# Biomarkers In tuberculosis a physician point of view

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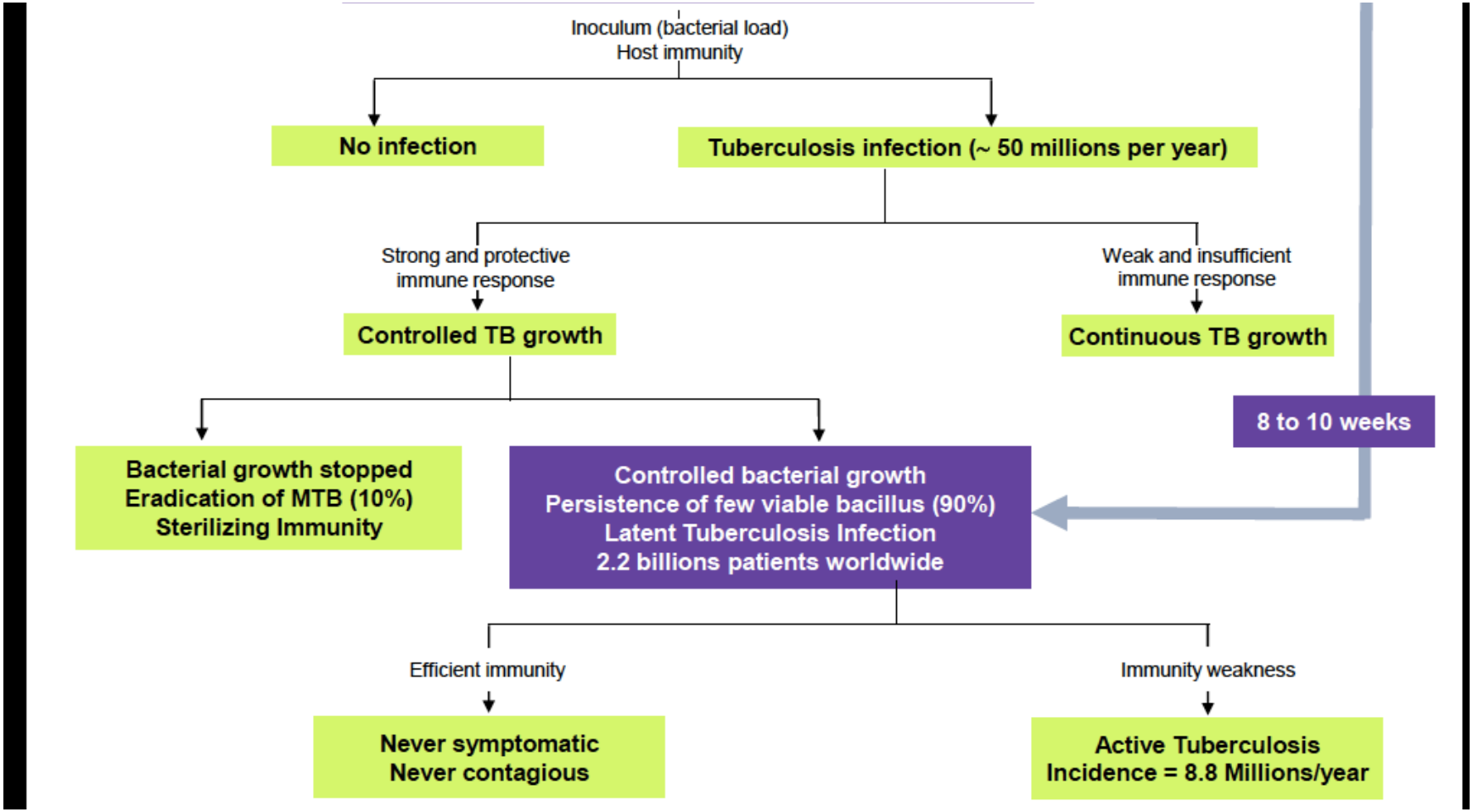
# Background

- Natural history of tuberculosis
  - Exposure
  - Latent tuberculosis infection
  - Incipient and active tuberculosis
- Which strategy to end TB ?
- Need for biomarkers to identify latent Tb at risk of progress to active Tb

