

Public Health Impact of Interferon Gamma Release Assays

- a 2007 U.S. Perspective -

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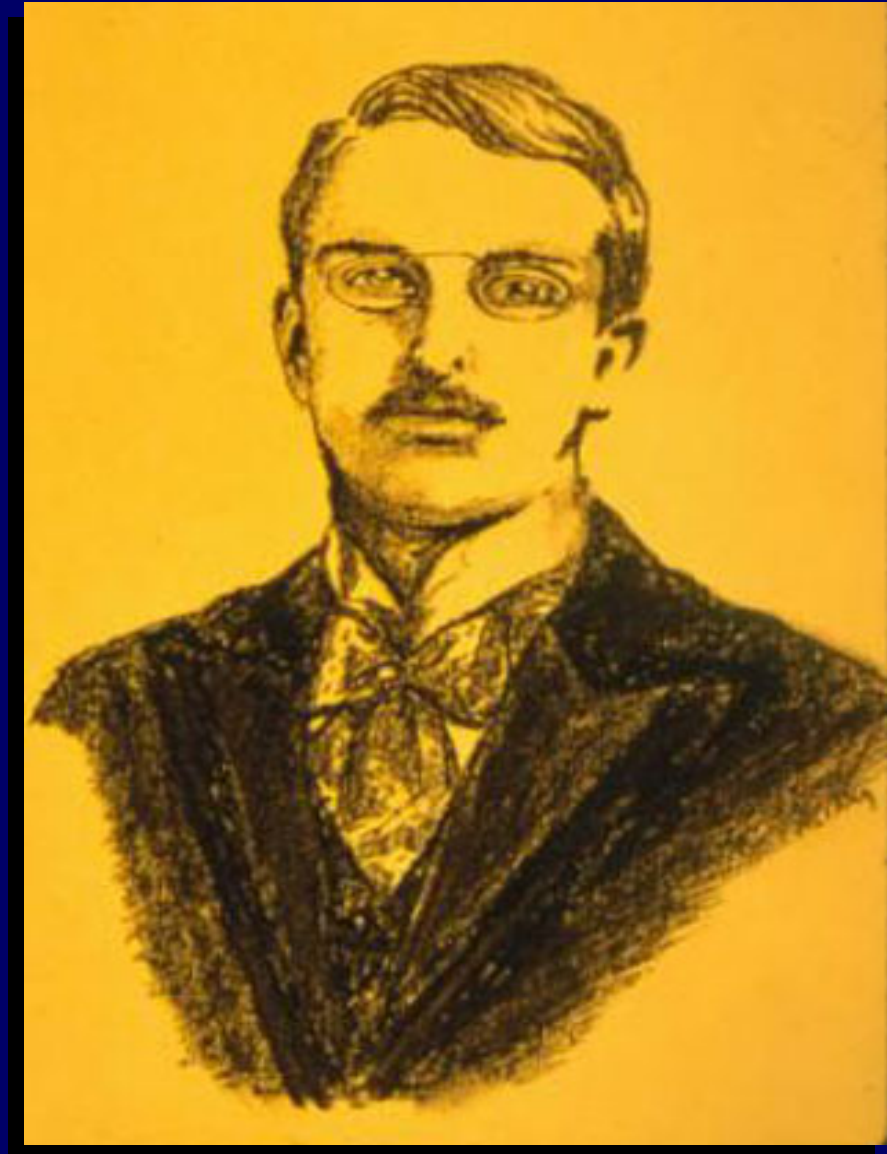




Koch

von Pirquet

**Allergist who
Developed
Method of
Testing with
Koch's tuberculin**





Florence Siebert (1897-1991)

- In 1934, Florence Seibert prepared PPD from OT by repeated precipitation with ammonium sulfate, using a protein-free culture medium
- PPD-S or Siebert's lot 49608 was adopted as the reference tuberculin in 1951; it was stored and released for use by the FDA
- A new lot PPD-S2 was prepared and standardized in the late 1990's



