



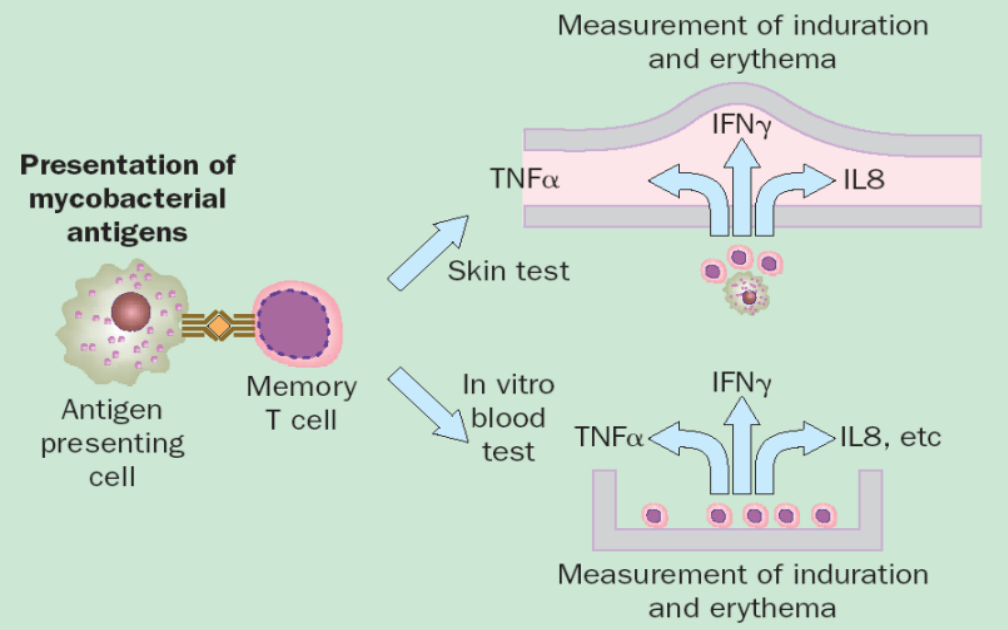
**"L'infettivologia del 3  
millennio:  
AIDS ed altro"**

# Interferon Gamma Release Assays (IGRA) VS Mantoux: *concordanza, discordanza o confusione?*

**Centro Congressi Hotel Ariston  
Paestum (SA)  
17 maggio 2014**

*Francesco Nicola Lauria  
INMI L. Spallanzani IRCCS, Roma*





## Test basati su IFN- $\gamma$

- test **in vitro** che misurano l'IFN- $\gamma$  rilasciato dai linfociti T in risposta ad antigeni tubercolari
- per la stimolazione vengono utilizzati gli antigeni **ESAT-6 e CFP-10, specifici per MTB ed assenti nel BCG**

Pai M. et al. *Lancet Infect Dis* 2004

### Tuberculosis complex

### Antigens

ESAT CFP

M tuberculosis

+ +

M africanum

+ +

M bovis

+ +

### BCG substrain

gothenburg

- -

moreau

- -

tice

- -

tokyo

- -

danish

- -

glaxo

- -

montreal

- -

pasteur

- -

- **QuantiFERON-TB Gold®**: misura la quantità di IFN- $\gamma$  prodotto, attraverso tecnica Elisa

- **T-SPOT.TB®**: misura il numero di linfociti T che producono IFN- $\gamma$ , attraverso tecnica Elispot

### Environmental strains

### Antigens

ESAT CFP

M abcessus

- -

M avium

- -

M branderi

- -

M celatum

- -

M chelonae

- -

M fortuitum

- -

M gordonii

- -

M intracellulare

- -

M kansasii

+ +

M malmoense

- -

M marinum

+ +

M oenavense

- -

M scrofulaceum

- -

M smegmatis

- -

M szulgai

+ +

M terrae

- -

M vaccae

- -

M xenopi

- -

# Test cutaneo con PPD

## falsi positivi

- legati a cross-reaione con altri micobatteri
- legati a vaccinazione con BCG
- effetto booster
- legati alla somministrazione
- legati alla lettura

## falsi negativi

### legati al soggetto testato

- infezioni
- disturbi metabolici
- fattori nutrizionali
- farmaci (corticosteroidi, agenti immunosoppressivi...)
- malattie degli organi linfoidi (HD, linfomi...)
- età (neonati, anziani)
- infezione di MTB recente
- stress (chirurgia, GVHD...)

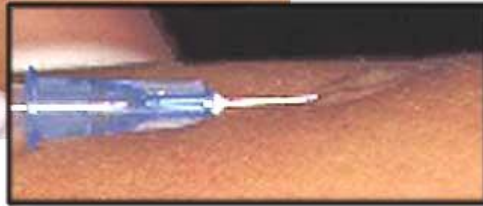
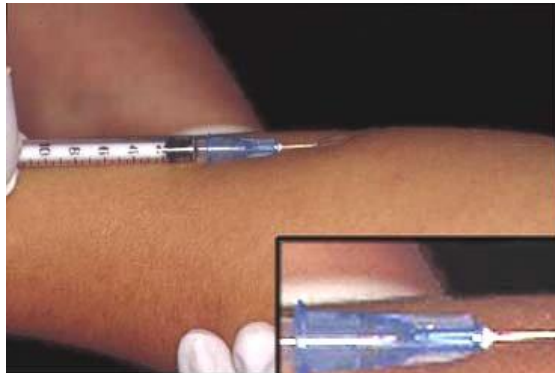
### legati alla somministrazione

- ritardo dopo la preparazione della siringa
- quantità insufficiente di antigene somministrato

### legati alla lettura

(6 mm variabilità tra lettori )

- inesperienza del lettore
- errori consci o inconsci



### legati alla tuberculina

- Improprio stoccaggio, diluizione...





**An IGRA may be used in place of (but not in addition to) a TST in all situations in which CDC recommends tuberculin skin testing as an aid in diagnosing *M. tuberculosis* infection, with preferences and special considerations noted below.**

### **Situations in which an IGRA is preferred but a TST is acceptable**

- Persons from groups that historically have low rates of returning to have TSTs read. (*i.e. homeless and drug-users*).
- Persons who have received BCG (*as a vaccine or for cancer therapy*).

*Use of IGRAs is expected to increase diagnostic specificity and improve acceptance of treatment for LTBI.*

### **Situations in which a TST is preferred but an IGRA is acceptable**

- A TST is preferred for testing children aged <5 years.

*Use of an IGRA in conjunction with TST has been advocated by some experts to increase diagnostic sensitivity in this age group.*

### **Situations in which Testing with both an IGRA and a TST may be considered**

Although routine testing with both a TST and an IGRA is not generally recommended, results from both tests might be useful when :

- **the initial test (TST or IGRA) is negative** but the risk for infection, the risk for progression, and the risk for a poor outcome are increased (e.g.: persons with HIV infection or children <5 years or *when clinical suspicion exists for active tuberculosis*).
- **the initial test is positive** and an additional evidence of infection is required to encourage compliance (e.g., *in foreign-born health-care workers who believe their positive TST result is attributable to BCG or in healthy persons who have a low risk for both infection and progression.*)
- **repeating an IGRA or performing a TST might be useful** (the initial IGRA result is indeterminate, borderline, or invalid and a reason for testing persists).

*Mazurek M et al.:MMWR Recomm Rep 2010; 59: 1–25.*

## IGRAs are not recommended for the diagnosis of active TB in adults.

IGRAs may be used as a supplementary diagnostic aid in combination with the TST and other investigations to help support a diagnosis of TB. ... *a negative test does not rule out active TB.*

IGRAs may be used as a confirmatory test for a positive TST in contacts (adult or child) who, are felt to have a low pretest probability of recently acquired LTBI and who have no other high risk factors for progression to active disease if infected. For close contacts or those contacts who have high or increased risk of progression to active disease if infected, a TST (or both TST and IGRA) should be used.

In an immunocompromised person (adult or child), the TST should be the initial test used to detect LTBI. If the TST is positive, the person should be considered to have LTBI.. However, in light of the known problem with false-negative TST results in immunocompromised populations, a clinician still concerned about the possibility of LTBI in an immuno-compromised person with a negative initial TST result may perform an IGRA test.

## To diagnose latent TB in:

*household contacts aged 5 years and older and non-household contacts of all people with active TB:*

- A Mantoux test should be performed. Those with positive results (or in whom Mantoux testing may be less reliable - for example, people who have had the BCG vaccination) should then be considered for IGT.
- If Mantoux testing is inconclusive, refer the person to a TB specialist.

**New entrants from high-incidence countries aged 5 - 15 years:**

- Offer a Mantoux test followed by IGT if positive.

**New entrants from high-incidence countries aged 16-34 years:**

- Offer either IGT alone or a dual strategy. For people aged 35 years or older, consider the individual risks and benefits of likely subsequent treatment before offering testing.

**New entrants from high-incidence countries aged under 5 years:**

- Use Mantoux as the initial test. If positive, taking into account BCG history, refer to a TB specialist to exclude active disease **and** consider treatment of latent TB.

**People who are immunocompromised:**

- If latent TB is suspected in children and young people who are immunocompromised, refer to a TB specialist.
- For people with HIV and CD4 counts of less than 200 cells/mm<sup>3</sup>, perform an IGT and a concurrent Mantoux test..
- For people with HIV and CD4 counts of 200-500 cells/mm<sup>3</sup>, perform an IGT alone or an IGT with concurrent Mantoux test. If either test is positive, assess for active TB **and** consider treating for latent TB.

