

Novel concepts in the epidemiology, diagnosis and prevention of childhood tuberculosis

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Summary

Tuberculosis (TB) is one of the oldest diseases known to mankind, yet it is the second leading cause of death from an infection worldwide today. In the absence of epidemiological data, TB in children was not considered significant and research efforts did not prioritise this group. More recently, there has been renewed interest in childhood TB, leading to improved understanding of the fundamental differences between children and adults in relation to both TB infection and disease. Children with TB infection have a high risk of rapid progression to disease which leads to challenges in diagnosis but also offers the opportunity to investigate biomarkers for infection, progression and protection. This review discusses the most relevant advances in the understanding of epidemiology, diagnosis and prevention of children with TB. It also highlights challenges and research priorities in childhood TB, which include better diagnostic tests, accurate correlates of protection and an improved vaccine.

Key words: strain; interferon gamma release assay; IGRA; BCG; Bacille Calm ette-Guerin; immune-diagnosis; latent TB

Tuberculosis and humans have a long shared history

Tuberculosis (TB) is one of the oldest diseases known to mankind. Evidence of TB has been found in lung and bone tissue from Egyptian mummies and Neolithic settlements. Recent work based on phylogenetic analysis of *Mycobacterium tuberculosis* strains goes further to suggest that *M. tuberculosis* has been infecting humans for more than 70,000 years [1]. The modern humans who migrated from Africa around 62,000 to 75,000 years ago were responsible for the early worldwide spread of TB. Given the long historical relationship between TB and humans, it may seem surprising that TB still ranks as the second leading cause of death from infection worldwide [2]. However, the long co-evolution of the human immune system with

M. tuberculosis may also explain the remarkable ability of this bacterium to evade the immune response.

The recent recognition of the global epidemiology of childhood tuberculosis

Before clinical diagnosis of TB started in the 18th century and *M. tuberculosis* was first described by Robert Koch in 1882, there is limited information on global TB incidence and mortality. However, it is believed that the incidence of TB increased with the global population and crowding during the Middle Ages to become a leading cause of death in the 18th and 19th century in Europe. In the 20th century, TB rates in industrialised countries dramatically declined only to resurge in the 1980s as a result of the human immunodeficiency virus (HIV) epidemic and a lack of interest in the medical community [3]. This eventually led to the declaration of TB as a 'global emergency' by the World Health Organization (WHO) in 1993. Renewed interest in TB resulted in the WHO publishing the first data on global TB incidence and mortality in 1997. However, it took another 15 years until data specifically relating to childhood TB was included in this report [2]. The absence of paediatric TB data in previous reports was predominantly the result of the WHO's definition of a TB case, which required a positive sputum smear as diagnostic criterion. As children often have a lower bacillary load, the majority of paediatric patients with TB disease are sputum smear negative and many are also culture negative (see below). Despite a number of limitations, the inclusion of data on childhood TB in the most recent two editions of the WHO TB report is a major improvement and provides a first estimate of the burden of TB in children worldwide.

The most recent WHO TB report, published in autumn 2013, states that of the 8.6 million new cases in 2012, an estimated 510,000 to 550,000 cases were in children under 15 years of age [4]. Despite being a curable disease, around 74,000 children died from TB in 2012. Compared to the first report on childhood TB published a year earlier, estimates of new cases in children have increased by 40,000

cases and those of children dying from TB by 10,000 per year [2, 4]. This is in marked contrast to the decline in new TB cases and TB mortality in the adult population and most likely reflects improved case finding and reporting rather than a true increase in disease burden in children.

The European Centre for Prevention and Diseases Control (ECDC) published their first paediatric TB report in 2011. In a 10-year-period, a total of 39'695 cases of paediatric TB were notified in Europe, which accounted for 4.3% of all notified TB cases [5]. As with the data published by the WHO, it is widely acknowledged that the estimates of the burden of TB disease in children is likely to be a substantial underestimate. The burden of TB disease in children is difficult to determine for a number of reasons:

- TB disease in children frequently presents with non-specific clinical features.
- A higher proportion of paediatric cases are extra-pulmonary forms of TB resulting in a different clinical presentation.
- Children with pulmonary TB are more likely to have paucibacillary disease which commonly results in a negative sputum smear microscopy and culture.
- Obtaining a sample for microbiological diagnosis is often difficult in children, as younger children are not able to produce sputum.
- For culture-negative children with TB there is no universally applicable and validated clinical diagnostic algorithm.

