Introduction

The World Health Organisation (WHO) reported an estimated 10.4 million incident cases of tuberculosis (TB) globally in 2015 including at least a million cases in children below 15 years of age, while an estimated 210,000 children died of TB (WHO, 2016a). About 75% of all childhood TB cases occur every year in the 22 high-burden countries, most of which are in sub-Saharan Africa (Nelson and Wells, 2004). However, routine data on childhood TB is likely to underestimate the true magnitude of the problem, principally because of the challenges in the diagnosis of TB in children; additionally, a low priority is traditionally given to childhood TB by most national TB control programs because children are not considered a substantial contributor to the transmission of Mycobacterium tuberculosis (M. tb) (Newton et al., 2008; Ahmed et al., 2008).

Until recently, pediatric TB disease estimates were derived from the proportions of smear-positive cases by age, resulting in a significant risk of underestimating the true burden in children given that most pulmonary TB disease in children is smear negative (Nelson and Wells, 2004; Corbett et al., 2003). In support of this assertion, a recently published mathematical model predicted that the annual incidence of pediatric TB in the 22 high TB-burden countries is greater than 650,000 cases, which is in fact much higher than the number of notifications in those countries (Dodd et al., 2014). Childhood TB is estimated to constitute approximately 5% of the TB caseload in low TB burden countries compared with high-burden countries where high transmission rates and large proportion of population under the age of 15 years mean children account for an estimated 10%–25% of the total TB case load (Nelson and Wells, 2004; Marais et al., 2006a).

Although the effect of the HIV epidemic on the burden of childhood TB is less well characterized than for adults (Newton et al., 2008), there is an HIV-related shift in TB disease burden to younger adults who are often parents of young children, putting children at particularly high risk of exposure and subsequent disease (Graham et al., 2001). The relative importance of TB as a preventable and treatable cause of childhood disease and death has been highlighted by the recent reductions in morbidity and mortality from vaccine-preventable infections such as measles, pneumococcus, and Haemophilus influenzae type b (Graham et al., 2014). Pediatric tuberculosis has since emerged as a leading cause of childhood morbidity and mortality. The 2012 World TB Day was dedicated to childhood TB, while the Stop TB partnership made a commitment to work towards zero deaths from TB in children worldwide (WHO, 2014a).
Pathogenesis of TB in Children

Children are most often exposed to infection from an adult with smear-positive pulmonary TB, with a high risk of infection, progression to active disease or extra pulmonary dissemination and death following exposure, particularly in infants and children less than two years of age (Guwatudde et al., 2003; Marais et al., 2004). Route of infection in children is similar to that in adults, and usually results from inhalation of aerosolized \( M.\text{tb} \) in infected droplets, usually from adults with cavitary disease (Mendez-Samperio, 2008). If the pathogen is successful in overcoming the initial barriers, it settles in the terminal alveoli where there is proliferation of single or multiple foci (primary focus) with a spread via the draining lymphatics to the hilar lymph nodes (primary complex). The infection can be contained at this stage and the primary complex might be discovered accidentally on routine chest radiograph (Marais et al., 2004). The only indication of \( M.\text{tb} \) infection in such patients could be established is by serial tuberculin skin test (TST) reactivity indicating delayed- type hypersensitization to \( M.\text{tb} \) proteins.

However, the infection could progress to primary disease in about 20%–40% of the children with hilar glandular enlargement and involvement of the bronchial tree and/or the pleural cavity. The typical cavitating lung disease is usually only seen in older children, primarily from early adolescent years onwards (Marais et al., 2003). There can also be haematogenous dissemination of the organism especially in infants and young children with a spread throughout the body resulting in acute disseminated (i.e., miliary) TB, affecting the bones, brain, and abdomen. The mechanisms that determine the differential outcomes following infection in children are not clearly understood but include age, nutritional status, underlying immunity, vaccination status, genetic susceptibility, microbial virulence, and comorbidities (Newton et al., 2008). The risk of disease progression is highest in the first 2 years of life when it is estimated to be 40%–50% while majority of children will develop disease within 2–12 months of the initial infection; pulmonary TB accounts for 60%–80% of all cases in the absence of prior BCG vaccination or prophylactic medication (Marais et al., 2004; Cruz and Starke, 2007).

