# Diagnosing Childhood Tuberculosis: Traditional and Innovative Modalities

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## Introduction and Epidemiology

Tuberculosis (TB) remains a leading cause of mortality in children worldwide. The diagnosis of tuberculosis in children is traditionally based on exposure history, symptoms, the tuberculin skin test, chest radiography, and mycobacterial staining and culture. However, these investigations lack in sensitivity and specificity and diagnosing childhood tuberculosis remains a major global challenge. Recent advances have improved our ability to diagnose tuberculosis, but many of the new modalities either have not been validated in the pediatric population or are inaccessible to tuberculosis endemic regions. Moreover, diagnosing tuberculosis in human immunodeficiency virus-coinfected children, a growing population, remains a major challenge. This review provides an overview of traditional and novel approaches used to diagnose *Mycobacterium tuberculosis* infection. As the overwhelming proportion of tuberculosis occurs in resource-limited regions, a global perspective on the feasibility of conventional and innovative diagnostics is provided in this review.

*Mycobacterium tuberculosis* (*M. tb*) is a significant global health challenge. *M. tb* is estimated to infect one-third of the world's population and 1.7 million deaths due to tuberculosis were reported in 2006.[1] While data on global prevalence of TB in children are sparse, the 2006 World Health Organization (WHO) Global Report estimates that there are approximately one million cases of tuberculosis disease and 400,000 deaths each year in children $<$15 years old.[2] However, this number is likely to be an underestimation of TB disease in children globally, as most data are derived from estimates of incidence, and most children have culture-negative TB. In addition, most published figures of the burden of childhood tuberculosis do not reflect the occurrence of extrapulmonary tuberculosis (20-30% of the caseload in some settings), which occurs much more commonly in young children than in adults with tuberculosis.

Early and accurate diagnosis of TB using currently available technology is particularly difficult in young children, especially in children coinfected with human immunodeficiency virus (HIV). New sensitive diagnostic modalities to rapidly identify TB in children are necessary because children often progress rapidly to disease, and adolescents with TB disease transmit infection to other community members. In the present article we discuss conventional and innovative approaches to diagnosing TB in children. Standard diagnostic tests performed in the USA are reviewed in addition to the diagnostic modalities used in resource-poor regions, as 95% of TB cases and 98% of TB deaths occur in these regions.[1]

### Immunopathogenesis of TB

Immunologic control of TB infection is a function of protective innate and cell-mediated responses. *M. tb* infection usually occurs after inhalation of the bacilli in infected respiratory secretions. Mycobacteria, inhaled as small aerosol particles, reach the alveoli where dendritic and macrophage cells process the bacteria and present antigens. Dendritic cells are particularly efficient at antigen presentation due to their robust surface expression of major histocompatibility complex molecules, which provide costimulatory signals for T-cell activation.[3] Antigen presentation mostly occurs in regional lymph nodes to CD4+ T-cells.

Alveolar macrophages often kill the mycobacteria that has been engulfed and accumulated by the phago-

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some. The phagosome-containing bacilli merge with the lysosome, forming a phagolysosome. The acidic environment and nitrogen radicals within the phagolysosome act to kill the mycobacteria.[4] However, mycobacteria have developed ways of circumventing this environment, making the macrophage less effective at killing the bacilli.[5,6] Consequently some bacilli may persist and replicate within the alveolar macrophage.

Cytokine IL-12 is released by infected macrophages and together with IL-23 and IL-27 orchestrate a Th1 immune response.[7,8] Neutrophils, monocytes, and lymphoctyes are recruited and interferon gamma (IFN-$\gamma$) is produced by the CD4 T-lymphocytes. Interferon gamma, in conjunction with TNF-$\alpha$ and 1,25-dihydroxy vitamin D3, induce killing of intracellular mycobacteria by up-regulating the nitrogen intermediates within the phagolysosome.[9] INF-$\gamma$ also activates dendritic cells, which make them more efficient at controlling mycobacteria.[10] Individuals lacking in receptors to IFN-$\gamma$ suffer from recurrent and sometimes lethal mycobacterial infections[11-13] and IFN-$\gamma$ knockout mice are highly susceptible to *M. tb*.[14] The significant role IFN-$\gamma$ plays in the cell-mediated response to *M. tb* make it a likely biomarker for mycobacterial infection. As such, *M. tb*-antigen-specific IFN-$\gamma$ production in vitro has recently been used as a surrogate marker of tuberculosis infection.

Protective immunity to mycobacterial infection is critically dependent on the CD4+ T-cell subset.[15] Individuals with poor CD4 lymphocyte numbers and function, such as HIV-infected individuals, are at much greater risk of disease progression. Other T-cell subsets, such as CD8+ T-cells and gamma-delta ($\gamma\delta$) T-lymphocytes, also recognize mycobacteria and act to kill infected macrophages and produce a broad spectrum of cytokines such as IFN-$\gamma$, IL-2, IL-4, IL-5, and IL-10.[16,17] The cytokines and chemokines also activate effector T-cells to produce granulomas to wall off the infection.

Granulomas, the primary pulmonary response to *M. tb* infection, limit initial replication and spread. Some of the recruited monocytes differentiate into mature tissue macrophages or epitheloid cells to form an organized granuloma. Tumor necrosis factor plays a significant role in granuloma formation and localization of infection. The importance of TNF-$\alpha$ has recently been demonstrated in patients who are receiving TNF-$\alpha$ inhibitors for autoimmune diseases. These patients are particularly vulnerable for developing TB disease.

**TABLE 1.** Risk of disease progression after infection with *M. tb* in children at different ages

| Age (yr) at *M. tb* infection | Percent of children with pulmonary disease after infection with *M. tb* | Percent of children that progress to miliary or CNS disease |
|---|---|---|
| <1 | 30-40 | 10-20 |
| 1-2 | 10-20 | 2-5 |
| 2-5 | 5 | 0.5 |
| 5-10 | 2 | <0.5 |
| >10 | 10-20 | <0.5 |

Adapted with permission from Marais and coworkers.[22]

The extent to which a protective immune response can develop against *M. tb* infection depends on the host's innate and cell-mediated immune response, *M. tb* virulence, and number of bacilli. Evidence suggests that the host immune response is rarely sufficient to eliminate all the mycobacteria from the human lung.[18] Moreover, young children are less efficient than adults at controlling bacilli replication and containing the bacilli within the walls of a granuloma. As the number of mycobacteria increases, dissemination throughout the body via the lymphatic system and bloodstream can occur, creating new foci of infection in other tissues or organs. If this occurs, a child can have primary active disease. In adults, the distinction between TB infection and disease is usually clear and often separated by period of years before the onset of reactivation-type disease.

## *Young Children Are More Susceptible to TB Disease Than Older Children and Adults*

Pediatric and adult TB differ markedly in epidemiological features, clinical appearance, and pathogenesis. Before the advent and use of prophylactic anti-TB medication, there were higher rates of disease progression in young children than in older children and adults infected with *M. tb* (Table 1). One study in Norway observed 73% of TB disease occurred in children less than 6 years of age.[19] Miller and coworkers observed that among children younger than 1 year of age with reactive tuberculin skin tests, pulmonary lesions developed in 43% and 15 to 20% had meningitis or miliary disease.[20] Even today in regions where access to health care and medication is often limited, a little more than half of the disease burden is carried by children less than 3 years of age.[21] Overall, the lifetime risk of progression from infection to active disease with *M. tb* is 5 to 10% for immunocompetent older children and

adults and 40 to 50% for children in the first 2 years of life.[22] Adolescents have a slightly higher risk of disease progression than adults.[23,24]

Not only is the risk of progression higher in young children, but also TB disease in this age group is often more severe and is already disseminated on clinical presentation. In children less than 5 years of age, early hematogenous and lymphatic spread of primary infection cause extrapulmonary manifestations such as miliary and meningitic disease[25] (Table 1). Miller and coworkers observed that TB meningitis developed in 15 to 20% of children younger than a year old, whereas central nervous system (CNS) disease was not observed in children over 4 years of age.[20] In a large Puerto Rican cohort miliary TB or meningitis developed in 10% of children less than 6 years of age, 1% between ages 7 and 12 years, and 0.4% in the adolescent group.[24] Overall, disseminated TB occurs in 40% of active TB cases in children less than 1 year of age and in less than 1% in adults with active TB.[16] Young children with severe and complicated disease have a much higher mortality rate than older children and adults. Some studies report a mortality rate exceeding 50% in children less than 1 year of age who have not received anti-TB medication.[26,27]

These differences reflect the inability of the developing immune system to mount a protective response to *M. tb*. Several studies have evaluated the innate and cell-mediated responses in children at various ages compared with adult. It has been observed that macrophages are quantitatively and functionally different in neonates than in adults.[9] In young children, delayed chemotaxis of monocytes and macrophages may slow the recruitment of activated cells to primary sites of infection.[28-30] Human cord blood dendritic cells are less efficient at antigen presentation and as stimulators of allogenic responses than their adult counterparts.[31]

Inefficient antigen presentation to naïve CD4+ T-cells may delay the initiation of an antigen-specific response. Phenotypic and functional lymphocyte differences exist in young children as compared with adults. T-cell memory phenotype CD45RO+ are minimal in infants[32] and are likely to contribute to a delayed or less robust antigen-specific response in young children. Moreover, regulatory T-cells are more prevalent[33] and suspected to down-regulate a protective Th1 cell-mediated response against *M. tb*.

INF-$\gamma$ production by natural killer and T-cells after mitogen stimulation is less than half of that in adult cells[34,35] and may not reach adult levels until age 5 years.[11,36] The amount of IFN-$\gamma$ release is directly correlated with age[37,38] and low levels of IFN-$\gamma$ transcription were observed at sites of *M. tb* infection in young children.[9,39] The resulting lower levels of IFN-$\gamma$ and other cytokines such as TNF-$\alpha$[16] may limit the extent to which macrophages are activated.[9,34] A weaker primary response to *M. tb* can prevent or delay the onset of protective immunity, allowing time for the bacilli to multiply.

## *M. tb* Infection

It is estimated that two billion people are infected with *M. tb*.[1] Specific data on children are lacking.[40] All countries except the United States and the Netherlands extensively use bacille Calmette-Guerin (BCG), limiting the population surveys for *M. tb* infection using the tuberculin skin test (TST). The prevalence rate is far less in the United States, where it is estimated that only 5% of all Americans and 1% of children are infected with *M. tb*.[41] In some urban populations, the risk is substantially higher. A 2003 study of New York City public schools showed that 9.7% of high-school students had positive TST reactions.[42] The most efficient method of finding children infected with *M. tb* is through contact investigations of adults with active TB. On average, 30 to 50% of household contacts of an index case have reactive TST results.[43]

A diagnosis of latent TB infection (LTBI) can be made solely on clinical grounds and a positive TST or INF-$\gamma$-releasing assay (IGRA). A healthy, asymptomatic child with a positive TST or IGRA and a normal chest radiograph is presumed to be infected with *M. tb*. This is, of course, an indirect way (based on a child's immune response) to determine infection status. There is no bacteriologic test for LTBI and as such there is no gold standard to confirm infection. Nevertheless, the past several decades have proven that diagnosing individuals with LTBI and providing chemoprophylaxis significantly protects from disease progression and acts to reduce prevalence of TB in all regions that treat LTBI.[1]

A child with LTBI has most likely already elicited an immunoprotective response to *M. tb* and most of the bacilli have been eliminated. Yet, small numbers of bacilli still do disseminate throughout the body and the risk of a child progressing to active TB is dependent on many factors, including age, immune status, and nutrition. Most children who develop disease do so within 2 to 12 months of initial infection.[44]

In older children and adults the distinction between TB infection and disease is usually clear and often separated by a period of years before the onset of reactivation-type disease. A major reason for making the distinction between infection and disease is because each is treated differently. Infection is treated with one medication, whereas disease is treated with at least three or more anti-TB drugs. The division between *M. tb* infection and disease in some children, especially children less than 2 years of age, may not be so obvious, since progression from infection to disease in children may occur rapidly.