# Natural history of tuberculosis

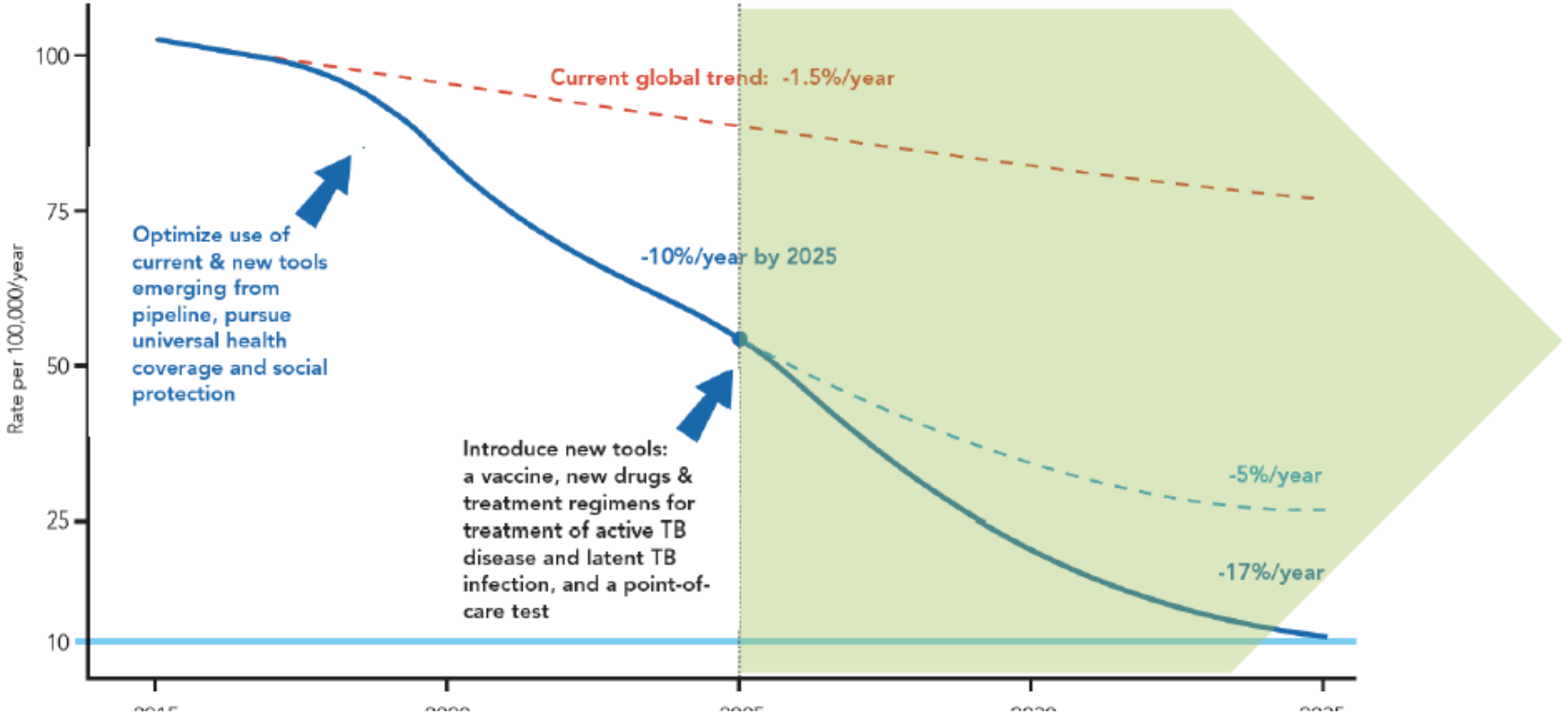


# Some conditions increase the risk to develop active Tb

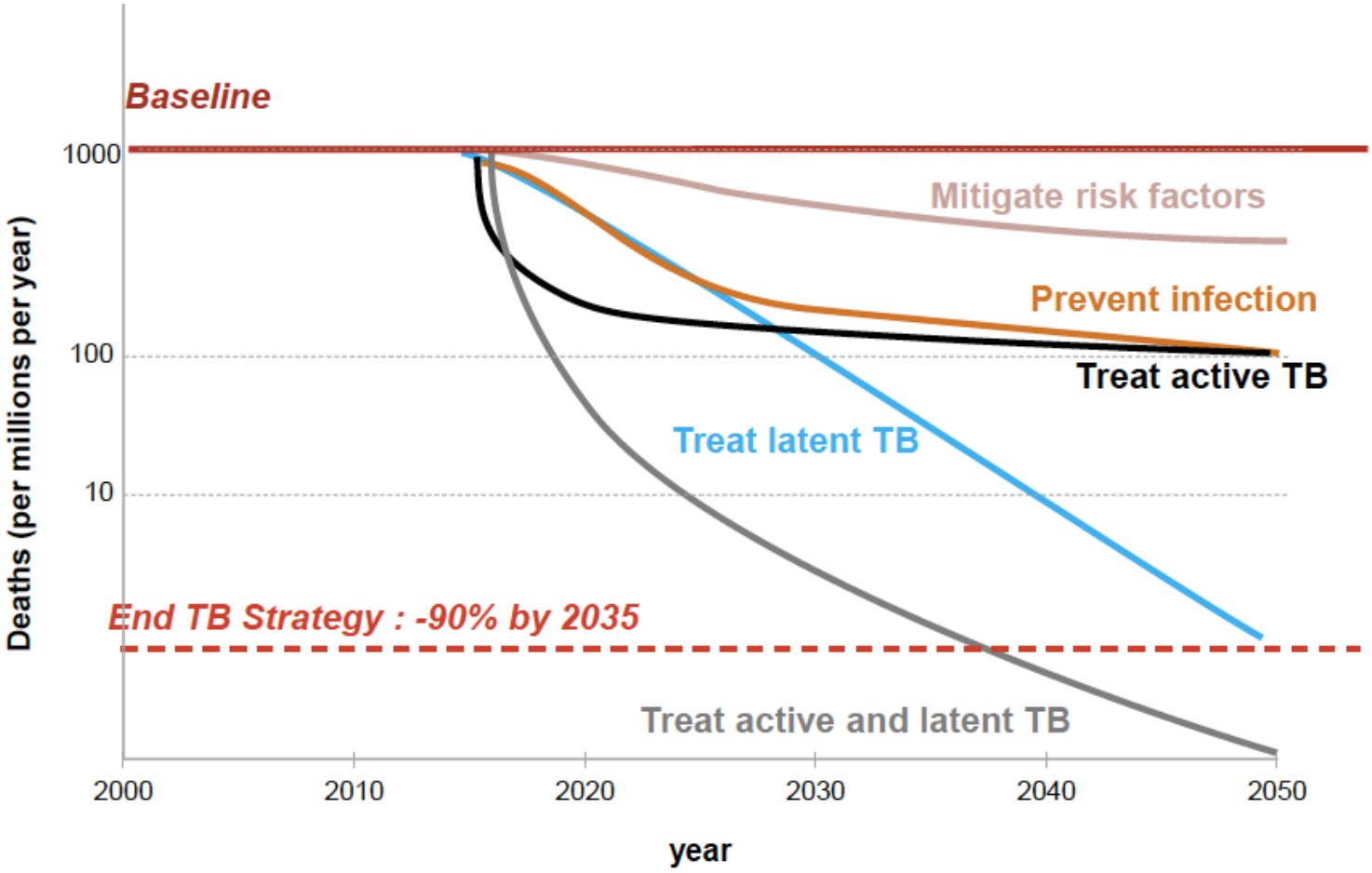
| Risk factors to develop active TB form Latent TB infection | |
|---|---|
| **Risk Factors** | **Estimation of relative risk*** |
| AIDS | 110 - 170 |
| Well controlled HIV infection | 50 - 110 |
| Solid Organ Transplantation | 20 - 74 |
| Chronic Hemodialysis | 10 - 25 |
| Head and neck cancer | 16 |
| Recent tuberculosis infection (<2 years) | 15 |
| Systemic prolonged corticosteroids therapy | 4.9 |
| Anti-TNF $\alpha$ treatment | 1.5 - 4 |
| Diabetes | 2 - 3.6 |
| Malnutrition (body mass index < 20 $kg/m^2$) | 2 - 3 |
| Passive smoking | 2 - 3 |

