T-cell assays for the diagnosis of latent tuberculosis infection: moving the research agenda forward

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For nearly a century, the tuberculin skin test was the only tool available for the detection of latent tuberculosis infection. A recent breakthrough has been the development of T-cell-based interferon-γ release assays. Current evidence suggests interferon-γ release assays have higher specificity than the tuberculin skin test, better correlation with surrogate markers of exposure to *Mycobacterium tuberculosis* in low-incidence settings, and less cross-reactivity as a result of BCG vaccination compared with the tuberculin skin test. The body of literature supporting the use of interferon-γ release assays has rapidly expanded. However, several unresolved and unexplained issues remain. To address these issues, a group of experts met in Geneva, Switzerland, in March, 2006, to discuss the research evidence on T-cell-based assays, their clinical usefulness, limitations, and directions for future research, with a specific focus on resource-limited and high HIV prevalence settings. On the basis of 2 days of discussions, a comprehensive research agenda was generated, which will propel the field forward by stimulating focused high-impact research and encourage the investment of resources needed to tackle priority research questions, especially in resource-limited settings. Ultimately, if adequately financed, the research findings will inform appropriate use of novel latent tuberculosis infection diagnostics in global tuberculosis control.

Introduction

An estimated third of the world's population is infected with *Mycobacterium tuberculosis*.[1–3] This large pool of individuals with latent tuberculosis infection poses a hurdle for tuberculosis elimination. Treatment of people with latent tuberculosis infection, including those with HIV co-infection, effectively reduces the risk of progression from latent tuberculosis infection to active disease,[4–7] but there is currently no accurate tool to predict which latently infected individuals are at greatest risk of disease progression. Until recently, the only diagnostic test for latent tuberculosis infection was the tuberculin skin test. Although the tuberculin skin test has proven to be useful in clinical practice, it has known limitations in accuracy and reliability.[8–10]

Figure 1: **Laboratory professionals at the University of Cape Town, South Africa, get ready to process clinical specimens for T-SPOT.TB and QuantiFERON-TB Gold assays**

A major breakthrough in recent years has been the development of in vitro assays that measure T-cell release of interferon γ in response to stimulation with antigens such as early secreted antigenic target 6 (ESAT6) and culture filtrate protein 10 (CFP10). These antigens are more specific to *M tuberculosis* than the purified protein derivative used for the tuberculin skin test. Within a short timeframe, two interferon-γ release assays have become commercially available: the QuantiFERON-TB Gold (Cellestis Ltd, Carnegie, Victoria, Australia) assay, and the T-SPOT.TB test (Oxford Immunotec, Oxford, UK; figure 1).

The QuantiFERON-TB Gold assay is available in two formats: a 24-well culture plate format that is approved by the US Food and Drug Administration (FDA), and currently used in the USA, and a more recent, simplified in tube format (not FDA approved as yet; but licensed in countries other than the USA). The T-SPOT.TB test is available in two formats: 96-well plate (T-SPOT.TB 96) or eight-well strips (T-SPOT.TB 8). Although not FDA approved, it is currently CE marked for use in Europe, and licensed for use in Canada.

With the availability of standardised interferon-γ release assays, there is great interest in using these assays in a variety of settings. Available research evidence on interferon-γ release assays has been extensively summarised in several reviews and guidelines.[11–23] Current evidence suggests interferon-γ release assays have higher specificity than the tuberculin skin test, better correlation with surrogate markers of exposure to *M tuberculosis* in low-incidence settings, and less cross-reactivity as a result of BCG vaccination than the tuberculin skin test. Interferon-γ release assays also appear to be at least as sensitive as the tuberculin skin test for active tuberculosis (used as a surrogate for latent tuberculosis infection), but concerns have been raised about suboptimal sensitivity in active disease.[18,24] In the

absence of a gold standard for latent tuberculosis infection diagnosis, the sensitivity and specificity for latent tuberculosis infection cannot be directly estimated. Besides high specificity, other potential advantages of interferon-γ release assays include logistical convenience, the need for fewer patient visits to complete testing, avoidance of unreliable and somewhat subjective measurements such as skin induration, and the ability to do serial testing without inducing the boosting phenomenon—ie, immunological recall of pre-existing hypersensitivity to tuberculosis.

Overall, because of their high specificity and other potential advantages, interferon-γ release assays are likely to replace the tuberculin skin test in low-incidence, high-income settings where cross-reactivity with BCG, particularly in immigrants, might adversely effect the interpretation and use of the tuberculin skin test. In fact, the US Centers for Disease Control and Prevention now recommends that the QuantiFERON-TB Gold assay may be used in place of the tuberculin skin test for all indications, including contact investigations, evaluation of immigrants, and serial testing of health-care workers.[18] The UK National Institute for Health and Clinical Excellence (NICE) tuberculosis guidelines recommend a hybrid, two-step approach for latent tuberculosis infection diagnosis, which includes initial screening with tuberculin skin test, and subsequent interferon-γ release assay testing, if available, of those who are tuberculin skin test positive (or in whom tuberculin skin test may be unreliable) to confirm tuberculin skin test results.[19] However, the efficacy and cost-effectiveness of this hybrid strategy is yet to be validated.