Pediatric Immune Response to \( M.\text{tb} \) and Differences to Responses in Adults

Newton et al. suggested that the immaturity of the immune response could largely account for the high rate of disease progression seen in young children following initial infection with \( M.\text{tb} \) (Newton et al., 2008). The initial immune response to \( M.\text{tb} \) is similar to that seen in adults with an early initiation of innate immunity required to limit the growth of the organism through a cascade of events leading to the recruitment of additional immune cells to the site of infection (Jones et al., 2011). The dendritic cells are the major antigen presenting cells, which process the \( M.\text{tb} \) antigens and migrate to regional draining lymph nodes where they present the processed antigens to naïve CD4\(^+\) T-cells via surface MHC-class II molecules (Giacomini et al., 2001). The major components involved in both the innate and adaptive immune responses to \( M.\text{tb} \) are as illustrated in Fig. 1.

However, a number of studies have reported an age-related functional impairment of both innate and adaptive immune responses in children (Holt, 1995; Sepulveda et al., 1997; Filias et al., 2011; Gold et al., 2007; Upham et al., 2006; White et al., 2002). Specifically, it has been reported that the alveolar macrophages in children show diminished phagocytosis, cell recruitment, and microbial killing when compared to adults, which could promote delayed initiation of antigen specific T-cell responses and disease progression (Holt, 1995; Sepulveda et al., 1997; Smith et al., 1997). Similarly, neonates and infants have fewer circulating dendritic cells with a reduced ability to synthesize IL-12 and to present antigens to naïve CD4\(^+\) T-cells (Upham et al., 2006; Smith et al., 1997). Neonatal CD4\(^+\) T-cells also have a diminished capacity to express Th 1-type effector function partly due to hypermethylation of the proximal promoter of the IFN-\(\gamma\) gene resulting in restriction of IFN-\(\gamma\) responses to variety of stimuli (White et al., 2002; Kampmann et al., 2006). There is also presentation of antigens to naïve CD8\(^+\) T-cells via the major histocompatibility complex (MHC) class I molecules resulting in their activation and proliferation into effector CD8\(^+\) cytolytic T lymphocytes by the less clearly understood phenomenon of cross presentation and/or cross priming (Schaible et al., 2003; van der Wel et al., 2007; Winau et al., 2006). Further studies are needed to define the role and importance of CD8\(^+\) T-cells in immune responses to \( M.\text{tb} \) in children and adults.

Diagnostic Challenges in Childhood TB

The challenges associated with the diagnosis of TB in children cannot be overemphasized. The clinical presentation of TB in children mimics other common childhood diseases such as HIV, pneumonia, viral and bacterial blood infection, and malnutrition (Edwards, 1987). The constitutional symptoms include failure to thrive, weight loss, and intermittent fever while involvement of the airway can result in persistent unremitting cough or wheeze; however, all of these symptoms lack specificity (Perez-Velez and Marais, 2012). Children produce smaller quantities of sputum when compared to adults, which is usually swallowed making it difficult to obtain good quality sputum samples for pathogen detection tests (Edwards et al., 2007). Facilities and expertise for sputum induction (with inhalation of nebulized hypertonic 3%–5% saline and subsequent aspiration or expectoration of mucus from the lower respiratory tract) are mostly unavailable in resource-limited settings while gastric lavage requires hospitalization with overnight fasting for up to three consecutive days in some cases.

The yield from sputum smear microscopy and \( M.\text{tb} \) culture is limited in childhood TB given the paucibacillary nature of TB disease in children, and this constitutes one of the major impediments to accurate diagnosis (Perez-Velez and Marais, 2012). Chest X-ray changes in pediatric TB cases are often nonspecific with wide intra- and inter-observer variations in interpretation. BCG vaccination given at birth, endemcility, of nontuberculous mycobacteria (NTM), and altered immune response especially in very
young children as a result of functional immaturity of immune cells, among other reasons, make TST of limited diagnostic value on its own, although a positive TST does suggest recent infection in children and must trigger action. (Newton et al., 2008; Haimi-Cohen et al., 2001). Although every effort must be made to seek a microbiological confirmation, the diagnosis of pediatric tuberculosis is often presumptive, based on epidemiological and clinical evidence such as history of exposure to an adult TB case, nonspecific clinical symptoms and signs, and results of investigations such as TST and Chest X-ray (Schaaf et al., 1995).

Clinical Diagnostic Approaches

The difficulty in microbiologically confirming the diagnosis of TB in children has led to the development of a number of alternative, symptom-based diagnostic approaches that represent potentially simple, stepwise, rational, and logical tools that aid health care workers in identifying children who are in need of TB treatment (Edwards et al., 2007). A diagnostic approach was defined as any published systematic method for diagnosis of childhood TB and includes point scoring systems, diagnostic classifications, and algorithms (Hesseling et al., 2002; Kabra et al., 2004). A “point scoring” system is a diagnostic approach in which a numerical value is assigned to each characteristic in the system and examples include the Keith-Edwards and Kenneth Jones point scoring systems (Edwards, 1987; Stegen et al., 1969). For “diagnostic classifications” the characteristics in the system are stratified into categories such as suspect, probable, or confirmed TB and examples include the Ghidey and Habte approach that was developed in Ethiopia (Ghidey and Habte, 1983), as well as a subsequent modification of this approach developed in Uganda.
In the “diagnostic algorithms,” a stepwise approach to TB diagnosis was advocated often in diagrammatic form, such as the IMCI/WHO guidelines on management of the child with serious infection or severe malnutrition and the Okeahialam diagnostic algorithm developed in Tanzania (WHO, 2000; Okeahialam, 1974).