**Diagnosis of
TB infection,
1907**



**Diagnosis of
TB infection,
2001**

Adapted with thanks to Mark Perkins, FIND

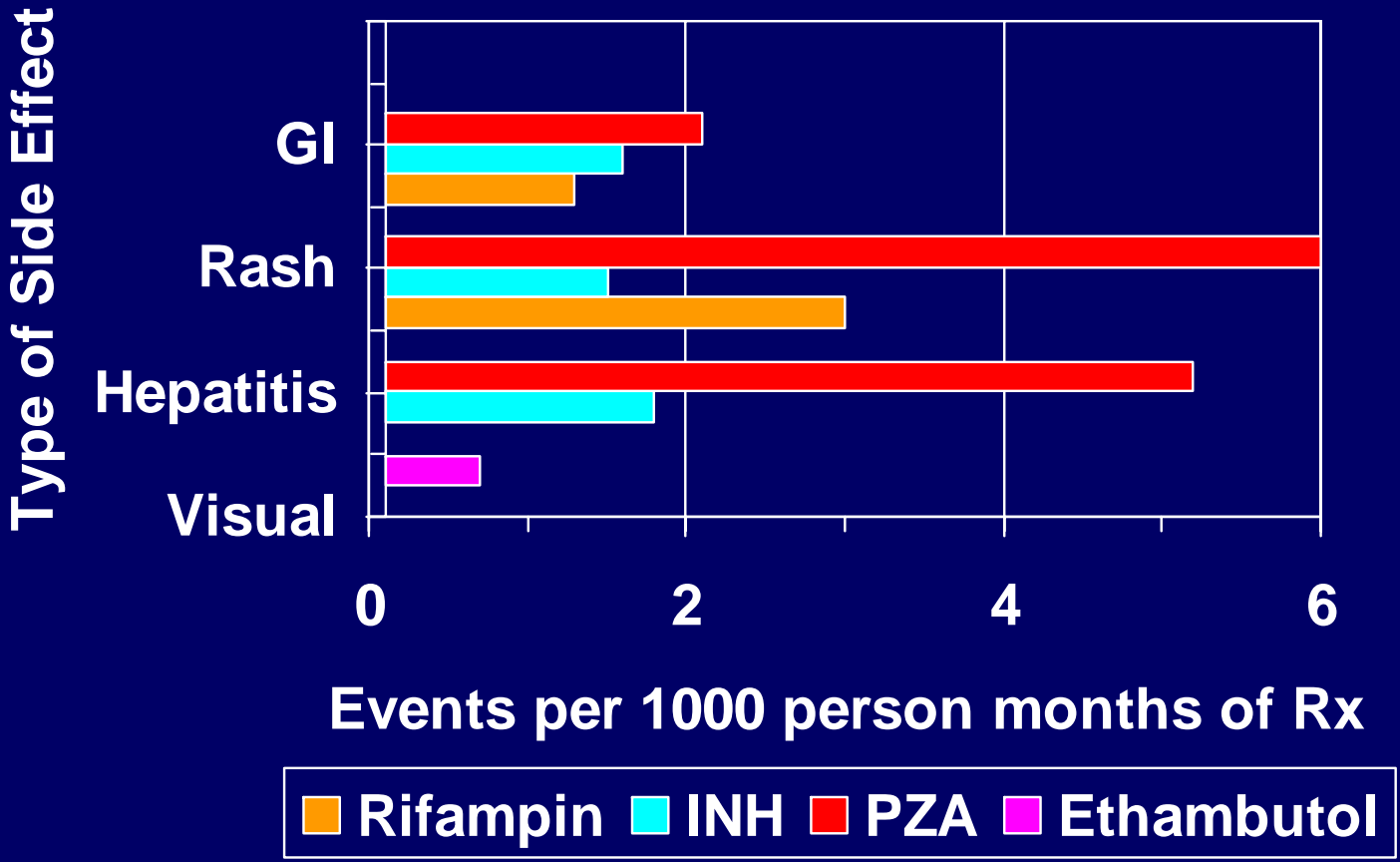
Priority activities for TB control in the U.S.

- 1. Diagnosis and treatment of active disease**
- 2. Diagnosis and preventive treatment of newly infected contacts**
- 3. Diagnosis and treatment of latent TB infection in other high-risk populations**

Outcome of contact evaluation

| | ARPES, 2001; S+ | Reichler, 1996; all | Marks, 1998; S+ close |
|--------------------|--|--------------------------------|--------------------------------------|
| Evaluated | 55-81% of contacts complete TST | | |
| <i>LTBI dx'd</i> | <i>22-36% found newly infected</i> | | |
| Start TLTBI | 63-74% begin treatment | | |
| Complete Rx | 51-62% finish treatment | | |
| OUTCOME | ~17-37% of the newly infected target population actually complete TLTBI | | |

Incidence of serious side effects, by type and drug



Yee et al., AJRCCM 2003;167:1472



National Survey to Measure Rates of Liver Injury, Hospitalization, and Death Associated with Rifampin and Pyrazinamide for Latent Tuberculosis Infection

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“However, since 1991, studies involving 11 million persons treated with isoniazid for LTBI have reported hospitalization rates of 0.1–0.2 hospitalizations per 1000 treatment initiations (median, 0.15 hospitalizations per 1000 treatment initiations) and mortality rates of 0–0.3 deaths per 1000 treatment initiations (median, 0.04 deaths per 1000 treatment initiations).”

Risk associated with treatment of LTBI

Roughly 300,000 new TLTBI starts per year in U.S.,
to prevent 10,000-20,000 cases

If median risk is 0.04 per 1,000 treatment starts,
then TLTBI is responsible for about a dozen deaths
a year in the U.S.

If 90-95% of those will never develop TB, then most
of these deaths are unnecessary and preventable.

Risk associated with active TB

Risk of death due to TB disease is now very low, and is generally associated with significant underlying co-morbidities.


Precise data are difficult to obtain because death due to TB (as distinct from “dead at diagnosis of TB) is not reportable in the U.S.

Concerns to be addressed with a “new” test:

1. Protection of individual patient interests:
 - reliable detection of “productive” infection
 - avoidance of unnecessary treatment
2. Promotion of program interests
 - focus efficiently on those truly at risk
 - a logistically easier test
3. Awareness of industry interests
 - a “commercially viable” test



World Health Organization

EXECUTIVE SUMMARY



Diagnosics for tuberculosis
Global demand and market potential

Diagnostico-economics

"This analysis indicates that annually over US\$ 1 billion is spent worldwide on TB diagnostics, a figure over twice as large as the current market for TB drugs.....Skin testing with purified protein derivative (PPD) is the highest volume TB diagnostic test used in the EME (40 million tests), where it makes up half the total market, reflecting the importance of detection of latent infection in those countries."



DIAGNOSTICS FOR ALL LEVELS OF THE HEALTH SYSTEM

TODAY

REFERENCE LABORATORY

Tb culture

Tb PCR

PERIPHERAL LABORATORY

Tb culture

Tb PCR

CLINIC / HEALTH POST

Tb culture

Tb PCR

TOMORROW

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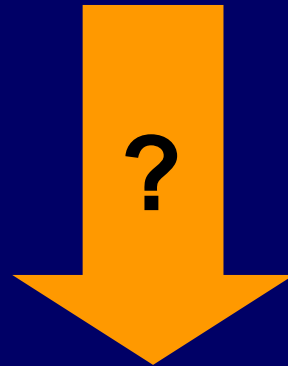
"Worldwide, we estimate that the largest potential available market for a new TB diagnostic would be for a test that both detects latent infection and predicts progression to active disease (767 million patient evaluations/year). Such a test, if widely implemented and accompanied by successful treatment, could revolutionize TB control."

The Holy Grail of TB Elimination (lacking a vaccine of course...)