NICE website at <http://guidance.nice.org.uk/CG117>

## Use of interferongamma release assays in support of tuberculosis diagnosis.

An expert scientific panel recently convened by the European Centers for Disease Prevention and Control concluded that IGRAs should not and cannot replace the existing standard diagnostic methods for the diagnosis of active tuberculosis .

The panel also emphasized that a negative IGRA result does not exclude active tuberculosis disease and in high-risk groups, a negative IGRA does not rule out *M. tuberculosis* infection.

The expert panel suggested that in order to identify individuals with latent *M. tuberculosis* infection for whom preventive treatment could be considered, IGRAs may be used only in conjunction with an overall risk assessment to provide supplementary information as part of a diagnostic workup.



*European Centre for Disease Prevention and Control. Use of interferon-gamma release assays in support of TB diagnosis. Stockholm: ECDC; 2011.*



# AGGIORNAMENTO DELLE RACCOMANDAZIONI PER LE ATTIVITÀ DI CONTROLLO DELLA TUBERCOLOSI

## “Gestione dei contatti e della tubercolosi in ambito assistenziale”

### Anno 2009

#### RACCOMANDAZIONI

**C 3.1** Come test di riferimento per la diagnosi di infezione tubercolare nei contatti va considerato attualmente il test tubercolinico con il metodo *Mantoux* (TST). Nello screening dei contatti, il test risulta positivo qualora si rilevi un infiltrato di diametro pari o superiore a 5mm a 48-72 ore dall'inoculazione. **A**

**C 3.2** Negli individui vaccinati con BCG, l'uso di test basati sul rilascio di interferon-gamma (IGRA) è raccomandato come test di conferma nei pazienti risultati positivi all'intradermoreazione. La negatività del test IGRA può essere considerata indicativa di assenza di infezione tubercolare anche in presenza di positività del TST. **A**

**C 3.3** Nelle persone con infezione da HIV+ e bassa conta dei linfociti CD4+ e negli altri pazienti con grave compromissione del sistema immunitario l'utilizzo dei test IGRA è raccomandato in tutti i soggetti TST negativi. **C**

**C 3.4** L'uso del test IGRA in alternativa al TST non è attualmente supportato dalle evidenze disponibili. **C**



Ministero del Lavoro, della Salute  
e delle Politiche Sociali



# Is It Time to Replace the Tuberculin Skin Test With a Blood Test?

*Discussed the reports and systematic reviews regarding the performance of interferon-gamma release assays (IGRAs) in comparison with the tuberculin skin test (TST) and highlight at least 2 important methodological limitations. In fact, the results of many of these studies are based on relatively small sample sizes with inadequate statistical power and there is not a sufficient gold standard .*

The authors noted that

“although no study or combination of studies has been definitive, the available data suggest IGRAs are at least as good as TST in predicting future incident TB and may be slightly better”

***This is consistent with the 2010 CDC guidelines: IGRAs can be used in the United States in place of the TST in all situations in which the TST is currently used***

## Different recommendations on the use of IGRAs with and without the TST

Surveyed the literature and contacted experts to identify 33 guidelines and position papers from 25 countries and two supranational organizations.

Considerable diversity in the recommendations on IGRAs,

Four approaches commonly proposed:

- Two-step approach of tuberculin skin test (TST) first, followed by IGRA either when the TST is negative (to increase sensitivity, mainly in immunocompromised individuals), or when the TST is positive (to increase specificity, mainly in bacillus Calmette–Gue rin-vaccinated individuals);
- Either TST or IGRA, but not both;
- IGRA and TST together (to increase sensitivity); and
- IGRA only, replacing the TST.

Denkinger CM et al.: *Clin Microbiol Infect.* 2011 Jun;17(6):806-14.

## Guidelines on interferon release assays (IGRAs): *recommendations for active tuberculosis*

Recommendation	Subgroup	Guideline or position statement <sup>a</sup>
For the use of IGRAs but only as an adjunct (some guidelines specify the use only when other diagnostic tests have been unrevealing)	In adults	ECDC, USA-CDC, UK, France (only for extrapulmonary TB), Australia, Slovakia, Japan, the Netherlands, Norway, Bulgaria, Portugal, Denmark, Austria
	In children	ECDC, Canada, USA (CDC and AAP), UK, Switzerland, Australia, Slovakia, Japan (children > 12 years of age), Saudi Arabia, the Netherlands, Norway, Bulgaria, Portugal, Croatia, Denmark, Austria
Against the use of IGRAs	In adults	WHO, Canada, Switzerland, Saudi Arabia, Croatia, Ireland, South Korea, Brazil
	In children	WHO, France, Ireland, South Korea, Brazil
No recommendations		Germany, Italy, Spain, Finland, Poland, Czech Republic

❑ ECDC: “ I test IGRAs non dovrebbero sostituire (*rimpiazzare*) I tradizionali metodi diagnostici considerati (*standard*) [...] per la diagnosi di TB attiva. [...], **basandosi su limitate evidenze, in determinate situazioni cliniche i test IGRA potrebbero fornire informazioni supplementari come parte di un iter diagnostico**“

❑ Alcune linee-guida (es. Canada) raccomandano in modo esplicito di non utilizzare I test IGRA nella diagnosi di TB attiva nei pazienti adulti, .. *ma... includono questi test in un algoritmo come esame diagnostici che forniscono evidenza di infezione tubercolare.*

❑ WHO raccomanda di non utilizzare I test IGRAs per la diagnosi di TB attiva nei paesi in via di sviluppo, in considerazione dell'alta prevalenza di LTBI.

# Guidelines on IGRAs:

## Recommendations for contact investigation in adults

Recommendation	Guideline or position statement <sup>a</sup>
TST alone	WHO, Brazil, ECDC (high-incidence countries)
TST followed by IGRA, if TST positive (either IGRA only in BCG-vaccinated persons or independent of BCG vaccine)	Canada (low-risk contacts), Germany, Italy, Switzerland, Spain, Saudi Arabia, the Netherlands, Norway, Bulgaria, Portugal, Ireland, ECDC (low-incidence countries), and for UK and South Korea only in adults <35 years old
Both TST and IGRA	Canada (high-risk contacts), Czech Republic, Croatia, Austria, Australia (IGRA may be considered in addition)
Either TST or IGRA	USA, Denmark, Finland (IGRA preferred if BCG-vaccinated in all three countries), South Korea (only in adults <35 years old), Austria
IGRA alone	Slovakia, Japan, France

BCG, bacille Calmette–Guérin; CDC, US Centers for Disease Control and Prevention; ECDC, European Centre for Disease Prevention and Control; IGRA, interferon-gamma release assay; TST, tuberculin skin test; WHO, World Health Organization.

<sup>a</sup>Some countries/organizations are listed more than once because their recommendations vary across risk groups.

- La maggior parte delle linee-guida raccomandano un approccio in due *step*. TST positivo seguito da test IGRA (*L'approccio con due step è utile principalmente per accrescere la specificità nei soggetti vaccinati con BCG*).

- Alcune Linee-guida ( es. Canada) raccomandano approccio basato sul grado di rischio del paziente.

- Altre Linee-Guida ( es. USA) raccomandano nelle screening dei contatti l'utilizzo sia dei test IGRA che TST e specificano determinati sottogruppi in cui i test dovrebbe essere preferibilmente usati..

- L'OMS raccomandano di non utilizzare i test IGRA nei paesi in via di sviluppo.

Denkinger CM et al.:

*Clin Microbiol Infect.* 2011 Jun;17(6):806-14.

# Guidelines on IGRAs:

## Recommendations for contact investigation in children

Recommendation	Guideline or position statement <sup>a</sup>
TST alone	WHO, ECDC, France, Brazil, Switzerland (IGRA in addition only in case of doubt), Slovakia (in BCG non-vaccinated children), South Korea (for children <5 years old)
TST alone (in children 0–4 years old); TST followed by IGRA, if TST positive (for children 5–17 years old)	Canada (low-risk contacts), Japan, Ireland, USA-AAP (for children >5 years old, IGRA may also replace TST)
TST followed by IGRA, if TST positive	Germany, Italy, Spain, Saudi Arabia, the Netherlands (dependent on BCG vaccination status and result of TST, only TST might be sufficient), Bulgaria, and for children >5 years of age only in Portugal and UK
TST followed by IGRA, if TST negative	Portugal (for children <5 years old), UK (for children 2–5 years old)
Either TST or IGRA	Denmark, USA-CDC (but TST is preferred in children <5 years old), South Korea (for children >5 years old, but TST preferred), Finland
Both TST and IGRA	Canada (high-risk contacts), Czech Republic, Croatia, Australia (IGRA may be considered in addition for children >2 years old)
IGRA alone	Norway

AAP, American Academy of Pediatrics; BCG, bacille Calmette–Guérin; CDC, US Centers for Disease Control and Prevention; ECDC, European Centre for Disease Prevention and Control; IGRA, Interferon-gamma release assay; TST, tuberculin skin test; WHO, World Health Organization.

<sup>a</sup>Some countries/organizations are listed more than once because their recommendations vary across risk groups.