Development of an agenda for research on T-cell-based tuberculosis diagnostics

The body of literature supporting the use of interferon-γ release assays has rapidly expanded, as evidenced by the plethora of reviews, commentaries, and guidelines.[11–20,22,23] However, despite a growing evidence base, several unresolved and unexplained issues remain. These include unexplained discordance between tuberculin skin test and interferon-γ release assay results, ill-defined correlation between bacterial burden and T-cell responses, unknown predictive value of interferon-γ release assays for the development of active tuberculosis, insufficient data on test performance in high-risk populations such as individuals with HIV infection and children, inconsistent results of studies on effect of tuberculosis treatment on T-cell responses, inadequate information on interferon-γ release assay performance in serial testing, and lack of evidence on the usefulness of interferon-γ release assays in epidemiological studies. Scientific knowledge gaps are matched by the paucity of data on feasibility, applicability, cost-effectiveness, and potential use of these assays in high-incidence and resource-limited settings.

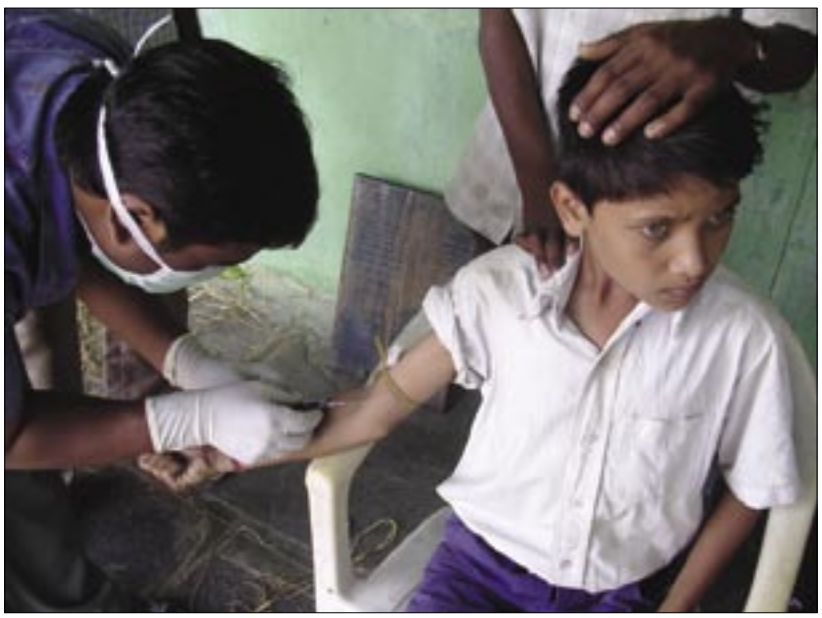

Figure 2: **A field worker collects blood specimens for a study on interferon-γ assays among household contacts of tuberculosis patients in a village in Maharashtra, India**

An international effort is required to address knowledge gaps efficiently, and to this end, an expert group (listed in the acknowledgments) was assembled in Geneva, Switzerland (March, 2006), by the Stop TB Working Group on New Diagnostics. The meeting was co-organised by the Foundation for Innovative New Diagnosis (FIND) and WHO. The group was charged with reviewing the research evidence supporting the use of interferon-γ release assays, their clinical use and limitations, and directions for future research, with a specific focus on resource-limited settings (figure 2). The overarching goal was to move the field forward by identifying crucial areas for research and implementation.

The proposed research agenda is derived from the workshop's scientific presentations and discussions, and a selection of the recent interferon-γ release assay reviews and guidelines.[13,14,17–19] The agenda is intended to be a useful resource for researchers and clinicians. In general, research questions can be grouped into seven areas (discussed below): (1) biological issues and assay development; (2) test performance in high-risk populations and poorly studied groups; (3) risk prediction and modelling; (4) test reproducibility and serial testing; (5) T-cell responses during treatment and role in treatment monitoring; (6) epidemiological and field applications; and (7) health systems, operational, and economic research. However, within each area, the research questions were not ranked or prioritised, since priorities may vary across countries or settings.