# What do we need to eliminate tuberculosis in 2050

- Better diagnostics, including new point-of care tests;
- Safer, easier and shorter treatment regimens;
- Safer and more effective treatment for latent TB infection;
- Effective pre- and post-exposure vaccines.

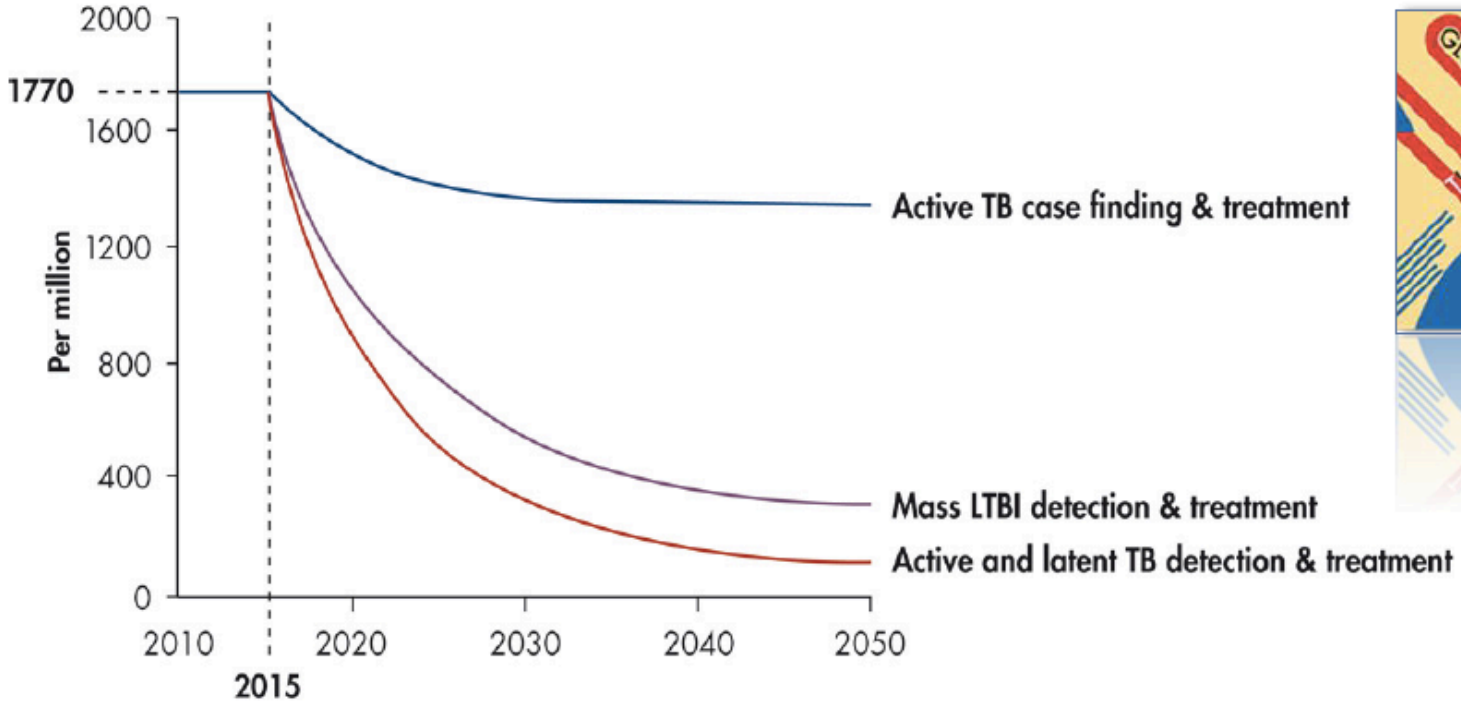


# Which Strategy to end TB ?

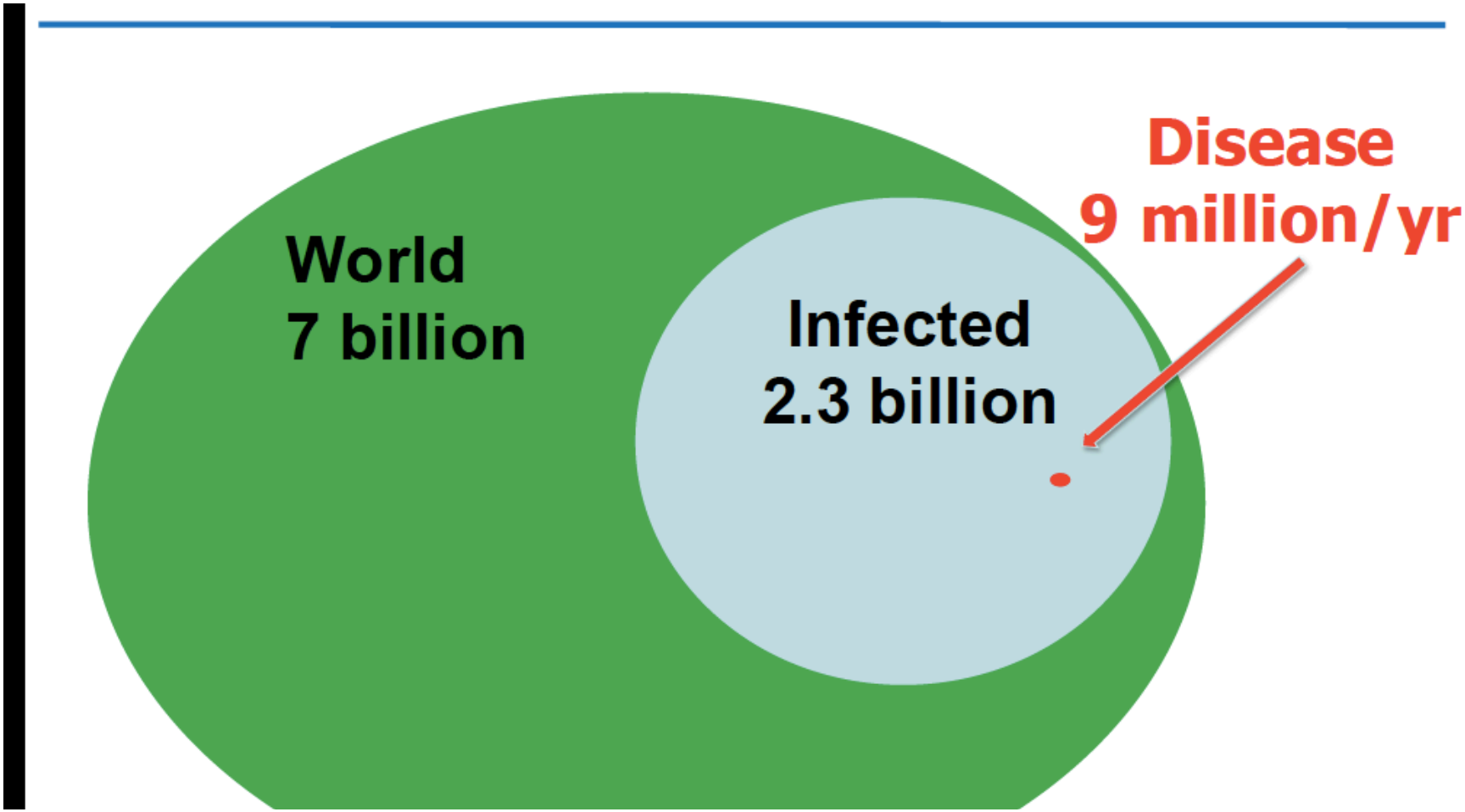


# Is it efficient enough to only consider active TB?

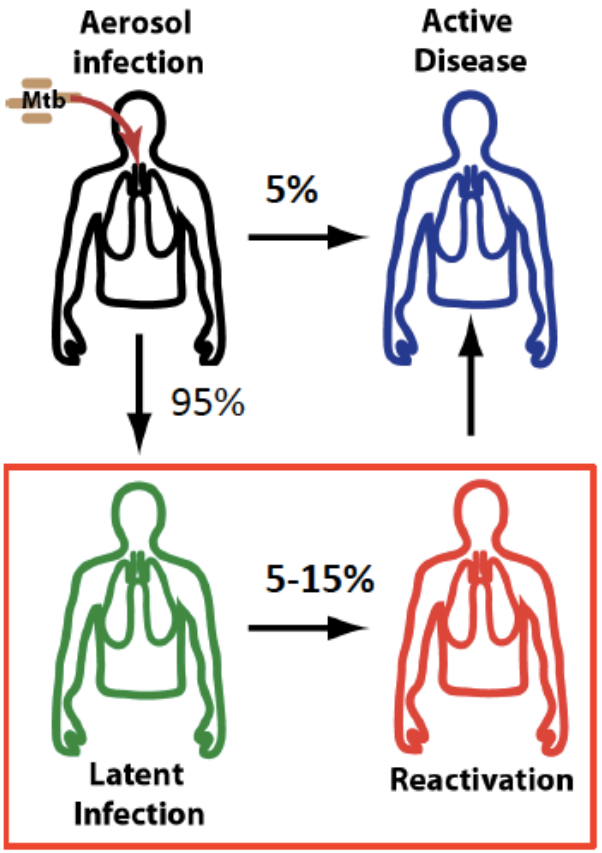
- **This will not be achievable if only the active TB cases are considered**