- LG molto eterogenee che riflettono l'incertezza delle evidenze su cui sono basate.
- **Molte LLGG continuano a preferire l'utilizzo del solo TST per tutti non solo per quelli < 5 anni.**
- Altre LLGG raccomandano l'utilizzo di TST in combinazione con IGRA se TST positivo, specialmente nei vaccinati con BCG.
- Pochi paesi raccomandano di utilizzare insieme i due test (*es. Canada- per contatti ad alto rischio*), oppure ... suggeriscono l'utilizzo di un test IGRA se TST negativo in determinati gruppi di età per aumentare la sensibilità.

## Guidelines on IGRAs: recommendations for screening of immigrants

Recommendation	Guideline or position statement <sup>a</sup>
TST followed by IGRA, if TST positive	UK (for children age 5–15 years), Italy, Switzerland, Spain, Norway, Ireland, Bulgaria, France (in children), Slovakia, the Netherlands (for children only; dependent on BCG vaccination status and result of TST, only TST might be sufficient)
Both TST and IGRA	Czech Republic, UK (for adults age 16–35 years; or IGRA alone alternatively)
Either TST or IGRA	USA (IGRA preferred in BCG-vaccinated persons), Canada, Australia
IGRA alone	France (in adults)
No recommendations/ not recommended	Germany, Japan, Saudi Arabia, Brazil, Portugal, Croatia, Denmark, South Korea, Finland, Poland, Austria

BCG, bacille Calmette–Guérin; CDC, US Centers for Disease Control and Prevention; ECDC, European Centre for Disease Prevention and Control; IGRA, interferon-gamma release assay; TST, tuberculin skin test; WHO, World Health Organization.

<sup>a</sup>Some countries/organizations are listed more than once because their recommendations vary across risk groups.

- Le maggior parte delle LLGG che prevedono raccomandazioni per lo screening dei soggetti immigrati sono prodotte da paesi con bassa incidenza di TB e sono focalizzate sugli immigrati provenienti da aree ad alta incidenza di TB:

- Altre LLGG prevedono raccomandazioni per lo screening in immigrati che hanno più probabilità di sviluppare una TB attiva (*es. bambini o soggetti con comorbidità che predispongono a una riattivazione di LTBI*) indipendentemente dal paese di origine..

- Tutte le LLGG per lo screening degli immigrati includono l'utilizzo di test IGRAs.

- L'algoritmo diagnostico più utilizzato prevede TST seguito da IGRA in caso di positività.  
*Per aumentare la sensibilità dato l'utilizzo esteso di BCG nelle aree endemiche per TB..*

Denkinger CM et al.:  
*Clin Microbiol Infect.* 2011 Jun;17(6):806-14.



## Guidelines on IGRAs: recommendations for HIV infected populations

Recommendation	Guideline or position statement <sup>a</sup>
TST alone	WHO, Brazil
TST followed by IGRA, if TST positive (and BCG-vaccinated)	Spain
TST followed by IGRA, if TST negative	Canada, Italy, Saudi Arabia, Spain, Ireland
Either TST or IGRA	Denmark, South Korea, Austria
Both TST and IGRA	ECDC, Portugal, Croatia, Slovakia, the Netherlands, USA (if either initial test negative), South Korea, UK
IGRA alone	Switzerland, Bulgaria, France, UK (if CD4 200–500)
No specific recommendations	Germany, Czech Republic, Norway, Japan, Finland, Australia

AAP, American Academy of Pediatrics; BCG, bacille Calmette–Guérin; CDC, US Centers for Disease Control and Prevention; ECDC, European Centre for Disease Prevention and Control; IGRA, interferon-gamma release assay; TST, tuberculin skin test; WHO, World Health Organization.

<sup>a</sup>Some countries/organizations are listed more than once because their recommendations vary across risk groups.

- Evidenze poco chiare si riflettono nell'ampia disponibilità di differenti raccomandazioni.
- **Le LLG di paesi in via di sviluppo, Brasile e OMS, raccomandano l'utilizzo di TST.**
- Altre LLG e *position papers* mostrano una chiara tendenza verso un maggior uso dei test IGRAs.
- Mentre alcuni paesi raccomandano l'utilizzo del solo test IGRA, altre organizzazioni nazionali (es. ECDC, USA, and UK per CD4 <200) suggeriscono l'uso contemporaneo di entrambi i test (direttamente o se il test inizialmente scelto è negativo) per accrescere la sensibilità dello screening..
- Altri ancora suggeriscono un approccio in due *step* con TST negativo seguito da test IGRA, sempre con l'obiettivo di accrescere la sensibilità..

Denkinger CM et al.: *Clin Microbiol Infect.* 2011 Jun;17(6):806-14.



# Guidelines on IGRAs: recommendations for LTBI screening in persons on TNF- $\alpha$ inhibitors

Recommendation	Guideline or position statement <sup>a</sup>
TST alone	Brazil
TST followed by IGRA, if TST positive	Spain, Norway
TST followed by IGRA, if TST negative	Canada, Italy, Spain, Saudi Arabia
Either TST or IGRA	Australia-ARA, Denmark (IGRA favoured), South Korea
Both TST and IGRA	ECDC, UK (alternatively IGRA alone), USA (if either initial test negative), Portugal, Croatia, Czech Republic, Slovakia, the Netherlands, South Korea, Ireland (TST preferred)
IGRA alone	Germany, Switzerland, Bulgaria, Japan, France, Poland, Austria
No recommendations	Finland, Australia-NTAC

ARA, Australia Rheumatology Association; CDC, US Centers for Disease Control and Prevention; ECDC, European Centre for Disease Prevention and Control; IGRA, interferon-gamma release assay; NTAC, National Tuberculosis Advisory Committee, Australia; TST, tuberculin skin test; WHO, World Health Organization.

<sup>a</sup>Some countries/organizations are listed more than once because their recommendations vary across risk groups.

- Come per HIV, le LLGG per lo screening di LTBI nei pazienti in trattamento con anti-TNF $\alpha$  riflettono la mancanza di dati definitivi e di conseguenza sono raccomandate differenti strategie.

- Tendenza verso un maggior utilizzo dei test IGRAS.

- In pochi paesi c'è indicazione ad un utilizzo del solo test iGRA

- La maggior parte delle LLGG prevede un suo combinato dei due test in ordine ad accrescere la sensibilità (es. ECDC, USA, UK) sia direttamente oppure se il test scelto inizialmente è negativo.

- Alternativamente, è raccomandato un approccio in due *step* con IGRA che segue TST, se questo è negativo.

*Per aumentare la sensibilità, limitando i costi (es. Canada).*

# Guidelines on IGRAs: recommendations for serial testing of healthcare workers

Recommendation	Guideline or position statement <sup>a</sup>
TST alone	Brazil, South Korea, Canada, Saudi Arabia, Ireland, Austria
TST followed by IGRA, if TST positive	Spain, the Netherlands, Bulgaria
Either TST or IGRA	USA, Switzerland, Italy (in BCG-vaccinated IGRA preferred)
IGRA alone	Slovakia, Japan, Switzerland, the Netherlands, Portugal, France
No recommendations/ not recommended	Australia, Czech Republic, Norway, Croatia, Denmark, Germany, UK, Finland, ECDC

BCG, bacille Calmette–Guérin; CDC, US Centers for Disease Control and Prevention; ECDC, European Centre for Disease Prevention and Control; IGRA, interferon-gamma release assay; TST, tuberculin skin test; WHO, World Health Organization.

<sup>a</sup>Some countries/organizations are listed more than once because their recommendations vary across risk groups.

- L'incertezza dei dati disponibili si riflette nelle LLGG prodotte
- **La maggior parte delle LLGG e PP non prevedono raccomandazioni per osservazioni seriali negli operatori sanitari..**
- Alcuni paesi suggeriscono l'utilizzo del test IGRA da solo o come alternativa al TST per i controlli seriali.
- **Alcune LLGG commentano in modo specifico riguardo le limitazioni dell'utilizzo dei test iGRAs per controlli seriali.**
- **LG Canada riportano che “ ci sono insufficienze evidenze disponibili per raccomandare un utilizzo seriale dei test IGRA negli operatori sanitari, reclusi ed addetti alle prigioni”.**
- **“CDC riportano che “ i criteri di un test IGRA per identificare una nuova infezione , rimangono incerti.”.**
- Diversi paesi sono attualmente è a favore del solo TST o di TST seguito da un secondo step con IGRA, se TST positivo.

Denkinger CM et al.: *Clin Microbiol Infect.* 2011 Jun;17(6):806-14.