- Smear or culture negative cases are not reported in many countries due to the lack to national surveillance systems.

In Switzerland, TB became a mandatory notifiable disease in 1988. The definition used in this country includes confirmed cases (*M. tuberculosis* detected by culture or nucleic acid amplification testing) as well as non-confirmed cases, which are defined as patients treated for TB disease with at least three anti-tuberculous drugs. This kind of reporting system is more suitable for the evaluation of TB in children. Based on these data collected by the Federal Office of Public Health, a recent study investigated the epidemiology of childhood TB in Switzerland between 1996 and 2011 [6]. The study showed that over the last 16 years, 20 to 30 cases of paediatric TB have been notified per year and incidence rates have remained at a consistently low level of 1.4 /100,000 children. This low rate results in reduced opportunities for clinicians to gain experience in childhood TB, and consequently diminished knowledge of diagnosis and prevention [7].

There are important differences between tuberculosis in children and adults

As childhood TB has been a neglected disease, knowledge about TB has largely been based on studies in adults. It is therefore not surprising that clinicians and scientists are

Feature	Children	Adults
Contact history	Contact history positive (parents, care-givers, teachers) in approximately 50% of cases	Contact history frequently negative
Most important risk factors for TB disease	Age <2 (5) years, HIV infection, malnutrition	HIV infection, immunosuppressive treatment, prior episode of TB, chronic renal insufficiency, diabetes mellitus, smoking, alcohol abuse
Social risk factors	Same bed, bedroom or household as a smear positive adult case, living in refugee camps, orphan	Homeless, iv-drug abuse, prisoner
Clinical clues	Weight loss or failure to thrive	Unwanted weight loss
	Chronic non-remitting cough (>2–3 weeks)	Chronic non-remitting cough (>2–3 weeks)
	Wheezing	
	Chronic fever	Chronic fever
Risk of progression from TB infection to disease	<1 year of age: up to 50% annual risk <5 years of age: 6–24% annual risk 6–15 years of age: 6–12% annual risk	HIV negative: 8–10% lifelong risk (5% in the first 2 years) HIV-positive: 10% annual risk
Frequency of extra-pulmonary TB	30%	15%
Most common radiologic findings	Hilar or mediastinal lymphadenopathy, Ghon focus, disseminated (miliary disease)	Cavitary disease, Ghon focus, Pleural effusion
Microbiological confirmation	1–15% smear positive 10–50% culture positive	50% smear positive 90% culture positive
Immuno-diagnostic testing	TST can be false positive, particularly after BCG immunisation	TST can be false positive after exposure to non-tuberculous mycobacteria
	IGRA may be false negative particularly in young children	IGRA has replaced TST testing in most resource-rich countries
Treatment	Weight-adapted dosing (dose per kg higher than in adults) Young children require special formulations (eg syrup) as unable to take tablets	Fixed dosing
	Hepatotoxicity less likely than in adults	Hepatotoxicity increases with age and in cases with underlying liver disease
	Pyridoxine only in malnourished children	Routine pyridoxine
Risk of transmission	Low in children <5 years because of absent force of cough and paucibacillary disease	Increased in sputum positive individuals
Protection afforded by BCG immunisation	77% for disseminated forms of TB 50% for pulmonary TB	Highly variable: 0–80% for all forms of TB

commonly unaware of the considerable differences between TB in children and adults. The spectrum of disease, risk factors, clinical features and diagnostic results differ considerably according to age. The following table summarises what we consider to be the most important differences between TB in children and in adults (table 1).

