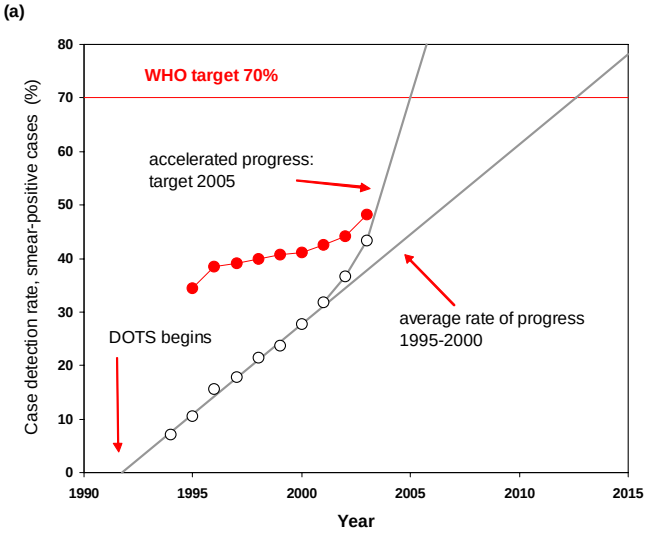
New tests for the diagnosis of tuberculosis infection

Madhukar Pai, MD, PhD
Dept. of Epidemiology & Biostatistics
McGill University
Montreal, Canada
Email: madhukar.pai@mcgill.ca

McGill

TB case detection: Achilles heel of TB control?



Source: WHO

Cette présentation a été effectuée le 24 octobre 2006, au cours du Symposium "L'utilisation des analyses de laboratoire en santé publique" dans le cadre des Journées annuelles de santé publique (JASP) 2006. L'ensemble des présentations est disponible sur le site Web des JASP, à l'adresse http://www.inspq.qc.ca/jasp.

Conventional TB diagnostics: badly in need of upgrade

- Latent TB (LTBI)
  - Tuberculin skin test [1890]
- Active TB
  - Sputum microscopy [1882]
  - Mycobacterial culture [1882]
  - Chest X-rays [1896]

TB diagnostics
1882

Stalled TB technology exemplifies a system-wide neglect of diagnostics for diseases of poverty

TB diagnostics
2003

Image: FIND

Tuberculin skin test

The end of tuberculin skin testing?

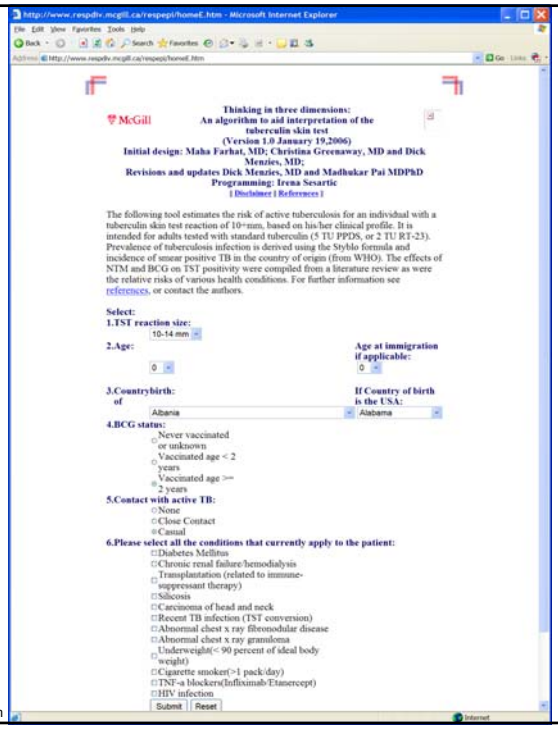
Image: Lancet Infect Dis

Tuberculin skin test (TST)

- TST
  - Measures cell-mediated immune response (CMI)
  - Uses PPD: a crude antigenic mixture
- Limitations of TST:
  - fairly high proportion of false positives and false negatives
  - technical problems in administration and interpretation
  - difficulty in separating true infection from the effects of BCG and non-tuberculous mycobacteria (NTM)
  - repeated TST boosts the immune response
  - requires a 3-dimensional interpretation