However, a review of the diagnostic approaches reported that the majority of these scoring systems, algorithms, and classifications were developed without being validated against a gold standard of diagnosis, that is, bacteriological confirmation of disease, while the few prospective studies conducted to validate these diagnostic approaches were all carried out in hospital based settings mostly without the use of control groups (Hesseling et al., 2002). Hatherill et al. reported only slight agreement and high variability in the number of TB cases diagnosed by nine structured approaches evaluated in a high-TB burden setting, and concluded that diagnostic approaches need to be tailored to their particular epidemiological context so as to avoid systematic errors in estimating disease burden or patient management in such setting (Hatherill et al., 2010). While there are general concerns about the diagnostic value of the symptoms-based diagnostic approaches, the natural history of childhood TB demonstrates that symptoms may have diagnostic value if appropriate risk stratification is applied, especially in immunocompetent children (Lopez Avalos, 2012; Marais and Pai, 2007).

The development of novel diagnostic tools that could give a rapid and reliable diagnosis of TB in children and the optimization of currently available diagnostic tools are major research priorities (WHO, 2011a). However, the lack of a perfect reference standard for TB in children and of standardized case definitions constitute major challenges to the assessment of accuracy of new diagnostic tools, comparison of findings between diagnostic studies or conduct of metaanalysis, which might provide evidence base to inform policy recommendations (Oliwa et al., 2015; WHO, 2013; Graham et al., 2015). As a result, an updated standardized case definition for the classification of intrathoracic TB in children when evaluating novel diagnostic tools was recently published by an international panel of experts (Graham et al., 2015). This revised case definition attempts to present broad symptomatic entry criteria that are compatible with TB. It could thus particularly address the challenge associated with the classification of TB in the majority of children who will be diagnosed with TB disease, among whom there will be no bacteriological confirmation of disease.

Table 1 shows the revised case definition and classifications into “confirmed tuberculosis,” “unconfirmed tuberculosis,” and “unlikely tuberculosis.” However, the consensus of the expert panel was that the revised case definition will support the diagnosis of TB in symptomatic children with suspected intrathoracic TB, much like in the original consensus (Graham et al., 2012). It may not be appropriate for studies that incorporate investigation for possible TB disease in children from active case finding, as in household contact tracing, for example.

### Clinical Case Definitions for Classification of Intrathoracic Tuberculosis in Children

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### Table 1 Revised classification of intrathoracic tuberculosis case definitions for diagnostic evaluation studies in children

<table>
<thead>
<tr>
<th>Case definition</th>
<th>Refine criteria[^a]</th>
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| **Confirmed tuberculosis** | - Bacteriological confirmation obtained  
- Requires Mycobacterium tuberculosis to be confirmed (culture or Xpert MTB/RIF assay) from at least 1 respiratory specimen |
| **Unconfirmed tuberculosis** | - Bacteriological confirmation NOT obtained AND at least 2 of the following:  
    - Symptoms/signs suggestive of tuberculosis (as defined)  
    - Chest radiograph consistent with tuberculosis  
    - Close tuberculosis exposure or immunologic evidence of *M*. *tuberculosis* infection  
    - Positive response to tuberculosis treatment (requires documented positive clinical response on tuberculosis treatment—no time duration specified)  
    - With *M*. *tuberculosis* infection  
    - Immunological evidence of *M*. *tuberculosis* infection (TST and/or IGRA positive)  
    - Without *M*. *tuberculosis* infection  
    - No immunological evidence of *M*. *tuberculosis* infection |
| **Unlikely tuberculosis**  | - Bacteriological confirmation NOT obtained AND Criteria for “unconfirmed tuberculosis” NOT met.  
    - With *M*. *tuberculosis* infection  
    - Immunological evidence of *M*. *tuberculosis* infection (TST and/or IGRA positive)  
    - Without *M*. *tuberculosis* infection  
    - No immunological evidence of *M*. *tuberculosis* infection |

[^a]: All children should have symptoms compatible with tuberculosis as determined by the treating clinician.