In the United States any child diagnosed with LTBI should be treated with isoniazid for 9 months to prevent active disease. An alternative treatment regimen is rifampin for 6 months, which can be used in infected children who have had known contact with an isoniazid-resistant strain. In TB-endemic regions where transmission is poorly controlled, TB exposure and infection are extremely common and the benefit of preventative treatment is reduced by the high likelihood of reinfection. However the provision of treatment of latent TB remains a high priority in groups at high risk of progressing to TB disease, such as very young children and children with HIV.

Childhood infection with *M. tb* represents a sentinel event within a community, suggesting recent transmission most commonly from an infectious adult. The public health dimensions of childhood TB are important for overall TB control in a population and for earlier diagnoses and treatment of children through identification of infectious cases. Current WHO, Center for Disease Control and Prevention (CDC), and American Academy of Physician (AAP) guidelines advise that all children <5 years of age (AAP suggests <4 years of age) and immunocompromised children who are in close contact with an index case should be actively traced, screened for TB, and provided preventive chemotherapy, even if the TST is negative, once active TB has been excluded.[1,45,46] Infected children may have a negative TST result initially because cellular immunity takes weeks to develop. Therefore, the AAP suggests replanting a TST in all children with negative TSTs who are immunocompromised or less than 4 years of age at 12 weeks after the last contact to the index case.[46] If the TST is still negative at that time, isoniazid can be discontinued in immunocompetent children. If the TST is positive at that time, isoniazid should be continued for a total of 9 months.

## *M. tb* Disease

The progression of *M. tb* from infection to disease over a relatively short amount of time is referred to as recent "primary infection." A child with TB disease may have pulmonary or extrapulmonary disease occurring alone or simultaneously. When a child, usually >5 years, initially controls infection with *M. tb* (enters latency) and then over a period of years progresses to active disease, it is referred to as "disease reactivation" or "post primary disease."

About 75% of childhood TB disease is pulmonary.[47] Infants and young children are more likely to be symptomatic and have physical signs of lung disease, whereas older children may initially have clinically silent disease but have findings on chest X-ray (CXR). Cavitary lesions in children are most commonly seen in adolescents. Caseating lesions may rupture into pleural or pericardial spaces leading to a pleural or pericardial effusion. Up to a third of all pediatric pulmonary TB disease cases may be complicated by pleural effusions.[48] However, pleural effusions may also occur without significant parenchymal disease. Erosion of caseating lesions into pulmonary vessels can result in hematogenous dissemination to other regions of the lung and distant anatomical sites.

Extrapulmonary TB disease occurs in approximately 25% of infants and young children less than 4 years of age.[49] The most serious complications of TB disease are miliary and TB meningitis and typically occur in the youngest children, less than 2 years of age. The overall mortality of TB meningitis is 13% with approximately half of the survivors sustaining permanent neurologic sequelae.[50] The most common form of nonpulmonary TB disease is lymphadenitis and typically involves supraclavicular, anterior cervical, and submandibular lymph nodes. If untreated, lymph nodes steadily enlarge in size and develop caseous necrosis. Rupture of the node causes a chronic draining sinus tract, called scrofula.

Joint and bone involvement usually involve weight-bearing bones and joints. Vertebral TB, also called Pott's disease, is the most common form of skeletal TB. Other extrapulmonary manifestations of TB, such as renal or gastrointestinal, are rare in children because of long incubation periods required to manifest disease.

Children diagnosed with active TB are placed on a multidrug regimen which either kills *M. tb* or inhibits replication of bacilli. Because *M. tb* bacilli are slow-

growing and often walled off in the body by granulomas, individuals are placed on multidrug regimens for at least 6 months. A multidrug regimen also hinders the emergence of drug-resistant strains. The first-line medications used to treat active TB are isoniazid, rifampin, ethambutol, and pyrazinamide. Specific treatment protocols are not the focus of this review and are well reviewed elsewhere.[46,51,52]

## Diagnostic Modalities for M. tb Infection and Disease

### *Tuberculin Skin Test*

For over 100 years the standard diagnostic test for *M. tb* infection has been the TST. In 1882 Robert Koch boiled the culture of tubercle bacilli and injected it into people as a means to treat tuberculosis. This experiment failed, as overwhelming inflammatory responses developed and resulted in several deaths. However, what emerged from this experience was a definitive means to identify *M. tb* infection. In 1934, an American scientist, Dr. Florence Siebert, developed a method of purifying the tuberculin and made a simple protein precipitate (purified protein derivative (PPD)), a solution of antigens produced by the metabolic activity of *M. tb*. Today the definitive TST uses five tuberculin units of PPD injected intradermally with the Mantoux technique. Multiple puncture tests are not as reliable as the Mantoux method and are not recommended for diagnosis.[53] A wheal of fluid measuring 6 to 10 mm in diameter is raised immediately when the tuberculin is injected properly. A delayed hypersensitivity reaction to the Mantoux skin test peaks in infected individuals at 48 to 72 hours after injection. In some individuals the reaction may occur after 72 hours and is considered a positive result. The diameter of induration, not erythema, is measured and recorded in millimeters. Occasionally, an allergic or Arthus-like reaction to skin test components may cause erythema and induration, peaking at 24 hours, and usually waning by 48 hours.

Skin test positivity is based on the size of induration and epidemiologic risk factors. The CDC and the AAP recommend specific interpretations of skin reactions (Table 2).[45,46] Children in the highest risk category (eg, had contact with an index case, are infected with HIV, or have clinical evidence of TB) are considered infected with *M. tb* if the skin test result is indurated at least 5 mm. For children with moderate risk factors a reactive diameter 10 mm or more is considered a positive result. Fifteen millimeters of induration or greater is considered to be positive for any child, including those children with low risk.

**TABLE 2.** Definitions of positive tuberculin skin test (TST) results in infants, children, and adolescents*

**Induration ≥ 5 mm**

Children in close contact with known or suspected contagious people with tuberculosis disease.

Children suspected to have tuberculosis disease:

- Findings on chest radiograph consistent with active or previously tuberculosis disease
- Clinical evidence of tuberculosis disease†

Children receiving immunosuppressive therapy‡ or with immunosuppressive conditions, including HIV infection.

**Induration ≥ 10 mm**

Children at increased risk of disseminated tuberculosis disease:

- Children younger than 4 years of age
- Children with other medical conditions, including Hodgkin disease, lymphoma, diabetes mellitus, chronic renal failure, or malnutrition

Children with increased exposure to tuberculosis disease:

- Children born in high-prevalence regions of the world
- Children frequently exposed to adults who are HIV infected, homeless, users of illicit drugs, residents of nursing homes, incarcerated or institutionalized, or migrant farm workers
- Children who travel to high-prevalence regions of the world.

**Induration ≥ 15 mm**

Children 4 years of age or older without any risk factors.

HIV indicates human immunodeficiency virus.
*These definitions apply regardless of previous bacille Calmette-Guérin (BCG) immunization (see also Interpretation of TST Results in Previous Recipients of BCG Vaccine, p 683); erythema at TST site does not indicate a positive test result. Tests should be read at 48 to 72 hours after placement.
†Evidence by physical examination or laboratory assessment that would include tuberculosis in the working differential diagnosis (eg, meningitis).
‡Including immunosuppressive doses of corticosteroids.
Reproduced with permission from the American Academy of Pediatrics.[46]

In the United States it is advisable to screen children for possible risk factors for *M. tb* exposure before administering a TST. Testing an immunocompetent child with no risk for *M. tb* infection is discouraged.[53] Factors that have consistently correlated with increased risk of *M. tb* infection include recent contact with a case of TB, family history of TB, positive TST reactions in current household members, and foreign born or prolonged travel to a country with high TB rates. The age of initiation of TSTs as well as intervals between tests depend on an individual's personal risk factors. Clinicians performing skin tests should be familiar with TB case rates in the community.

A negative TST does not necessarily exclude infection with *M. tb*. There are many factors that can cause a decreased response to TST including concurrent illnesses or infections, along with active TB. Up to 25% of people with active TB are nonreactive to 5 TU of tuberculin.[54] It is likely that these individuals have

suppressed immune responses and may convert to a positive skin test after several months of anti-TB medication.[55] In addition, if a child was exposed to *M. tb* within a month of implanting a skin test, it may be too early to elicit an delayed hypersensitivity reaction, as it can take up to 3 months for tuberculin reactivity to become apparent. The false-negative rate is also increased by the failure to administer the PPD intradermally and the complexity encountered in reading the indurated reaction of the test. Globally, malnourishment and HIV infection are the more common reasons for an anergic reaction to tuberculin. Historically control antigens (eg, mumps, Candida, tetanus toxoid) have been applied to detect cutaneous anergy. However, a child can be infected with *M. tb*, be anergic to tuberculin, and still respond to other antigens. Therefore, anergy testing is no longer recommended.

A drawback of the TST is that its specificity is compromised because PPD contains over 200 antigens shared with the BCG vaccine and most nontuberculosis mycobacteria.[56] Cross-reactions from non-TB mycobacteria usually cause tuberculin induration sizes of 10 mm or less[57] and reactions are usually transient, lasting for several months. A meta-analysis found that the TST false-positive rate in children from non-TB mycobacteria is about 2%.[58] Concurrent skin testing with a variety of mycobacterial antigens can help to differentiate between true *M. tb* infection and reactions due to infection with environmental mycobacteria. However, these antigens are not readily available.

Tuberculin reactions caused by BCG cannot be distinguished from infection with *M. tb*. Very young children are most likely to experience cross-reaction. About 50% of infants vaccinated with BCG will have a positive TST,[59] yet 80 to 90% lose such reactivity within 2 to 3 years. In fact, if BCG is given in the newborn period, less than 5% of children will demonstrate a cross-reaction by 10 years of age.[58,59] However, if BCG vaccination is given after 12 months of age, up to 42% can have false-positive reactions secondary to BCG.[58] Therefore, BCG has a much greater effect on TST if given after infancy.[60,61] However, prior receipt of BCG vaccine is not a contraindication to tuberculin testing. In the USA, a TST result is interpreted similarly for children with and without a history of prior BCG vaccination.[46]

Most children with a true positive TST will remain positive for many years, if not lifelong. However, without frequent exposure to *M. tb* or environmental mycobacteria, a positive tuberculin test can revert to negative. In one survey, 22% of TST-positive children reverted to negative when retested 1 year later.[62] Children with a history of a positive TST result can be safely retested. Two negative skin tests in a healthy asymptomatic child a week apart (to exclude boosting) most likely indicates true negativity.

### INF-γ-Releasing Assays

A new generation of immune-based rapid blood tests for the diagnosis of LTBI, called IGRAs, offers particular advantages over the century-old TST. These tests rely on the host response to *M. tb* infection by measuring the IFN-γ produced by T-cell responses to *M. tb*-specific antigens called early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10. These antigens are transcribed from the region of difference−1, which is a region on the mycobacterium genome specific for *M. tb* and absent in BCG and most other mycobacteria. Two IGRAs are available as commercial kits and have recently been approved by the Food and Drug Administration (FDA). The Quantiferon TB Gold® assay (Cellestis, Carnegie, Australia) is based on a whole-blood enzyme-linked immunosorbent assay (ELISA). The newest version of the assay, the Quantiferon-TB Gold In-tube® (QFT), includes an additional *M. tb* specific antigen TB7.7, requires only 3 mL blood, and is the preferred version of the assay. The T-Spot.TB ® (T-Spot) test (Oxford Immunotec, Abingdon, UK) is based on the ex vivo overnight enzyme-linked immunospot assay. The T-Spot enumerates individual T-cells producing IFN-γ after antigenic stimulation, while the QFT measures the level of IFN-γ in the supernatant of the stimulated whole blood. Both the QFT and the T-Spot have an internal positive control (a mitogen) that elicits robust IFN-γ responses in immunocompetent people. The mitogen provides information regarding the validity of the assays in a subject with questionable immune status, such as HIV-infected individuals or very young children.