1. A highly specific diagnostic for LTBI that is highly predictive of subsequent disease

+

2. A highly active ultra-short regimen for TLTBI



IGRA + 12 doses HP
“mini-Grail”

1st generation QFT-TB (QFT-1)

At an FDA advisory meeting held on October 12, 2001, the Microbiology Devices Panel recommended that the Cellestis' QuantiFERON-TB test be approved with conditions.

CDC guidelines for 1st generation QFT appeared in MMWR in January 2003

Guidelines for Using the QuantiFERON[®]-TB Test for Diagnosing Latent Mycobacterium tuberculosis Infection

Prepared by
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Division of Tuberculosis Elimination
National Center for HIV, AIDS, STD, and TB Prevention

Summary

Until 2001, the only test used to diagnose latent tuberculosis infection (LTBI) was the tuberculin skin test (TST). However, in 2001, a new test (QuantiFERON[®]-TB or QFT) manufactured by Cellestis Limited, Carnegie, Victoria, Australia that measures the release of interferon-gamma in whole blood in response to stimulation by purified protein derivative was approved by the Food and Drug Administration. This statement provides interim recommendations for using and interpreting QFT. As with TST, interpretation and indicated applications of QFT differ for persons according to their risk for LTBI and for detecting tuberculosis (TB). This report provides guidance for public health officials, health-care providers, and laboratorians with responsibility for TB control activities in the United States to help them to incorporate QFT testing for detecting and treating LTBI. Regardless of the test used to identify LTBI, testing should be primarily targeted at diagnosing infectious patients who will benefit from treatment.

Introduction

In 2001, the QuantiFERON[®]-TB test (QFT) (manufactured by Cellestis Limited, Carnegie, Victoria, Australia) was approved by the Food and Drug Administration (FDA) as an aid for detecting latent *Mycobacterium tuberculosis* infection (1). This test is an *in vitro* diagnostic aid that measures a component of cell-mediated immune reactivity to *M. tuberculosis*. The test is based on the quantification of interferon-gamma (IFN- γ) released from sensitized lymphocytes in whole blood incubated overnight with purified protein derivative (PPD) from *M. tuberculosis* and control antigens.

Tuberculin skin testing (TST) has been used for years as an aid in diagnosing latent tuberculosis infection (LTBI) and includes measurement of the delayed type hypersensitivity response 48–72 hours after intradermal injection of PPD. TST and QFT do not measure the same components of the immunologic response and are not interchangeable. Assessment of the accuracy of these tests is limited by lack of a standard for conferring LTBI.

As a diagnostic test, QFT (1) requires phlebotomy; (2) can be accomplished after a single patient visit; (3) assumes responses to multiple antigens simultaneously; and (4) does not boost mucosal immune responses. Compared with TST, QFT results are less subject to reader bias and error. In a CDC-sponsored multicenter trial, QFT and TST results were mod-

estly concordant (overall kappa value = 0.60). The level of concordance was adversely affected by prior bacille Calmette-Guérin (BCG) vaccination, immune reactivity to nontuberculous mycobacteria (NTM), and a prior positive TST (2). In addition to the multicenter study, two other published studies have demonstrated moderate concordance between TST and QFT (3,4). However, one of the five sites involved in the CDC study reported less agreement (5). Limitations of QFT include the need to draw blood and process it within 12 hours after collection and limited laboratory and clinical experience with the assay. The utility of QFT in predicting the progression to active tuberculosis has not been evaluated.

This report provides interim recommendations for using and interpreting QFT results based on available data. As with TST, interpretation and indicated applications of QFT differ between those persons at low risk and those at increased risk for LTBI. This report should assist public health officials, health-care providers, and laboratorians who are responsible for TB control activities in the United States in their efforts to incorporate QFT testing for detecting and treating LTBI.

QFT Performance, Interpretation, and Use

Tuberculin testing is performed for persons who are 1) suspected as having active TB; 2) at increased risk for progression to active TB; 3) at increased risk for LTBI; or 4) at low risk for LTBI, but are tested for other reasons (Table 1).

The material in this report originated in the National Center for HIV, AIDS, STD, and TB Prevention, Harold W. Jaffe, M.D., Director, and the Division of Tuberculosis Elimination, Kenneth G. Conry, M.D., Director.



Limited uptake in the U.S. for QFT-1

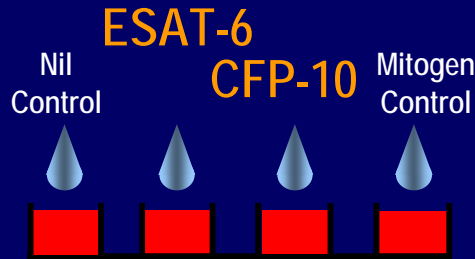
- a. Guidelines did not endorse use in contact investigations**
- b. Specificity remained a challenge – frequent false positives due to BCG, NTM, etc.**
- c. Logistic challenges: required same day lab manipulation, overnight incubation, further testing day 2**

QuantiFERON[®]-TB Gold (QFT-2g)

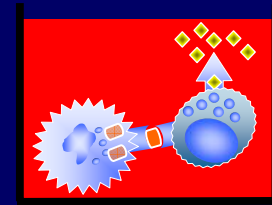
Stage 1 Whole Blood Culture



Draw blood
with heparin

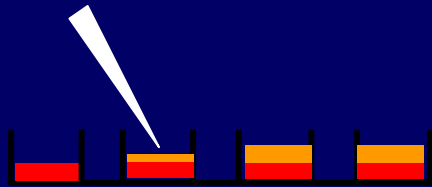


Make 1 ml aliquots &
add antigen

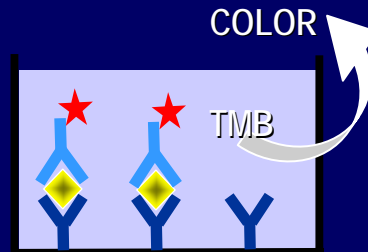


Incubate overnight. $\text{INF-}\gamma$
from sensitized lymphs

Stage 2 IFN-gamma ELISA



Harvest plasma from
above settled cells



Measure [$\text{INF-}\gamma$] in
'Sandwich' ELISA



Computerized
interpretation

2nd Generation of QFT

- 1st: used PPD
 - FDA approved Nov 2001
 - No longer commercially available
- 2nd: uses TB-specific antigens (ESAT-6 & CFP-10), to avoid cross reaction with BCG and most NTM