- **WHO Global Tuberculosis 2016 Report clearly states that treatment of LTBI is key for prevention of new infections**




# Tuberculosis infection



# Reservoir = latent TB Infection



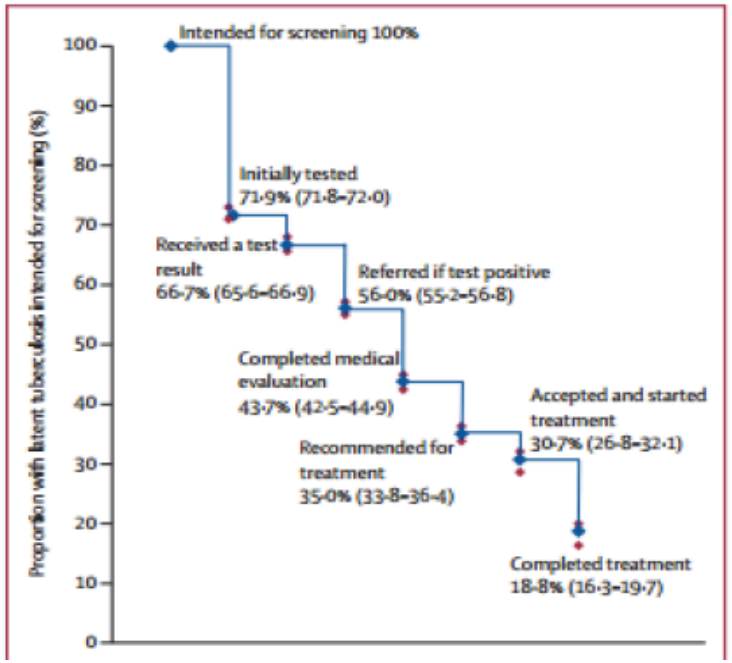

9 million TB cases per year

2 billion population with latent TB infection

# Latent Tb diagnosis and treatment : poor adherence and completion

**The cascade of care in diagnosis and treatment of latent tuberculosis infection: a systematic review and meta-analysis**

*Hannah Alsdurf, Philip C Hill, Alberto Matteelli, Haileyesus Getahun, Dick Menzies*