IGRAs have several important advantages over the TST. Testing requires only one patient visit and these assays are ex vivo tests that eliminate the potential of boosting when testing is repeated (Table 3). Results can be obtained within a day of blood draw. However, IGRAs do not distinguish between latent and active disease. It has been suggested by scientists that very high or rising levels of IFN-γ in IGRAs may be able to predict the asymptomatic individual with LTBI that is at highest risk of progressing to disease[63,64] but

**TABLE 3.** Performance characteristics of tests to diagnose LTBI

| Performance characteristic | TST | Quantiferon | T-SPOT.TB |
|---|---|---|---|
| Technique | In vivo skin test | ELISA | ELISPOT |
| Results given in | mm of induration | IFN-γ units | Spot-forming units |
| Antigen used | PPD | ESAT-6, CFP-10, TB7.7 | ESAT-6, CFP-10 |
| Time for result (d) | 2-3 | 2 | 2 |
| Mitogen control to distinguish indeterminate versus false-negative response | No | Yes | Yes |
| Cost per test | Low | High | High |
| Influenced by prior BCG | Yes | No | No |
| Influenced by atypical mycobacteria | Yes | Rarely | Rarely |
| Booster effect if repeated | Yes | No | No |
| Sensitivity for LTBI | 85-90%[a] | 58-80%[c] | 62-93%[b] |
| Specificity for LTBI | 60-95%[c] | 98%[c] | 92[c] |

[a]Huebner and coworkers.[56]
[b]Menzies and coworkers.[65]
[c]Farhat and coworkers.[58]

long-term prospective studies are needed to investigate this interesting finding. A significant limiting factor of IGRAs in resource-challenged regions is the high cost of IGRAs and trained personnel needed to run the assays.

Determining the sensitivity of IGRAs is complicated by the fact there is no gold standard for testing LTBI. As such, IGRA sensitivity has been estimated from studies including patients with active TB, persons in close contact to index cases who were categorized into gradients of exposure, and concordance of IGRA with the TST. A meta-analysis by Menzies and coworkers examined 59 IGRA studies that included the aforementioned ways to assess IGRA sensitivity. The sensitivity of the IGRAs was estimated to be between 58-80% and 62-93% for QFT and T-Spot, respectively.[65] Moreover, several studies that evaluated IGRA sensitivity in children found IGRAs correlated better with *M. tb* exposure risk than the TST.[38,66-69] A study performed in a low-prevalence region confirmed that IGRAs allow a distinction between infection with *M. tb* and with non-TB mycobacteria in children.[70] Improved sensitivity in diagnosing TB infection by IGRAs was also shown in HIV-infected and malnourished children, although the improved performance in malnourished children was not substantiated after correction for the HIV status of the child.[71] IGRA sensitivity may be compromised in HIV-infected individuals with low CD4 counts and there is a high reported indeterminate rate in asymptomatic HIV+ individuals with CD4 counts less than 100 cells/mm$^3$.[72-74]

Two studies found the amount of IFN-γ released from the mitogen in the QFT directly correlated with age, indicating the possibility of less sensitivity in very young children (<2 years of age).[38,66] Moreover, some studies reported high indeterminate results in the older version of the Quantiferon in children less than 5 years of age.[75] It has been suggested that cutoffs of positivity in very young children may need to be adjusted to optimize their accuracy in this age group.[38,76]

Two recent studies investigated individuals with high rates of discordant TST positive/IGRA negative results.[38,77] Both studies suggested that as time from initial infection increases, IGRA sensitivity may decrease. A few studies explored increasing IGRA sensitivity by either adding a novel antigen such as heparin-binding-hemagglutinin in individuals with remote *M. tb* exposure[78] or measuring chemokine IP-10 in addition to INF-γ in children <5 years.[79,80]

IGRA specificity is estimated from studies of healthy persons with a very low likelihood of *M. tb* exposure. Pooled average specificity was 97.7% and 92.5% in the QFT and T-Spot assays, respectively.[65] The IGRAs results are unaffected by prior BCG vaccination.

Recently, the U.S. CDC recommended the use of QFTs for diagnosing LTBI in all circumstances in which the TST is currently used, including in screening children. However, the AAP has withheld recommending using the Quantiferon assay until more data are available in children.[81] The main application of these assays seems to be in nonendemic regions where disease elimination is a realistic target as suspected contacts are identified and treated. IGRAs are highly specific and correlate well with known exposure history. IGRAs appear to be sensitive, at least in children over 2 years of age. It is reasonable to hold off on treatment in a TST+, IGRA− asymptomatic child who is over age 2 years and has a normal CXR. The

authors suggest that until long-term studies are available, IGRAs can be repeated in these children yearly for up to 2 years to ensure no misdiagnosis.

## Clinical Diagnosis of TB Disease

In resource-limited settings, where the vast majority of TB cases are diagnosed, expensive and inaccessible diagnostics are often inaccessible. The diagnosis of childhood TB is further complicated by the absence of a practical gold standard. In endemic regions, the only test usually available is sputum microscopy, and this is positive in <10 to 15% of children with probable pulmonary TB.[82] Given this situation, the diagnosis of childhood TB is often made solely on the key clinical features of chronic symptoms, physical signs, a positive TST, and/or a CXR suggestive of TB.[83]

In some countries score charts are used for TB diagnosis, although they have rarely been evaluated against a "gold standard." Attempts to validate score charts showed poor performance in children with pulmonary TB and in HIV-infected children. The WHO recommends that score charts should therefore be used as screening tools and not as the means of making a firm diagnosis.[2]

Worldwide the most common symptoms of pediatric TB disease are a chronic cough for more than 21 days, a fever >38°C for 14 days (after common causes such as malaria and pneumonia have been excluded), and weight loss or failure to thrive.[1] Any child with any of these symptoms for a shorter duration than described above and a history of contact to an index case should have a TST planted and diagnostic workup for TB, which includes an HIV test. In young children, weight loss may be the only sign of disease. Adolescents, however, can exhibit typical B-cell symptoms such as night sweats, fever, and lymphadenopathy. Additionally, hemoptysis and fatigue are more commonly experienced in adolescents than younger children.

A prospective study in South Africa found that symptoms of cough >2 weeks and failure to thrive for at least 3 months provided a 68% sensitivity and 80% specificity in diagnosing HIV-uninfected children with tuberculosis, suggesting that simple symptom-based screening has considerable value in resource-limited settings.[40] A reduced sensitivity in symptom-based TB diagnosis was observed in children <3 years of age and those coinfected with HIV, both groups in which TB disease often is characterized by acute symptom onset.

There are no pathognomonic signs on the physical examination that can confirm a diagnosis of TB. Some uncommon but highly suggestive signs of pediatric TB include gibbus deformity of the spine, nonpainful lymphadenopathy, pleural or pericardial effusion, enlarged joints, and distended abdomen with ascites. A pneumonia or meningitis unresponsive to antibiotic treatment should also raise the suspicion of TB in endemic regions. Any documented weight loss, such as on a growth curve, is a good indicator of chronic disease in children and may suggest TB as a cause.

A study of young symptomatic children in South Africa found the vast majority of TB patients be accurately diagnosed on clinical grounds alone.[84] In this study 80% of the children were clinically diagnosed, usually within a day of presentation, with what eventually was culture-confirmed TB. The other 20% of children were diagnosed only after culture results were known, at a median time of 73 days after presentation. This retrospective study highlights that clinical diagnosis is a fairly sensitive way to diagnose TB, yet no information is provided on specificity and how many children were originally clinically misdiagnosed as having TB.

### *Clinical Diagnosis in HIV-Prevalent Regions*

The incidence of TB is low among HIV-infected children living in the United States.[85] Globally, however, both TB and HIV are leading causes of morbidity and mortality in children. In regions of high HIV prevalence, coinfection with TB is common. In 2004, at least 50% of children aged 0 to 9 years who developed TB were also HIV-infected.[2] TB is a major cause of death among HIV-infected children over 1 year of age, accounting for 32% of deaths among HIV-infected children over age 1 year in Zambia.[86] There is a six times higher mortality in HIV-infected children with TB compared with HIV-uninfected children.[87]

TB and HIV are inextricably linked. Susceptibility to TB is enhanced by HIV infection[88] and TB hastens the progression of HIV disease by increasing viral replication and further reducing CD4 counts. Reports suggest that HIV-infected children are more likely than HIV-uninfected children to be exposed to contacts with smear-positive TB.[89,90] Therefore, HIV-infected children are more likely than uninfected children to have TB disease secondary to high exposure risk, immunosuppression from the HIV, and HIV-related malnutrition.

HIV and TB disease share many clinical and radiologic features, making recognition of TB in a coinfected child particularly challenging. A prospective cohort study of children with TB found that HIV-infected children were at risk of diagnostic error as well as a delayed diagnosis of TB, and that weight for age could be used to identify children at high risk of a fatal outcome.[87] TB manifestations are more severe in HIV-infected children, with lower cure rates.[91] Young children with HIV are at high risk of morbidity from other respiratory diseases making symptom-based diagnostic approaches for TB less useful in HIV-infected children. A recent autopsy study from Zambia found that multiple pathogens could commonly be found in a single child and that the four most common findings were acute pyogenic pneumonia, *P. jiroveci* pneumonia, TB, and cytomegalovirus.[86] Thus, there is a risk that TB disease may initially be missed in an HIV-infected child who improves with empirical antibiotics.

A triad often used to identify children with TB (index case contact, positive TST, and findings on CXR) is less helpful in HIV-infected children. Due to immune suppression, TST is frequently negative in children with HIV infection, despite using a reduced induration cutoff of $\geq$5 mm. One study found $<$20% of coinfected children had positive skin tests. There have been only a handful of IGRA studies performed on HIV-infected children. The T-spot assay demonstrated better correlation with the degree of TB exposure[92] and had improved sensitivity in HIV-infected children treated for probable TB compared with the TST.[71]

A chest radiograph has well-recognized limitations in a coinfected child and is often unavailable in many resource-limited settings.[93] If a child has severe immunosuppression, 10 to 20% will have normal chest films despite having pulmonary TB disease.[94] Interpretation of chest radiographs is complicated by HIV-related comorbidities such as bacterial pneumonia, lymphoid interstitial pneumonitis, pulmonary Kaposi sarcoma, and bronchiectasis.[91,95]

## Radiologic Findings

Despite some subjectivity and reader variability,[96] the chest roentgenogram remains the most widely used diagnostic test in clinical practice.[97,98] A calcified lesion found in the parenchyma of the lungs indicates an old granuloma (Fig. 1). In this scenario the immune system has adequately walled off the *M. tb* infection.