ESAT-6 & CFP-10 Overlapping Peptides

- ESAT-6: 7 overlapping sequences

MTEGQWNFAGIEAAASAIGG
GIEAAASAIGNVITS
SAIQGNVTSIHSLDEGKOSLTKLA
EGKQSLTKLAAAWGSSGSEAYGVQ
SGSEAYGVQKWDATATELNALQ
TATELNALQNLARTISEAGOAMAS
NLARTISEAGOAMASTEGNVGMFA

- CFP-10: 6 overlapping sequences

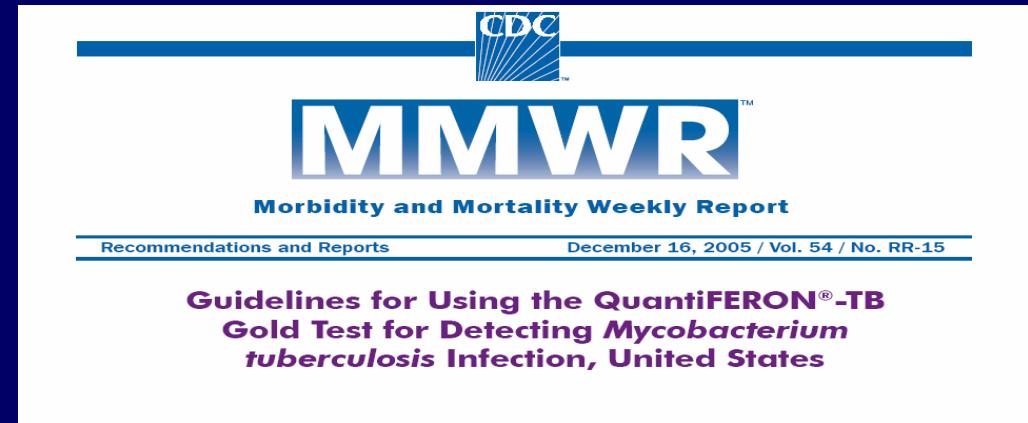
MAEMKTDAAATLAQEAQGNFERISGDL
GNFERISGDLKTDQDQVESTAGSLQ
DOVESTAGSLQGWIRGAAGTAAQAAV
AAGTAAQAAVRFQEAANKKOELD
AANKKOELDEISTNIRQAGVQVSR
IRQAGVQVSRADDEEQQALSSQMGF

QFT-TB Gold (QFT-2)



1. QFT-TB GOLD preliminarily approved by FDA as a diagnostic aid in December 2004; CDC was consulted often in process
2. Application was a Supplement to original QFT-TB approval -- no FDA panel process.
3. Approval finalized in March 2005
4. CDC released in Dec 2005 guidelines developed thru an expert panel process

Guideline Summary



- “QFT-G may be used in all circumstances in which the TST is currently used....”
- “This report provides specific cautions for interpreting negative QFT-G results in persons from selected populations.”
- “Further research is needed regarding use of QFT-G in multiple clinical circumstances.”

Cautions

- “Sensitivity [of QFT-G] for particular groups of patients (e.g., young children and immunocompromised patients) has not been determined”
- “The optimal follow-up of persons with indeterminate QFT-G results has not been determined”

Additional Cautions

- “Each QFT-G result...should be considered in conjunction with other epidemiologic, historic, physical and diagnostic findings.”
- “Whenever *M. tuberculosis* infection or disease is being diagnosed by any method, the optimal approach includes coordination with the local or regional public health TB control program”

Limitations

- Inadequate diagnostic standards
 - Unable to confirm “clinical TB”
 - Unable to confirm LTBI
- Few reports using QFT-G or similar tests
 - Variations in antigens used
 - Variations in methods

Addressing Limitations

- Sensitivity in people with culture + TB
 $\text{Sen} = \# \text{ positives} / \# \text{ tested}$
- Specificity in people at low risk for TB infection
 $\text{Spec} = \# \text{ negative} / \# \text{ tested}$
- Test agreement in various populations
 $\text{Agreement} = \# \text{ concordant} / \# \text{ tested}$

Sensitivity Estimates

| Reference | Population | + IFN- γ (n) | + TST (n) | Exclude |
|-------------|--------------------------|---------------------|-----------|---------|
| Mori; 2004 | Untreated Cult+TB; Japan | 89% (118) | 66% (76) | 1 |
| Kang; 2005 | Pulmonary TB; Korea | 81% (54) | 78% (54) | 4 |
| CDC; Unpub. | Untreated Cult+TB; US | 81% (41) | 81% (41) | 6 |
| Ravn; 2005 | Active TB; Denmark | 85% (48) | Not done | 3 |

- 81 to 89% IFN + excluding “indeterminates”
- 71 to 88% IFN + including “indeterminates”

Specificity Estimates

| Reference | Population | + IFN- γ (n) | + TST (n) | Exclude |
|-------------------|-------------------------|---------------------|-----------|---------|
| Mori; 2004 | Nursing Students; Japan | 2% (213) | 65% (113) | 0 |
| Kang; 2005 | Med Students; Korea | 4% (99) | 51% (99) | 0 |
| CDC; Submitted | Navy recruits; US | .2% (532) | .9% (532) | 31 |

- 96 to 99.8% specificity w/o “indeterminates”
- 96 to 98.9% specificity including “indeterminates”



***National Institute for
Health and Clinical Excellence***

*The National Collaborating Centre
for Chronic Conditions*

Funded to produce guidelines for the NHS by NICE

TUBERCULOSIS

Clinical diagnosis and management of tuberculosis,
and measures for its prevention and control

● <http://www.nice.org.uk/>

Published by



**Royal College
of Physicians**

Setting higher medical standards



A decision model was used to compare the expected cost-effectiveness of four strategies of testing for latent infection in the context of a contact tracing programme in England and Wales.