Beyond the concept of latent and active tuberculosis

For many years TB has been categorised into two distinct stages: “latent” and “active”. The understanding was that in “latent TB”, *M. tuberculosis* bacilli were dormant or non-replicating. However, in recent years, evidence has emerged that in both stages there are non-replicating and replicating *M. tuberculosis* bacilli but that in TB infection (“latent TB”) the number of replicating *M. tuberculosis* bacilli is low and in TB disease (“active TB”) it is high [8]. To highlight this change in understanding, we prefer to use the terms TB infection and disease rather “latent” and “active TB”. It is therefore now hypothesised that in TB infection, non-replicating *M. tuberculosis* bacilli sporadically return to a replicating stage [8]. This would explain why isoniazid, which is used as treatment of TB infection but which is only active against replicating *M. tuberculosis*, is effective in reducing the risk of TB disease. It has therefore been suggested by a number of experts that TB is better envisaged as a continuum between infection and disease rather than there being distinct binary stages [9–11]. A proposed novel classification of this continuum of different stages is illustrated in table 2. Although currently this classification does not impact clinical management, in the future it may be possible to determine whether *M. tuberculosis* infection has been cleared in individuals currently classified as “latent TB” for example and therefore suggest different management.

Clinical features and diagnosis of childhood tuberculosis

Confirmation of TB in both children and adults involves detection of *M. tuberculosis* by culture or by nucleic acid amplification testing (NAAT). For individuals with TB who have negative culture and/or NAAT, there is currently no accurate diagnostic test for TB. This scenario is typically more common in children. In these situations, the diagnosis of TB disease is usually based on a combination of clinical, radiological and immunodiagnostic criteria.

Classical TB stage classification	Proposed TB stage	Clinical features (Symptoms and signs)	Microbiology (Culture/PCR for <i>M. tuberculosis</i>)	Immunodiagnostic tests (TST/IGRA)	Imaging (Chest X-ray)
Latent TB	Cleared TB infection	-	-	+/-	-
	Contained TB infection	-	-	+/-	-
	Local TB disease	-	-	+/-	-
Active TB	Disseminated TB disease	+	+	+/-	+/-
	Cleared TB disease	+	+	+/-	+
Healed prior TB	Cleared TB disease	-	-	+/-	+

Table 2

Proposed continuum of stages between infection and disease in childhood tuberculosis (adapted from [9]).

Clinical diagnosis and diagnostic scores

The symptoms classically associated with TB diseases are cough, fever and weight loss. A landmark study in children with TB disease, in a high prevalence setting in South Africa, combining the classic symptoms of persisting cough for more than two weeks, failure to thrive and prolonged fatigue had an overall sensitivity of 63% [12]. Particularly worrying, however, is the finding that below the age of three years, and in children infected with HIV, the sensitivity declined to 52–56% [12]. Several other approaches using clinical scores have reported similar results. A recent evaluation including nine different scores developed to improve and standardise diagnosis of TB showed variable results with between 7% and 89% of children identified by different scoring systems [13]. Importantly, scores relying on clinical and radiological features only had the lowest sensitivity.

Mycobacterial culture or molecular techniques

Culture confirmation of presumed pulmonary TB disease is usually done using a sputum sample. Younger children, particularly those under the age of five years, are unable to expectorate sputum and therefore alternative samples are commonly used including gastric aspirates, induced sputum, nasopharyngeal aspirates, string test samples or bronchoalveolar lavage (BAL) fluid.

These sample collection techniques need specifically-trained staff and facilities, and some are associated with a high degree of invasiveness and discomfort for the child. Regardless of the sample technique, microbiological confirmation of TB disease in children is generally limited and at best reaches 50% [6, 14]. In adults, improved confirmation rates in pulmonary TB disease have recently been achieved using NAAT from sputum. Based on this, the WHO has advocated universal use of Xpert MTB/RIF (Cepheid, CA, USA) for the diagnosis of TB. The clear ad-