Biological issues and assay development

This area is focused on biological issues related to immunology, test interpretation, and improvement of

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***Panel 1*: Biological issues and assay development**

Research question

1 What type of T-cell responses are detected by interferon-γ release assays—effector or memory T-cell responses?
2 To what extent does a positive interferon-γ release assay result suggest previous (remote) infection (either cleared or still persistent) versus recent infection?
3 Can the identification and validation of novel tuberculosis-specific antigens help to increase sensitivity of T-cell-based assays without compromising their high specificity?
4 Can the identification and validation of novel tuberculosis-specific antigens (or biomarkers) help to distinguish between latent tuberculosis infection and active disease?
5 Can the tuberculin skin test reagent be improved? Can ESAT6 and CFP10 be used as skin test reagents?
6 What is the biological basis for discordance between tuberculin skin test and interferon-γ release assay results?
7 After exposure to *M tuberculosis*, how long does it take for the interferon-γ release assay test to become positive? How soon after tuberculosis exposure can the interferon-γ release assay detect latent infection?
8 In head-to-head comparisons, what is the difference in performance characteristics (eg, sensitivity and indeterminate rates) of the commercial interferon-γ release assays (QuantiFERON-TB Gold *vs* T-SPOT.TB)? What is the difference in performance characteristics of different versions of the same commercial assay (QuantiFERON-TB Gold *vs* QuantiFERON-TB Gold In Tube)?
9 What is the best approach to determining appropriate cut-off points for interferon-γ release assays? In high-risk groups (eg, HIV-infected individuals) do interferon-γ release assay cut-off points need to be set lower?
10 What is the correlation between total lymphocyte count and T-cell responses to specific antigens? Is the performance of whole-blood interferon-γ release assays likely to be affected by variations in total lymphocyte counts?
11 Is there an association between mitogen response and sensitivity of interferon-γ release assays? Are interferon-γ release assays likely to be more sensitive in tuberculosis patients with strong mitogen responses?
12 What is the effect of delay in processing of blood specimens on interferon-γ release assay performance? What is the effect of longer incubation periods on assay sensitivity?
13 What is the effect of bacterial strain type on T-cell responses? Does exposure to certain strains affect immune responses to ESAT6 and CFP10?
14 What is the effect of host genetic factors on T-cell responses?
15 Can interferon-γ release assay technology be simplified to enhance its applicability in resource-limited settings—eg, testing with smaller quantities of blood, such as fingerstick, or testing with lateral flow or strip formats.

Adapted in part, with permission from reference 17. ESAT6=early secreted antigenic target 6. CFP10=culture filtrate protein 10.

existing interferon-γ release assays. As shown in panel 1, research questions covered issues such as biological basis for discordance between the tuberculin skin test and interferon-γ release assays, selection of appropriate cut-off points (thresholds) for interferon-γ release assay positivity in different populations, correlation between bacterial burden and T-cell responses, and the need to understand to what extent a positive interferon-γ release assay result suggests previous (remote) infection (either cleared or still persistent) versus recent infection.

These issues stem from research observations that interferon-γ release assays and the tuberculin skin test probably do not measure the same components of the cellular immune response. This, in turn, might explain why several studies have found unexplained discordance between tuberculin skin test results and interferon-γ release assay results, which is reviewed elsewhere.[13,25] T-cell assay results appear to correlate with bacterial burden in a way that has not been previously demonstrated for the tuberculin skin test.[26–29] It has been hypothesised that short incubation interferon-γ release assays (eg, both commercial assays use 16–24 h incubation) detect responses of activated effector T cells that have recently encountered antigens in vivo, and can therefore rapidly release interferon γ when stimulated in vitro.[26,27,30] By contrast, long-lived central memory T cells that may persist even after clearance of the organism (eg, previously treated tuberculosis) may be less likely to release interferon γ during short incubation times. Effector response may be driven by the antigen (bacterial) load, and there is some evidence that reduction of the antigen load by treatment decreases T-cell responses.[26–29] However, other studies have shown no change, inconsistent changes, or stronger T-cell responses after treatment.[31–37] Thus, there is a need to study the dynamic nature of T-cell responses, especially since it relates to the interpretation of interferon-γ release assays. Traditionally, a positive tuberculin skin test has been used to diagnose and define latent tuberculosis infection.[38] With the emergence of T-cell assays, this conventional definition of latent tuberculosis infection will need to be reconsidered.

Although several studies have shown that interferon-γ release assays have higher specificity, there is some concern that interferon-γ release assays may be less sensitive than the tuberculin skin test,[18,24] especially if single *M tuberculosis*-specific antigens are used (eg, only ESAT6).[11,13,39] Thus, there is a need to identify and validate novel *M tuberculosis*-specific antigen combinations that can increase sensitivity of T-cell-based assays without compromising their high specificity.[11,17] Currently available interferon-γ release assays cannot distinguish between latent infection and active disease.[24] Therefore, the identification and validation of novel antigens or biomarkers with this discriminative capacity is required,[40,41] and will be particularly helpful in areas of high endemicity.

With the increasing range of commercial interferon-γ release assays and their variants, there is a need for head-to-head evaluations in target populations to identify differences in performance and operational feasibility. Such data are emerging and will greatly assist in the selection of the appropriate test for a specific indication or population.[42–44]

Lastly, there is a need to simplify interferon-γ release assay technology or develop alternative platforms that will enhance applicability in resource-limited and field settings. This might include development of an improved tuberculin skin test reagent[45] (where purified protein derivative is

replaced with a cocktail of *M tuberculosis*-specific antigens) and simplification of interferon-γ release assay technology (eg, lateral flow dipstick formats with smaller blood volume requirements).