Diagnostic Tests for TB and Their Application in Childhood TB Diagnosis

Over the last decade, a number of new, rapid, and sensitive diagnostic tools for TB have been developed. These include tests based on molecular methods such as the Xpert MTB/RIF ("Xpert") Cepheid, Sunnyvale, USA, the loop-mediated isothermal amplification test (TB-LAMP, Eiken Chemical, Tokyo, Japan), and the line probe assays (LPAs). These tests could either replace or complement the existing conventional tests used for diagnosis of TB and/or for detecting drug resistance including sputum smear microscopy and solid or liquid culture methods. Although the WHO has endorsed several of these tools for diagnosis of TB, we discuss their applicability to the diagnosis of TB in children.

Smear Microscopy and Cultures

Diagnosis of TB is traditionally confirmed by identification of AFB in stained smears by microscopy and/or the isolation of \( M. tb \) from culture of clinical specimens. In resource-limited settings however, most cases of TB continue to be confirmed by sputum smear microscopy. Young children are unable to expectorate and facilities and expertise for sputum induction are mostly unavailable in high-TB burden countries. 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 (Newton et al., 2008; Hesseling et al., 2002; Marais et al., 2006b). In children, the sensitivity of smear microscopy 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 (Nicol and Zar, 2011).

While culture of \( M. tb \) in biological samples including sputum is more sensitive than smear microscopy, both liquid and solid culture facilities are mostly unavailable in the laboratories within the national public health systems in resource-limited settings because of cost and the inherent technical demands. Where culture is available, 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 \( M. tb \) in children seldom exceeds 30% even when using gastric aspirates, induced sputum, liquid culture media, and molecular diagnostic tools (Nelson and Wells, 2004; Edwards et al., 2007; Nelson et al., 2004; Nicol et al., 2005). The yield of these methods increases with increasing age of the child.

Xpert MTB/RIF

The Xpert is a diagnostic tool recommended by the WHO for the diagnosis of TB disease and multidrug-resistant (MDR) TB in both adults and children, aimed at reducing the time to bacteriological confirmation and ability to detect MDR cases rapidly (WHO, 2014b). It is based on nucleic acid amplification and detection of \( M. tb \) DNA and mutations associated with rifampicin resistance simultaneously. This is done through the amplification of the 81 base-pair core region of the RNA polymerase \( rpoB \) gene of \( M. tb \), using real-time polymerase chain reaction with molecular beacons. Xpert is a closed system that integrates automated sample processing, nucleic acid amplification, and detection of target sequences and exhibits high sensitivity and specificity for detecting \( M. tb \) DNA in sputum samples (Fig. 2). Estimates of pooled sensitivity and specificity of Xpert in a systematic review and

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**Fig. 2** A four-module GeneXpert ("Xpert") MTB/RIF system. The Xpert is a WHO endorsed rapid diagnostic device recommended for the diagnosis of TB disease and multidrug-resistant (MDR) TB in both adults and children, aimed at reducing the time to bacteriological confirmation and ability to detect MDR cases rapidly. Picture courtesy of Professor Madhukar Pai, McGill University, Montreal, Canada.
metaanalysis of 22 studies that recruited 8998 adult subjects with presumed TB disease are 89% (95% CI 85%–92%) and 99% (95% CI 98%–99%) respectively (Steingart et al., 2014).

A separate metaanalysis of pediatric Xpert studies showed that compared with culture, the pooled sensitivities and specificities of Xpert for TB detection were 62% (95% credible interval 51–73) and 98% (97–99), respectively, with use of expectorated or induced sputum samples and 66% (51–81) and 98% (96–99), respectively, with use of samples from gastric lavage. Xpert sensitivity was 36%–44% higher than the sensitivity for microscopy. Xpert’s pooled sensitivity and specificity to detect rifampicin resistance was 86% (95% credible interval 53–98) and 98% (94–100), respectively.

Based on the metaanalysis of data from pediatric studies and the limitations of microbiological diagnosis of TB in children, the Xpert was recommended in a policy statement in 2013 by the World Health Organisation (WHO) as the initial diagnostic tool for children with suspected HIV-associated TB or MDR-TB (WHO, 2013). More recently, in 2017, WHO endorsed the use of Xpert MTB/RIF Ultra cartridge, based on the findings from a large multicenter noninferiority diagnostic accuracy study in adults with signs and symptoms of pulmonary TB (WHO, 2017a). The Ultra assay was designed to have a higher sensitivity, by the incorporation of two different multiplex amplification targets (IS6110 and IS1081) as well as a relatively larger DNA reaction chamber, when compared to Xpert MTB/Rif. The study reported that Ultra had a 5% higher sensitivity relative to Xpert MTB/Rif (95% CI: +2.7, +7.8) but 3.2% lower specificity (95% CI: −2.1, −4.7), with sensitivity increases being highest among smear-negative culture-positive patients and among HIV-infected patients (FIND, 2017). Data on the performance of Xpert Ultra cartridge in children is not yet available.