# Comparative accuracy: assessing new tests against existing diagnostic pathways

Patrick M Bossuyt, Les Irwig, Jonathan Craig and Paul Glasziou

*BMJ* 2006;332;1089-1092  
doi:10.1136/bmj.332.7549.1089

## Features

Accuracy

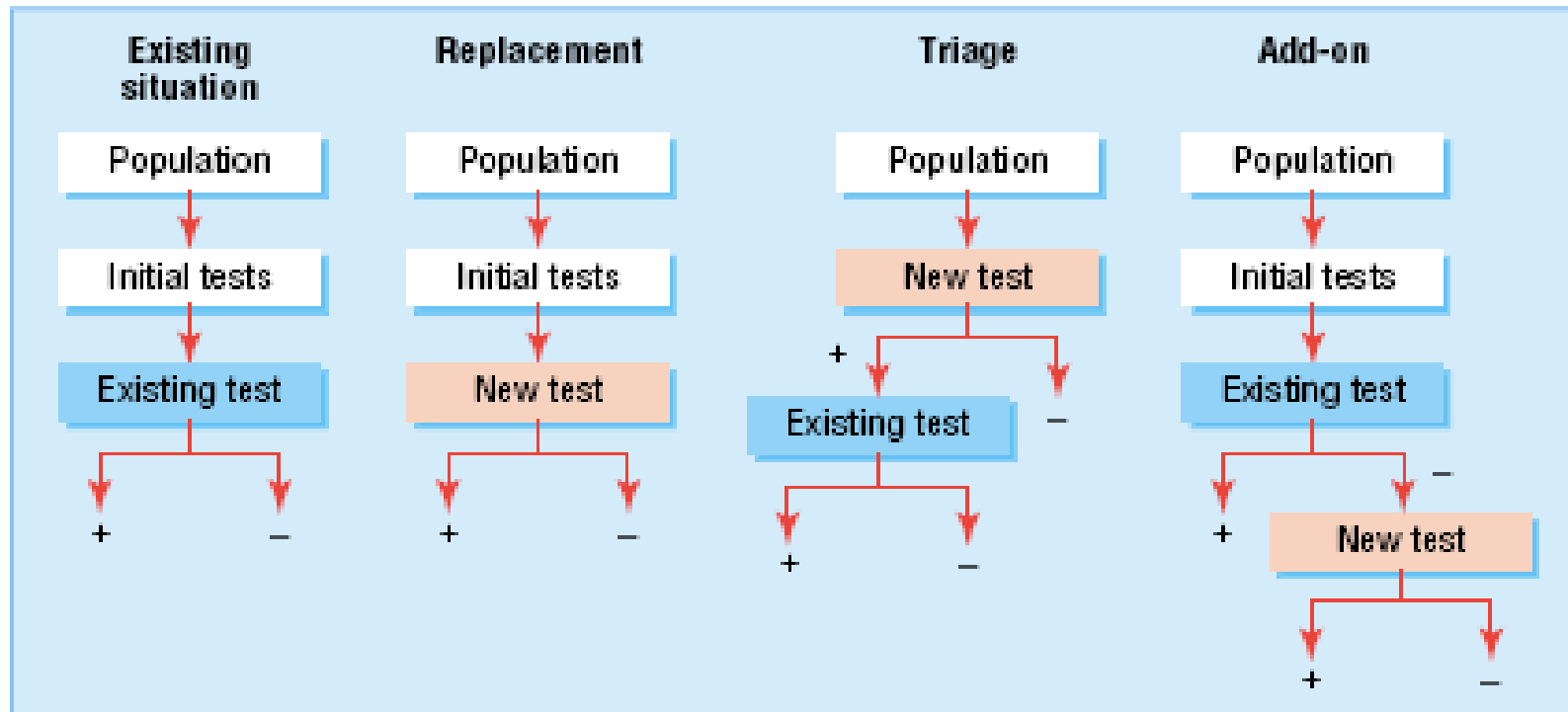
Invasiveness

Waiting time

Knowledge and skills  
needed

Interpretable

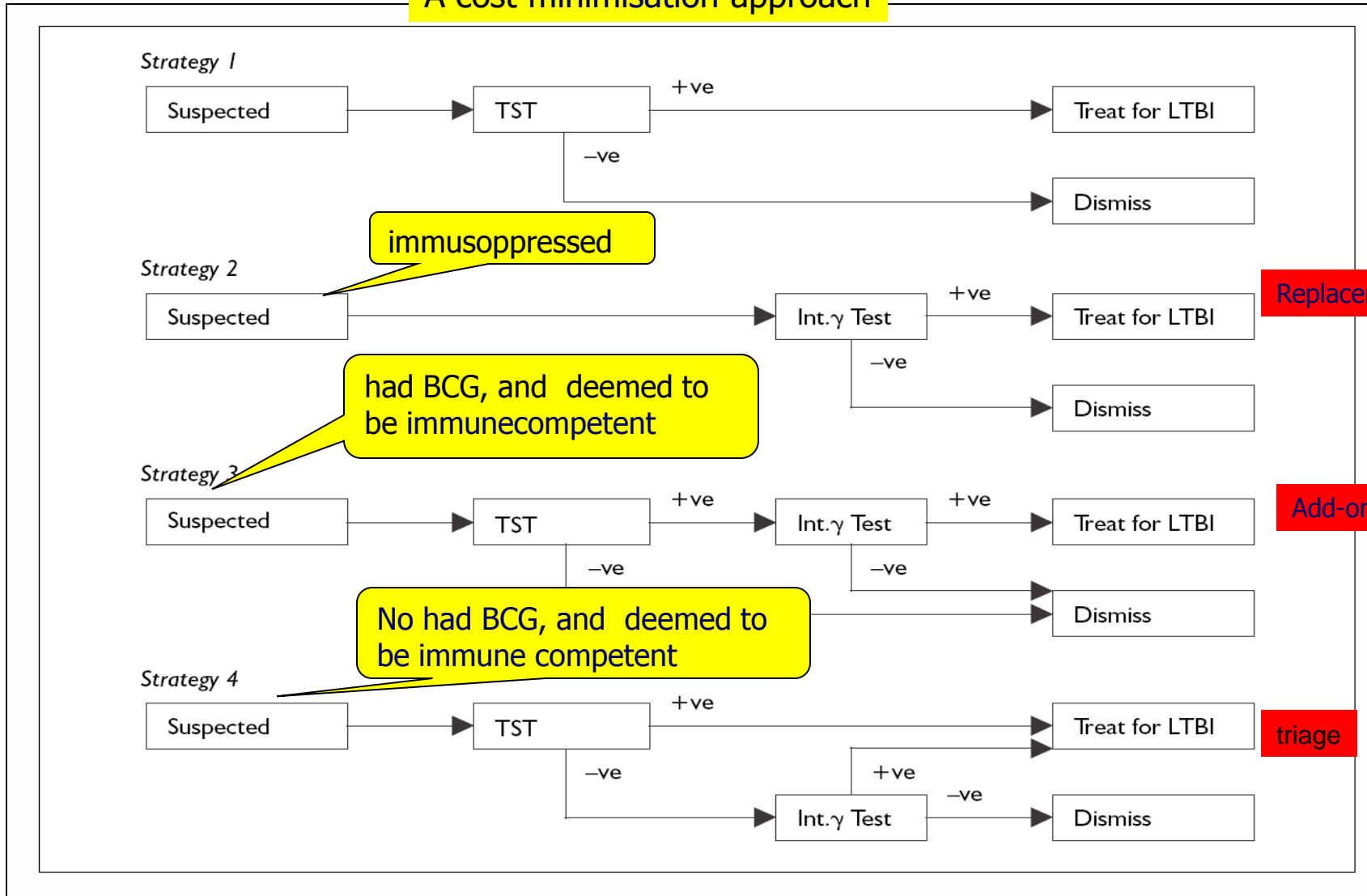
Cost



Roles of tests and positions in existing diagnostic pathways

# Economic aspects to the introduction of new tests for tuberculosis: Alternative strategies for screening for LTBI

## A cost minimisation approach



Dinnes J *et al.*. *Health Technol Assess* 2007;11(3).

## Interferon--Release Assays for Latent Tuberculosis

### To the Editor:

*The Viewpoint by Drs LoBue and Castro regarding latent tuberculosis infection and the use of interferon--release assays (IGRAs) does not discuss another potential advantage of these blood tests: to reduce the possibility of false-negative or uninterpretable nonreactive purified protein derivative (PPD) tests due to anergy*

*I believe that...the IGRAs may find a niche in this area, in which a lone negative PPD test, lacking specificity, may not provide the necessary information.*