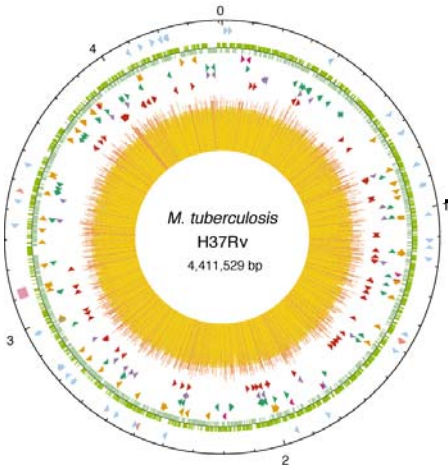
McGill

TST in 3D



http://www.respdiv.mcgill.ca/respepi/homeE.htm

Recent advances in the development of antigens specific to *M. tuberculosis*



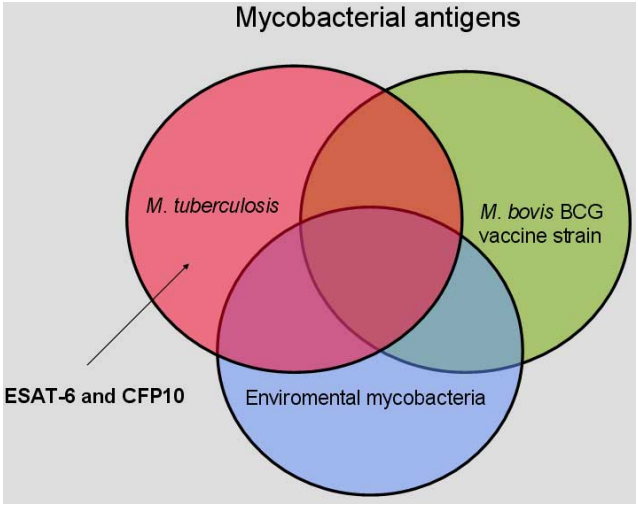
Genomics
Proteomics

ESAT-6

CFP-10

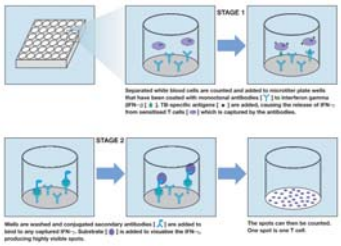
Cole et al, Nature 1998

Recent advances in the development of antigens specific to *M. tuberculosis*

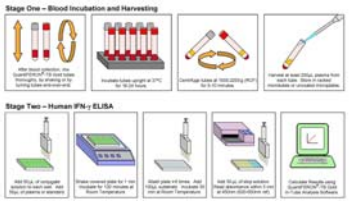


Source: Statens Serum Institute, Denmark

Interferon-gamma release assays (IGRA)



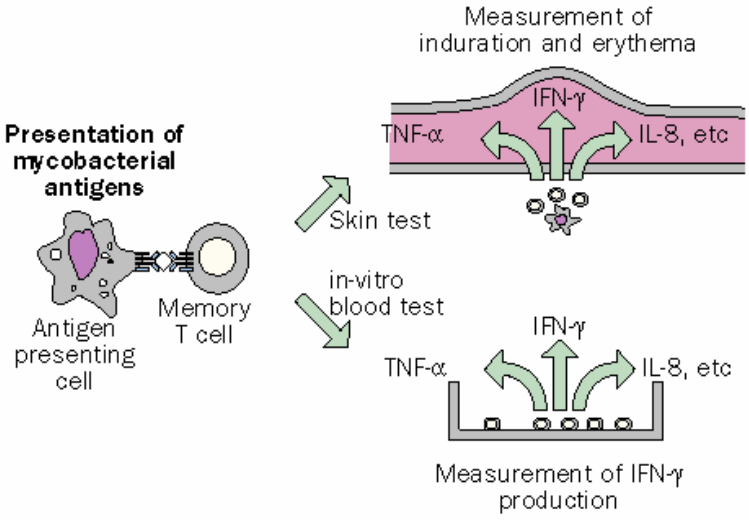
T-SPOT.*TB*® [Oxford Immunotec, UK]