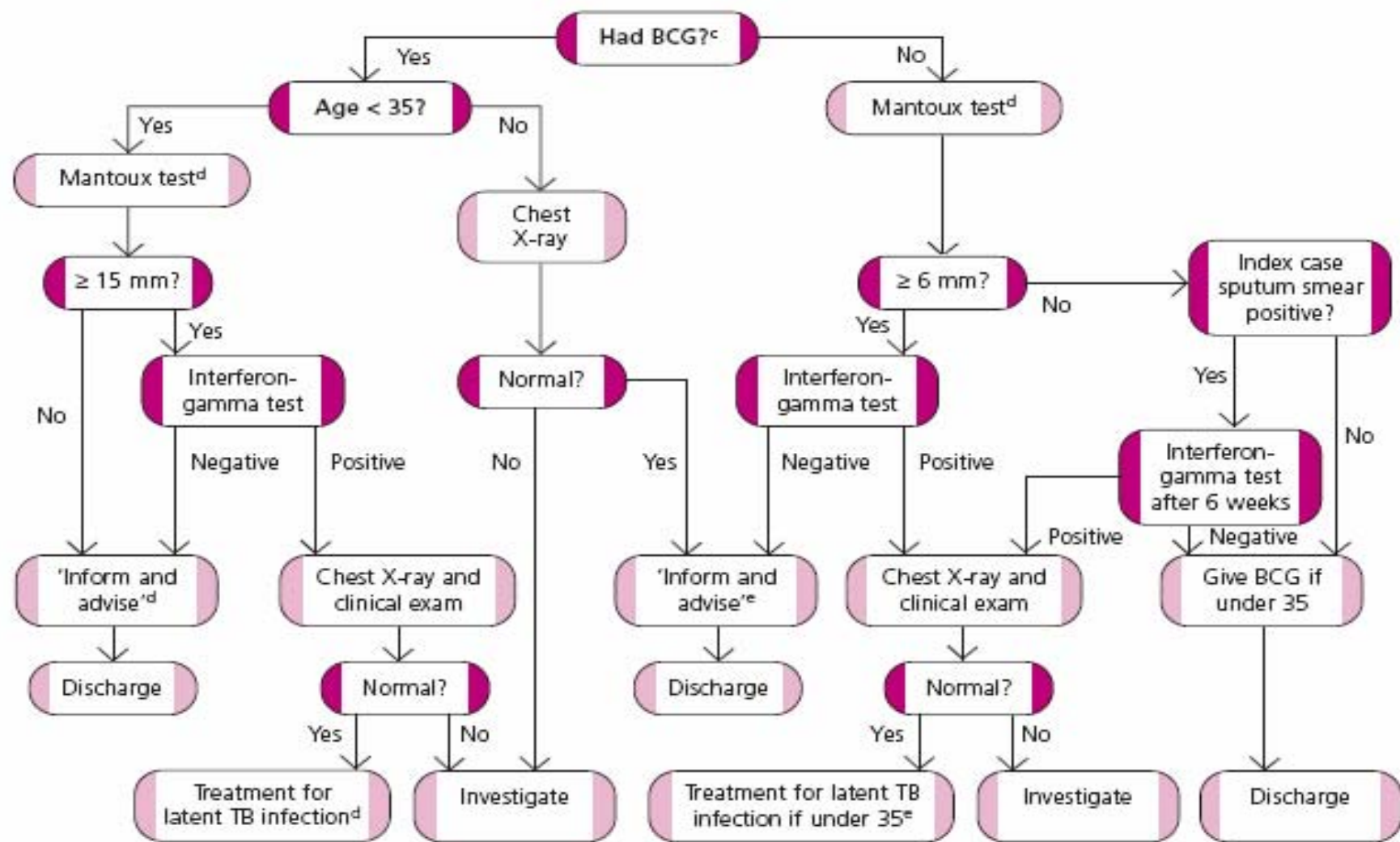
The strategies compared were:

- TST
- IGT
- TST followed by IGT for patients with a positive TST
- no test (inform and advise only).

The basecase economic analysis suggests that the two-stage strategy (TST/IGT) is within the range usually considered 'cost-effective', at around £26,000 per quality-adjusted life-year (QALY) gained. Compared with this, IGT is not cost-effective (over £150,000 per QALY gained). TST is both less effective and more expensive than all of the other options (it is 'dominated').

The results of the economic analysis were highly dependent on the context of the contact tracing scheme – with a higher-risk cohort of contacts, the expected benefits of early diagnosis of active cases, treatment of latent infection, and vaccination will be greater. Below a prevalence of about 10% none of the testing strategies is cost-effective. At intermediate levels of prevalence (between about 10% and 40%), the two-stage TST/IGT strategy is cost effective. Above 40% IGT on its own is the most cost-effective option.

Testing and treating asymptomatic household and other close contacts of all cases of active TB^b



