performances are lower among children presenting intrathoracic lymph-node disease that is also classified as pulmonary TB. Adolescents usually present with adult-type pulmonary TB with a higher bacillary load, while younger children usually have different presentations and lower bacillary load.

Is nasopharyngeal aspiration a safe and feasible procedure?

NPA has been tested in different pediatric populations through the UNITAID funded TB-Speed project - children with presumptive TB severe pneumonia, severe malnourishment, or living with HIV - with good preliminary tolerance, feasibility and acceptability findings. Preliminary results were presented at the last annual meeting of Child and Adolescent TB working group and additional results will be available by the end of 2021. [http://stoptb.org/wg/dots_expansion/childhoodtb/assets/documents/am2020/09.%20Chishala%20Chabala_TB-Speed_Experience_NPA&stool.pdf](http://stoptb.org/wg/dots_expansion/childhoodtb/assets/documents/am2020/09.%20Chishala%20Chabala_TB-Speed_Experience_NPA&stool.pdf)

What resources are needed to introduce the different specimen collection and testing methods for children with presumptive TB?

To assist NTPs in estimating accurate costs related to the procurement of devices and consumables, the POSEE Task Force developed a budgeting tool for sputum induction, gastric aspiration, nasopharyngeal aspiration and stool specimen collection. These tools are available on the WHO Child and Adolescent Working Group. [http://stoptb.org/wg/dots_expansion/childhoodtb/posee.asp](http://stoptb.org/wg/dots_expansion/childhoodtb/posee.asp) In addition to equipment, NTPs should assess the need for training of providers and laboratory technicians to implement updated clinical and microbiological diagnosis algorithms.

What are the benefits of microbiological diagnosis of TB in children, given the challenges and limitations? Is it worth the effort?

Although challenging, microbiological diagnosis of TB is beneficial because it can more quickly identify drug resistance and likely also reduce time to a treatment decision which can reduce mortality and potentially catastrophic costs associated with care seeking. Where microbiological diagnosis of TB
disease is not feasible or not obtained, treatment decisions should be based on clinical history and presentation and/or radiological examination, with regimen selection based on the resistance pattern of the most likely index case if DR-TB is suspected.

**What other types of specimens can be used for pediatric TB diagnostic testing?**

In children with signs and symptoms of pulmonary or extrapulmonary TB, advanced HIV disease, serious illness, a CD4 cell count of less than 200 cells/mm$^3$ in inpatient settings (irrespective of signs and symptoms) or a CD4 cell count less than 100 cells/mm$^3$ in outpatient settings (irrespective of signs and symptoms), WHO strongly recommends the Alere Determine TB LAM (lipoarabinomannan) Ag (antigen) assay be used to test urine to rule-in TB diagnosis$^{11}$. This test only requires a small amount (60ul) of urine be applied to a lateral flow test strip that is read by eye after 25 minutes. Pediatric urine samples should preferably be collected early in the morning after urogenital cleaning with a cleansing wipe and tested as soon as possible. Importantly, use of the urine-based TB LAM test does not replace the need for mWRD diagnostic and drug susceptibility testing, and should instead be conducted in parallel with respiratory specimen testing with an mWRD whenever and wherever possible, to maximize chances of laboratory confirmation of TB. Lastly, TB LAM should not be used to test urine from children that lack signs and symptoms of TB and do not have CD4 cell count information available.

In addition to the additive value of urine, blood is also now endorsed by WHO for Xpert MTB/RIF diagnostic testing among children living with HIV that have signs and symptoms of disseminated TB. Given that this recommendation is generalized from adults and the certainty of evidence supporting the endorsement was very low, mWRD testing of blood among this high-risk pediatric population may provide an opportunity for countries to conduct operational research that could inform future global policy updates$^4$. Xpert MTB/RIF is also recommended for extra-pulmonary samples such as CSF, FNAB, pleural fluid, peritoneal fluid, pericardial fluid, synovial fluid or urine specimen and Ultra is recommended for CSF and FNAB$^4,30$. In available at referral centers, broncho-alveolar lavage for mWRD and mycobacterial culture can be considered for diagnosis of difficult cases of pulmonary TB and examination of tissue/biopsy with histology, microscopy and culture for diagnosis of extra-pulmonary TB.

**What diagnostic algorithms are available for use in children?**

Although the evidence base for scoring systems and algorithms has improved over time, there is no single preferred algorithm or score that has been recommended for widespread use. The WHO is currently reviewing the use of treatment decision algorithms for pediatric diagnosis, and updated guidance is expected at the end of 2021. Several new approaches have attempted to further improve and standardize clinical diagnosis of TB in children. The Ugandan National TB Leprosy Program is using a stepwise approach to diagnosis for frontline clinicians inspired by the Union Desk Guide for diagnosis and Management of TB in children$^{31}$. A diagnostic score specifically dedicated to CLHIV has been developed in the PAANTHER study implemented in Burkina Faso, Cambodia, Cameroon, and Vietnam$^{28}$. Based on simple clinical features, CXR features, bacteriology (Xpert), and abdominal ultrasound findings, the score had a sensitivity of 89% and a specificity of 61% in these very sick children with a high mortality risk. It has been proposed and integrated in an algorithm, the PAANTHER TB treatment decision algorithm that is currently undergoing external validation in the TB-Speed HIV study. A similar approach has recently been used for the development of a treatment decision algorithm for HIV-uninfected children, based on data collected in children assessed for TB in South Africa$^{32}$. Based on simple clinical and CXR features, bacteriology (Xpert), this algorithm was 90.1% sensitive and 52.1% specific, and maintained a sensitivity of above 90% among children <2 years or with low weight-for-age$^{31}$. To further cover specific needs of children with SAM in terms of TB diagnosis, the ongoing TB-Speed SAM study$^2$ is aiming to develop a screening and diagnostic algorithm for children hospitalized with SAM. Results are expected mid-2022.

---

$^4$ https://clinicaltrials.gov/ct2/show/NCT04121026
Where can training materials for diagnosis of TB in children be found?

A CXR training module was developed by the TB-Speed project for clinicians at primary health care level focusing on six major radiological findings associated with TB in children. The module is available on the project website.  https://www.tb-speed.com/wp-content/uploads/2020/09/TB-Speed_Interpret-Child-CHR.pdf.

Under the CaP TB project, The UNION and EGPAF have collaborated to update the training package for pediatric TB, targeting front-line health care workers. Those training materials are aligned with the current WHO guidelines (available as of May 2021). The training material will be available on The UNION and on EGPAF websites in Q3 2021.

ACKNOWLEDGMENT

The Posee group would like to thank CDC, USAID, UNICEF and WHO for their collaboration in the development of this note. The CaP TB project, TB-Speed project and IMPAACT4TB project are funded and supported by UNITAID.