QuantiFERON-TB Gold® [Cellestis Ltd, Australia]

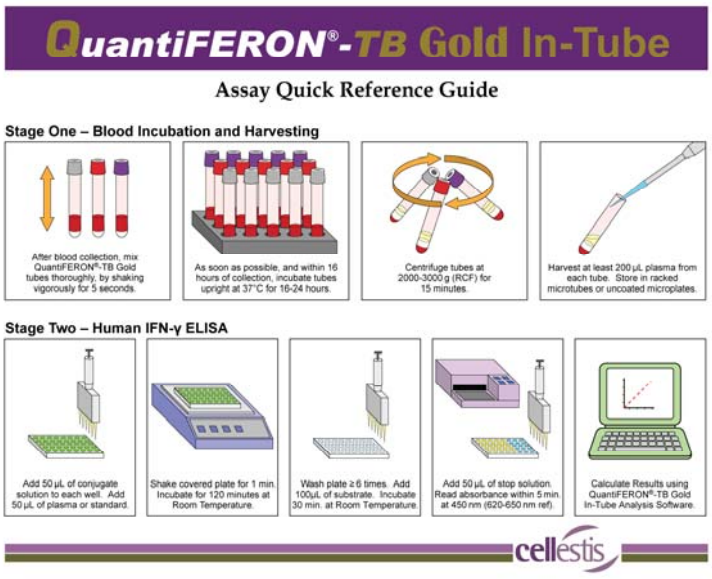
Pai M et al. Lancet Infect Dis 2004;4:761-76