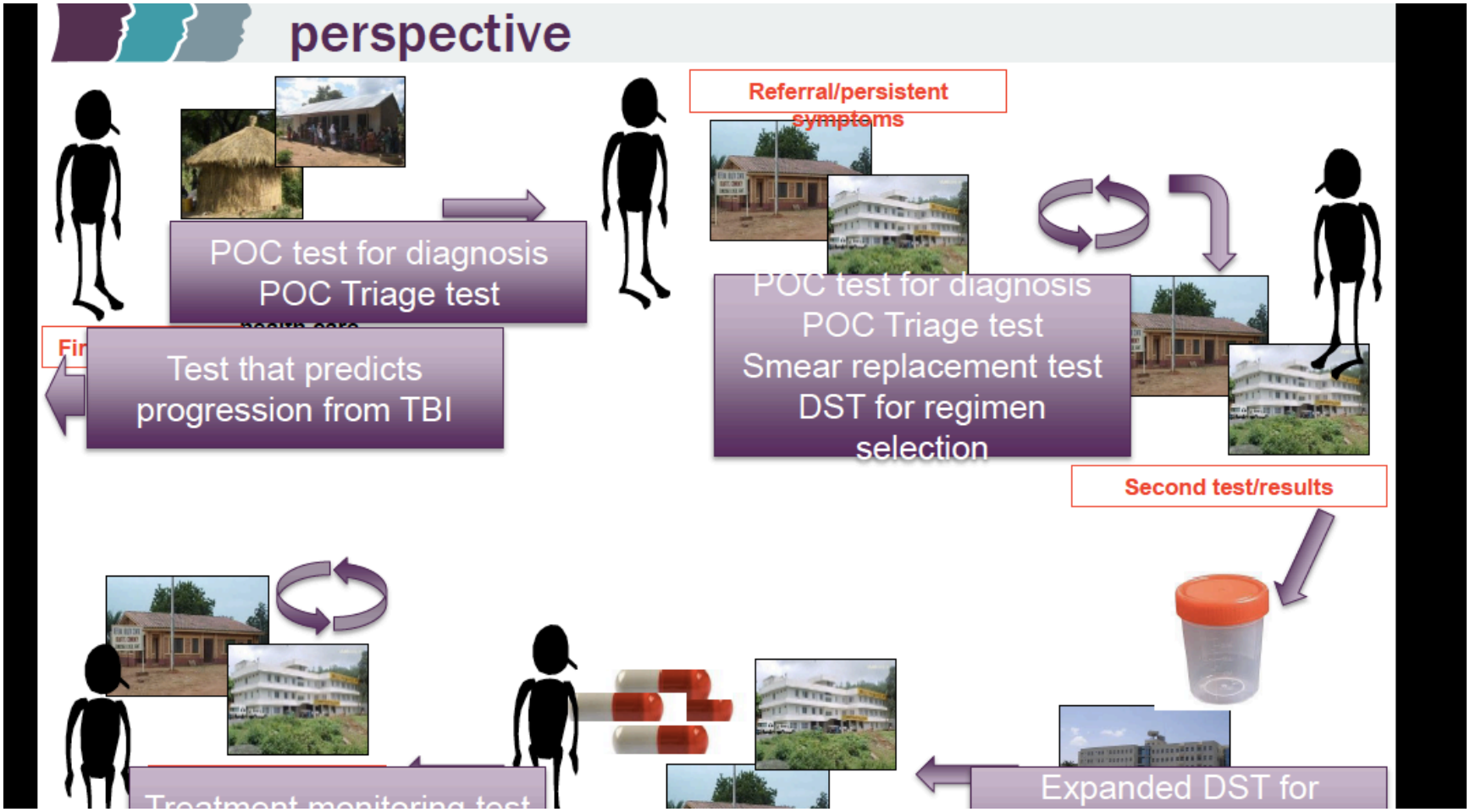

**Pooled completion rate**

Contact: 29.3% (19–40)

Medical (including PLHIV):50.4% (20–81)

Lancet Infect Dis 2016; 16: 1269–78

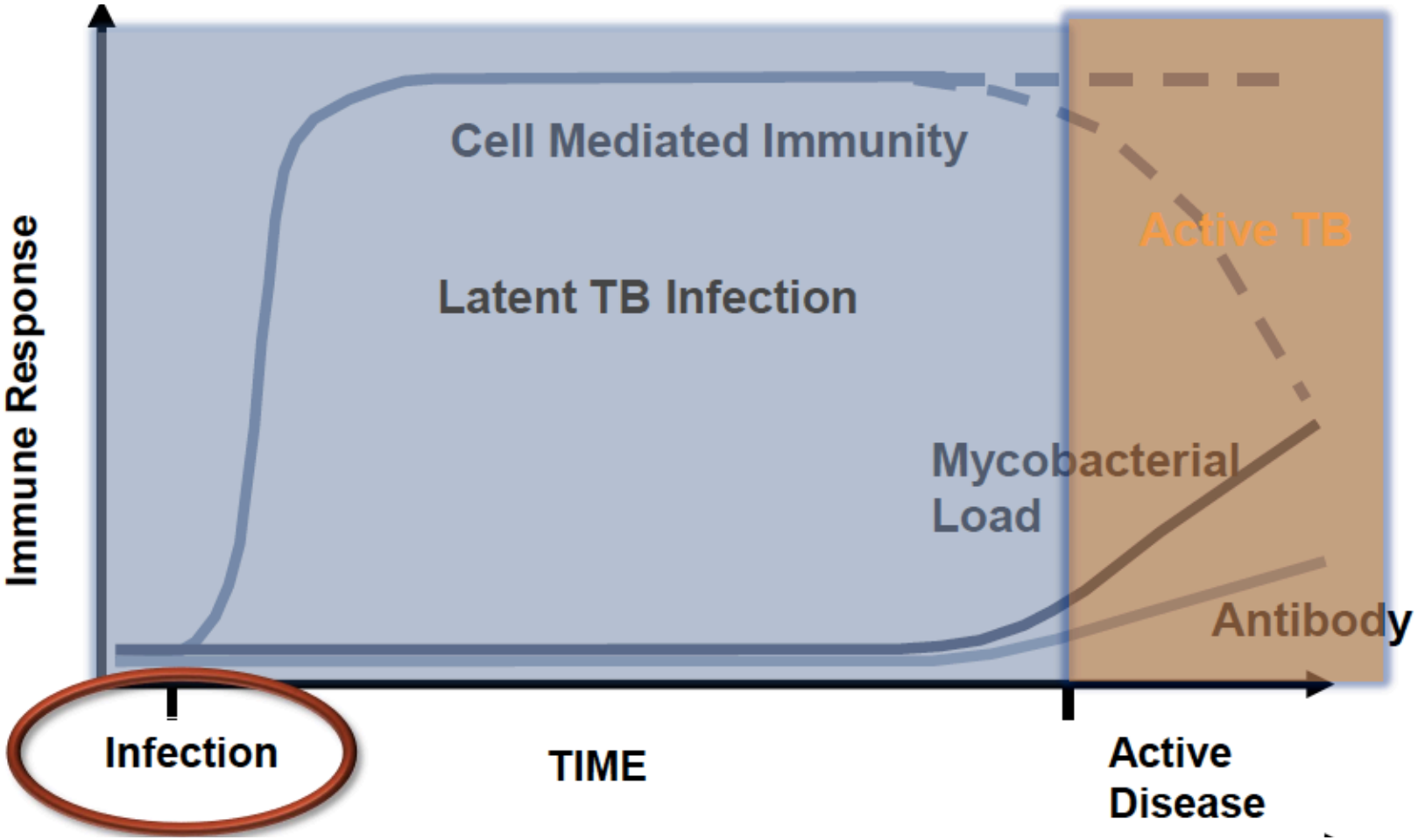
# Point of care test for LTB infection and test that predicts progression from LTBI