While Xpert is now the front-line test for TB in adults and children, access to this technology continues to be a problem in many settings, and the technology is still expensive for low-income settings. While the existing GeneXpert platform is not easy to decentralize, work is ongoing to develop more robust, molecular solutions that could be decentralized. Furthermore, for children, the requirement for a sputum sample poses additional challenges, since sputum induction or gastric aspiration is not easy to implement in many settings. Thus, there is a need for nonsputum based TB tests and target product profiles for such tests have been published (Denkinger et al., 2015).

Other Molecular Methods (TB-LAMP, LPAs)

The TB-LAMP in a commercial molecular assay that requires minimal laboratory infrastructure and biosafety requirement, has been evaluated as a replacement to sputum smear microscopy in resource-limited settings. It is a manual assay that provides an isothermal amplification of M. tb DNA from sputum samples and results could be available in less than one hour (Gray et al., 2016). Thus, it is potentially applicable as a rapid point of care test at peripheral health care facilities where microscopy is often performed. However, studies to evaluate the performance of TB-LAMP either as a replacement for smear microscopy or comparison to Xpert MTB/RIF, have all been carried out in adult populations.

In a metaanalysis of 7 studies with 1810 adult patients suspected of having TB, the pooled sensitivity of TB-LAMP was 15% higher than smear microscopy (78% [95% CI: 71%–83%] vs. 63% [95% CI: 56%–69%]), while a separate metaanalysis of 4 studies with 1075 patients reported that the pooled sensitivity of TB-LAMP (78% [95% CI: 66.6%–86.4%]) is lower than the pooled sensitivity of Xpert MTB/Rif (89% [95% CI: 85%–92%]), but the specificity of all three tests is similar (WHO, 2016b). Based on these metaanalyses from the adult studies, the WHO gave a conditional policy recommendation that TB-LAMP may be used as a replacement test for sputum smear microscopy for the diagnosis of pulmonary TB in adults and extrapolated the recommendation to the use of TB-LAMP assay in children by generalization of the data in adults (WHO, 2016b).

LPAs is a high throughput genotypic technology that provides opportunity for rapid detection, programmatic management, and surveillance of drug-resistant TB. It has been endorsed by the WHO for the detection of resistance to both first-line (i.e., INH and rifampicin), and second-line anti-TB drugs (i.e., fluoroquinolones and second-line injectable drugs) (WHO, 2016c). However, LPAs require smear-positive samples or culture isolates. This makes it challenging to apply it in pediatric populations.

Urine LAM Detection Assay

The lateral flow urine lipoarabinomannan (LAM) assay is based on the detection of LAM, which is a key lipopolysaccharide present in mycobacterial cell wall, in urine samples. This urine-based test would have been advantageous particularly in children given that it is a nonsputum based test that uses urine, which is a biological sample relatively easy to collect. However, several studies have found that the urine LAM assay has a low sensitivity for diagnosis of TB, except in HIV-infected patients with advanced disease who have very low CD4 T-cell counts (Hanifa et al., 2016; Shah et al., 2016). Accordingly, WHO strongly recommended the use of urine LAM assay for diagnosis of TB only in HIV-infected persons with low CD4 counts or who are seriously ill (WHO, 2015a).

Host Response Tests: Tuberculin Skin Test or Interferon-gamma Release Assay

The WHO guidelines on latent TB testing endorse the use of both TST and IGRA as tests for latent TB infection (LTBI) (WHO, 2015b). For active TB, neither test is optimal, but can be helpful as one of several tests in an algorithm or case definition.


**Tuberculin skin test (TST)**

The TST is the most widely used test based on host response rather than detection of causative organism and can detect evidence of prior exposure and sensitization to *M. tb*. It is based on detection of delayed-type hypersensitivity reaction to intradermal injection of purified protein derivative (PPD), which is a heterogeneous mixture of antigens present in *M. tb*, BCG-strain of *M. bovis*, and several NTMs (Lalvani and Millington, 2007). The TST can establish if the individual has previously been sensitized by mycobacteria, as it measures the immune response, but it cannot confirm the presence or absence of *M. tb*. As such it cannot distinguish between TB disease and LTBI. Its utility is also hampered by technical and logistical problems including potential for false-positive and false-negative results (Lopez Avalos, 2012). False-positive reactions could be attributed to prior BCG vaccination or asymptomatic NTM infection while false-negative reactions could occur in young children aged less than 2 years because of functional immaturity in cell-mediated immune responses, and in immunosuppressive illnesses, malnutrition, or recent measles infection or simply because the test was done too early following exposure (Dogra et al., 2007). It is also known that around 10% of any given population have immune anergy and will not respond to the intradermally applied mycobacterial or control antigens.