*Elliott C. Rosch, Mt Sinai-Riverside Medical Group, Yonkers, New York*

### In Reply:

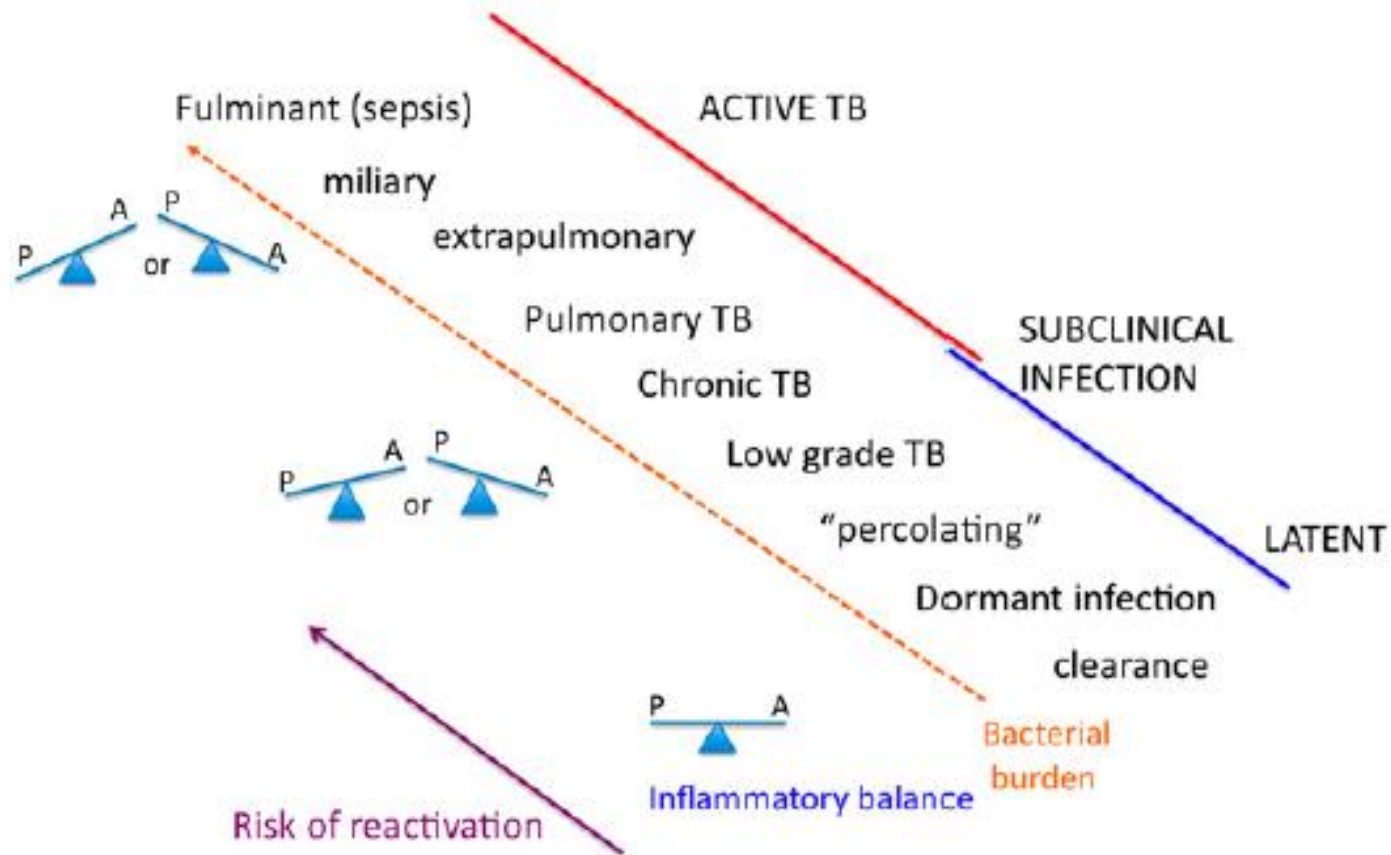
... at the present time there is no evidence to suggest that this is the case.

A review of 14 studies found that 19% of patients with tuberculosis had valid negative IGRA test results ....

It is, however, possible that knowing about an individual patient's ability to respond to nonspecific immune stimuli may be useful in making diagnostic and treatment decisions when used in conjunction with all other clinical and testing information

*Philip A. LoBue, MD and Kenneth G. Castro, MD Division of Tuberculosis Elimination, CDC Atlanta*

# SPECTRUM of M. TUBERCULOSIS INFECTION OUTCOMES



## Understanding Latent Tuberculosis: A Moving Target

Philana Ling Lin and JoAnne L. Flynn

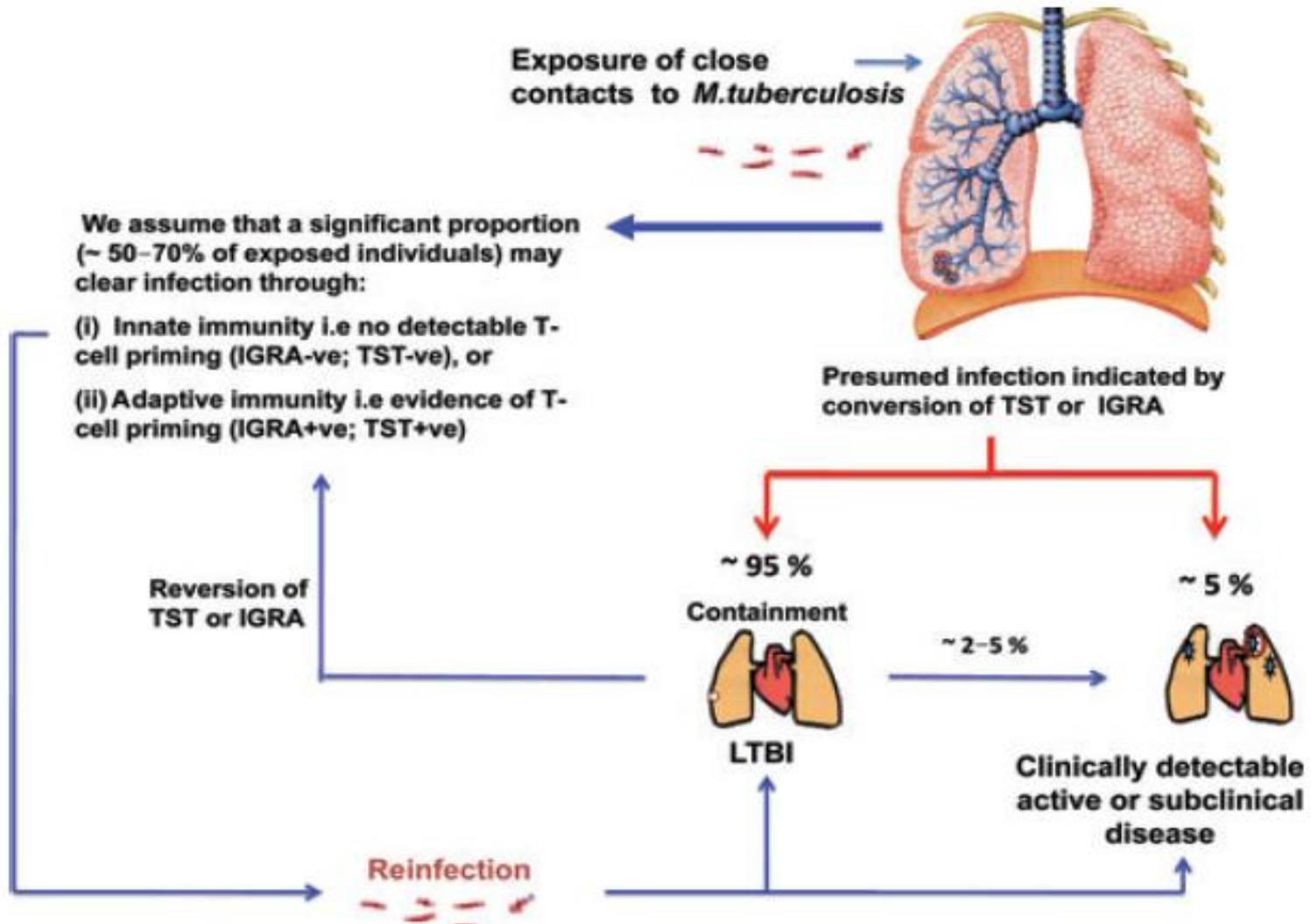
*J. Immunol.* 2010;185;15-22

doi:10.4049/jimmunol.0903856

<http://www.jimmunol.org/cgi/content/full/185/1/15>

# The immunology of tuberculosis: From bench to bedside

KEERTAN DHEDA,<sup>1,2,3</sup> STEPHAN K. SCHWANDER,<sup>4</sup> BINGDONG ZHU,<sup>5</sup> RICHARD N. VAN ZYL-SMIT<sup>1</sup> AND YING ZHANG<sup>6</sup>





## No sufficient gold standard for diagnosing LTBI

Latency, as assayed by the tuberculin skin test and IGRA, is:  
A state of persistent mycobacteria-specific T-cell responses, in the absence of clinical evidence for tuberculosis.

*The diagnostic tests used to identify individuals latently infected with M. tuberculosis (TST and IGRAs) are designed to identify an adaptive immune response against, but not necessarily a latent infection with M. tuberculosis.:*

- *LTBI depends on the presence of living mycobacteria is controversially discussed ;*
- *the proportion of individuals who truly remain infected with M. tuberculosis after TST or IGRA conversion is unknown.;*
- *it is also uncertain how long adaptive immune responses towards mycobacterial antigens persist in the absence of live mycobacteria.*

No sufficient gold standard for diagnosing LTBI, only considering the percentage of persons exposed who become positive to the tests, because sensitivity was usually measured using persons with TB disease.

It is unknown what proportion of those with latent infection of M. tuberculosis will not develop tuberculosis because either their immune system persistently controls dormant living mycobacteria or because they are no longer infected with living bacteria.

Mack U, Migliori GB et al.:  
Latent tuberculosis infection or lasting immune responses to M. tuberculosis? A TBNET consensus statement  
*Eur Respir J* 2009; 33: 956–97.

## How do we define the cut-off points for IGRAs and the TST?

- The sensitivity and specificity, and consequently the positive and negative predictive values of these tests are influenced by the cut-off point used.
- We have decades of history immersed in the cut-off points used for the TST but we have less “access” to the cut-off equivalents with the two new tests.  
*A lower cut-off point will result in a higher sensitivity and a lower specificity for M. tuberculosis infection.*

□ The positive predictive value of IGRAs for the development of active tuberculosis is likely to be equal or higher than that of the TST test for immunocompetent individuals.

□ The negative predictive value of IGRA for active tuberculosis is very high in immunocompetent hosts if combined with the TST .