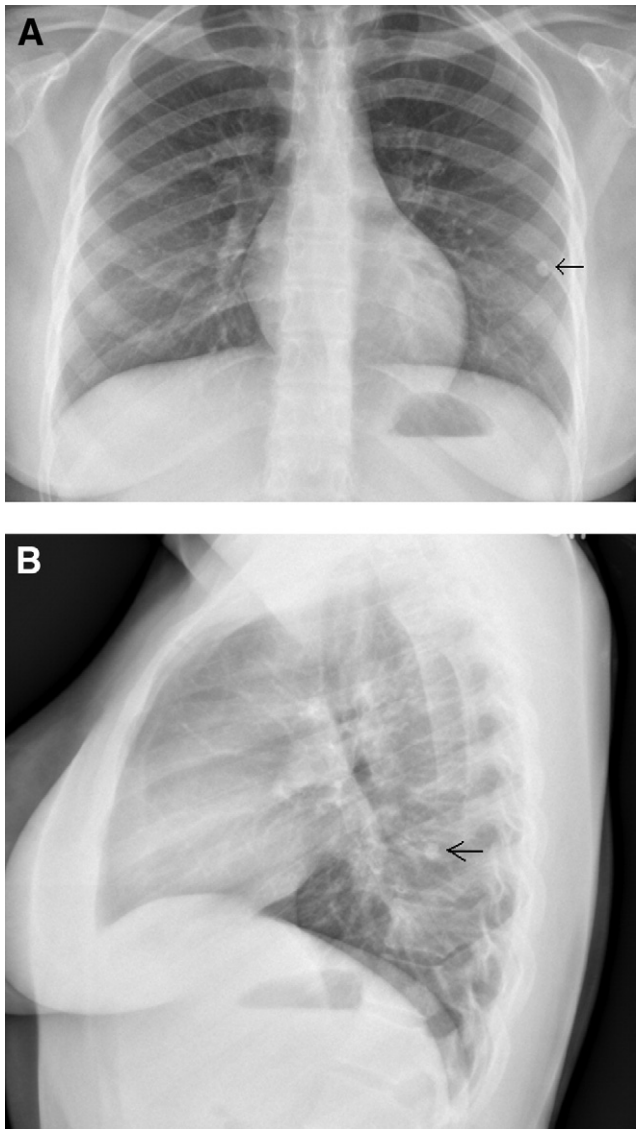


**FIG 1.** (A and B) Posteroanterior and lateral chest radiograph images of an adolescent showing a 7-mm calcified granuloma in the left lower lobe (arrows).

An asymptomatic child with these findings on CXR and a positive skin test or IGRA can be treated as a case of *M. tb* infection and not active disease.

The most common radiological feature found in children with TB disease is hilar or mediastinal lymphadenopathy (Fig. 2). On plain radiographs, hilar lymphadenopathy is best seen on a posteroanterior radiograph as a lobulated density but can also be viewed in lateral films posterior to the bronchus intermedius.[99] Associated parenchymal infiltrates are encountered on the same side as nodal enlargement in approximately two-thirds of pediatric cases of primary

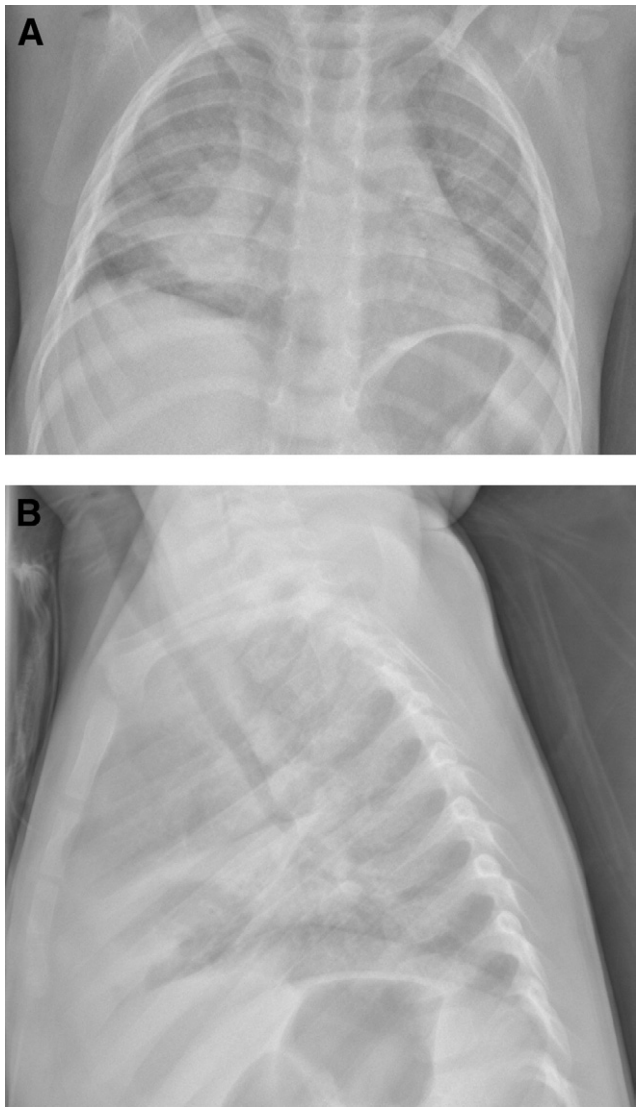


**FIG 2.** (A and B) Posteroanterior and lateral chest radiograph of 22-month-old girl with right middle lobe consolidation and mediastinal widening.

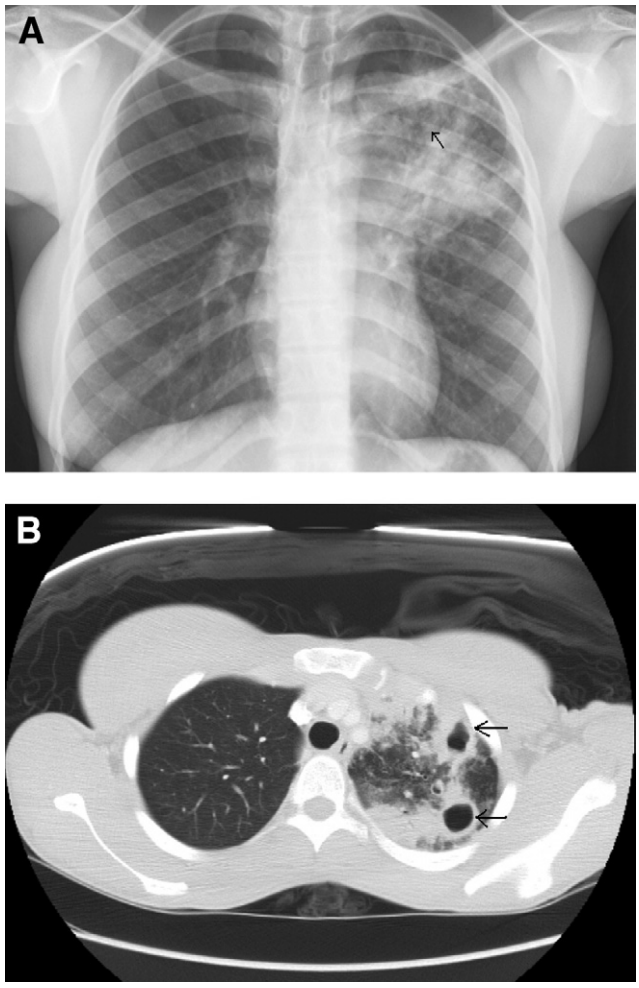


**FIG 3.** (A) Posteroanterior chest radiograph of an adolescent showing a consolidation in the left upper lobe with suggestion of a cavitary lesion (arrow). (B) CT scan of chest confirms consolidation with two cavitary lesions (arrows) in the left upper lobe.

pulmonary TB.[100] Common parenchymal changes that can be seen in a child with TB include alveolar consolidation (Fig. 2), pleural effusion or empyema, segmental hyperinflation, atelectasis, or a focal mass. Miliary TB, resulting from an acute hematogenous dissemination of bacilli in the lungs, has the only distinct radiologically diagnostic feature of TB disease and is characterized by fine bilateral reticular shadowing, sometimes called a snowstorm appearance.

Adolescents can present with adult-type reactivation disease. Post primary disease usually occurs in the apical, posterior segments of the upper lobes and apical segment of the lower lobe because the oxygen tension is highest in the upper lung zones. Cavitation in one or multiple sites is radiographically evident in 40% of cases of disease reactivation[101] (Fig. 3). Bronchogenic spread is radiographically identified in 20% of cases of TB reactivation, manifested as multiple, ill-defined micronodules, involving the lower zones of the lungs, usually distant from the site of cavitation.[102] Pleural TB is also more commonly seen in adolescents than in younger children (Fig. 4).

When available, computed tomography (CT) imaging can be helpful in a symptomatic child with a normal CXR or an asymptomatic TST- or IGRA-positive child with equivocal findings on CXR. Intravenous contrast medium should be used to distinguish lymph nodes from normal blood vessels. On CT scans,

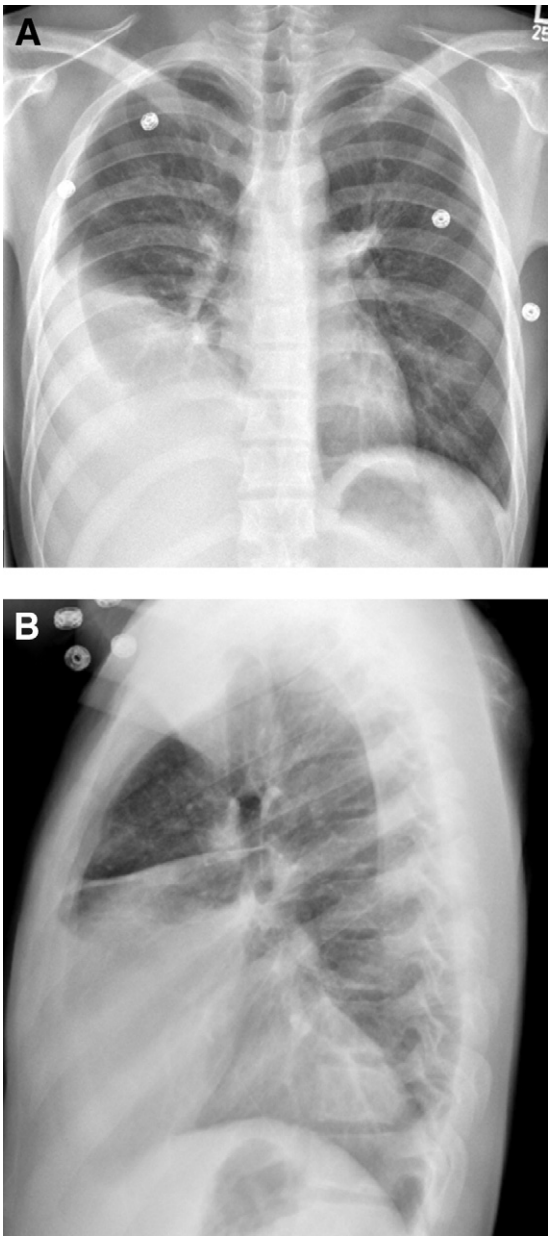


**FIG 4.** (A and B) Posteroanterior and lateral chest radiographs showing a right pleural effusion and consolidation of the right middle and lower lobe in an adolescent.

tuberculosis adenopathies often show the presence of conglomerated nodal masses with central lucency. Lymph nodes measuring over 2 cm with thick irregular rims of contrast enhancement and inner nodularity may suggest mycobacterial cervical lymphadenitis (Fig. 5). Particular manifestations such as endobronchial involvement, bronchiectasis, and early cavitation are more apparent on CT imaging than CXR (Fig. 3B). CT imaging is also useful in demonstrating pleural or

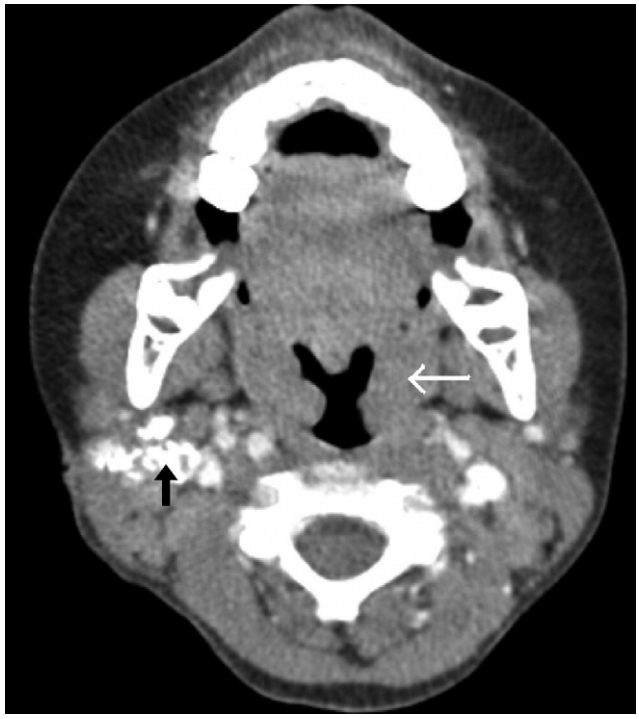

**FIG 5.** Axial CT image of the neck in an 8-year-old male shows calcified right cervical lymphadenopathy (black arrow) with tonsillar swelling (white arrow).

pericardial disease.[102] When CNS involvement is suspected, a CT scan may show hydrocephalus and basal enhancement, indicating meningeal involvement. Tuberculomas, focal CNS lesions, are also readily viewed on CT imaging and may appear as ring-enhancing or nodular-enhancing with surrounding edema.