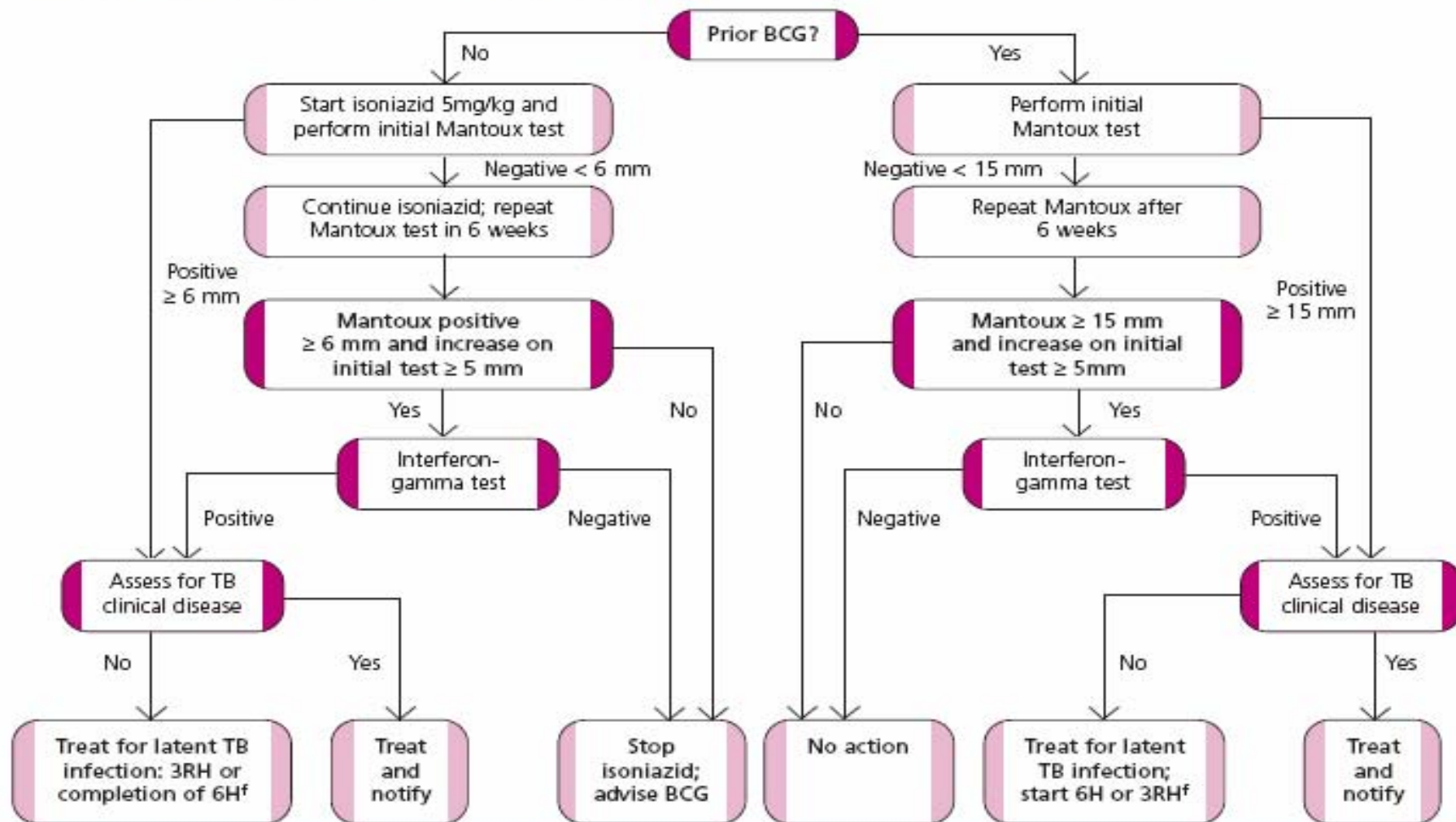
^b For children older than 4 weeks and younger than 2 years who are contacts of people with sputum smear-positive TB, see algorithm on page 15.

^c Previous BCG vaccination cannot be accepted as evidence of immunity in HIV-infected patients.

^d A negative test in immunocompromised people does not exclude TB infection.

^e People advised to have treatment for latent TB infection, but who decline, should have 'Inform and advise' information reinforced and chest X-ray follow-up at 3 and 12 months.

Testing and treating asymptomatic children older than 4 weeks but younger than 2 years who are contacts of people with sputum smear-positive TB



^f Drug regimens are often abbreviated to the number of months a phase of treatment lasts, followed by letters for the drugs administered in that phase: H is isoniazid, R rifampicin, Z pyrazinamide, E ethambutol, S streptomycin.

So 3RH is three months of rifampicin and isoniazid, 6H is 6 months of isoniazid.

Research recommendation 1

A diagnostic and qualitative study, assessing whether interferon-gamma tests are acceptable to patients and more effective than tuberculin skin tests for:

- predicting subsequent development of active TB , *or*
- diagnosing or ruling out current active TB

when undertaking TB screening in:

- new immigrants from high TB prevalence countries
- healthcare workers
- children in high-risk areas who missed neonatal BCG
- contacts of sputum smear-positive TB
- HIV-positive patients.

This study should compare strategies of TST only, TST then IGT if positive, and IGT only.

Population

- New immigrants from high TB prevalence countries.
- Healthcare workers.
- Children in high-risk areas who missed neonatal BCG.
- Contacts of sputum-positive TB.
- HIV-positive patients.

Intervention

Interferon-gamma tests.

Comparison

Tuberculin skin tests.

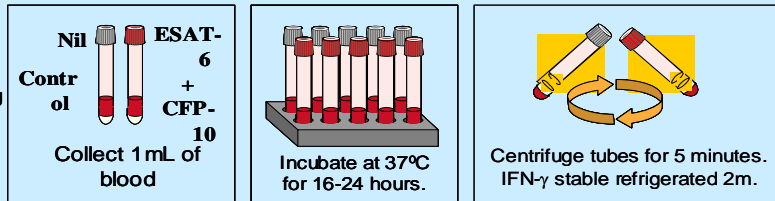
Outcome

Subsequent development of active TB. Qualitative patient acceptability outcome.