Test performance in high-risk populations and poorly studied groups

Although several studies have evaluated interferon-γ release assays, there are few published studies on high-risk populations, including immunocompromised individuals (eg, those with HIV infection, diabetes, cancer, or renal failure, people taking immunosuppressive medications, or organ transplant recipients), children, elderly, health-care workers, and individuals with extra-pulmonary tuberculosis and disease by non-tuberculous mycobacteria. Recent studies suggest that interferon-γ release assays are promising in HIV-infected and immunocompromised individuals[46–50] and children,[51–54] but further evaluation is urgently needed to determine assay performance and feasibility in such high-risk groups.

New tests are an urgent priority for many of the above mentioned groups because the tuberculin skin test is likely to be less sensitive because of anergy.[4,55] This poses serious difficulties for effective implementation of preventive therapy in settings with high HIV prevalence. There is some evidence that interferon-γ release assays may be less affected by anergy than the tuberculin skin test, but this requires confirmation.[47–50] If interferon-γ release assays are shown to retain their sensitivity and specificity in immunocompromised individuals, they have the potential to appropriately direct preventive therapy and have a positive epidemiological impact. The correlation between degree of immunosuppression (eg, CD4+ count) and indeterminate interferon-γ release assay results (ie, lack of T-cell response to mitogen) is another issue that deserves further study, because of its clinical implications for use in individuals with severe immunosuppression.[46,48,56,57] It is important to recognise that antigen-specific anergy may occur and therefore response to tuberculosis antigens may not necessarily correlate with response to mitogen.

In children, establishing a microbiologically confirmed diagnosis of active tuberculosis is difficult.[58,59] A test for tuberculosis infection can serve two purposes: (1) to diagnose latent tuberculosis infection—eg, as part of contact investigation or immigrant screening, and (2) in conjunction with other tests, to support or refute the diagnosis of active disease. In children, studies have shown that interferon-γ release assays are feasible and potentially useful for both purposes,[51–54,60] although concerns remain about indeterminate results and failed phlebotomy.[61,62] Further study is required to determine their role as tests used to rule out active disease, especially in children with HIV infection.[22] Panel 2 lists other research questions identified under this area.

Risk prediction and modelling

One of the greatest advantages of the tuberculin skin test is that risk of development of active tuberculosis has been established in many cohort studies for tuberculin skin test reactions of different sizes, for various populations, and associated clinical conditions.[9,63,64] Furthermore, in many randomised trials, treatment of tuberculin skin test-positive people reduced the risk of active disease.[4–6] This wealth of research evidence has resulted in guidelines for targeted tuberculin skin test testing and latent tuberculosis infection treatment.[4] Currently, there are no equivalent data for interferon-γ release assays. Thus, a crucial unresolved issue is whether interferon-γ release assays have the ability to identify latently infected individuals who are most likely to progress to active disease, and, therefore, most likely to benefit from preventive therapy. Although there are limited data on the basis of one small study[65] of an association between interferon-γ response to ESAT6 and subsequent progression to active tuberculosis in contacts of tuberculosis patients, the prognostic value of a positive interferon-γ release assay test is largely unknown. Large, long-term cohort studies are urgently needed to address this essential knowledge gap, although these may pose ethical issues.

***Panel 2*: Test performance in high-risk populations and poorly studied groups**

Research question

1. What is the accuracy and reliability of T-cell-based assays in the diagnosis of active and latent tuberculosis infection in children? In children with extra-pulmonary or severe/disseminated tuberculosis, are interferon-γ release assays likely to be less sensitive?
2. What is the accuracy and reliability of T-cell-based assays in the diagnosis of active and latent tuberculosis infection in individuals with HIV infection? Can interferon-γ release assays be used to detect subclinical tuberculosis in HIV-infected individuals? Will the use of interferon-γ release assays enhance the applicability and effectiveness of preventive therapy in populations with high HIV prevalence?
3. In individuals with HIV infection, are T-cell-based assays more likely to produce indeterminate results? Is there an association between degree of immunosuppression (eg, CD4 counts) and antigen-specific T-cell responses?
4. What is the accuracy and reliability of T-cell-based assays in the diagnosis of active and latent tuberculosis infection in individuals on immunosuppressive therapies (eg, TNFα blockers, steroids), and other immunocompromising conditions (eg, diabetes, cancer, renal failure, organ transplantation)?
5. What is the accuracy and reliability of T-cell-based assays in the diagnosis of extra-pulmonary tuberculosis? Are T-cell assays likely to be less accurate in paucibacillary forms of extrapulmonary tuberculosis?
6. In close contacts of active tuberculosis, do T-cell-based assays have a stronger correlation with surrogate markers of exposure than tuberculin skin tests?
7. What is the effect of non-tuberculous mycobacterial infections on interferon-γ release assay performance?
8. What is the correlation between degree of immunosuppression and indeterminate and/or negative interferon-γ release assay results? What is the effect of anergy on interferon-γ release assay results?