The use of MRI for demonstrating intrathoracic lymphadenopathy is limited because of the sedation and respiratory gating requirements, as well as limited availability. MRI is the preferred study when chronic osteomyelitis is suspected because it enables evaluation of early marrow involvement and soft-tissue extension of the lesion (Fig. 6).

If brain or spinal TB is a possible diagnosis, MRI is an excellent tool for detecting basal enhancement and the spinal cord itself. Potts disease often involves a slow-growing abscess, called a cold abscess, located in the anterioinferior part of the vertebrae (Fig. 7). Over time bony destruction occurs, causing vertebral collapse and subsequent kyphosis. As the vertebral periosteum becomes destroyed (Fig. 7B), cord compression can occur, resulting in paraesthesia, paralysis, and cauda equine syndrome.

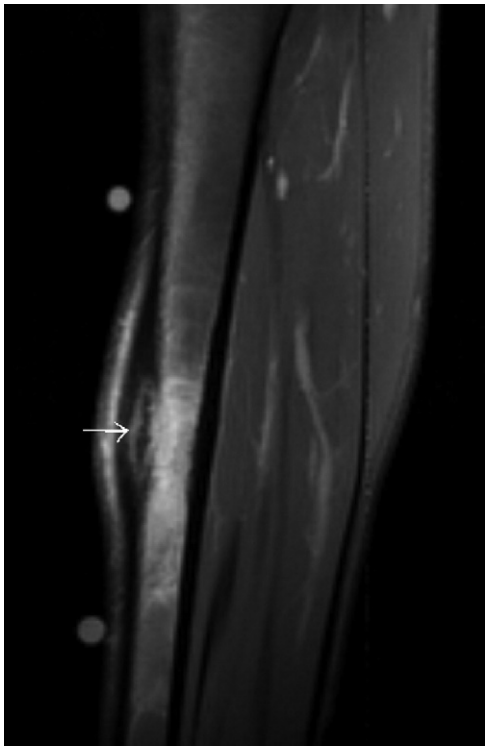

**FIG 6.** MRI image of an adolescent with a growing mass over 4 weeks. Sagittal post gadolinium image of the tibia showing periosteal elevation (arrow) corresponding to the palpable mass. There is involvement of the bone marrow that extends beyond the level of the periosteal reaction.

Ultrasound plays a role in the diagnostic imaging of abdominal and urogenital TB. Ultrasound is better than CT for detecting ascites and can also demonstrate organ lesions, lymph nodes, and masses.

## Pathogen-Based Diagnosis of TB Disease

### *Specimen Collection in Children*

In children, TB is often paucibacillary and extrapulmonary and it is therefore challenging to obtain adequate specimen for culture. Effort should be made to obtain specimens from any body cavity in which the organism may reside before starting antimicrobials. In addition, several specimens should be sent for evaluation to increase the chances of capturing the bacilli. Possible microscopy and culture sites include the following: whole blood, bone marrow (in miliary disease), biopsy specimens (ie, lymph node or bone), cerebral spinal fluid, sterile fluids (ie, pleural), feces, midstream urine, and bronchoalveolar lavage fluid. Sterile leak-proof containers without fixative agents

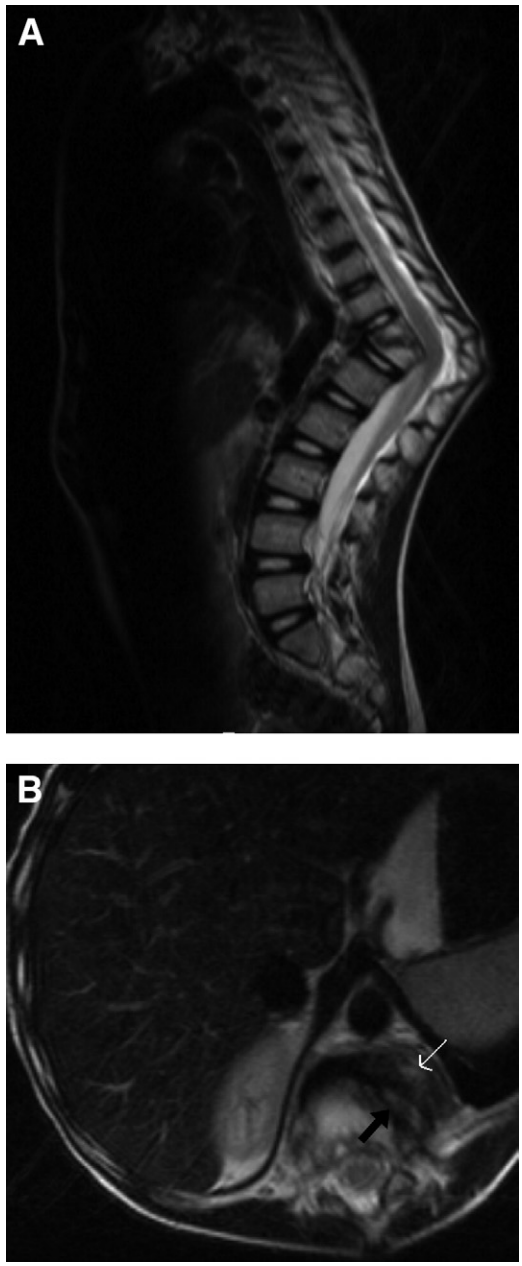


**FIG 7.** (A) A 6-year-old girl presented with severe kyphosis. Her sagittal T2-weighted image of the thoracic spine showed destruction of the T11-T12 intevebral disc space as well as destruction of the vertebral bodies resulting in kyphosis and compression of the spinal cord. (B) Axial T2-weighted image at the T11-T12 level shows a left-sided paraspinal mass (white arrow) causing destruction of the cortex of the vertebral body (black arrow).

should be used for collection and quickly transported to the laboratory where examination should be performed on the day of receipt. If brief storage is necessary, specimens other than blood should be

**TABLE 4.** Specimens for examination and diagnostic studies

| Specimen | Procedure |
|---|---|
| Aspirates, fluids | Sterile syringe, container, or direct inoculation to culture media |
| Bone, bone marrow | Direct inoculation to broth culture media designed for mycobacterial blood cultures or ISOLATOR tube |
| Bronchial washings | Sterile container |
| Gastric lavage | Obtain when patient awakes<br>50 mL gastric aspirate in sterile container with 100 mg of sodium carbonate |
| Sputum | Exudates from lungs by a productive cough (not saliva), sterile container |
| Stool | Clean container |
| Tissues, biopsy specimens | Sterile container |
| Urine | First morning void, do not pool |

Adapted from Hanna.[115]

refrigerated to inhibit the growth of contaminating microorganisms. General guidelines for collection of various types of specimens are listed in Table 4. Specimens that are normally expected to be sterile, such as blood and urine, may be inoculated directly to culture media. Conversely, specimens that may be contaminated with normal flora microorganisms require digestion and decontamination before inoculation to culture media to prevent the overgrowth of these more rapidly growing microbes.

## Obtaining Sputum in Children

Different types of collected specimens have been used for confirmation of pulmonary TB including gastric aspirates, induced sputum, bronchoalveolar lavage specimens, or specimens collected using the string test. Whichever technique is used, at least three specimen samples should be evaluated to ensure recovery of low numbers of mycobacteria. On arrival in the laboratory, most specimens are homogenized with a mucolytic agent and decontaminated before inoculation to culture media to inhibit the normal flora.

The stain and culture yield from three properly obtained gastric aspirates is higher than from bronchoalveolar lavage.[103-105] Collection of gastric content is best performed after a child has fasted for at least 8 hours and is still in bed. Aspiration is performed via a silastic nasogastric tube, which is inserted before the child has taken anything by mouth. When aspiration of gastric secretions is unsuccessful, sterile distilled water can be instilled and aspirated. If gastric aspiration is performed under these conditions, *M. tb* can be recovered in up to 50% of children with TB disease.[106] Nonhospitalized children can also undergo gastric aspiration for Acid-fast bacilli (AFB) staining and culture, but the yield is lower.[107]

In a study of young children (median age, 13 months) living in South Africa with suspected pulmonary TB, the yield from a single induced sputum was equivalent to that from three gastric lavages.[108] In addition, almost 40% who were culture positive on sputum were also smear positive, enabling rapid diagnosis of pulmonary TB.[108] Sputum production was stimulated by inhalation of sterile hypertonic saline (3-5%). Unfortunately, the safety and feasibility of this technique has not been studied outside the hospital setting and further studies are needed to validate the sensitivity in children less than 5 years of age.

Induced sputum in older children is easily performed in an outpatient setting, which is an advantage over the hospitalization required when obtaining specimens via gastric lavage. Induced sputum should be collected after the child has rinsed the oral cavity with water to remove excess oral flora. Infection control measures should be used to reduce exposure to other patients and staff.

The string test is a novel approach that has recently been evaluated for its ability to retrieve *M. tb* from sputum smear-negative adults infected with HIV.[109] A gelatin capsule that adheres to gastric contents over a couple of hours is swallowed and then pulled back up for investigation. In a recent study the string test was shown to be well tolerated in children over age 4 years, yet there were no positive TB cultures detected within this small study.[110]

## Microscopy

Microscopic examination of acid-fast-stained smears is one of the easiest, most inexpensive, and rapid methods for demonstrating the presence of mycobacteria in clinical specimens and cultures. Fluid and solid specimens that have been liquefied can be stained. Stains are used to penetrate the bacilli's cell wall. Once stains are applied, they are difficult to remove, even when mineral acids are applied, hence the name "acid-fast." Fuchsin-stained smears are examined with a Brightfield microscope under oil immersion. AFB appear as red and rod-shaped filaments. The most common staining technique used to identify AFB is the Ziehl-Neelsen stain, in which the bacteria are

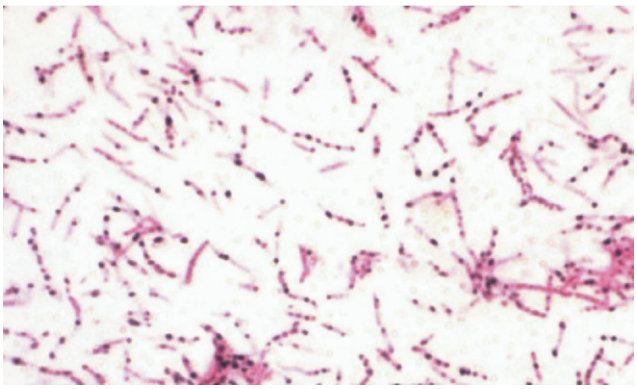

**FIG 8.** Kinyoun-stained smear of specimen concentrate showing numerous acid-fast bacilli displaying the typical beaded appearance of *M. tb*. (Photograph courtesy of Bruce Hanna, PhD.) (Color version of figure is available online.)