## Diagnostic Accuracy of TST and IGRA: sensitivity and specificity

Test		Sensitivity for active TB	Specificity for TB infection	Specificity for active TB
		Percentage		
TST	Pai et al, 2008	77	59/97	
	Sester et Sotgiu et al, 2010	65		75
QFT-IT	Pai et al, 2008	70	96	-
	Sester et Sotgiu et al, 2010	80	-	79
T-SPOT.TB	Pai et al, 2008	90	93	-
	Sester et Sotgiu et al, 2010	81	-	59

Interferon- $\gamma$  release assays for the diagnosis of active tuberculosis: a systematic review and meta-analysis

M. Sester\*, G. Sotgiu\*, C. Lange, C. Giehl, E. Girardi, G.B. Migliori, A. Bossink, K. Dheda, R. Diel, J. Dominguez, M. Lipman, J. Nemeth, P. Ravn, S. Winkler, E. Hultic, A. Sandgren and D. Manissero

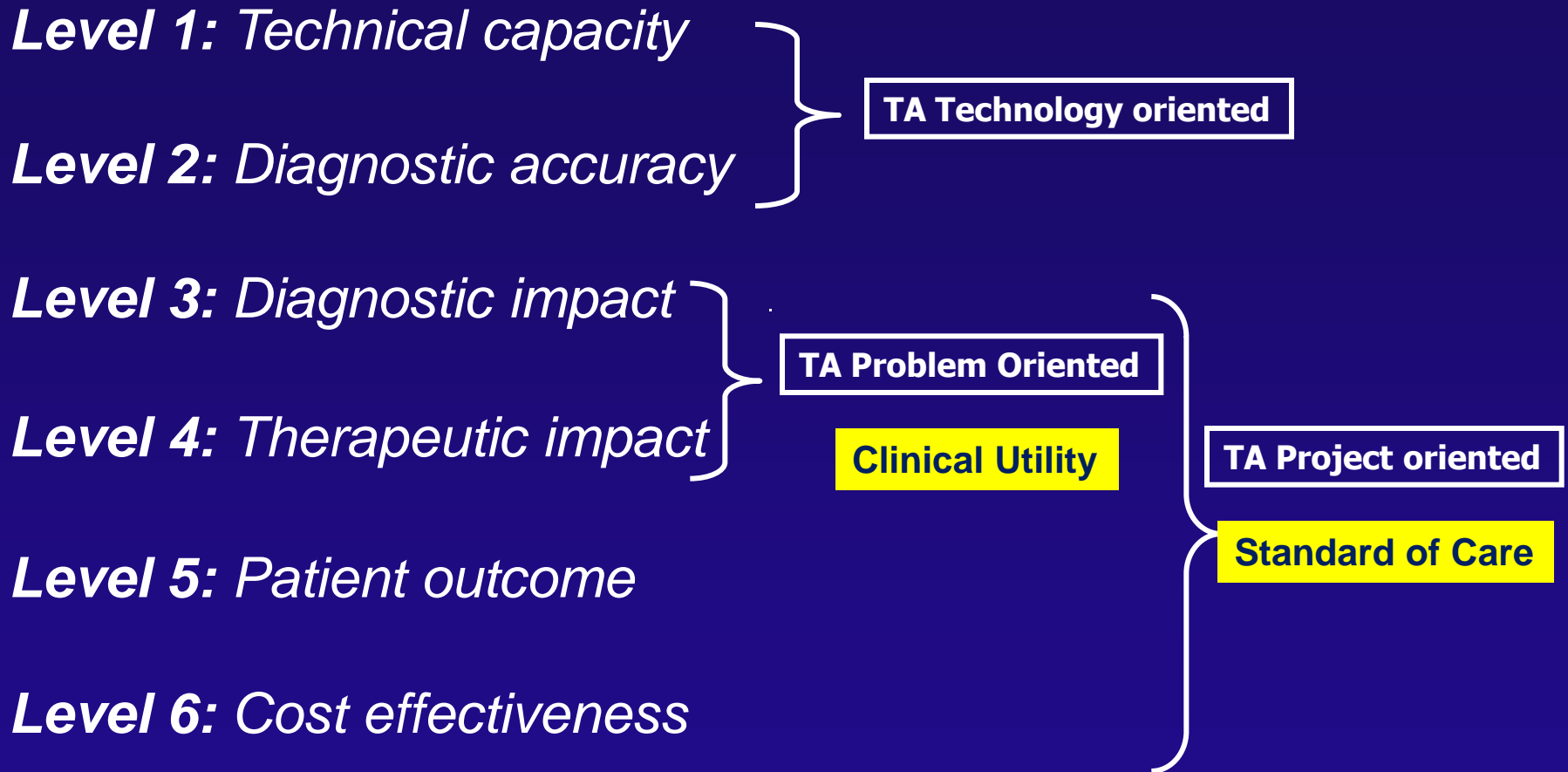
Eur Respir J 2011; 37: 100–111

**Systematic Review: T-Cell–based Assays for the Diagnosis of Latent Tuberculosis Infection: An Update**

Madhukar Pai, MD, PhD, Alice Zwerling, MSc, and Dick Menzies, MD, MSc  
McGill University and Montreal Chest Institute, Montreal, Quebec, Canada

*Ann Intern Med.* 2008 August 5; 149(3): 177–184.

# Hierarchical Model of Efficacy for Diagnostic Tests



National Information Center on Health Services Research and Health Care Technology (NICHSR)- OTA

## Clinical Application and Limitations of IGRAs ...

### ***Reproducibility of Results After Serial Testing***

Studies examining the reproducibility of IGRAs after serial testing have found variability in IFN- $\gamma$  responses. **Both conversions in the apparent absence of TB exposure and reversions in the absence of therapy have been observed, with frequencies of 12%–50%**

Pai M et al.: *Int J Tuberc Lung Dis* 2009; 13:84–92.

Ewer K et al. : *Am J Respir Crit Care Med* 2006;174:831–9.

Conversions and reversions are thought to be secondary to within-subject fluctuations and/or attributable to variations in laboratory procedures, but biological and environmental causes remain possible .

Veerapathran A, et al.: *PLoS One* 2008; 3:e1850.

Herrera V. et al.: *J Clin Microbiol* 2010; 48:2672–6.

To date, there is no consensus on how to interpret conversions and reversions in terms of their accuracy. The main challenge is to differentiate nonspecific variations from true conversions. **For this reason, a grey zone has been proposed for individuals with fluctuating QFT-GIT results close to the cutoff value of .35 IU/mL . Results outside this zone are presumed to be true reversions or conversions. A borderline category already exists for T-SPOT.**

Pai M et al: *Am J Respir Crit Care Med* 2006; 174:349–55.

**Table 1. Interpretation criteria for the QuantiFERON-TB Gold In-Tube assay (QFT-GIT)<sup>20</sup>**

Result	IFN- $\gamma$ concentration (International Units per ml, IU/ml)		
	<i>M. tuberculosis</i> antigens	Nil	PHA nil
Positive	$\geq 0.35$ IU/ml and $\geq 25\%$ over nil	$\leq 8.0$ IU/ml	Any
Negative	$< 0.35$ IU/ml or $< 25\%$ over nil	$\leq 8.0$ IU/ml	$\geq 0.5$ IU/ml
Indeterminate	$< 0.35$ IU/ml or $< 25\%$ over nil	$\leq 8.0$ IU/ml	$< 0.5$ IU/ml
	Any	$> 8.0$ IU/ml	Any

*M. tuberculosis* antigens: mixture of peptides representing the entire amino acid sequences of ESAT-6 and CFP-10, and partially TB7.7; negative control (i.e. nil), positive control (phytohemagglutinin A, PHA).

**Table 2. Interpretation criteria for the T-SPOT.TB assay<sup>21</sup>**

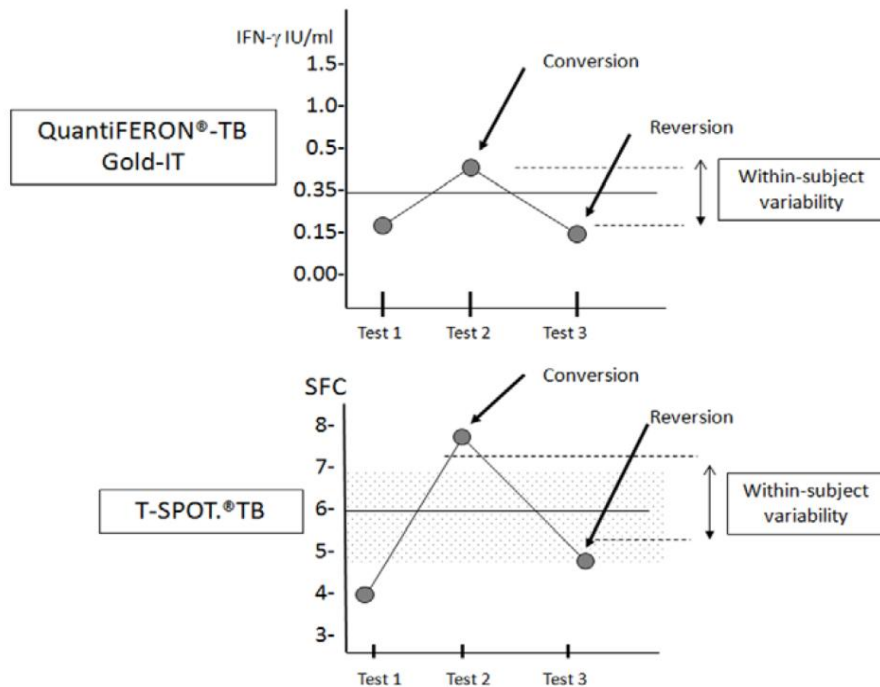
Result	Spot count				
	<i>M. tuberculosis</i> antigens			Nil	PHA
	ESAT-6		CFP-10		
Positive	$\geq 6$ over nil	and/or	$\geq 6$ over nil	$\leq 10$	Any
Negative	$\leq 5$ over nil	and/or	$\leq 5$ over nil	$\leq 10$	$\geq 20$
Borderline*	If for any antigen highest is 5 - 7 over nil			$< 10$	$\geq 20$
Indeterminate	$\leq 6$ over nil	and	$\leq 6$ over nil	$\leq 10$	$< 20$
	Any		Any	$> 10$	Any

\* Retesting of patient is recommended

# Within-Subject Variability of Interferon-gamma Assay Results for Tuberculosis and Boosting Effect of Tuberculin Skin Testing: A Systematic Review.