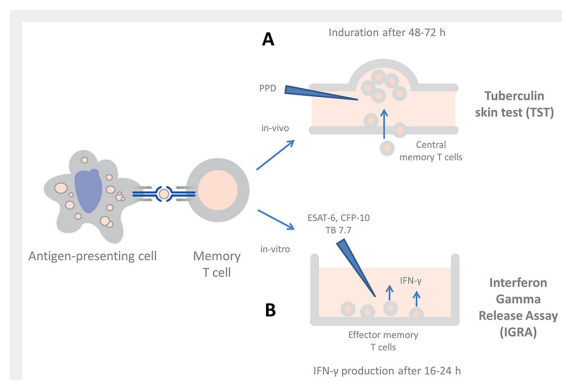


Figure 1

The current immunodiagnostic tests in clinical use for the diagnosis of tuberculosis (adapted from: Andersen P, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. Lancet. 2000;356(9235):1099–104, with permission from Elsevier). Exposure to *M. tuberculosis* leads to uptake by antigen-presenting cells and primes antigen-specific T cells in the local lymph node. (A) In the TST, after re-stimulation with PPD, mycobacterial-specific central memory T cells infiltrate the skin at the injection site and cause a palpable induration. (B) In IGRAs, blood is stimulated *in vitro* with ESAT-6, CFP-10 and (in the QFT-GIT only) TB 7.7, resulting in IFN- γ production by circulating effector memory T cells, which is measured by ELISA or ELISPOT.

vantage of this assay is that it detects significantly more cases than sputum smear microscopy and, importantly, results are available within one day, including detection of rifampicin resistance. However, studies in children show that Xpert MTB/RIF is unfortunately unable to detect a significant proportion of children with TB disease who are culture negative. This new technique therefore does not improve the overall detection rate and will not increase the proportion of cases of confirmed TB disease in children [15].

Immunodiagnosis of tuberculosis

The immunodiagnosis of TB infection and disease relies on the detection of a cell mediated immune response (CMI). The tuberculin skin test (TST), which was developed more than a century ago, is still the standard test in children. A recent study shows that the skin induration in the TST results from a cellular infiltrate of central memory T cells (CD4⁺/CD45RO⁺) (fig. 1) [16]. There are numerous disadvantages of TST including low specificity, particularly in BCG-immunised children, sub-optimal sensitivity, the need for a technically-demanding and difficult-to-standardise intradermal injection, the requirement for the patient to return for the reading of the test and an unsophisticated error-prone reading technique using a ruler to measure the palpable induration.

To overcome the shortcomings of the TST, *in vitro* cytokine-based immunodiagnostic tests were developed in the 1990s. These tests measure the concentration of IFN- γ in stimulated blood in response to mycobacterial antigens (fig. 1) [17, 18]. Two commercial kits are licensed for use: the QuantiFERON-TB Gold In-Tube test (QFT-GIT, Cellestis/Qiagen) and the T-SPOT.TB test (Oxford Immunotech, Oxford, UK). Rather than purified protein derivative (PPD), both tests use more specific TB antigens. Sensitivity and specificity is similar for both IGRAs, with the QFT being more frequently used in resource-rich countries [19]. The QFT offers additional advantages over the T-SPOT.TB test including no requirement for peripheral blood mononuclear cell (PBMC) separation and importantly the potential to measure additional cytokines from the same supernatant. It is now clear, that many other expressed cytokines might be suitable biomarkers for TB infection and disease (reviewed in [20, 21]). Interferon gamma-inducible protein (IP)-10 has been most studied. It is produced by antigen-presenting cells in response to IFN- γ , has similar kinetics to IFN- γ but is expressed in higher concentrations. In studies in children, the addition of IP-10 has been shown to increase the sensitivity of commercial IGRAs [22–24]. In one study, although children under the age of five years had reduced IFN- γ responses, IP-10 responses were not affected by age [22]. Several other cytokines, including interleukin (IL)-2, IL-1 receptor antagonist (IL-1 ra) and tumour-necrosis factor (TNF)- α are currently being evaluated as potential immunodiagnostic markers.

IGRAs were developed, optimised and standardised for adults. They are now well-established tests for the immunodiagnosis of TB in adults and have replaced the TST in many resource-rich countries [25]. Evidence for the performance of IGRAs in children is limited but recent meta-analyses have failed to show improved sensitivity com-

pared to TST and in resource-limited, high burden TB-countries sensitivity was lower for IGRAs than for TSTs [19, 26]. Therefore the majority of national guidelines – including Switzerland – and the two supranational guidelines (WHO and ECDC) recommend using a TST as the immunodiagnostic test of choice for TB in children [27–30]. The underlying reason for the lower performance of IGRAs in children is uncertain but the age of the child, the immune status, nutrition and co-infections all likely play an important role [31–33].