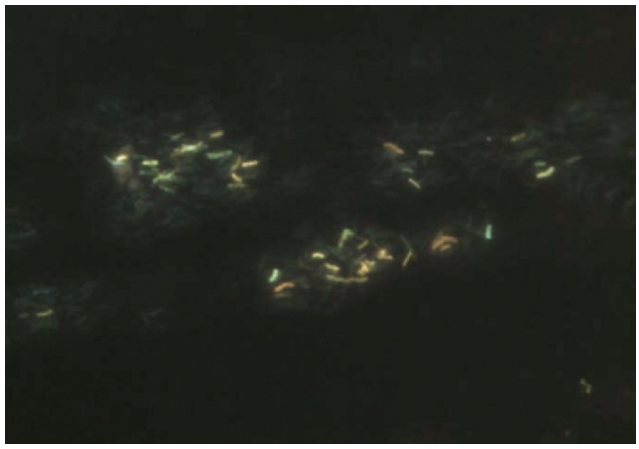

**FIG 9.** Auramine O-stained smear showing numerous acid-fast bacilli. (Photograph courtesy of Bruce Hanna, PhD.) (Color version of figure is available online.)

stained bright red and stand out clearly against a blue background. Another method is the Kinyoun method, in which the bacteria are stained bright red and stand out clearly against a green background (Fig. 8). AFB can also be visualized by fluorescence microscopy using specific fluorescent dyes, such as auramine and rhodamine. Fluorescence-stained AFB smears have similar morphology and will fluoresce yellow when examined with a fluorescent microscope (Fig. 9). The advantages in sensitivity of fluorescence over light microscopy for detection of pulmonary TB have recently been confirmed in a systematic review of 45 studies comparing the two methods that found that fluorescence microscopy yielded an average increase in sensitivity of 10%, without loss of specificity.[111] The equipment costs for fluorescence microscopy are high, so utility has been limited to regions that can afford it.

All mycobacteria exhibit various degrees of acid-fastness so microscopy cannot be used to determine the individual species of mycobacteria including *M. tb*. Despite this, staining has significant advantages because it remains the most rapid technique and is of value in identifying the most infectious patients for hospital and community infection control. The burden of bacterial infection is usually reflected by the number of organisms seen on microscopic examination of stained smears. As a general rule, a positive sputum smear examination indicates a concentration of at least $10^4$ bacteria/mL.[53] Higher grading of results (1+ to 4+) indicates greater concentration of bacteria. Environmental contamination, which usually involves only a few organisms, rarely results in a positive smear examination.

Given the paucibacillary nature of pediatric TB, most children are smear-negative. Less than 10 to 15% of children with proven TB will have a sputum or gastric aspirate stain positive for AFB.[112] The rates of positive smears from other specimen sources are even lower.[113] Adolescents, however, often develop adult-type disease and have higher bacteriological yield.[95] Further, microscopy evaluation in low-income settings is often performed on unconcentrated sputum and the sensitivity of this method for detecting mycobacteria is limited.

## *Traditional Culture Methods*

Culture is more sensitive than microscopy and can detect as few as 10 to 100 bacteria/mL of material[114] but is positive in less than 50% of children with active disease (Table 5). There are two major forms of media which are routinely used in mycobacteriology laboratories, solid and liquid media. The main advantage of solid media is that it allows the determination of characteristic features of colonial morphology, growth rate, and pigment production, which can yield significant preliminary information. The two types of solid media commonly used are egg-based and agar-based media. Lowenstein-Jensen (LJ) is an egg-based media and most commonly used globally. LJ contains malachite green that suppresses growth of contaminating bacteria and fungi and is used for both detection and susceptibility testing. In resource-challenged countries

**TABLE 5.** Diagnostic modalities for detection of *M. tb infection*

| Diagnostic modality | *M. tb* stage | Accuracy in children | Cost | Turn around time | Drug sensitivity testing |
|---|---|---|---|---|---|
| Clinical diagnosis | Active | Moderate sensitivity, low specificity, poor performance in HIV-infected children | Low | Immediate | No |
| TST | LTBI & active | High sensitivity and moderate specificity | Low | 2-3 d | No |
| IGRAs | LTBI & active | High sensitivity in children >2 y, highly specific | High | 2 d | No |
| Radiologic | Active | High sensitivity, low specificity | Moderate | Hours | No |
| Direct smear | Active | 10-15% sensitivity, not specific for *M. tb* | Low | Hours | No |
| Conventional culture media. (Gold standard) | Active | 30-40% sensitivity, high specificity | Low | 3-4 wks | Yes |
| Liquid culture systems | Active | <50% sensitivity, high specificity | High | 2-3 wks | Yes |
| MODS | Active | No data in children | Low | 1 wk | Yes |
| Phage-based tests | Active | No data in children | Low | 2-3 d | Yes |
| Colorimetric culture systems | Active | No data in children | High | 1-2 wks | Yes |
| NAAT | LTBI & active | Moderately sensitive, highly specific | High | 2 d | Yes |
| Antibody-based | LTBI & active | Moderate sensitivity, moderate specificity | Low | 1 d | No |
| LAM-ELISA | Active | No data in children | Low | 1 d | No |
| *M. tb*-specific skin tests | LTBI & active | Phase I trials underway | Low | 2-4 d | No |

Abbreviations: TST, Tuberculin skin test; IGRAs, interferon-gamma-releasing assays; MODS, microscopic observation drug-susceptibilty assay; NAAT, nucleic acid amplification test; LAM-ELISA, lipoarabinomannan enzyme-linked immunosorbent assay test.

LJ is often the only media used, while in the USA it is used in conjunction with liquid media.

It is easier to observe colony morphology and enumerate colonies on Middlebrook 7H10 and 7H11, agar-based media. Middlebrook 7H11 is better than LJ at establishing resistant *M. tb* isolates. The average time for *M. tb* detection on LJ and Middlebrook 7H11 media is 4 and 3 weeks, respectively. Once a colony appears, physiologic tests are performed to identify mycobacterial species. Such tests include pigment inspection, microscopy examination for cording, colony morphology, and biochemical tests. A reliable method for identifying mycobacteria is the use of chromatography for the analysis of fatty acids extracted from the mycobacterial cell wall. Molecular methods used to speciate mycobacterium include the use of gene probes in hybridization assays, amplification with subsequent DNA sequencing, or restriction fragment length polymorphism analysis.

## Liquid-Based Culture Systems

Liquid media support growth of the *M. tb* complex better than solid media with an increased recovery of positive cultures,[115] even in cases in which a child was started on anti-TB medication before obtaining cultures.[116] An important advantage of the liquid media is the shorter time to detection of positive cultures than in solid media. Isolation and susceptibility testing of *M. tb* in liquid media can be reported on the average of 2 and 4 weeks, respectively.[117] The average time to recovery for smear-positive specimens is only 8 days compared with 18 days for solid conventional media.[118] Because of the significant increase in the positive culture rates and the timesaving, the CDC has recommended the use of commercially available liquid media for detection as well as for drug susceptibility testing of *M. tb* in the USA.[119] The first broth-based mycobacterial detection system was the BACTEC 460, which uses modified Middlebrook broth and a novel radiometric detection scheme. Mycobacterial growth can be periodically ascertained by the liberation of $^{14}CO_2$ as metabolized by the mycobacteria and detected by the BACTEC instrument. Most recently, nonradiometric liquid culture systems have been developed to minimize the handling and disposal of radioactive waste.[115]

The Mycobacterial Growth Indicator Tube (MGIT; Becton Dickinson, Baltimore, MD) is supplemented with enriched growth media and antibiotics and processes all types of clinical specimens other than blood and urine; however, a recent study in India found high sensitivity when using concentrated urine specimens.[120] The automated MGIT system has slightly superior sensitivity and reduced turnaround time compared with conventional LJ culture.[121] There is a manual MGIT system that allows anti-TB drug susceptibility testing. Tubes are examined for fluorescence by placing them on a 365-nm UV transilluminator. However, the high cost of even the manual MGIT system and

laboratory infrastructure required remain major limitations in resource-challenged regions.

It is customary to use at least two types of media to maximize the recovery of *M. tb*: liquid media to assure rapid results and solid media to examine clinical morphology (which is not possible in liquid media). Moreover, using solid media provides a backup in case of contamination of the liquid media.[116]

Because mycobacteremia and miliary TB occur more often in children with TB than adults, it is always important to obtain blood cultures in a child with suspected TB disease. The ISOLATOR tube (Wampole Laboratories, Princeton, NJ) provides a unique approach to the recovery of mycobacteria, which tend to circulate intracellularly. The ISOLATOR system uses saponin to lyse leukocytes, thus releasing any intracellular microbes. After centrifugation, the sediment can be used to inoculate a variety of mycobacterial media.

## Susceptibility Testing

The incidence of multidrug resistance (MDR) TB, defined as resistance to both isoniazid and rifampin, is increasing in many parts of the world. As children have lower rates of TB isolation, MDR TB is often initially identified exclusively in the adult index case.

Chromosomal alterations such as mutations or deletions are the unique mechanisms by which mycobacteria acquire drug resistance. These chromosomal alterations affect either the drug target itself or the bacterial enzymes activating the prodrug. In the United States, drug susceptibility is performed on all initial isolates and on any isolate from patients with persistent positive cultures or relapse of clinical symptoms. In addition to the immediate benefit in planning a therapeutic regimen, drug susceptibility information is valuable for public health. Understanding resistance patterns in a particular region provides strategic policy advantage for the treatment and control of TB.

There are a variety of methods to determine the susceptibility of *M. tb* to anti-TB drugs. From a technical perspective, drug susceptibility is determined on the basis of growth (or metabolic) inhibition induced by the drug by means of (1) macroscopic observation of growth in drug-free and drug-containing media; (2) detection or measurement of the metabolic activity or products; (3) lysis with mycobacteriophage; and (4) detection of genetic mutations using molecular techniques.

A direct test uses the processed clinical specimen for susceptibility testing, whereas the indirect method uses the primary isolate culture for testing. Both direct and indirect methods can be performed using either conventional or rapid techniques. Conventional techniques involve inoculating the microorganisms to a solid medium that contains a known concentration of the test drug. The most commonly used conventional technique in the USA is the proportion method. Dilutions of the inoculum are made so that growth on control media results in the production of a number of colonies that is counted and compared to the number of colonies that grow on the drug containing media. Thus, the proportion of organisms resistant to a drug can be measured and expressed as a percentage of the total population. If more than 1% of the test population is resistant to the drug being tested, it is established that resistance to that drug has developed. Results usually take about 3 weeks by the conventional method. In contrast, BACTEC susceptibility results are usually available in as little as 4 to 6 days after inoculation and provide results with a high degree of correlation to the proportion method.

Newer techniques including the microscopic observation drug susceptibility (MODS) and the phage-based assays enable laboratories, particularly those in resource-limited regions where the incidence of MDR TB is the highest, to have access to modern drug susceptibility methods (Table 5). The luciferase reporter mycobacteriophage is reported to be a rapid method that is highly accurate,[122] but its specificity may vary.[123] The MODS technique is a liquid-based methodology that also enables rapid (within 10 days) detection of drug resistance.[124,125]

## Nucleic Acid Amplification Techniques (NAAT)

NAATs are diagnostic methods based on the amplification of *M. tb* DNA. During the past decade NAAT techniques have been developed for a more rapid identification of *M. tb* on direct or cultured specimens, for genotypic drug-resistance testing, and for molecular typing used in epidemic investigations.[126] However, NAATs have not been routinely applied to clinical specimens due to variations in methodology, limited accuracy, and the high cost of running the assays.

The DNA sequence most frequently used to detect *M. tb* has been the insertion element IS6110. Primers amplify a target fragment of 200 base pairs from the

insertion-like *M. tb* sequences element IS6110. Various NAATs are commercially available, including polymerase chain reaction (PCR) (Amplicor, Roche, Basel, Switzerland); transcription-mediated amplification (GenProbe, San Diego, CA), strand displacement amplification (ProbeTec-SDA, Becton Dickinson, Franklin Lakes, NJ), and ligase chain reaction (Abbott, Abbott Park, IL). These tests offer high specificity for *M. tb*, usually ranging between 80 and 95%.[127] Recently, two commercial molecular assays have used conventional PCR followed by amplicon hydridization onto a series of oligonucleotide probes on nitrocelullose strips to detect rifampin resistance with a 1-day turnaround time. A number of trials have shown good correlation with conventional rifampin susceptibility testing.[128,129]

The FDA has recently approved two NAAT tests, Gen-Probe's Enhanced Amplified *M. tb* Direct Test and Amplicor *M. tb* test. Both assays were approved for adults with smear-positive and smear-negative respiratory samples for patients clinically suspected of having TB. However, most of these techniques have not been applied in routine clinical care because of the presumed high false-positive rate due to contamination.