## Objectives of the Review

- 1) What is the within-person reproducibility (i.e. variability) of T cell responses over time?
- 2) What is the effect of a TST on subsequent IGRA results ?



**Figure 2. Schematic of the concept of "conversion and reversion" and "within-subject variability".** The conversion and reversion points depicted are based on the manufacturer's definitions with a hypothetical within-subject variability or borderline/grey zone indicated. The shaded area for the T-SPOT.TB diagram is the FDA defined grey zone.

- 4 studies :within-subject variability;
- 13 studies :TST effects on subsequent IGRA .

Based on limited data, **within-subject variability was present in all studies but the magnitude varied (16-80%).** TST induced "boosting" of IGRA responses in several studies ,**more pronounced in IGRA-positive** , also in a smaller but not insignificant proportion of IGRA-negative subjects.

TST appeared to affect IGRA only after 3 days and may apparently persist for several months, but evidence for this is **weak.**

van Zyl-Smit RN et al.: *PLoS ONE* 2009; 4(12): e8517



## Clinical Application and Limitations of IGRAs ...

### ***Prognostic Value for Progression to Active TB***

The risk of developing active TB after a positive TST result has been defined in large longitudinal studies . **It is known that only a small proportion of infected individuals develop active TB; therefore, only this subset would theoretically benefit from receiving prophylaxis .**

Horsburgh CR Jr. *N Engl J Med* 2004; 350:2060–7+115.

IGRAs could provide prognostic insight in identifying cases that are most likely to progress to active disease. Thus far, **the quantitative results from IGRAs have not been shown to have prognostic value and, therefore, should not be used for that purpose in clinical practice.**

Andersen P et al.: *Trends Mol Med* 2007; 13:175–82

It is possible that a unique signature of differential cell surface markers and secreted cytokines in functional T cell assays could identify a protective immunophenotype and be of prognostic value for clinical decision making

Pai M. : *Nat Rev Microbiol* 2010; 8:242

# Could IGRAs have a sufficiently high accuracy for predicting active TB?

Reported a pooled specificity of 99% among non-BCG vaccinated and 96% among BCG-vaccinated low-risk groups

Pai M et al.: *Ann Intern Med* 2008; 149:177–184.

Assessed the diagnostic accuracy (21% of controls showed test results above 0.35 IU/mL) of the latest generation IGRA in low-incidence areas in Germany

Vesenbeckh SM et al. *Tuberc Res Treat.* 2012;2012:768 -723.

Not restricting studies on specificity to low-risk groups (a situation that is closer to the clinical setting), the specificity of QFT-GIT was only 0.79 (95% CI 0.75–0.82). This finding is also consistent with the expected number of false positives assuming a specificity of around 0.8 .

Sester M, et al.. *Eur Respir J.* 2011;37(1):100-11.

Sensitivity in the above- reported studies was also found to be highly dependent on the study population, notably local TB prevalence, and ranged from 0.58 in a high-prevalence country to 1.00 in a low-prevalence country .

Baba K, et al.: *BMC Infect Dis.* 2008 Mar 14;8:35.

Sauzullo I, et al. : *PLoS One.* 2009;4(4):e5187.

**Systematic review and meta-analysis to evaluate the predictive value of IGRA for incident-active TB. Neither IGRAs nor the TST have a sufficiently high accuracy for predicting active TB, although use of IGRAs in some populations might reduce the number of people considered for preventive treatment.**

Rangaka MX, Wilkinson KA, Glynn JR : *Lancet Infect Dis.* 2012;12(1):45-55.

## Correlation between tuberculin skin test and IGRAs with risk factors for the spread of infection in close contacts with sputum smear positive in pulmonary tuberculosis

**Table 5 Risk factors associated with the positivity of the TST, QFN-G-IT and T-SPOT.TB in adults**

Risk factors	TST			QFN-G-IT			T-SPOT.TB		
	n (%)		p	n (%)		p	n (%)		p
	Positive	Negative		Positive	Negative		Positive	Negative	
<b>Cough</b>									
Yes	34 (94.4)	2 (5.6)	0.929	22 (61.1)	14 (38.9)	0.001	25 (69.5)	11 (30.5)	0.007
Not	31 (93.9)	2 (6.1)		7 (21.2)	26 (78.8)		12 (36.4)	21 (63.6)	
<b>Diagnostic delay</b>									
<30	35 (97.2)	1 (2.8)	0.244	11 (30.5)	25 (69.5)	0.565	14 (38.9)	22 (61.1)	0.077
30-59	13 (76.5)	4 (23.5)		7 (41.2)	10 (58.8)		7 (41.2)	10 (58.8)	
60-89	17 (85)	3 (15)		7 (35)	13 (65)		9 (45)	11 (55)	
>89	25 (100)	0 (0)		12 (48)	13 (52)		18 (72)	7 (28)	
<b>Room's size (m<sup>2</sup>)</b>									
≤50	2 (66.7)	1 (33.3)	0.462	1 (33.3)	2 (66.7)	0.020	2 (66.7)	1 (33.3)	0.023
51-79	15 (78.9)	4 (21.1)		4 (21.1)	15 (78.9)		6 (31.6)	13 (68.4)	
≥80	19 (90.5)	2 (9.5)		14 (66.7)	7 (33.3)		16 (76.2)	5 (23.8)	
<b>Overcrowding</b>									
≤15	7 (87.5)	1 (12.5)	0.800	2 (28.6)	5 (71.4)	0.480	5 (62.5)	3 (37.5)	0.820
>15	26 (83.9)	5 (16.1)		16 (51.6)	15 (48.4)		18 (58.1)	13 (41.9)	

TST: tuberculin skin test. QFN-G-IT: Quantiferon-TB Gold in tube.

Diagnostic delay: days between first symptoms and TB diagnostic. Overcrowding: floor area index per person (square meters per person).

Two-tailed statistical test was used to compare the qualitative results of TST, QFN-G-IT and T-SPOT.TB, respectively, between the different categories of the risk factors studied.

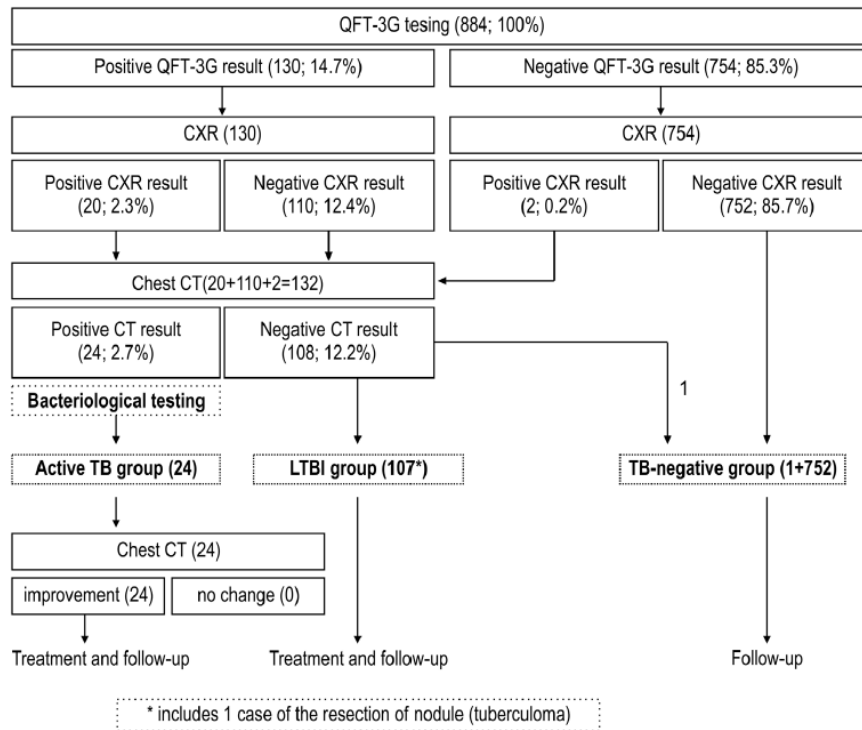
The agreement between IGRA tests and TST was slight (especially in BCG-vaccinated), while the agreement between both IGRA tests was substantial and similar in BCG-unvaccinated and BCG-vaccinated contacts

**Both IGRAs were associated with certain host-related risk factors involved in the transmission of disease, such as the presence of cough**

# Tuberculosis Contact Investigation Using Interferon-Gamma Release Assay with Chest X-Ray and Computed Tomography

Akira Fujikawa<sup>1,2\*</sup>, Tatsuya Fujii<sup>3\*</sup>, Satoshi Mimura<sup>4</sup>, Ryota Takahashi<sup>5</sup>, Masao Sakai<sup>4</sup>, Shinya Suzuki<sup>1,4</sup>,

Screening TB with IGRA, Chest X-Ray & CT



Contact investigation of all members who had come into contact with the infected members of the Japanese Eastern Army,  
 - 884 subjects underwent interferongamma release assay (IGRA) and chest X-ray.