# Priorities in Research

- More investment in LTBI diagnostic to developp a POC test with improved performance to predict progression from LTBI to active TB disease
- Optimize the performance and utility of existing LTBI tests

# Principle of immunological tests



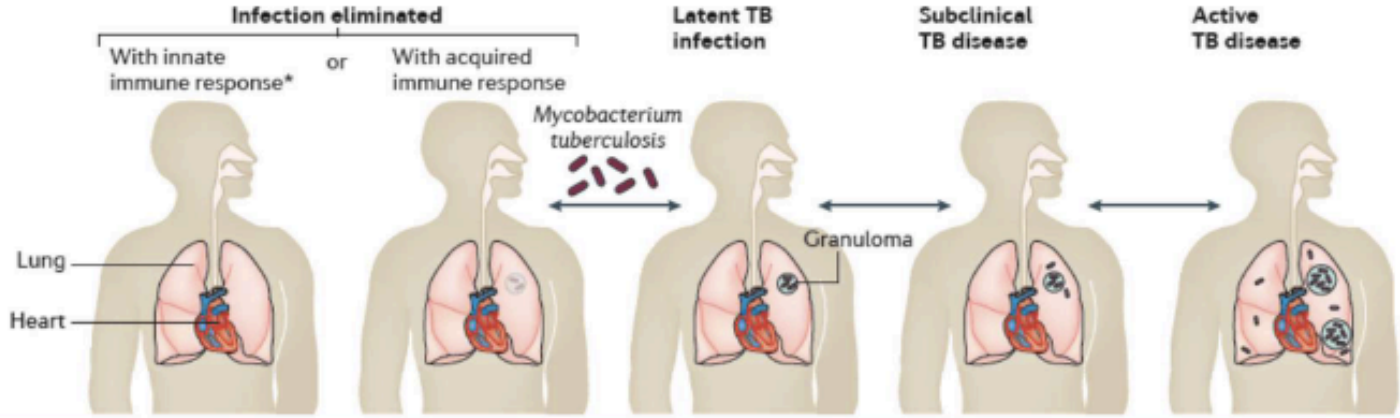
# Biomarkers ?

- Biomarkers for active TB
  - Breath markers ( not yet avalaible)

- Biomarkers for TB infection
  - Immunological tests : CD4 CD8 response
  - QFG and QF Plus

- Biomarkers to identify individuals with infection who will develop active tuberculosis
  - QF Plus ?
  - Transcriptional : RNA signature ( works in progress)

# *Pai et al, Nature Review, 2016*




| | | | | | |
|---|---|---|---|---|---|
| **TST** | Negative | Positive | Positive | Positive | Usually positive |
| **IGRA** | Negative | Positive | Positive | Positive | Usually positive |
| **Culture** | Negative | Negative | Negative | Intermittently positive | Positive |
| **Sputum smear** | Negative | Negative | Negative | Usually negative | Positive or negative |
| **Infectious** | No | No | No | Sporadically | Yes |
| **Symptoms** | None | None | None | Mild or none | Mild to severe |
| **Preferred treatment** | None | None | Preventive therapy | Multidrug therapy | Multidrug therapy |

# Defining criteria for the five categorical states of tuberculosis

from Drain and al Clin Microbiol Rev 07/2018

| Categ state of TB | Exposure to M.tub | Viable M.tub pathogen | M.Tub has metabolic activity : progression ? | RX ab or microb evidence of active MT | Symptoms of tuberculosis Microb+ |
|---|---|---|---|---|---|
| Eliminated TB | + | | | | |
| Latent TB | + | + | | | |
| **Incipient TB** | + | + | + | | |
| **Subclinical TB** | + | + | + | + | |
| Active TB disease | + | + | + | + | + |

# Biomarkers to identify people with latent infection ?

- No microbiological markers : PCR and cultures are negative
- Immunological witnesses of contact with M.tuberculosis ?
- Among them those with Specific markers for detecting metabolic activity to ongoing of impending progression of infection ( incipient tuberculosis)

# Immune correlates of incipient and subclinical tuberculosis

- Upregulation of Interferon signaling
- Decreased B and T cell signaling

- Humoral immunity ?
- Interferon gamma release assay ( IGRAs)
  - M tuberculosis specific CD4 T cell immunity ( Qf gold)
  - Using both CD4 and CD8 ( Q Gold +)
- Blood RNA signature ( 16 genes)