IGRA: rationale



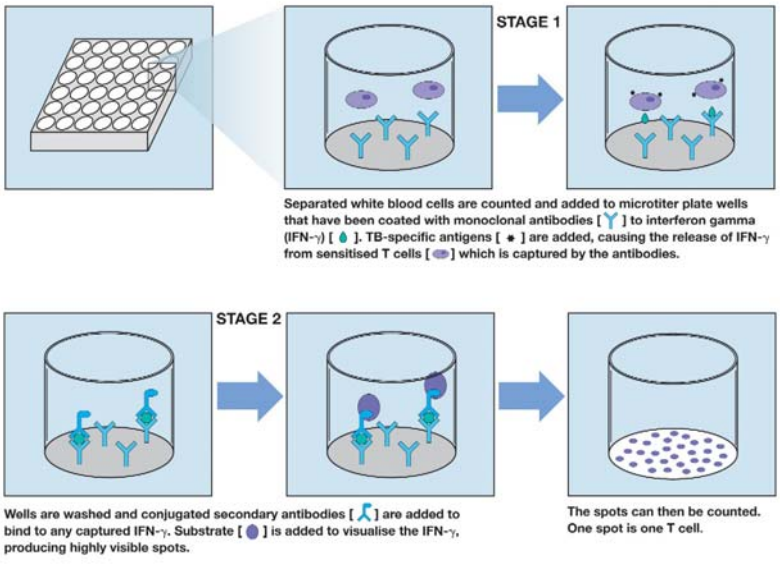
Andersen P et al, Lancet 2000

QFT-Gold In Tube®

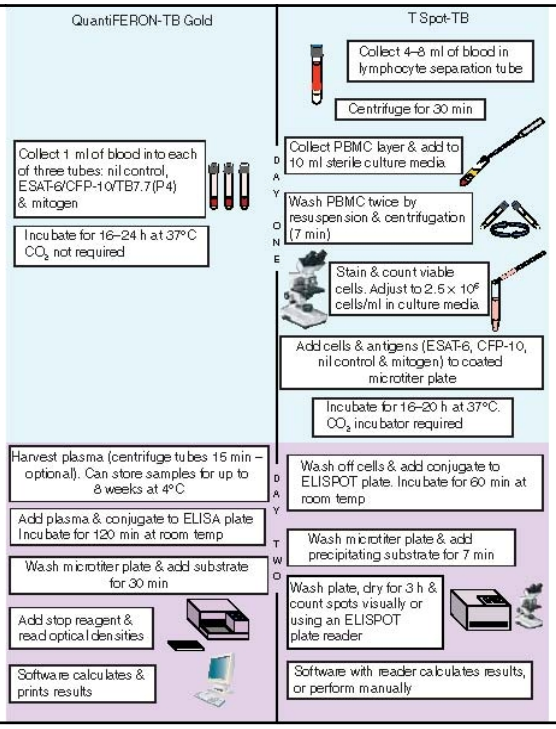


Courtesy: Cellestis Ltd, Australia

T-SPOT.*TB*®



Courtesy: Oxford Immunotec, UK

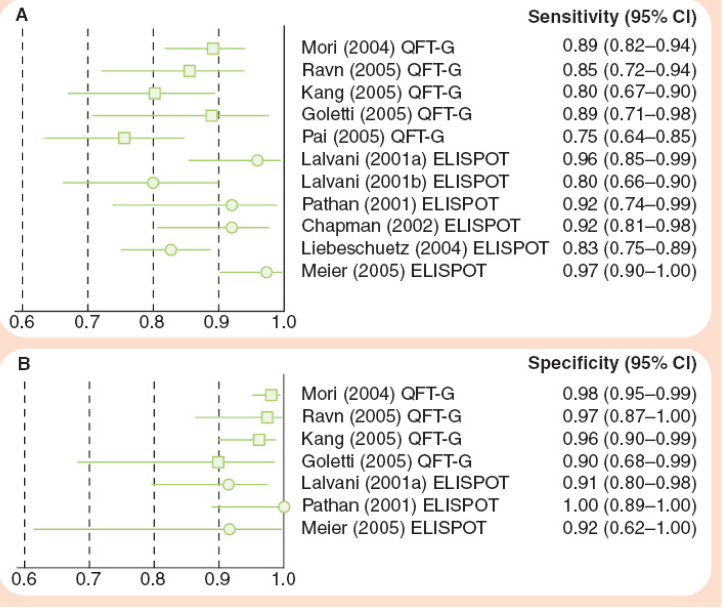


Rothel J, et al. Exp Rev Anti Infect Ther 2005;3:981-993

Methodological issues in the evaluation of IGRAs

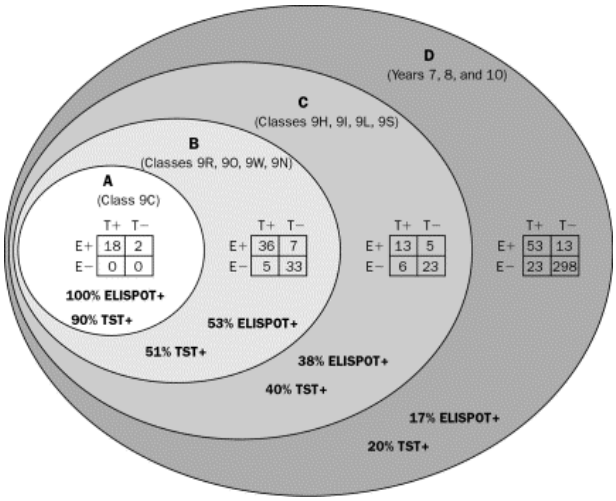
- In the absence of a gold standard for LTBI, direct estimation of sensitivity and specificity is not possible
- Hypotheses that allow for an indirect ranking of TST and IGRA: if IGRA is superior to TST, then IGRA should
  - have higher sensitivity and specificity for active TB than the TST
  - correlate better with exposure to *M. tuberculosis* than TST;
  - be less influenced by BCG vaccination and NTM infection;
  - be able to predict better who will develop active TB among those latently infected
- Studies have measured agreement between TST and the IGRA, and identifying factors associated with discordance.