Adapted in part, with permission from reference 17. TNFα=tumour necrosis factor α.

Although interferon-γ release assays are designed to detect latent tuberculosis infection, it has been suggested that they can serve as "rule out" tests for active tuberculosis, where a negative interferon-γ release assay can be used to exclude the presence of infection, and, consequently, active disease.[14,17] Research is needed to estimate the negative predictive value of interferon-γ release assays for active disease. This will be particularly helpful in special populations (eg, children, patients with smear-negative pulmonary tuberculosis, and HIV-infected individuals) where the diagnosis of active tuberculosis is difficult to establish using conventional tests.

Because there is no gold standard for diagnosis of latent tuberculosis infection, there is a need to explore mathematical modelling techniques that can be used to estimate sensitivity and specificity of interferon-γ release assays and latent tuberculosis infection prevalence when the true infection state is unknown.[66] This includes Bayesian methods such as mixture models[67] and latent class model analyses.[68] Modelling approaches may also be helpful in the determination of cut-off points for interferon-γ release assays.[69] Like the tuberculin skin test, interferon-γ release assay results are inherently continuous, and cut-off points are used to convert them into dichotomous results. However, most studies have analysed interferon-γ release assay results as dichotomous outcomes, and little work has been done on validation of cut-off points in diverse populations.[25] Although there is epidemiological evidence to support the risk stratified

Panel 3: Risk prediction and modelling

Research question

1. What is the risk (incidence) of active disease in those with positive and negative interferon-γ release assay results? Are individuals with positive interferon-γ responses at greater or lower risk for developing active disease? What is the predictive value of a positive interferon-γ release assay test relative to a positive tuberculin skin test?
2. What is the importance and predictive value of absolute interferon-γ responses? Among individuals with a positive interferon-γ release assay, are individuals with higher and/or rising levels of interferon-γ responses more likely to progress from latency to active disease?
3. Is it possible to identify an interferon-γ release assay cut-off point that is predictive of incipient or subclinical tuberculosis disease?
4. What is the accuracy and role of interferon-γ release assays as a "rule out" test for active tuberculosis? What is the negative predictive value of interferon-γ release assays for active disease?
5. In the absence of a gold standard for latent tuberculosis infection, what is the role of mathematical modelling approaches to deriving appropriate cut-off points for the interferon-γ release assay and the tuberculin skin test in various populations?
6. In the absence of a gold standard for latent tuberculosis infection, what is the role of Bayesian modelling approaches (eg, latent class and mixture models) to determining interferon-γ release assay sensitivity and specificity, and prevalence of latent tuberculosis infection?
7. What are the ethical issues pertinent to the conduct of longitudinal studies on predictive value of interferon-γ release assays?

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Panel 4: Test reproducibility and serial testing

Research question

1. What is the amount of test-related variability in the T-cell responses—ie, variations in interferon γ because of variability of factors such as operators, laboratories, sample processing interval, incubation times, antigens (proteins *vs* peptides), assay formats (ELISA *vs* enzyme-linked immunospot assay [ELISPOT]), use of fresh versus frozen samples (for ELISPOT), etc?
2. What is the amount of random, biological variability of interferon-γ responses over time, within the same individuals, including day-to-day, week-to-week, and month-to-month variability of interferon-γ levels in the absence of tuberculosis exposure?
3. For serial testing of health-care workers with interferon-γ release assays, which threshold for interferon γ (cut-off point) is best for distinguishing between true infection (ie, conversion) and non-specific, random variation?
4. Among health-care workers screened with serial tuberculin skin test and interferon-γ release assay, what is the concordance between interferon-γ release assay and tuberculin skin test conversions? What is the correlation between changes in absolute tuberculin skin test reactions and interferon-γ levels?
5. How should an interferon-γ release assay reversion be defined, how commonly do reversions occur, and what is the clinical/epidemiological significance of reversions? What factors are associated with interferon-γ release assay reversions, including treatment, baseline interferon-γ levels, variability around cut-off points, etc?
6. What is the effect of a tuberculin skin test on subsequent interferon-γ release assay results—ie, can a tuberculin skin test boost a subsequent interferon-γ release assay result?
7. When discordance between tuberculin skin test and interferon-γ release assay occurs, what proportion of the overall discordance is caused by variations around tuberculin skin test and interferon-γ release assay cut-off points? When discordant cases are re-tested, what proportion become concordant?
8. In serial testing, are those with strong increases in T-cell responses more likely to develop active tuberculosis? Is the strong increase more likely to be seen in those with recent exposure?