**Interferon (IFN)-γ release assays (IGRAs)**

IGRAs are an alternative test of the host immune response as a measure of prior exposure and sensitization to *M. tb*. IGRAs are known to be at least as sensitive—but more specific—than TST for the detection of *M. tb* infection (Pai et al., 2004), and are based on measurement of IFN-γ production by antigen-specific T-cells following in vitro stimulation of whole blood or peripheral blood mononuclear cells by *M. tb*-specific antigens such as ESAT-6, CFP-10, and/or TB 7.7 (Walzl et al., 2011). These antigens are known to be highly immunogenic and much more specific for *M. tb* than PPD because they are not present in the BCG vaccine strain of *M. bovis* or most NTM (Dogra et al., 2007). There are currently two commercial IGRA kits that are available; these include the QuantiFeron-TB Gold In-Tube (QFT-GIT; Cellestis, Australia) and the T_SPOT.TB assay (Oxford Immunotec, UK).

Both TST and IGRAs detect evidence of immunological sensitization to *M. tb*, but neither of the two is able to distinguish between active TB and LTBI or directly measure the presence of mycobacteria (Pollock et al., 2013; Kampmann et al., 2009). Neither TST nor IGRAs are able to adequately resolve the “spectrum of TB” as depicted in Fig. 3, where TB can be viewed as a dynamic continuum from *M. tb* infection to active TB disease (Pai et al., 2016). Therefore, while IGRAs are designed to be more specific than TST for diagnosis of LTBI in BCG vaccinated populations and/or in those infected with NTM, neither TST nor IGRA has been demonstrated to be superior in the diagnosis of LTBI in children. IGRAs do not have sufficient sensitivity or specificity to confirm or exclude the diagnosis of active TB. Both TST and IGRAs are known to have low predictive value (for progression from latent to active TB) and their availability in resource-limited settings remains limited (Pollock et al., 2013). In this context, there have been some promising developments in trying to predict TB using novel biosignatures (Zak et al., 2016), and use of IGRA conversions to identify those that are likely to progress in the near future that is, detect incipient TB disease (Andrews et al., 2017).

### Diagnostic Algorithm

The current preferred algorithm for universal patient access to rapid testing to detect MTB and rifampicin resistance is shown in Fig. 4. In this algorithm, Xpert MTB/RIF test is used as the initial diagnostic test for all adults and children (regardless of HIV status) with signs or symptoms of pulmonary TB or with a chest X-ray with abnormalities suggestive of TB.

### Future Prospects

**Diagnostic biomarkers**

The International Roadmap for TB Research published in 2011 by the WHO highlighted the investigation and development of new diagnostics suitable for children as a research priority (WHO, 2011a). Being able to accurately distinguish between children with latent TB infection, who have respiratory symptoms due to another pathogen, and children with active TB disease represent the most pressing issue in the management of children presenting with symptoms consistent with but not specific for TB. Research into host TB biomarkers has gained more prominence due to the lack of suitable tests based on detection of the *M. tb* or its products in clinical samples (Walzl et al., 2011). A recently published blueprint for pediatric TB biomarkers listed the characteristics of an “ideal” biomarker for TB in children to include: (i) measurable in small volumes of readily obtainable samples such as blood, urine, stool, saliva, etc.; (ii) identify *M. tb* with high sensitivity and specificity regardless of age, nutritional status, or HIV status; (iii) distinguish children with active TB disease from latently infected children with other respiratory infections; and (iv) suitable for incorporation into a diagnostic platform that would provide rapid results at or near the point of care (Nicol et al., 2015).

Studies investigating TB biomarkers involve immunological approaches including the use of antigen stimulated peripheral blood as well as the relatively more advanced “omics” approaches including transcriptomics, metabolomics, lipidomics, and proteomic markers. Peripheral blood is currently the most widely used source of biomarkers as genes, transcripts, proteins, and metabolites can all be measured in blood, although biomarkers from urine, sputum, saliva, and breath have all been shown to have clinical diagnostic potential (Parida and Kaufmann, 2010). Immunological markers are based on antigen-specific T-cell responses, notably the releases of cytokines that are currently considered relevant for protection against *M. tb* such as IFN-γ, IL-2, and TNF-α as well as the release of cytolytic molecules such as granulysin, granzyme, and perforin (Kaufmann and Parida, 2008). Several studies have reported that different combinations of TNF-α, IL-12p40, IL-17, sCD40L, EGF, VEGF, IL-1α, IP-10, MCP-1, and IL-15 could...
differentiate between TB disease and LTBI in adults in TB endemic settings (Sutherland et al., 2010; Chegou et al., 2009; Harari et al., 2011; Frahm et al., 2011). Given the report that distinct cytokine expression profiles of CD4+ T-cells are associated with bacterial loads (Caccamo et al., 2010), different cytokine expression profiles could be expected in childhood TB cases which are paucibacillary when compared to adult TB and represent primary infection rather than reactivation disease. The presence of antigen-specific T-cells and their respective cytokines can therefore be expected to be lower.