In research laboratories, nucleic acid amplification and hybridization are very sensitive and detect as few as 10 bacilli.[130] Although it was expected these tests would be highly sensitive in clinical samples, studies have shown that sensitivity is relatively poor, ranging as high as 90-100% in smear-positive sputum, but only 48-70% in smear-negative, culture-positive respiratory samples in adults.[53,131] A negative NAAT result, therefore, does not rule out TB. NAATs may be tested on any specimen thought to contain bacilli (blood, urine, cerebral spinal fluid (CSF)) but there is even less sensitivity reported in nonrespiratory samples.[132] Sensitivity is improved when multiple samples are tested, because not all samples necessarily contain detectable nucleic acid. An increase in sensitivity can also be achieved if DNA purification is performed before amplification, but some specificity is compromised.[133]

Performing PCR on urine has the potential to be a rapid and noninvasive technique to diagnose TB. However, several studies found sensitivity of the test to be between 8 and 56% in cultured-confirmed patients with pulmonary TB.[134,135] A novel PCR test that identifies short[67] base pairs specific for *M. tb* using primers selected from the IS6110 region was recently reported to have a sensitivity approaching 80% in patients with active TB using transrenal DNA.[136] Yet, this technique has not been validated in other studies, although it does hold promise for an alternative technique in children.

Several recent meta-analysis studies on NAATs have shown that they are not consistently accurate to be routinely recommended for the diagnosis of smear-negative pulmonary TB or extrapulmonary TB, which of course represents the majority of childhood TB cases. However, the study authors suggest that NAATs could be useful in smear-positive or bronchial specimens (as opposed to sputum or gastric aspirates).[131,137,138]

Experience with NAATs on pediatric samples is limited. Several studies in children have found the PCR test on clinical samples to have a sensitivity of 40-60%,[127,139,141] which is better than standard culture sensitivities. However, up to 39% of children with no radiologic or clinical evidence of TB disease had positive PCR results.[140] It was impossible to determine if these results were secondary to the PCR detecting LTBI or false-positive results from contamination. PCR use in other body fluids or tissues, such as CSF, appears to have been even less successful.[137] PCR is less sensitive than AFB smear and culture for children with TB meningitis.[142] PCR may be most helpful in a symptomatic child when the diagnosis of active TB is difficult and an early diagnosis can help exclude other etiologies. Its routine use is limited by its expense and complexity. Overall, NAATs have not performed to expectations, but efforts are underway to simplify testing protocols and increase accuracy. The immediate application of NAATs in childhood TB is for rapid drug-resistance mutation screening in patients with culture-confirmed TB.

## Novel Assays That Are Field Friendly

***MODS.*** To have an impact on TB control and patient care in resource-limited settings with high TB burden, diagnostic tools need to be affordable, rapid, robust, and technically straightforward (Table 5). The MODS assay was developed by Luz Caviedes in the late 1990s in Peru as a method to rapidly diagnose pulmonary TB and multi-drug-resistant strains.[125] MODS development was based on the following three key principles: (1) *M. tb* grows faster in liquid (broth) than on solid media; (2) in liquid cultures *M. tb* grows in a visually characteristic manner (tangles, cording), which can be observed under the microscope long before the naked eye could visualize colonies on solid

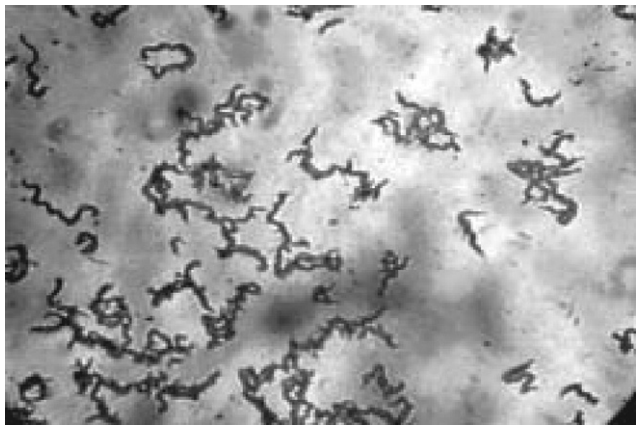

**FIG 10.** In the microscopic observation drug susceptibility (MODS) technique positive cultures are identified by cord formation, characteristic of *M. tb* growth in liquid medium in drug-free control wells. (Photograph courtesy of Robert Gilman, MD.)

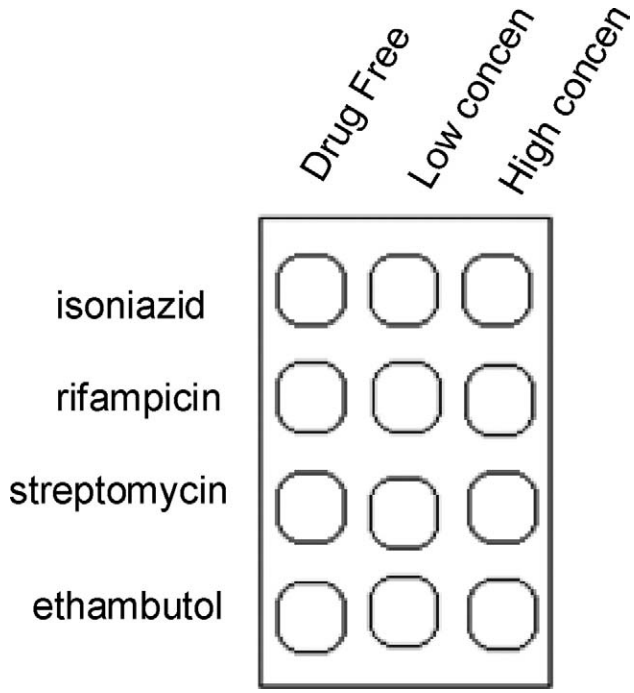


**FIG 11.** Schematic of sample layout of MODS plate, which allows for culture and susceptibility testing. If multi-drug-resistant TB is identified, any anti-TB drugs can be used in MODS.

agar (Fig. 10); and (3) incorporation of anti-TB drugs into broth cultures at the outset enables direct susceptibility testing from sputum samples. MODS is a methodology usable to all and not a product. As such, all consumables and reagents are available from standard laboratory suppliers. This keeps the cost of the assay down to as low as $2.00 a specimen.

MODS is performed on sputum samples that are decontaminated (to minimize occupational exposure) and placed into 12 aliquots (Fig. 11). Antimicrobial and nutritional supplements are added to the Middlebrook 7H9 culture medium, which is then placed into each of the wells. Four of the wells are drug-free and the other eight have low and high concentrations of anti-TB medications. A plastic cover is then placed on the plate and the whole plate is sealed in a ziplock plastic bag for safety. The plate is read through the bag under a microscope. Positive cultures are identified in drug-free control wells by cord formation, characteristic of *M. tb* growth in liquid medium (Fig. 10). Concurrent growth in drug-containing wells indicates resistance. Non-TB mycobacteria are recognized by their lack of cording, except for *Mycobacterium chelonae*, which is the only non-TB mycobacteria that does form cords. *M. chelonae* is ubiquitously in the environment (soil, water, and dust particles) but rarely causes infection. Fungal or bacterial contamination of a MODS assay is recognized by rapid overgrowth and clouding of wells.

Plates are permanently sealed in ziplock bags through which microscopic examination is made, thus spillage of mycobacterial "soup" cannot occur, as experienced at times with other liquid culture systems is limited. Furthermore, as no secondary subculture is needed, and because this is a direct susceptibility testing, no further manipulation is required and there is little potential for aerosolization.

MODS has been evaluated recently in several studies comprising more than 3000 adults, including HIV-infected persons, and 5600 sputum specimens.[124,143,144] These studies reveal that MODS is at least as good a test as traditional culture methods, while requiring less time for diagnosis and drug-sensitivity testing. In several large prospective studies using sputum testing for pulmonary TB, the overall sensitivity of the MODS method was 97 to 98%[124,143,144] and 92 to 97% for MDR TB.[144,145] In fact, MODS had a similar sensitivity than traditional methods (automated mycobacterial culture or LJ culture) in each of the studies. Specificity was between 94 and 99%. A major advantage of MODS over standard culture techniques is the rapid time to TB diagnosis. The median time to detection for MODS is 7 to 9 days compared with 21 to 26 days for standard LJ culture medium.[124,143,144] Pleural TB can also be diagnosed more efficiently and accurately than by using standard mycobacterial culture

techniques.[146] Unfortunately, there have never been any clinical trials in children.

There are several limitations to the MODS technique. Although originally developed to be applicable in a laboratory with minimal infrastructure, the lack of a kit format has limited the application of this promising assay in the field. Moreover, the qualitative nature of the MODS assay limits a resistance proportion evaluation of cultures. In solid-agar methods there are discrete colonies to count, and therefore, prevents a resistance proportion evaluation of cultures.

***Colorimetric Systems.*** Colorimetric systems depend on the metabolites and enzymes produced by different species to change color, allowing for early mycobacterial identification before visible bacterial colonies appear. Colorimetric tests are based on broth cultures and the simple procedure, safety profile, and low cost make them field friendly. Moreover, the rapid identification time and ability to test for drug susceptibility provide advantages over standard solid culture. Only a handful of studies have evaluated each system, and none are FDA-approved. A recent meta-analysis found that colorimetric methods are highly sensitive and specific for rapid detection of rifampin and isoniazid resistance in culture isolates.[147] However, only one of these studies in the meta-analysis performed the assay directly to clinical specimens, so there is still a great need to validate these novel systems on actual specimens. In addition, there have been no colorimetric studies performed in pediatric populations.

***Phage-Based Tests.*** Mycobacteriophages are DNA viruses that infect mycobacteria. Following phage replication, lysis of the cell wall occurs, eventually destroying the host bacterium and releasing the progeny phage. Although attempts at using mycobacteriophages for TB therapy have been unsuccessful,[148] implementation into diagnostics has provided a useful, cost-effective, and easy tool for diagnosing *M. tb* and for drug susceptibility testing. The techniques use a mycobacteriophage to infect live *M. tb* and detect the bacilli using either a phage-amplification method or a light detection method. The underlying principle of the first method is the amplification of phages after infecting *M. tb*, followed by the detection of progeny phages as plaques on a lawn of *Mycobacterium smegmatis*. In the second method, the principle is the detection of light produced by luciferase reporter phages after infecting live *M. tb*.[149] This assay uses recombinant DNA technology to insert the gene for luciferase (an enzyme found in fireflies) into mycobacteriophage. In the presence of luciferin substrate, phage-infected bacilli emit light that can be detected with a luminometer or by a photosensitive film.

A commercial bacteriophage kit, FASTPlaque TB® (Biotech Laboratories Ltd, Ipswich, UK), is not FDA approved but has CE-licensure for use in Europe. Results with this commercial test have been mixed and show that it may not be as sensitive as traditional culture methods.[150-152] While some studies report higher sensitivity than microscopy,[152-154] further trials are needed to evaluate this technology to include assessment of economic and logistical factors. It is likely that improved sensitivity will be required before bacteriophage tests are judged suitable for routine diagnostic screening. A new version of FASTPlaque has been developed for direct use on sputum specimens to detect rifampicin resistance. The test is in a single-tube format and takes 48 hours to perform. The results of several studies showed promising results as 100% of the rifampicin-resistant strains were detected.[153,155,156] However, some false-positive results were observed where susceptibility strains were wrongly classified as resistant.