More accurate diagnostic tests for TB in children are therefore urgently needed. There is growing recognition that there are fundamental differences in children and adults in relation to the underlying immunology and consequent diagnosis of TB. The recently published roadmap for childhood TB by the WHO highlights that the evaluation of new diagnostics is a research priority with particular emphasis on assays that are suitable for paediatric samples and that have the ability to identify disease progression in children [34].

Immunisation against tuberculosis: misconceptions and knowledge gaps

The Bacille Calmette-Guérin (BCG) vaccine is one of the oldest vaccines still in use today. Although it is no longer used or licensed in many Western European countries, more than 100 million children, or approximately 90% of all children born worldwide each year, receive a BCG immunisation. A common misconception about BCG is that the vaccine has little or no protective efficacy. The vaccine is most effective for the prevention of severe forms of TB that particularly affect infants and young children, including TB meningitis and miliary TB. In the 1980s and 1990s, many studies in different settings repeatedly confirmed the protective efficacy of BCG for TB meningitis (67% to 79%) and for miliary TB (58% to 87%) (reviewed in [35]). However, BCG has limited efficacy for the prevention of pulmonary TB disease in children and limited to no efficacy for the prevention of adult pulmonary TB disease. It is unclear whether this is the result of waning immunity of the vaccine's protective efficacy or if BCG is less effective in preventing pulmonary forms of TB disease. In addition, in children infected with HIV, BCG immunisation does not induce protective immunity and potentially leads to severe adverse effects, including disseminated BCG disease [36]. HIV infection or immunosuppression is therefore a contraindication to BCG immunisation. In particular, WHO now recommends that BCG immunisation is delayed in infants born to HIV-infected mothers until these infants are confirmed to be HIV negative.

Another common misconception is that BCG is one single product and that children immunised worldwide all receive the same vaccine. For historical reasons, however, a number of different BCG-vaccine strains are produced and licensed worldwide today [37, 38]. Two recent studies provide evidence that the specific BCG vaccine strain influences both the immune response and protection against TB [39, 40]. Knowledge of the BCG vaccine strain used for immunisation is also important in the treatment of potential BCG adverse effects. All BCG vaccine strains are resist-

ant to pyrazinamide. In addition, at least two currently-used strains also exhibit low level resistance to isoniazid [41]. Given the fact that BCG vaccine strain influence immune response, protection and anti-mycobacterial resistance, it seems surprising that in a recent European survey in two TB specialist networks, one third of participants did not know which BCG vaccine strain was used in their country [42].

In addition to protection against TB, BCG has a number of other beneficial heterologous ('non-specific') effects on the immune system [43, 44]. The immunomodulatory and anti-tumour effect of BCG is used in the treatment of bladder cancer [45]. In addition, BCG influences vaccine response to routine immunisations [46, 47]. Moreover, there are a large number of reports showing that BCG is associated with reduced all-cause mortality, independent of TB, in resource limited settings [48, 49].

Development of novel TB vaccines

After the first use of BCG in humans in 1921 in France, despite limited efficacy data, the vaccine was distributed worldwide. Encouraging studies in the 1950s and 60s led to the inclusion of BCG in the expanded immunisation programme (EPI) by WHO in 1974. Subsequent studies from high-prevalence TB countries suggesting variable, sometimes absent, protection by BCG prompted the search for improved TB vaccines. A number of other factors paved the way for the development of novel vaccines against TB including the improved understanding of the complex mechanisms underlying the adaptive immune response against TB in the early 1990s, the analysis of the complete genome sequence of *M. tuberculosis* in 1998 [50] and the comparative analysis of the genome of several BCG vaccine strains in 1999 [51]. This led to the first novel TB vaccine candidates being tested in animal models and, over the last two decades, 14 TB vaccine candidates have advanced to clinical trials. There are now three different types of novel TB vaccines: priming vaccines, boosting vaccines and therapeutic vaccines (table 3).