Test performance: sensitivity and specificity



Pai M et al. Exp Rev Mol Diagn 2006;6(3):413-422

Correlation with TB exposure: example from a school outbreak in UK



P-value for difference between TST and ELISPOT trend = 0.03

Ewer et al, Lancet 2003;361:1168-73

Ability to predict active disease among latently infected

RR 10 [95% CI 1.42, 70.22]

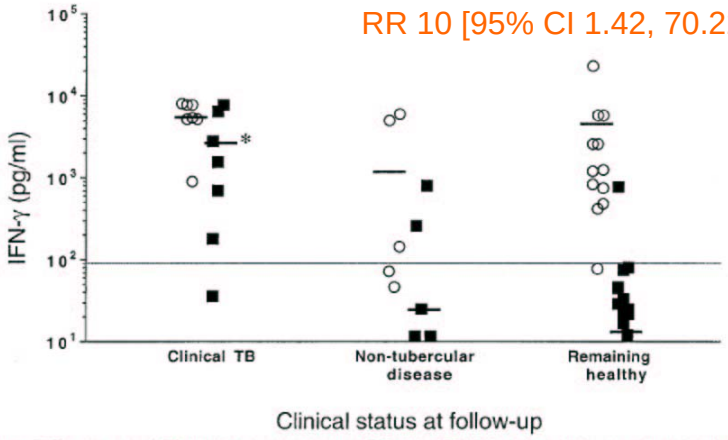


FIG. 1. In vitro IFN-γ responses of PBMCs after restimulation with PPD (○) or ESAT-6 (■) at the time of entry into the study. The results are for individual contacts segregated by their clinical status at the end of the follow-up period (2 years). The cutoff point for positivity in the assay is indicated by the solid line. Median in vitro IFN-γ responses are indicated by the heavy horizontal bar. Results significantly different from those for the contacts who remained healthy are indicated (*, $P < 0.001$).

Doherty et al. J Clin Microbiol 2002;40(2):704-6

Table 1. Comparison of tuberculin skin test and interferon-γ release assays.

Performance and operational characteristics	Tuberculin skin test	Interferon-γ release assays
Estimated sensitivity (in patients with active tuberculosis)	75–90% (lower in immunocompromised populations)	75–95% (inadequate data in immunocompromised populations, but appears promising)
Estimated specificity (in healthy individuals with no known tuberculosis disease or exposure)	70–95% (lower in BCG-vaccinated, especially if BCG is given after infancy)	90–100% (maintained in BCG vaccinated)
Cross-reactivity with BCG	Yes	Less likely
Cross-reactivity with nontuberculous mycobacteria	Yes	Less likely, but limited evidence
Association between test positivity and subsequent risk of active tuberculosis during follow-up	Moderate-to-strong positive association	Insufficient evidence
Correlation with *Mycobacterium tuberculosis* exposure	Yes	Yes (correlated better with exposure than tuberculin skin test in some, but not all, head-to-head comparisons)
Benefits of treating test positives (based on randomized controlled trials)	Yes	No evidence
Reliability (reproducibility)	Moderate and variable	Limited evidence, but appears high; no evidence on within subject variability during serial testing
Boosting phenomenon	Yes	No
Potential for conversions and reversions	Yes	Insufficient evidence
Adverse reactions	Rare	Rare
Material costs	Low	Moderate to high
Patient visits to complete testing	Two	One
Laboratory infrastructure required	No	Yes
Time to obtain a result	2–3 days	1–2 days, but longer if run as batches
Trained personnel required	Yes	Yes

Adapted with permission from references [10,16].

Pai M et al. Exp Rev Mol Diagn 2006;6(3):413-422

QFT-G vs T-SPOT.*TB*: head to head

- Ferrara et al (Lancet 2006):
  - 393 patients in routine clinical practice in Italy.
    - T-SPOT.TB and QFT-G had higher specificity than the TST.
    - Rates of indeterminate and positive results, however, differed between the blood tests
    - Indeterminate results were significantly more frequent with QFT-G (11%) than with T-SPOT.TB (3%; p<0.0001) and were associated with immunosuppressive treatments for both tests
    - T-SPOT.TB produced significantly more positive results than QFT-G (38% vs 26%; p<0.0001)