- 132 subjects IGRA positive or with X-ray findings suggestive of TB and subsequently chest computer tomography (CT)  
 24 (2.7%) active TB,  
 107 (12.1%) LTBI and  
 753 (85.2%) non-TB group.

All 3 groups were followed for 2 years after treatment.  
 No patient in the LTBI or non-TB groups developed TB.

**Figure 1. Contact investigation protocol for tuberculosis.** The subjects were stratified into 3 groups according to the results of QFT-3G, chest X-ray, and chest CT.

## LTBI in Individuals with HIV Infection

- Data on the use of IGRAs in people living with HIV were summarized in a recent systematic review.
- The sensitivity estimate in HIV-infected patients with culture confirmed TB was higher for T-SPOT.TB (72%) than for QFT-GIT (61%), but not consistently more sensitive than the TST in head-to-head comparisons.
- The agreement between the two IGRA and TST was higher in the high-income countries where BCG-vaccination was used less frequently.
- Some evidence suggests that IGRAs, and especially the T-SPOT.TB assay, are less affected by HIV-related immunosuppression than the TST, but the differences between the tests were small.
- Overall, the data thus far indicates that IGRAs perform similarly to the TST in identifying HIV-infected individuals with LTBI.

Cattamanichi A. et al.: *J Acquir Immune Defic Syndr* 2011;56:230–238

## LTBI in persons on TNF- $\alpha$ inhibitors

- Only **few and very heterogeneous studies** have evaluated the performance of IGRAs in screening for LTBI in patients with IMIDs.

Two recent reviews have synthesized the data. The differences in the studies relate to::

- the level of immunosuppression
- the types of TNF $\alpha$ -inhibitors used
- the IMIDs treated
- the tests that were evaluated
- the rate of BCG-vaccination in the population.
- In addition, the lack of any data on predictive value of IGRA limited the studies.

The authors concluded that **the current evidence does not suggest superiority of IGRAs over the TST in identifying latent TB in individuals with IMIDs.**

Winthrop KL : *Int J Adv Rheumatol* 2010;**8**(2):43–52.  
Smith R. et al.: *Current Opinion in Rheumatology* 2011,23:377–384

## Clinical Application and Limitations of IGRAs ...

### ***Efficacy in Children***

In a study of 204 children, 81% of 99 TST-positive (induration, >10 mm) children considered to be at low to moderate risk of TB were QFT-GIT negative, as were 100% of 5 close contacts of a patient with TB .

Lighter J et al.: *Pediatrics* 2009; 123:30–7.

Outside the United States, sensitivities of QFT and T-SPOT in children are generally reported to be similar to or lower than that of TST .

Connell TG et al.: *PloS one* 2008; 3:e2624.

Bamford AR, et al.: *Arch Dis Child* 2010; 95:180–6.

Adetifa IM, et al.: *Pediatr Infect Dis JPediatr* 2010; 29:439–43.

Reports of lower mitogen levels in children and higher rates of indeterminate results are consistent with the concern that age-related immunologic factors may affect the sensitivity of these tests .

Lighter J et al.: *Pediatrics* 2009; 123:30–7

Kampmann B et al. : *Eur Respir J* 2009; 33:1374–82.

Lewinsohn DA et al.: *Curr Opin Pediatr* 2010; 22:71–6.

**Because young children are at greater risk of disseminated disease , a negative IGRA result should not be used to exclude infection.**

Newton SM et al. : *Lancet Infect Dis* 2008; 8:498–510.



## Clinical Application and Limitations of IGRAs ...

### **Conversions and Reversions**

In the event that a test is performed for an individual at low risk of LTBI, conversion from a negative to a positive result may represent a false conversion. A reasonable approach is to withhold treatment and repeat testing. In individuals at high risk, results close to the cutoff for QFT-GIT are more likely to revert and convert during repeat testing .

Perry S et al.: *lin Vaccine Immunol* 2008; 15:425–32.

Therefore, a high positive result (ie, .1.0 IU/mL) is more likely to remain positive and, therefore, should be confirmed and treated as if it is still positive. It is not clear why false positive results occur, but postulated reasons include concomitant illness at the time of testing, laboratory factors, and nonspecific boosting of IFN- $\gamma$  responses .

Pai M et al.: *Int J Tuberc Lung Dis* 2009; 13:84–92

van Zyl-Smit RN, et al: *Am J Respir Crit Care Med* 2009; 180:49–58.

Reversions from positive to negative come to attention when the positive result is suspected to be a false positive and the test is repeated. Reversions can also occur spontaneously or after therapy and are postulated to represent immune clearing of the infection .

Andersen P et al.: *Trends Mol Med* 2007; 13:175–82

Studies evaluating the effect of LTBI treatment on positive IGRAs results have not confirmed that therapy increases the rate of reversions, although a decrease in quantitative results has been reported .

Lee SH et al. *Respir Med* 2010; 104:448–53.

Bosshard V et al.: *Respir Med* 2009; 103:30–4.

## Clinical Application and Limitations of IGRAs ...

### ***Discordant Results Between IGRAs and TST***

Longitudinal studies suggest that the TST is more sensitive than IGRAs in high-risk populations .

Dheda K et al.: *Curr Opin Pulm Med* 2009; 15:188–200.

However, IGRAs may be more sensitive at detecting recent TB exposure than is the TST .

Arend Smet al.: *Am J Respir Crit Care Med* 2007; 175:618–27.

In the case of an individual with a history of **BCG vaccination and a positive TST and negative IGRA results**, if risk for TB infection is otherwise low, it is reasonable to assume that the TST result is false positive and to withhold further evaluations. **In the case of a negative TST and positive IGRA results, if the individual is considered to be at high risk for TB infection, the negative TST result alone should not prevent further examination.**

Another consideration when interpreting discordant TST and IGRA results is the observation that TST preceding IGRA testing could boost IGRAs . This effect appears more pronounced on the days after the TST and could wane with time.

van Zyl-Smit RN, et al: *Am J Respir Crit Care Med* 2009; 180:49–58

## Clinical Application and Limitations of IGRAs ...

### ***Interpretation of Quantitative IGRA Results***

According to recent recommendations by the CDC, “both the qualitative results and the quantitative assay measurements for IGRAs should be reported” *Reporting IFN- $\gamma$  measurements does provide useful information in individuals undergoing serial IGRA testing* .

Arend SM et al.: *Am J Respir Crit Care Med* 2007; 175:618–27.

There are limited data on the significance of changing IFN- $\gamma$  levels .

van Zyl-Smit RN et al.: *PLoS One* 2009; 4:e8517

It is not clear whether higher IFN- $\gamma$  responses correlate with greater risk of progression to active TB. Pre-analytical factors, such as delays in incubation and sample processing, are also known to negatively affect IFN- $\gamma$  responses .

Herrera V. et al.: *J Clin Microbiol* 2010; 48:2672–6

Smith SG et al.: *PLoS One* 2009; 4:e7972.

The following approach to interpretation of quantitative results is recommended:

- (1) The quantitative results should not be used for prognostic or therapeutic monitoring purposes at this time, because evidence is lacking or non supportive and
- (2) the quantitative results are useful for predicting the likelihood for reversion or conversion of test results when the IFN- $\gamma$  signal is close to the assay cutoff.

## Serial Testing of Healthcare Workers

- The value of IGRAs in the testing of healthcare workers has been investigated in over 50 studies, summarized in a recent systematic review.
- The review differentiates between initial testing (e.g. preemployment) and serial (repeated) testing of healthcare workers.
- Overall, the review concluded that **the use of IGRAs instead of TST for one-time screening may result in a lower prevalence of positive tests and fewer healthcare workers who require LTBI treatment, particularly in low TB incidence settings.**
- However, the use of IGRAs for serial testing is complicated by lack of data on optimal cut-offs for serial testing, unclear interpretation and prognosis of conversions and reversions

Zwerling A, et al. *Thorax* 2012;67:62-70

Critical is the problem of using IGRAs in serial screenings in health care settings. In fact, high **rates of conversion and reversion as well as growing evidence of substantial within-subject variability of interferon-gamma responses complicate their interpretation in the serial testing of HCWs.**

Ringshausen FC, Schablon A, Nienhaus A : *Journal of Occupational Medicine and Toxicology* 2012 7:6.

## Conclusioni

- ❑ C'è una considerevole eterogeneità nelle LLGG prodotte..
- ❑ Un numero crescente di LG e *PP* da indicazioni riguardo l'utilizzo dei test IGRAs..
- ❑ Nei paesi in via di sviluppo ed ad alta incidenza di TB, il TST è ancora il test preferito, dal momento che non ci sono forti evidenze che I test IGRA siano superiori al TST in tali contesti.
- .
- ❑ Nei paesi sviluppati e bassa incidenza di TB, la più alta specificità degli IGRA e I loro vantaggi logistici sembrano incoraggiarne l'adozione e l'utilizzo crescente
- ❑ Una maggiore disponibilità di dati recentemente prodotti specialmente riguardo l'utilizzo degli IGRA in particolari contesti clinici (*pazienti che iniziano un trattamento con anti-TNF $\alpha$ - o in bambini <5 anni*), rendono superate le LLGG meno recenti.