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cut-off points used to interpret the tuberculin skin test,[4] such data do not currently exist for the interferon-γ release assay. Interferon-γ release assay thresholds, therefore, need validation in diverse populations. Some of the observed discordance between the tuberculin skin test and interferon-γ release assay may in part be resolved through further exploration of interferon-γ cut-off points, and by analysing both the tuberculin skin test and interferon-γ release assays as continuous outcomes.[25] Panel 3 lists the other research issues identified under this area.

Test reproducibility and serial testing

Tuberculosis is an important occupational health problem among health-care workers.[70,71] Periodic screening of health-care workers for latent tuberculosis infection is an important component of tuberculosis infection control programmes.[72] In addition to its known limitations, the interpretation of serial tuberculin skin tests is particularly complicated because of boosting, conversions, and reversions.[63] Interferon-γ release assays have several features that are ideal for serial testing: they are more specific than the tuberculin skin test, can be repeated without sensitisation and boosting, reduce the need for repeat visits, and eliminate the requirement for two-step baseline testing. However, there are virtually no data on the short-term and long-term reproducibility of interferon-γ release assays, particularly within-subject variability in serial testing, where conversions and reversions can occur. Without data on longitudinal changes and biological variability, the results of serial interferon-γ release assay testing will be difficult to interpret.[73]

Although the QuantiFERON-TB Gold test has been recommended for serial testing,[18,72] there are currently limited data to support this practice. There are limited data on how much interferon-γ responses will change with new tuberculosis infection (ie, conversion) and how to differentiate this from changes caused by test-related error (ie, reproducibility) or biological variations over time. One study showed that conversions, reversions, and non-specific variations occur with interferon-γ release assay serial testing, just as they do with tuberculin skin test serial testing.[73] This study highlighted the need for studies on within-subject variability of interferon-γ responses during serial testing, frequency of interferon-γ release assay conversions and reversions, and optimum thresholds (cut-off points) to distinguish new infection from non-specific variation.[73]

In the context of serial testing, there is some evidence that tuberculin skin test conversions may be associated with strong increases in interferon-γ responses. A study from India showed that highly exposed health-care workers who had tuberculin skin test conversions had massive increases in interferon-γ responses to ESAT6 and CFP10 (measured using the QuantiFERON-TB Gold In Tube assay).[73] The QuantiFERON-TB Gold assay successfully detected all cases of tuberculin skin test conversion, and every health-care worker who had a large increase in tuberculin skin test induration had a huge increase in interferon-γ response, orders of magnitude higher than the diagnostic cut-off point.[73] Another study from Uganda in household contacts found a strong correlation between tuberculin skin test conversions and increased interferon-γ responses among exposed individuals.[74] Thus, it is plausible that individuals with recent exposure have vigorous increases in T-cell responses, probably because of active bacterial replication. Because it is well documented that individuals with tuberculin skin test conversions have a high likelihood of progressing to active disease,[63] it is plausible that strong increases in interferon-γ responses after recent exposure might be predictive of progression to active disease.

Studies also need to distinguish between the biological variability of positive responses (ie, whether they often fluctuate above and below the limit of detection or cut-off for positivity), and the frequency of reversions and false-positive results as a result of such fluctuations. Thus, cohort studies are needed to better define the role of interferon-γ release assays in serial testing. Panel 4 lists the specific questions relevant to this area.

***Panel 5*: T-cell responses during treatment and role in treatment monitoring**

Research question

1. What is the association between bacterial burden and T-cell responses?
2. How do T-cell responses change during and after treatment for latent tuberculosis infection? What factors, including host, disease, and assay characteristics, influence variability in responses after treatment?
3. How do T-cell responses change during and after treatment for active tuberculosis? What factors—including host, disease, and assay characteristics—influence variability in responses after treatment?
4. Can T-cell-based assays have a useful role in monitoring response to latent and active tuberculosis treatment?
5. Is failure to modulate T-cell responses during the initial phase of treatment predictive of subsequent relapse?
6. Will treatment of patients with positive interferon-γ release assay results reduce the future probability of active tuberculosis?
7. What is the ability of interferon-γ release assays to detect new infection after treatment for both latent tuberculosis infection and tuberculosis disease? If interferon-γ release assay results become negative after treatment, and become positive after a new exposure, does this indicate new infection?

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Panel 6: Epidemiological and field applications