QFT-G vs T-SPOT.*TB*: head to head

- Lee et al (ERJ 2006):
  - 218 patients in a tertiary hospital in Korea
    - Using 10 mm as a cut-off for TST, SPOT sensitivity (96.6%) was significantly higher than that seen for TST (66.7%) and QFT-G (70.1%)
    - QFT-G showed superior specificity over TST (91.6 vs 78.6%).
    - Although the specificity of QFT-G was higher than that of SPOT (91.6 vs 84.7%), the difference was not statistically significant

Guidelines on IGRAs

- CDC 2005 guidelines (MMWR 2005;54(RR-15):49-55):
  - recommends that QFT-G may be used in all circumstances in which the TST is currently used, including contact investigations, evaluation of immigrants, and serial testing of healthcare workers
    - QFT-G can be used in place of (and not in addition to) the TST
- CDC also published its updated guidelines for preventing the transmission of TB in healthcare settings (MMWR 2005; 54(RR-17):1-141)
    - QFT-G can be used in place of the TST for infection control surveillance


Vol. 54 / RR-15 Recommendations and Reports 49

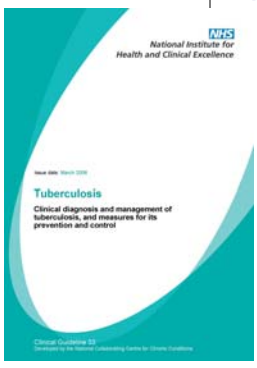
Guidelines for Using the QuantiFERON®-TB Gold Test for Detecting *Mycobacterium tuberculosis* Infection, United States

Summary

MMWR 2005

Guidelines on IGRAs

- UK National Institute for Health and Clinical Excellence (NICE) 2006 guidelines:
  - recommends a two-step (hybrid) strategy for LTBI diagnosis: initial screen with TST, and those who are positive (or in whom TST may be unreliable) should then be considered for IGRA testing, if available, to confirm positive TST results.



NICE, 2006

Other guidelines in preparation

- Canadian guidelines by the Public Health Agency of Canada and the Canadian Tuberculosis Committee (to be released later this year)
- Japanese guidelines on QFT-2G (JATA guidelines)

1) QFT-2G as a replacement for the tuberculin skin test (TST) for three areas:

   (i) Contact investigation following exposure to an active TB case (but not mass exposure situations where for logistic reasons the TST is recommended for initial contact screening and QFT-2G to confirm exposure)

   (ii) Initial and serial screening of health care workers; and

   (iii) For clinical diagnosis of patients at highest risk of contracting TB disease, including diabetics, and patients receiving immunosuppressive medication.

2) QFT-2G is also recommended as a diagnostic tool to assist active TB diagnosis.

3) At this time, the test is not recommended for diagnosis of TB in children five years and under.

Japanese guidelines: Cellestis Ltd, Australia

Conclusions

- Overall, because of its high specificity and other potential advantages, IGRAs are likely to replace the TST in low-incidence, high income settings where cross-reactivity due to BCG might adversely impact the utility of the TST
  - In high income settings, the evidence base is still weak on issues such as active TB, HIV+, children, immunocompromised, and serial testing
- In high incidence settings, active TB is the first priority, and the role of LTBI diagnostics in currently limited
  - However, as active TB case rates decrease with the rapid expansion of global DOTS coverage, LTBI diagnosis and treatment will become increasingly important to eliminate TB
  - In high incidence settings, current applicability may be restricted to high risk groups such as HIV+, children, and contacts.
- In addition to clinical utility, these tests are promising as research tools to advance our knowledge of LTBI and its epidemiology
- Despite the growing evidence base, areas of uncertainty remain and future studies should address them

Unresolved issues

- Discordance between the TST and IGRAs results
- Correlation between bacterial burden and T cell responses; effect of TB treatment on T cell responses
- Predictive value of IGRAs for the development of active TB
- Test performance in high-risk populations such as HIV+, children, immunocompromised
- Performance in serial testing
- Utility of IGRAs in epidemiologic studies
- Feasibility, applicability, cost effectiveness
- Utility in high incidence and resource limited settings

Pai M et al. Lancet Infect Dis 2004;4:761-76 Pai M et al. Exp Rev Mol Diagn 2006;6(3):413-422