The luciferase reporter phage can also be used to screen for drug resistance in cultures of *M. tb*. It may be tested against rifampicin within hours, whereas slow-acting drugs such as ethambutol, isoniazid, and ciprofloxacin require 2 to 3 days.[157] The luciferase-reported phage assay can be performed in an automated 96-well plate format using a luminometer to detect the emitted light. Thus, the method could be convenient for large-scale screening programs.[157] However, the high cost of such equipment would restrict its use in the poorly resourced laboratories of high-burden countries. Detection of emitted light by photosensitive film is a less expensive alternative and a device using dental radiograph film has been developed by Riska and coworkers,[157,158] and Jacobs and coworkers,[159] and evaluation studies are in progress (Sequella Inc, Rockville, MD).

The advantages of phage-based assays include short turnaround time (2-3 days) and the opportunity for drug-susceptibility testing and require a laboratory infrastructure similar to that used for performing mycobacterial cultures. A disadvantage to the phage-based assays is that they are not specific for *M. tb* and can cross-react with environmental mycobacteria. Moreover, most studies to date have been performed on culture isolates (reducing rapidity) or sputum, which is, of course, a significant limitation in pediatric

**TABLE 6.** Chemistry and cellular characteristics commonly observed in fluid specimens infected with *M. tb*

| Fluid specimen | Glucose level (g/dL) | Protein level (g/dL) | Leukocyte count (cells/mm$^{3)}$ | Predominant leukocyte | Adenosine deaminase level (IU/L) |
|---|---|---|---|---|---|
| Pleural[a] | <70 | >4 | 1000-5000 | Lymphocytes | >36 |
| CSF[b] | <40 | 200-300 | <300 | Lymphocytes | >7 |
| Synovial[c] | Low | High | 10,000-20,000 | Neutrophils | — |

[a]Merino and coworkers,[48] and Andersen and coworkers.[64]
[b]Kashyap and coworkers,[167] and Xavier and coworkers.[186]
[c]Wallace and Cohen,[187] and Allali and coworkers.[188]

patients. These assays have also not yet been validated in any studies involving children.

***Antigen-Based Assay: Lipoarabinomannan (LAM) Detection.*** Attempts have been made to detect *M. tb* antigens directly in body fluids. Assays using serum or urine may have particular application in extrapulmonary disease, which tends to occur more often in children or people coinfected with HIV and TB. Recently, monoclonal antibodies have been developed to target proteins and glycolipids such as LAM, a heat-stable high-molecular-weight glycolipid present in the cell wall of mycobacteria. Several groups have demonstrated elevated LAM levels in the sputum,[160] serum,[160] and urine[161,162] of TB patients. Recently a large study in Ethiopia found a urine LAM ELISA was slightly more sensitive than smear microscopy (74% versus 69%) in culture-proven TB patients.[161] A U.S. company, Chemogen, developed a urine test called LAM-ELISA and in 2005 it was evaluated in Tanzania in 231 subjects, many of whom were HIV-infected.[163] There was 80% sensitivity (76% of the smear-negative, culture-positive patients were LAM-positive) and 99% specificity among the healthy volunteers also screened using LAM-ELISA. However, similar studies have yet to be undertaken, and it is suspected that the relatively good sensitivity reported in this study may be related to higher LAM concentrations in patients coinfected with TB and HIV compared with those who have TB infection alone.

## Immune-Based Diagnosis of TB Disease

### *Chemistry and Cellular Characteristics in Fluid Specimens*

Markers of inflammation often provide valuable information when determining if *M. tb* is a cause of infection. Liquid specimens commonly obtained for diagnostic evaluation include pleural fluid, CSF, and synovial fluid. Significant inflammation in the pleural tissues from *M. tb* causes an accumulation of exudative fluid that is usually secondary to the inflammatory process rather than the bacillus itself. Given the paucibacillary state of the infection of the pleura, AFB smears and culture are rarely positive and the disease is mostly diagnosed by examining the chemistry and the cellular characteristics of the pleural fluid specimen (Table 6). A common finding in *M. tb* infected fluid is a low-glucose and high-protein level secondary to the leukocytes recruited to the site of infection. The doubling time of mycobacteria is much slower than more common causes of pyogenic infections and hence the glucose levels are usually not as low as that observed in other bacterial infections. The chronic nature of TB infection also causes a lymphocytic predominance in CSF and pleural fluid.

Adenosine deaminase (ADA) is an enzyme found at the cell surface of lymphocytes and macrophages. It catalyzes the conversion of adenosine to inosine and is generally elevated in regions of active lymphocyte proliferation. Several studies have consistently shown high ADA levels in the pleural, peritoneal, and CSF in the respective anatomic patients in patients infected with *M. tb*.[48,164-166] One meta-analysis found ADA levels over 36 to 40 IU/L had a sensitivity of 100% and specificity of 97%.[166] A study in adults with meningitis found an ADA level above 5 U/L in the CSF had a 81% sensitivity in culture-confirmed TB meningitis and 86% specificity in the non-TB meningitis group.[167] ADA is usually determined by the Giusti colorimetric method, which is based on a simple reaction to form ammonia. Since ADA determination is a fast, inexpensive, and discriminating test, effusions and CSF should be analyzed when working up a patient with active TB.

### *Serology*

Serologic testing can detect specific antibodies to mycobacteria in serum and is an attractive diagnostic method due to its ease of application, depen-

dence on humoral immunity (an important factor in HIV-infected, T-cell-depleted patients), and possible future applicability to determine stage of infection. A number of serologic tests for TB have been developed recently using a variety of antigens to detect certain antibodies in the blood, including complement fixation tests, hemagglutination tests, radioimmunoassay, and ELISAs. Serologic tests are relatively simple and inexpensive to perform, so the effort to develop a reliable test has been extensively explored. Most studies to date have involved adults living in resource-poor regions. As yet, none of these tests have shown adequate accuracy due to great heterogeneity of humoral responses in patients with TB and cross-reactivity with other mycobacterial species. Therefore, no serologic test has been widely implemented in clinical care.[168] A particular problem in pediatrics is that children tend to have lower antibody titers than adults.[169] A recent comparison of seven serologic tests for active TB in adults found poor to moderate sensitivity ranging from 16 to 57% and variable specificity of 62 to 100%.[170] The combination of two serological tests had a higher accuracy: sensitivity 66% and specificity 86%.[170]

A systematic review was conducted by Steingart and coworkers to assess the accuracy of commercial serologic antibody detection tests for the diagnosis of extrapulmonary TB.[111] In that review, 21 studies reported sensitivities between 26 and 100% and specificity estimates from 59 to 100% for extrapulmonary TB. Among the 21 studies there were no data on children, a group for which TB detection would be most useful. The article concluded that, at present, commercial antibody testing for extrapulmonary TB has no role in clinical care.[111]

Most recently researchers have purified naïve or recombinant antigens and this has led to the increased specificity of serologic TB testing. Various antigens, such as antigen 85A, antigen 85B, 38-kDa protein, alpha-crystallin (16 kDa), and 19-kDa lipoprotein, have been recognized to have diagnostic value. It has been found that specific antibody profiles vary according to the stage of TB disease. For example, antibodies to the 14-kDa and ESAT-6 antigens have been associated with LTBI, previous TB treatment, and immigration from TB endemic areas.[171-174] By contrast, the antibody to the 38-kDa antigen was found to correlate with advanced, smear positive, and multibacillary TB in adults.[174,175] Some antigens, such as TB9.7 and 81/88 kDa, may be preferentially expressed in HIV-coinfected individuals and have specific diagnostic utility in that population.[176]

Only a few published studies report on antibody detection in primary infection, a condition that predominantly occurs in children. Raja and coworkers examined serologic response to only two antigens, the 30 kDa and 16 kDa, in 26 children with biologically proven or suspected active TB and found a sensitivity of 84 and 73% for the 30- and 16-kDa antigens, respectively.[177] A study performed in India with the 38-kDa IgG antibody was found to be positive in 37% of children with pulmonary TB, 86% of children with TB lymphadenitis, and 27% of controls (who may have been latently infected).[178] It is believed that in the future serologic assays will contain various specific antigens to evaluate the humoral immune response to *M. tb* during different stages of infection.

## Novel Skin Test: ESAT-6 and MPB64 Skin Patch Test

The discovery of antigens specific to *M. tb* that do not cross-react with BCG has led to innovative tests that seem feasible for all regions of the world. A skin test based on ESAT-6 combines the advantages of a high specificity without the need for a laboratory facility and high cost of an assay. Two recent phase I studies found intradermal recombinant ESAT-6 to be safe and feasible.[179,180] It is expected that in the near future more studies will explore the sensitivity, the specificity, and the utility of the assay.

The mycobacterial antigen MPB-64 was formulated in a transdermal patch delivery system to be used for the diagnosis active TB. Two studies in the Philippines found the skin patch to be highly sensitive and specific for clinically diagnosed tuberculosis.[181,182] In patients with active TB, a localized inflammatory response consisting of erythema and/or vesiculation appears less than 4 days after application. Both studies were performed by the same group and these findings have yet to be reproduced in a large cohort. To simultaneously detect LTBI and active TB, MPB-64 and recombinant PPD proteins have been incorporated into one skin patch, and the company (Sequella, Inc) is currently enrolling subjects worldwide to test the system. Both the ESAT-6 and the MPB64 skin tests appear safe, but implementation is still years away as large-scale phase II and III trials have yet to determine efficacy of each test.

## Conclusion

In summary, diagnostic modalities currently available to diagnose *M. tb* infection include the TST and IGRAs. IGRAs appear to be sensitive in children over 2 years of age, and unlike the TST, are specific for *M. tb*. Both these tests are also used, in conjunction with clinical and radiologic findings, to aid in the diagnosis of active TB. An effort should be made to obtain several specimen samples in any child being evaluated for active TB, as the paucibacillary nature of pediatric TB limits the rates of positive AFB smear and culture. The gold standard remains solid traditional culture, and it is recommended to perform the liquid-based culture in conjunction with solid agar to decrease the time to detection. In the United States susceptibility testing is performed on any positive culture using the proportion method. Although less precise, drug-susceptibility testing can also be performed in novel assays such as PCR, MODS, and phage-based tests, which is needed as MDR TB is on the rise. The novel assays that are field-friendly offer great hope, but such assays have yet to be validated on any large scale in diversified populations.

In TB-endemic settings, limited access to diagnostic services results in substantial diagnostic delay and patients often receive a diagnosis after as many as 3 to 6 months of symptoms.[183,184] This delay fuels disease transmission and increases severity of disease when it is finally discovered. In Malawi >6 visits to a health care center is made before TB therapy is initiated in TB patients with smear-negative disease.[185] The greatest need is for tests that can improve the detection of active TB among symptomatic children, including HIV-infected children. A suitable TB diagnostic would need to be sensitive, be simple, have a rapid turnaround time, and be able to detect MDR TB. Such a test would combine these features in a simple point-of-care format that could replace microscopy and culture in the first clinic visit. In general, assays based on the immunological responses to *M. tb* are preferred to the current bacteriologic methods of TB diagnosis because they do not depend on the detection of mycobacteria and exposure risk is minimized. A urine dipstick or a similar format would have vast potential.

The ideal test would be able to diagnose asymptomatic active disease, thereby enabling the delivery of treatment to children in endemic regions before they become sick. There are a handful of studies that describe rising or very high levels of IGRAs as predictors of disease progression.[63,64] There is also promise in the serologic approach, as newly discovered purified antigens expressed during mycobacterial replication are being investigated in prospective studies.

To date there is no test that is highly accurate in children and, although many promising advances have been made, none of these tests are currently in a position to replace microscopy or culture in TB-endemic regions. There is a great need for innovative and novel assays to be tested in children, a population most vulnerable to severe disease. As discussed previously, the pathophysiologic response to *M. tb* in young children can be different from that observed in adults, and these distinctions should be considered as future diagnostic tests are developed and evaluated in clinical trials.

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