Research question

1 Can interferon-γ release assays be used in community surveys to estimate annual risk of tuberculosis infection? Can they be used for community-based prevalence surveys?
2 What is the accuracy and use of screening strategies that use combinations of tuberculin skin test and interferon-γ release assays—eg, first screen with tuberculin skin test and confirmation of positive results by interferon-γ release assay?
3 How does interferon-γ release assay performance vary between high and low tuberculosis incidence settings? In addition to geographical variability, are there racial/ethnic differences in interferon-γ release assay performance and accuracy?
4 In tropical, high-burden settings, what is the effect of immune modulating factors such as malnutrition, BCG vaccination, non-tuberculous mycobacterial exposure, leprosy, and helminth infections on T-cell-based assays?
5 In vaccine trials, can interferon-γ release assays serve as correlates of protective immunity? Can these be used to measure "vaccine take"? Can they help diagnose active tuberculosis cases during follow-up in vaccine trials?
6 In high-burden, developing countries, which patient or population subgroups are most likely to benefit from the use of T-cell-based assays—eg, HIV-infected people, children under 5 years, contacts, health-care workers, and those who are most likely to be anergic with tuberculin skin test?
7 Can interferon-γ release assays enable researchers to revisit and revise some of the risk and rate estimates traditionally used in tuberculosis epidemiology including, for example, the global prevalence of tuberculosis infection, the lifetime risk of reactivation tuberculosis, and the Styblo rule on ratio of the annual risk of infection to the incidence of new smear-positive tuberculosis cases?
8 Can interferon-γ release assay results be used to improve tuberculin skin test cut-off points in prevalence/annual risk of tuberculosis infection surveys?

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T-cell responses during treatment and role in treatment monitoring

Another controversial topic is T-cell response kinetics during and after treatment for latent and active tuberculosis. This issue closely overlaps with the ill-defined correlation between T-cell assay results and bacterial burden.[26–29] As reviewed elsewhere,[13,14] some studies have shown declining responses after treatment (mainly the ELISPOT assay), whereas others have shown unchanging, fluctuating, or increasing responses during treatment. Variations in disease severity, test reproducibility, incubation periods, antigens (proteins versus peptides), possible endotoxin contamination, non-tuberculous mycobacterial exposure, and assay formats might explain some of the discrepancies.[17] Although not proven, there is emerging evidence that T-cell-based assays might provide a more quantitative and dynamic assessment of latent tuberculosis than the tuberculin skin test, and this potential may be exploited to study the effect of new vaccines and therapeutic agents.[28,73,75,76]

Overall, further work is necessary to determine if interferon-γ release assays can be used for monitoring response to latent tuberculosis infection and active tuberculosis treatment. If shown to be useful, then interferon-γ release assays might be helpful as surrogate markers for long-term outcomes in the evaluation of new drugs and therapies. Panel 5 lists the research issues identified under this area.

Epidemiological and field applications

Epidemiological studies contribute to our understanding of disease burden and disease risk factors in the community, and they permit impact assessments following targeted interventions.[77] Historically, the tuberculin skin test has proven to be a useful tool for these purposes. Community surveys using the tuberculin skin test have been used to estimate the prevalence of latent tuberculosis infection, and the annual risk of tuberculosis infection, mostly in high-burden countries.[67,77–80] To date, no community based surveys have been done using interferon-γ release assays. Although the higher specificity of interferon-γ release assays will be a major advantage, especially in populations with high BCG vaccine coverage, the need for laboratory personnel and infrastructure, and venepuncture under field conditions do pose serious practical limitations, especially if children are participants.[62,67]

To date, most studies on interferon-γ release assays have been done in low-incidence countries, and the few studies undertaken in high-incidence settings have reported findings that are somewhat inconsistent with the findings from studies in low-incidence settings.[43,51,81,82] Thus, it is conceivable that T-cell assay performance may vary across populations, depending on background disease prevalence and other factors such as HIV prevalence, malnutrition, BCG vaccination, non-tuberculosis mycobacteria exposure, leprosy, and helminth and other tropical infections that can modulate immune responses.[17,83] Therefore, more studies are needed in geographically diverse, tuberculosis endemic settings, with a special focus on patient or population subgroups most likely to benefit from the use of T-cell-based assays. Studies in high-incidence and tropical countries will need to evaluate the effect of *Mycobacterium leprae*, and non-tuberculosis mycobacteria such as *Mycobacterium kansasii* and *Mycobacterium marinum* on inducing false-positive interferon-γ release assay results, because homologues of ESAT6 and CFP10 are found in these organisms.[84–87] Panel 6 lists other research questions in this area.

Health systems, operational, and economic research

One potential rate-limiting factor for interferon-γ release assay uptake, particularly for high-burden, resource-limited countries, is their higher material costs and the need for laboratory infrastructure and trained personnel. Economic evaluations and decision analyses are needed to better delineate the role of interferon-γ release assays in public-health and routine clinical settings (panel 7). It is possible that, at least in some settings, the advantages

of a more logistically convenient and highly specific blood test might outweigh the higher costs.[88] It has been suggested that hybrid strategies that combine tuberculin skin test and interferon-γ release assay might be more cost effective.[19,89,90] Further research is needed to confirm this. From a control programme perspective, it is essential to determine what resources are required to increase laboratory capacity in developing countries to enable implementation of new tools such as interferon-γ release assays. In parallel, modelling is needed to predict the potential effect of improved latent tuberculosis infection diagnosis and treatment on global tuberculosis burden and the role of these tests in supporting tuberculosis elimination targets.