Biologic issues and assay development

No.	Research question
1	To what extent does a positive IGRA result suggest previous (remote) infection (either cleared or still persistent) versus recent infection? What type of responses are detected by IGRAs - effector or memory T cell responses?
2	Can the identification and validation of novel TB specific antigens help to increase sensitivity of IGRAs without compromising their high specificity?
3	Can the identification and validation of novel TB specific antigens (or biomarkers) help to distinguish between LTBI and active disease?
4	What is the biological basis for discordance between TST and IGRA results?
5	After exposure to *M. tuberculosis*, how long does it take for the IGRA test to become positive? Can IGRAs detect spontaneous clearance of infection?
6	In head to head comparisons, what is the difference in performance characteristics (e.g. sensitivity and indeterminate rates) of the commercial IGRAs?
7	What is the best approach to determining appropriate cut-points for IGRAs? In high-risk groups (e.g. HIV+), do IGRA cut-points need to be set lower?

Test performance in high risk populations and poorly studied groups

No	Research question
1	What is the accuracy of IGRAs in the diagnosis of active TB and LTBI in children? In children with extra-pulm or severe TB, are IGRAs less sensitive?
2	What is the accuracy of IGRAs in the diagnosis of active TB and LTBI in HIV infected? Can IGRAs be used to detect sub-clinical TB in HIV+? Will IGRAs enhance the effectiveness of preventive therapy?
3	In HIV+, are IGRAs more likely to produce indeterminate results? Is there an association between degree of immunosuppression and antigen-specific T cell responses?
4	What is the accuracy of IGRAs in the diagnosis of active TB and LTBI in immunosuppressed individuals (e.g. TNF-a blockers, steroids, diabetes, cancer, renal failure, organ transplantation)?
5	What is the accuracy of IGRAs in the diagnosis of extra-pulmonary TB?
6	What is the impact of NTM infections on IGRA performance?

Risk prediction and modeling

No.	Research question
1	What is the risk (incidence) of active disease in those with positive and negative IGRA results? Are individuals with positive IFN-g responses at greater or lower risk for developing active disease? What is the predictive value of a positive IGRA test relative to a positive TST?
2	What is the importance and predictive value of absolute IFN-g responses? Among individuals with a positive IGRA, are individuals with higher levels of IFN-g responses more or less likely to progress from latency to active disease?
3	What is the accuracy and role of IGRAs as a “rule out” test for active TB? What is the negative predictive value of IGRAs for active disease?
4	In the absence of a gold standard for LTBI, what is the role of mathematic modeling approaches to deriving appropriate cut-points for IGRA and TST in various populations?
5	In the absence of a gold standard for LTBI, what is the role of Bayesian modeling approaches (e.g. latent class and mixture models) to determining IGRA sensitivity and specificity, and prevalence of LTBI?

Reproducibility and serial testing

No.	Research question
1	What is amount of test-related variability in the T cell responses?
2	What is the amount of random, biological variability of IFN-g responses over time, within the same individuals?
3	For serial testing of HCWs, which IFN-g cut-point is optimal for distinguishing between true infection (i.e. conversion) and non-specific, random variation?
4	Among HCWs screened with serial TST and IGRA, what is the concordance between IGRA and TST conversions?
5	How should a IGRA reversion be defined, how commonly do reversions occur, and what is the significance of reversions? What factors are associated with IGRA reversions?
6	What is the effect of a TST on subsequent IGRA results?
7	In serial testing, are those with dramatic increases in T cell responses more likely to develop reactivation TB? Is the dramatic increase more likely to be seen in those with recent exposure?

T cell responses during treatment and role in treatment monitoring

No.	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 TB infection? What factors influence variability in responses after treatment?
3	How do T cell responses change during and after treatment for active TB? What factors influence variability in responses after treatment?
4	Can T cell based assays play a useful role in monitoring response to latent and active TB treatment?
5	Will treatment of IGRA positive subjects reduce the future probability of active TB?
6	What is the ability of IGRAs to detect reinfection after treatment for both LTBI and TB disease?