One pediatric study reported that childhood TB was associated with elevated plasma levels of biomarkers at homeostasis including TGF-β, IL-21, and IL-23 when compared to health controls (Pavan Kumar et al., 2013). Dhanasekaran et al. reported that the combination of IL-2 and IL-8 in QFT-supernatants of children could differentiate between TB disease and LTBI, while Armand et al. did not identify any discriminant biomarker between the same groups in another study that used similar methods (Dhanasekaran et al., 2013; Armand et al., 2014). Tebruegge and colleagues reported that a M.tb-specific biosignature, comprising the combination of TNF-α, IL-1ra, and IL-10, showed the best discriminatory ability between active TB and LTBI pediatric cases (Tebruegge et al., 2015). In contrast, Chegou et al. reported that unstimulated levels of IL-1ra and IP-10 and antigen-specific levels of VEGF in QFT supernatants may be useful for diagnosing TB disease and differentiating between TB disease and M.tb infection respectively in children investigated in a high HIV/TB prevalence setting (Chegou et al., 2013). Although serological tests have not been found useful in diagnosis of TB and discouraged by the WHO (WHO, 2011b), Thomas et al. reported a high diagnostic accuracy for childhood TB of a novel assay called antibodies in lymphocyte supernatant, which was based on measurement of IgG production by activated plasma cells in BCG-stimulated cultures of PBMCs (Thomas et al., 2011). Studies using the transcriptomic approach to TB biomarker discovery in children are limited but currently emerging. A TB-specific whole blood 51-transcript signature with reasonable diagnostic accuracy in distinguishing TB from other diseases with similar clinical features was identified in HIV-infected and -uninfected Africa children (Anderson et al., 2014), while Verhagen et al.
Fig. 4  Preferred algorithm for universal patient access to rapid testing to detect MTB and rifampicin resistance (reproduced with permission from GLI) (WHO, 2017b). 1. Persons to be evaluated for TB include adults and children with signs or symptoms suggestive of TB or with a chest X-ray with abnormalities suggestive of TB. This algorithm may also be followed for the detection of MTB using CSF, lymph node, and other tissue specimen from persons being evaluated for extra pulmonary TB. For persons being evaluated for TB who are HIV positive and have CD4 counts ≤100 cells/μL or are seriously ill, see Algorithm 4. 2. Programs may consider collecting two specimens upfront. The first specimen should be promptly tested using the Xpert MTB/RIF test. The second specimen may be used for the additional testing described in this algorithm. For persons being evaluated for pulmonary TB, sputum is the preferred specimen. (3). Patients at high risk for multidrug-resistant TB (MDR-TB) include previously treated patients including those who had been lost to follow-up, relapsed, and failed a treatment regimen; nonconverters (sme–positive at end of intensive phase); MDR-TB contacts; and any other MDR-TB risk groups identified in the country. (4). Patients should be initiated on a first-line regimen according to national guidelines. A sample may be sent for molecular or phenotypic DST for isoniazid if the patient has been previously treated with isoniazid or if there is a high prevalence of isoniazid resistance not associated with rifampicin resistance (i.e., isoniazid mono- or poly-resistance) in this setting or for DST for rifampicin if rifampicin resistance is still suspected. (5). Repeat Xpert MTB/RIF test at the same testing site with a fresh specimen. Interpret the result of the repeat test as shown in this algorithm. Use the result of the second Xpert MTB/RIF test for clinical decisions. (6). Further investigations for TB may include chest X-ray, additional clinical assessments, clinical response following treatment with broad-spectrum antimicrobial agents, repeat Xpert MTB/RIF testing, or culture. (7). Repeat Xpert MTB/RIF test at the same testing site with a fresh specimen. Use the rifampicin result of the second Xpert MTB/RIF test in this algorithm for a decision(s) regarding choice of regimen (first line or second line regimen).
reported a whole blood 42-transcript signature for TB among Warao Amerindian children in the United States (Verhagen et al., 2013). Recently, Zhou and colleagues applying a microarray assay of micro RNA (miRNA) reported a combination of eight miRNA that may be a novel early diagnostic biomarker for childhood TB (Zhou et al., 2016).