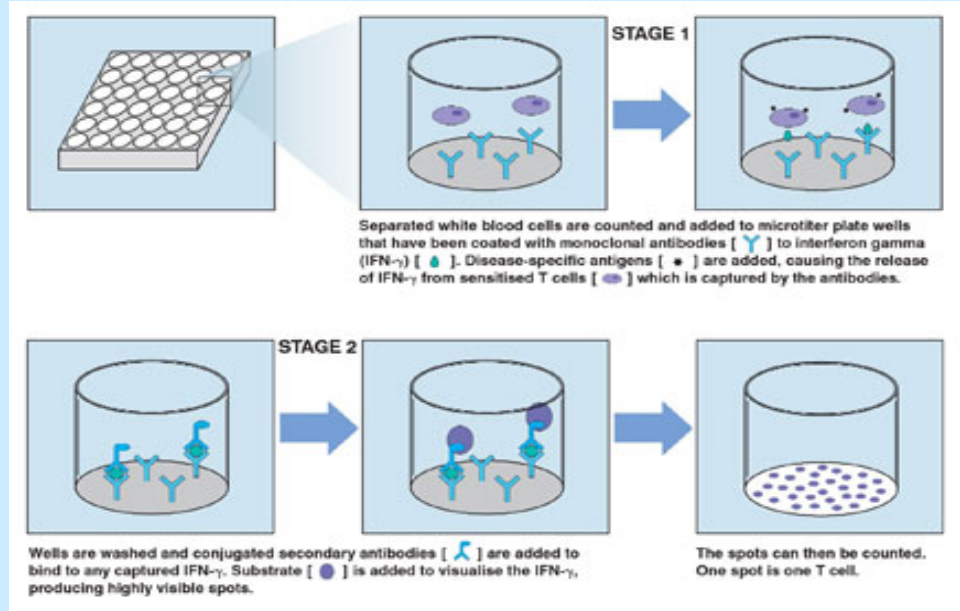
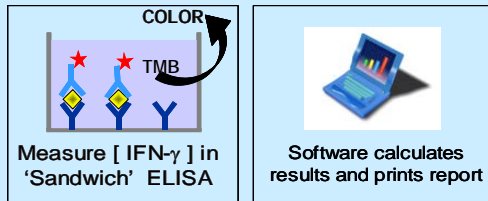


QuantiFERON®-TB Gold “In-tube” (QFT-3g)

**Stage 1:
Blood
Incubation
and Harvesting**



**Stage 2:
IFN- γ ELISA
&
Interpretation**



Two tests “new” to the U.S.

- QFT-Gold In Tube offers greater practicality, and perhaps increased sensitivity. It is being submitted as a supplement (i.e., no FDA panel)
- T-spot TB will likely be the first commercial Elispot test available in the U.S. As the first submission, it is likely to be reviewed by an FDA panel
- CDC will develop guidelines for each.

Pulmonary Perspective

An Update on the Diagnosis of Tuberculosis Infection

Luca Richeldi

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Am J Respir Crit Care Med Vol 174. pp 736–742, 2006

In summary, published studies categorically demonstrate that T-SPOT.TB and QuantiFERON-TB Gold are more specific than the TST for the diagnosis of LTBI in BCG-vaccinated populations. T-SPOT.TB, in addition, seems to be more sensitive than the TST in immunocompetent people with LTBI and in patients with active TB, including those with impaired cellular immunity at high risk of false-negative TST results. QuantiFERON-TB Gold probably has a sensitivity similar to the TST in immunocompetent people with LTBI. As with T-SPOT.TB, QuantiFERON-TB Gold has a higher sensitivity than TST in immunocompetent patients with active TB

Comparison of two interferon-gamma assays and tuberculin skin test for tracing TB contacts.

Sandra M. Arend², Ph.D., Steven F.T. Thijsen⁴, Ph.D., Eliane M.S. Leyten², M.D., John J.M. Bouwman⁴, Willeke P.J. Franken², M.Sc., Ben F.P.J. Koster³, M.D., Frank G.J. Cobelens^{6,7}, Ph.D., Arend-Jan van Houte^{4,5}, Ph.D., Ailko W.J. Bossink^{1,8*}, Ph.D.

When a **supermarket** employee with smear positive TB had infected most close contacts, a contact investigation among >20,000 customers was performed.....

Performance of IGRAs in persons with TST* cutoff 15 mm, Supermarket study, Netherlands

| IGRA | Sensitivity | Specificity |
|-----------------------------|--------------------|--------------------|
| Quantiferon Gold In Tube | 68/161 (42%) | 611/624 (98%) |
| T spot TB | 80/156 (51%) | 541/603 (90%) |

QFT-GIT

-

+

| TST | |
|------------|-----------|
| < 15 mm | ≥ 15 mm |
| 611 (86.8) | 93 (13.2) |
| 13 (16.0) | 68 (84.0) |

T-spot TB

-

+

| TST | |
|------------|-----------|
| < 15 mm | ≥ 15 mm |
| 541 (87.7) | 76 (12.3) |
| 62 (43.7) | 80 (56.3) |

Performance of IGRAs in persons with TST* cutoff 05 mm, Supermarket study, Netherlands

| IGRA | Sensitivity | Specificity |
|-----------------------------|--------------------|--------------------|
| Quantiferon Gold In Tube | 80/336 (24%) | 447/448 (99%) |
| T spot TB | 121/330 (37%) | 408/429 (95%) |

| QuantiFERON-TB Gold in-tube (N=785) | TST | |
|--|-------------|------------|
| | < 5 mm | ≥ 5 mm |
| negative (N=704) | 448* (63.6) | 256 (36.4) |
| positive (N=81) | 1 (1.2) | 80 (98.8) |

| T-SPOT.TB (N=759) † | TST | |
|---------------------|------------|------------|
| | < 5 mm | ≥ 5 mm |
| negative (N=617) | 408 (66.1) | 209 (33.9) |
| positive (N=142) | 21 (14.8) | 121 (85.2) |

Arend et al, AJRCCM 2006 Epub

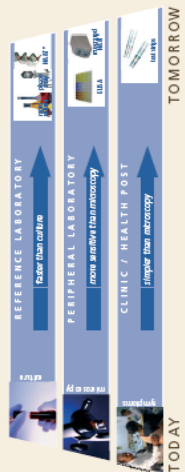


An agenda for research on T-Cell Based Diagnosis of Latent Tuberculosis Infection

Compiled by
Madhukar Pai, Keertan Dheda & Rick O'Brien



DIAGNOSTICS FOR ALL LEVELS OF THE HEALTH SYSTEM



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Risk prediction

- What is the predictive value of a positive IGRA test for development of active disease, relative to a positive TST?
 - Among IGRA positive individuals, are individuals with higher levels of IFN-g responses more or less likely to progress to active disease?
- What is the accuracy and role of IGRAs as a “rule out” test for active TB?