Epidemiologic and field applications

No.	Research question
1	Can IGRAs be used in community surveys to estimate annual risk of TB infection? Can they be used for community based prevalence surveys?
2	What is the accuracy and utility of screening strategies that use combinations of TST and IGRAs: e.g. first screen with TST, and confirmation of positive results by IGRAs?
3	How does IGRA performance vary between high and low TB incidence settings?
4	In high burden settings, what is the impact of factors such as malnutrition, BCG, NTM exposure, leprosy, and helminthic infections on T cell based assays?
5	In vaccine trials, can IGRAs serve as correlates of protective immunity? Can these be used to measure “vaccine take” or diagnose active TB at follow up?
6	In high burden, developing countries, which subgroups are most likely to benefit from the use of T cell based assays? E.g. HIV+, children under 5 years, contacts, health care workers, and those who are most likely to be anergic with TST.
7	Can IGRAs help us revise risk and rate estimates traditionally used in TB epidemiology, including, for e.g., the global prevalence of TB infection, the lifetime risk of reactivation TB, and the Styblo rule on ratio of the ARI to the incidence of new smear-positive TB cases?

Health systems, operational and economic research

No.	Research question
1	How do IGRAs and TST compare in economic and decision analyses for various screening programs (e.g. immigrant screening, contact investigations, serial testing of health-care workers, etc.)
2	What is the impact of switching from TST to IGRA on laboratory/clinic work load, staff work load, program costs, patient convenience, compliance with testing and follow-up, etc.?
3	How acceptable are IGRAs to various commonly screened populations (e.g. contacts, immigrants, individuals with HIV infection, healthcare workers)?
4	What is the impact of LTBI diagnosis and treatment on global TB control? What LTBI test characteristics will enhance the impact?
5	What resources are needed to increase lab capacity in developing countries to enable implementation of new tools such as IGRAs?

Prospects for the future

- Several IGRA studies are ongoing or being launched
  - Foundation for Innovative New Diagnostics (FIND) is planning a series of demonstration projects in high burden countries
  - Agencies such as CDC (TBESC) are launching larger scale cohort studies
  - McGill TB Group: CIHR funded cohort study among healthcare workers and household contacts
- Research during the next 5 years will help settle unresolved issues, and define the exact role for these assays in clinical and public health settings
- Further refinement (e.g., inclusion of additional antigens to increase sensitivity) and standardization of these commercial assays will also likely occur, which will enhance their utility and applicability
- At this time, the role for IGRAs in low-income, high-burden settings is rather limited
  - Simplification of the test format and reduction of costs might enhance applicability in such settings, particularly in selected subgroups, such as HIV-infected individuals, children and other high-risk groups
  - Until such time, the TST will continue to be a useful, simple, low-cost tool in developing countries where BCG vaccination is given in infancy, and thus has limited impact on TST results

Further reading

- Pai M, Riley LW, Colford JM. Interferon-g assays in the immunodiagnosis of tuberculosis: a systematic review. *Lancet Infect Dis* 2004;4:761-76.
- Pai M, Lewinsohn D. Can immunosuppression and anergy affect interferon-g assays for tuberculosis. *Am J Respir Crit Care Med* 2005;172:519-21.
- Pai M, Kalantri SP, Dheda K. New tools and emerging technologies for the diagnosis of tuberculosis: Part I. Latent tuberculosis. *Exp Rev Mol Diag* 2006;6(3):413-422.
- Pai M, Kalantri SP, Dheda K. New tools and emerging technologies for the diagnosis of tuberculosis: Part II. Active tuberculosis and drug resistance. *Exp Rev Mol Diag* 2006;6(3):423-32.
- Farhat M, Greenaway C, Pai M, Menzies D. False positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria? *Int J Tuberc Lung Dis* 2006;10(11):1-13.
- Pai M, Menzies D. Interferon-gamma release assays: what is their role in the diagnosis of active tuberculosis? *Clin Infect Dis* 2006 (in press).
- Mazurek GH, Jereb J, Lobue P, Iademarco MF, Metchock B, Vernon A. Guidelines for using the QuantiFERON-TB Gold test for detecting Mycobacterium tuberculosis infection, United States. MMWR Recomm Rep. 2005 Dec 16;54(RR-15):49-55.