Priorities for resource-limited and high HIV prevalence settings

Detection and treatment of latent tuberculosis infection is an important component of tuberculosis control efforts in low-incidence settings.[4,91] However, in high-incidence settings, the diagnosis and treatment of active tuberculosis cases is the first priority,[92] and the role of latent tuberculosis infection diagnostics is currently limited. However, as active tuberculosis case rates decrease with the rapid expansion of global DOTS (directly observed treatment, short course) coverage, latent tuberculosis infection diagnosis and treatment may become increasingly important to eliminate tuberculosis as a public-health problem by 2050. Furthermore, as described in the Global Plan to Stop TB 2006–2015,[93] management of latent tuberculosis infection in high HIV prevalence settings will be of paramount importance, together with DOTS expansion and provision of a tuberculosis/HIV package of prevention and care, to control and eventually eliminate tuberculosis. Research should be promoted to address the unresolved issues around the use of interferon-γ release assays in such settings and modelling studies should be done to better understand the effect of improved latent tuberculosis infection management on global tuberculosis and HIV epidemics.

Simplification of the current interferon-γ release assay formats and reduction of costs through public–private partnerships and collaborations,[94,95] and bulk purchasing might increase the likelihood of uptake in high tuberculosis burden settings, particularly in selected populations such as children, HIV-infected individuals, and contacts of infectious tuberculosis cases. Additionally, if interferon-γ release assays are shown to be more predictive of active tuberculosis than the tuberculin skin test, then their use can be expected to be expand exponentially, with the potential to revolutionise our approach to tuberculosis diagnosis and treatment.

Interferon-γ release assays may also serve as useful research tools, especially in epidemiological studies. For nearly 100 years, researchers had to rely on a single test for studying latent tuberculosis infection. Interferon-γ release assays now provide a second window into the biology and epidemiology of latent tuberculosis infection, and may enable researchers to revisit and revise some of the risk and rate estimates traditionally used in tuberculosis epidemiology, including the global prevalence of tuberculosis infection,[1,78,96] the lifetime risk of reactivation tuberculosis,[64,91] and the Styblo rule[97] on the relation between annual risk of tuberculosis infection and incidence of new smear-positive tuberculosis cases, especially in high HIV prevalence settings. These risk estimates were determined using tests such as the tuberculin skin test, before the global HIV epidemic, and therefore need to be revised in light of the effect the HIV epidemic has had on the course of the tuberculosis epidemic.[2,80] Refined estimates of these key epidemiological parameters will enable better surveillance and monitoring of the global tuberculosis/HIV burden, and allow policymakers to evaluate the effectiveness of the new global tuberculosis control strategy.[98]

Conclusions

The lack of accurate and rapid diagnostics for latent and active tuberculosis is a major impediment for effective tuberculosis control.[94,95,99] The engagement of agencies such as the Stop TB Partnership, WHO, FIND, and the Bill & Melinda Gates Foundation has led to a revival of interest in the development of new tuberculosis diagnostics.[94,95,100] Indeed, the development of new tools and evaluation of existing tools figure prominently in the Global Plan to Stop TB, 2006–2015[93] and the new global

***Panel 7*: Health systems, operational, and economic research**

Research question

1. How do interferon-γ release assays and the tuberculin skin test compare in economic and decision analyses for various screening programmes—eg, immigrant screening, contact investigations, serial testing of health-care workers, etc?
2. What is the effect of switching from tuberculin skin test to interferon-γ release assay on laboratory/clinic work load, staff work load, programme costs, patient convenience, compliance with testing and follow-up, etc?
3. How acceptable are interferon-γ release assays to various commonly screened populations—eg, contacts, immigrants, individuals with HIV infection, health-care workers?
4. What is the effect of latent tuberculosis infection diagnosis and treatment on global tuberculosis control? What latent tuberculosis infection test characteristics will enhance the effect?
5. What resources are needed to increase laboratory capacity in developing countries to enable implementation of new tools such as interferon-γ release assays?

Adapted in part, with permission, from reference 17.

strategy to Stop TB (2006).[98] Thus, the emergence of novel tools such as the interferon-γ release assay is a welcome development because, for the first time, these assays have expanded the armamentarium of diagnostics available for latent tuberculosis infection. In addition to clinical use, these tests are highly promising as research tools to advance our knowledge of latent tuberculosis infection and its epidemiology. The proposed research agenda provides a comprehensive compilation of key research questions that deserve attention to ensure appropriate and best possible use of latent tuberculosis infection diagnostics in tuberculosis control, especially in the context of the HIV epidemic. This agenda aims to advance the field by stimulating focused high-impact research and engage a wider network of researchers and institutions. It should also encourage the investment of resources needed to tackle research questions of high importance and potential effect, especially in resource-limited settings with high tuberculosis and HIV burden.

Conflicts of interest

We declare that we have no conflicts of interest. ROB works for Foundation for Innovative New Diagnostics (FIND), a non-profit agency that collaborates with several industry partners (including those that manufacture interferon-γ release assays) for the development of new diagnostics. No industry was involved in the preparation, review, or submission of this manuscript.

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