Immune Responses to the *Mycobacterium tuberculosis*-Specific Antigen ESAT-6 Signal Subclinical Infection among Contacts of Tuberculosis Patients

T. Mark Doherty,^{1*} Abebech Demissie,² Joseph Olobo,² Dawit Wolday,³ Sven Britton,⁴
Tewodros Eguale,⁵ Pernille Ravn,⁶ and Peter Andersen¹

Department of Tuberculosis Immunology, Statens Serum Institute,¹ and Hvidovre Hospital,⁶ Copenhagen, Denmark; Armauer Hansen Research Institute,² Black Lion Hospital,³ and Hossana Regional Hospital, Ministry of Health,⁵ Hossana, Ethiopia; and Karolinska Institute, Stockholm, Sweden⁴

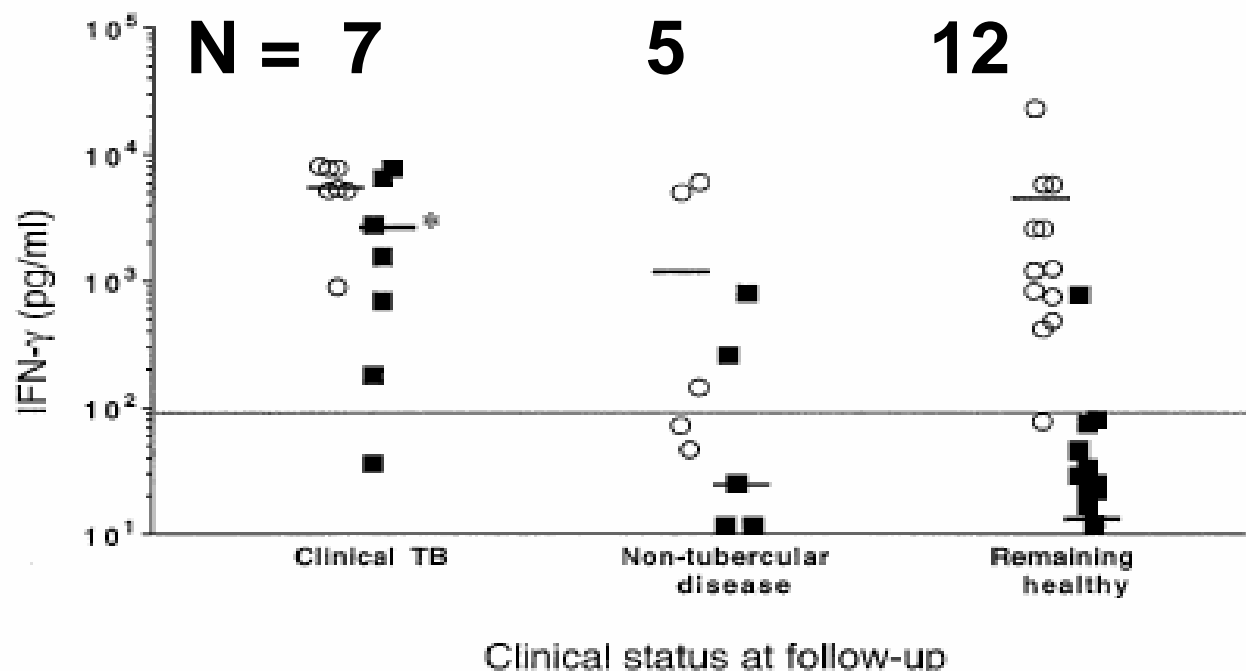


FIG. 1. In vitro IFN- γ responses of PBMCs after restimulation with PPD (○) or ESAT-6 (■) at the time of entry into the study. The results are for individual contacts segregated by their clinical status at the end of the follow-up period (2 years). The cutoff point for positivity in the assay is indicated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

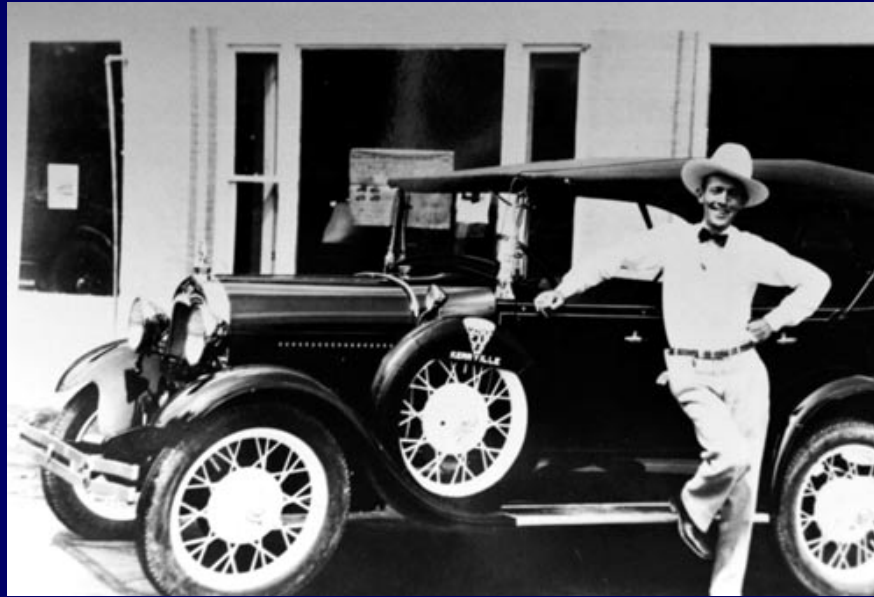
**Continued challenge of prediction,
esp. for vulnerable populations
(eg., children, immunocompromised)**

**Cost of study (\$2-3 million) slows or
prevents progress**

**All other issues (conversion, reversion,
cut-off points, variability), while important,
are secondary**



Jimmy Rodgers
1897-1933



Thank you
For your
Attention