Table 2 summarizes studies that investigated potential biomarker candidates in childhood TB infection and disease states. These reports suggest that available data in children are limited and that there is a marked heterogeneity in the methodology of the studies often with overlapping or conflicting results. Research into TB biomarkers using the metabolomic and/or proteomic approaches is still relatively new. To date, proteomic and metabolomic studies that have investigated novel TB biomarker were carried out mostly in adult populations and rarely involved children. In general, the cost effectiveness and real world applicability of high throughput “omics” assays in high TB burden countries is still very doubtful, and major translational work will be required to develop simple and affordable assays that can be used for TB control efforts in resource-limited settings (Togun and Pai, 2017).

### Conclusion

The recent WHO End TB Strategy sets ambitious targets for TB control including the achievement of a 90% reduction in TB incidence rate by the year 2035 compared to the current levels (WHO, 2016d). This strategy provides added opportunity for global TB control efforts to address childhood TB, which is now increasingly being recognized as an important cause of childhood morbidity and mortality caused by a preventable and treatable infectious disease. Increased funding and policy support should be made available for concerted research efforts to improve the diagnosis and treatment of TB infection and disease in this particularly vulnerable group. This will ultimately contribute towards the global effort to eradicated TB disease, as many cases of adult TB are likely to arise from undiscovered and untreated primary infection in childhood.

### Table 2  Potential biomarker candidates in childhood TB infection and disease

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Study design</th>
<th>Methods used</th>
<th>Groups</th>
<th>Discriminant biomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thomas et al.</td>
<td>Case—control</td>
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</tr>
<tr>
<td>Verhagen et al.</td>
<td>Case—control</td>
<td>Microarray analysis of whole blood</td>
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<tr>
<td></td>
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<td></td>
<td>PTB vs. LTBI</td>
<td>42-gene transcript signature</td>
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<td></td>
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<td></td>
<td>PTB vs. HC</td>
<td>RAB33A</td>
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<td></td>
<td>Multiplex assay in QFT supernatants</td>
<td>PTB vs. HC</td>
<td>IL-2 &amp; IL-8</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>PTB vs. HC</td>
<td>IL-2 &amp; IL-13</td>
</tr>
<tr>
<td>Dhanasekaran et</td>
<td>Prospective</td>
<td>dcRT-MLPA in whole blood</td>
<td>PTB vs. HC</td>
<td>IP-1, IP-10, &amp; VEGF</td>
</tr>
<tr>
<td>al. (2013)</td>
<td>study</td>
<td></td>
<td>PTB vs. HC</td>
<td>IFN-α2, IL-1Ra, sCD40IL, &amp; VEGF</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PTB vs. HC</td>
<td>MMP/TMPs, CRP, s2M, Haptoglobin &amp; TGF-β</td>
</tr>
<tr>
<td>Pavan Kumar et</td>
<td>Case—control</td>
<td>Multiplex assay in Plasma</td>
<td>PTB vs. HC</td>
<td>IL-2, IL-5, IL-8, IL-10</td>
</tr>
<tr>
<td>al. (2013)</td>
<td></td>
<td>Multiplex assay in QFT supernatants</td>
<td>PTB vs. HC</td>
<td>IL-2, IL-13</td>
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<tr>
<td>Anderson et al.</td>
<td>Prospective</td>
<td>Microarray analysis of whole blood RNA expression</td>
<td>PTB vs. HC</td>
<td>51-gene transcripts signature</td>
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<tr>
<td>(2014)</td>
<td>study</td>
<td></td>
<td>PTB vs. HC</td>
<td>42-gene transcripts signature</td>
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<td>Armand et al.</td>
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<td>PTB vs. LCBI</td>
<td>IP-10, IL-2, IL-5 &amp; IL-13</td>
</tr>
<tr>
<td>(2014)</td>
<td></td>
<td>Multiplex assay in QFT supernatants</td>
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<tr>
<td>Tebruegge et al.</td>
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<td>Multiplex assay WBA supernatants</td>
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<td>TNF-α, IL-1Ra &amp; IL-10</td>
</tr>
<tr>
<td>(2015)</td>
<td>study</td>
<td>Multiplex assay WBA supernatants</td>
<td>PTB vs. LCBI</td>
<td>TNF-α, IL-1Ra &amp; IL-10</td>
</tr>
<tr>
<td>Zhou et al.</td>
<td>Case—control</td>
<td>miRNA microarray analysis</td>
<td>PTB vs. LCBI</td>
<td>miR-1, miR-155, miR-31, miR-146a, miR-10a, miR-125, miR-150 &amp; miR-29</td>
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</tbody>
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References