The most advanced of these vaccines is a boosting vaccine called MVA85A that uses a modified vaccinia virus expressing *M. tuberculosis*-85A antigen. In a recently completed, phase 2b, randomised placebo-controlled trial (RCT), MVA85A was given as a booster vaccine at the age of 4 to 6 months following BCG immunisation at birth [52]. In the clinical follow-up of the nearly 2800 HIV-negative infant participants over two years, there was no

evidence that MVA85 offered additional protection over BCG immunisation. While the results are disappointing, the completion of this trial is a milestone in the history of TB vaccines. It is the first large-scale RCT investigating the efficacy of a novel TB vaccine in infants. The capacity building that allowed such a trial to be completed efficiently and according to good clinical practice standards in a resource limited, high TB-prevalence country should not be underestimated.

The results of this trial have also stimulated the discussion about current knowledge gaps which will have a profound influence on future research into novel TB vaccines [53, 54]. Firstly, it has become very clear that the mouse model – which was the most commonly-used animal model for preclinical testing of TB vaccine candidates – has several shortcomings and may fail to predict efficacy of a TB vaccine candidate in humans. There is therefore now debate whether other animal models, including non-human primate and bovine models, need to be included in future pre-clinical trials of novel TB vaccine candidates [53]. Secondly, unlike in other infectious diseases, there is currently no marker or set of markers which clearly predict protection against TB. Correlates of protection against TB have been a research priority for many years, yet many attempts to define accurate biomarkers, though increasing our knowledge about the complex immune response to TB, have failed to produce a clear answer [55–57]. Whilst the MVA85 vaccine induced A85-specific T cells that produced several cytokines four weeks after immunisation, this immune response did not correspond to protective efficacy. Many factors could have contributed to this including measurement of the wrong immunological outcome (ie other cells or cell-products are responsible for protection) or the *in-vitro* stimulation with A85 does not predict protection against *M. tuberculosis*. Lastly, the diagnosis of TB, particularly confirmation of disease by novel accurate immunodiagnostic tests, would considerably improve the diagnostic certainty in the follow-up of all children included in future TB vaccine trials.

In summary, a number of candidates are currently being evaluated as novel vaccines against TB representing a major achievement in this research field. However, the development of novel TB vaccines and clinical trials are significantly hampered by the absence of biomarkers that correlate with protection and the lack of an ideal animal model. It is therefore important to improve the diagnostic accuracy of all tests used to diagnose TB disease in children included in TB vaccine trials. In the light of these challenges, it is

Vaccine type	Intended use	Intended endpoint	Example vaccines in phase 2–3 clinical trials
Prime	Replace BCG in infants, before the individual's immune system has been exposed to <i>M. tuberculosis</i> or other mycobacteria	Prevent infection and disease	H56/AERAS-456 +IC31 H1+IC31 VPM 1002
Boost	Given as a booster vaccine either after BCG immunisation at birth as a 'primer' or after exposure to non-tuberculous mycobacteria later in life	Prevent infection, disease and/or reactivation	Crucell Ad35/AERAS-402 H1+IC31 H4/AERAS-404 +IC31 H56/AERAS-456 +IC31 M72+AS01
Therapeutic	Adjunctive treatment for individuals with TB disease in addition to standard anti-tuberculous medication	Shorten treatment duration, prevention of transmission	<i>M. vaccae</i> RUTI

likely that BCG will remain the only licensed TB vaccine for at least the next 10 years.

Conclusion

A number of major advances have been made in the understanding of the epidemiology, diagnosis and protection of childhood TB in the last few years. This has led to childhood TB being lifted out of the shadow of adult TB. The increased public attention, commitment of clinicians and researchers and increased funding for treatment and research at both local and global levels is invaluable. Addressing research priorities, including the lack of accurate diagnostic tests that can detect all TB cases in children and improved TB vaccines, is now the responsibility of the current generation of clinicians and researchers alike.

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Figures (large format)

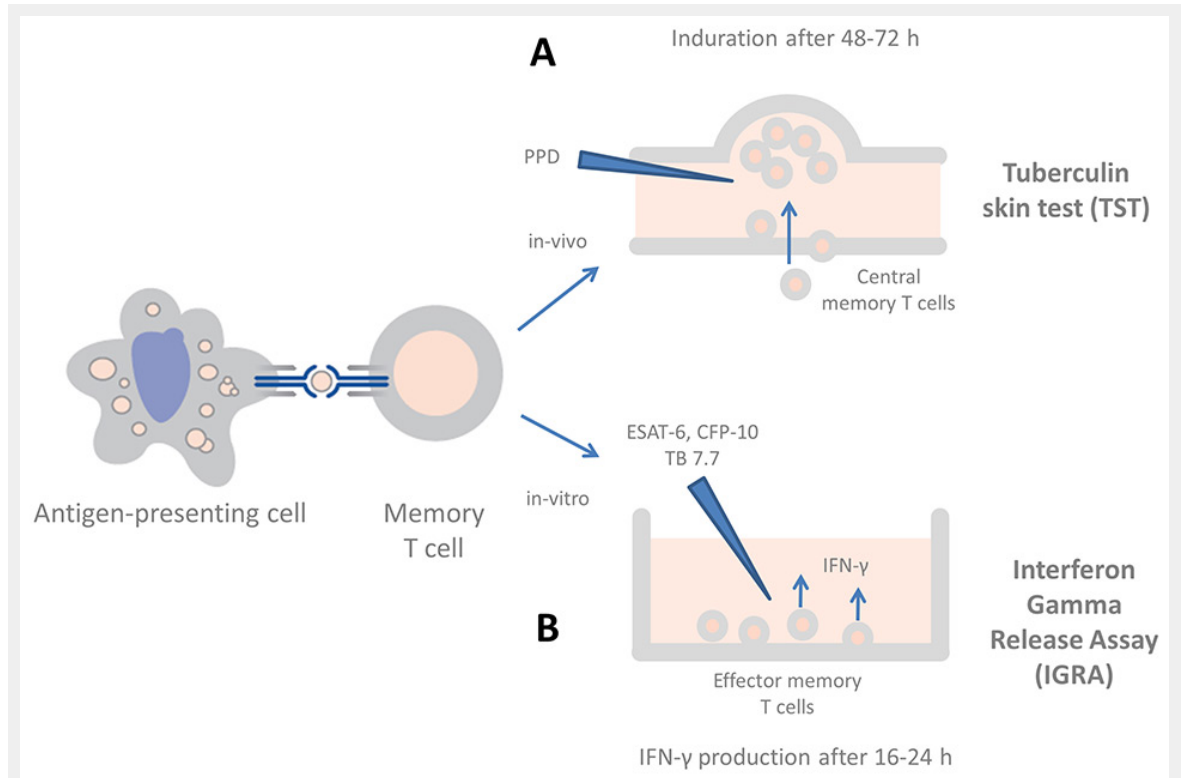


Figure 1

The current immunodiagnostic tests in clinical use for the diagnosis of tuberculosis (adapted from: Andersen P, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. Lancet. 2000;356(9235):1099–104, with permission from Elsevier). Exposure to *M. tuberculosis* leads to uptake by antigen-presenting cells and primes antigen-specific T cells in the local lymph node. (A) In the TST, after re-stimulation with PPD, mycobacterial-specific central memory T cells infiltrate the skin at the injection site and cause a palpable induration. (B) In IGRAs, blood is stimulated *in vitro* with ESAT-6, CFP-10 and (in the QFT-GIT only) TB 7.7, resulting in IFN- γ production by circulating effector memory T cells, which is measured by ELISA or ELISPOT.

Classical TB stage classification	Proposed TB stage	Clinical features (Symptoms and signs)	Microbiology (Culture/PCR for <i>M. tuberculosis</i>)	Immunodiagnostic tests (TST/IGRA)	Imaging (Chest X-ray)
Latent TB	Cleared TB infection	-	-	+/-	-
	Contained TB infection	-	-	+/-	-
	Local TB disease	-	-	+/-	-
Active TB	Disseminated TB disease	+	+	+/-	+/-
	Cleared TB disease	+	+	+/-	+
Healed prior TB	Cleared TB disease	-	-	+/-	+

Table 2

Proposed continuum of stages between infection and disease in childhood tuberculosis (adapted from [9]).