# Serology for tuberculosis

Meta-analysis : KR steingart PLoS Medicine 2011

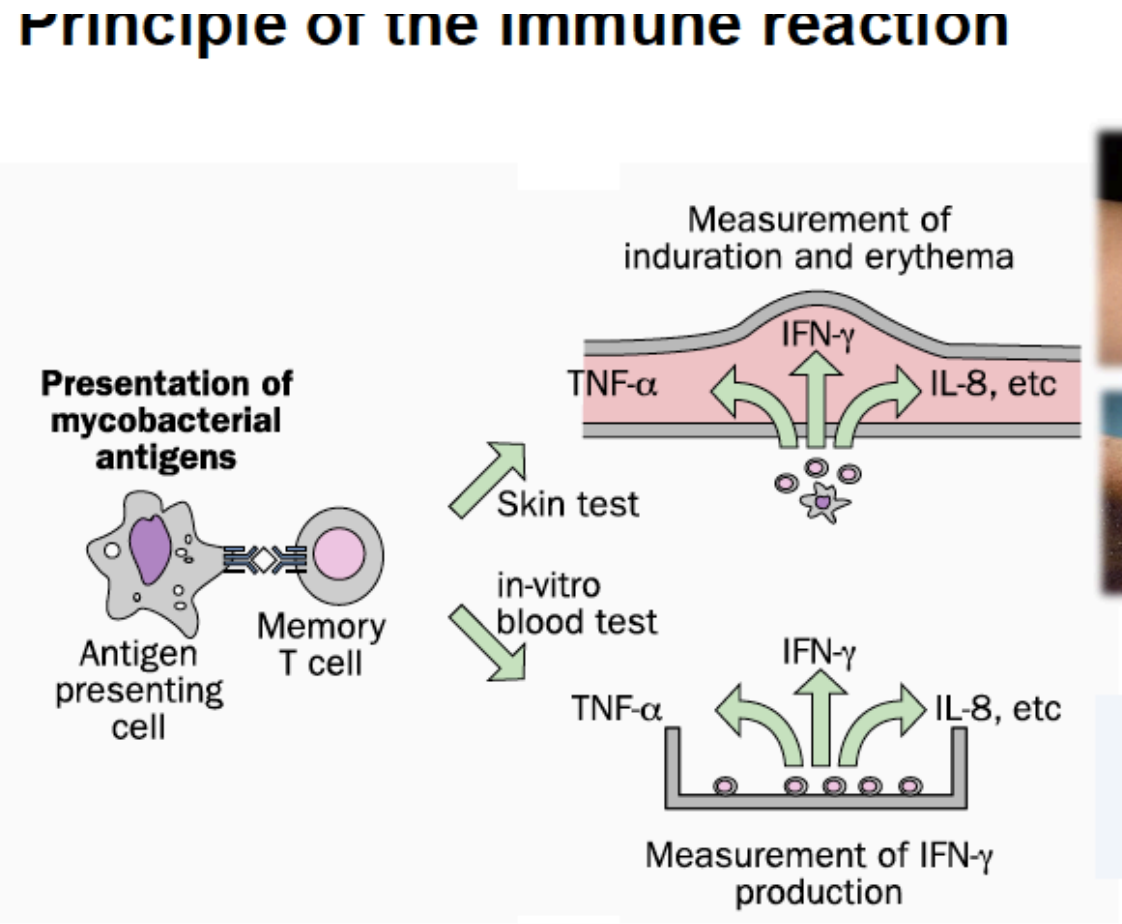
- Pulmonary TB (67 studies; 5,147 patients)
- Extra pulmonary TB (25 studies;1,809 patients)

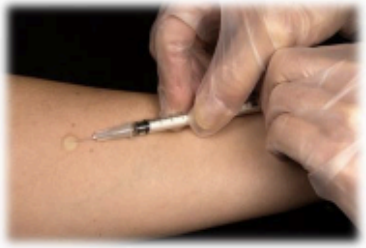
**- Sensitivity : 0 to 100%**

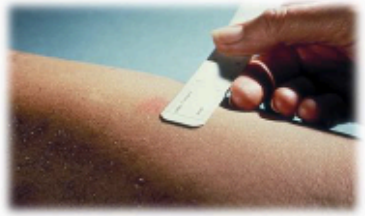
**- Specificity : 31-59% to 100%**

*Conclusions:* Despite expansion of the literature since 2006, commercial serological tests continue to produce inconsistent and imprecise estimates of sensitivity and specificity. Quality of evidence remains very low. These data informed a recently published World Health Organization policy statement against serological tests.

# Immunological tests : IGRA /TST



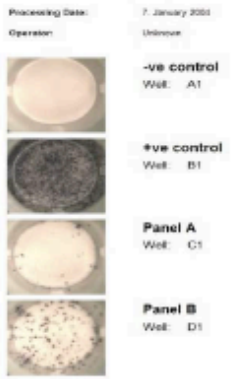





# IGRA tests

IFN-γ

ESAT-6, CFP-10



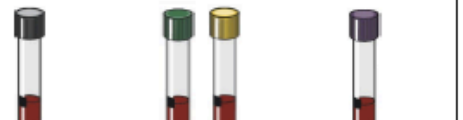

Sang Total

PBMC

QuantiFERON® TB Plus

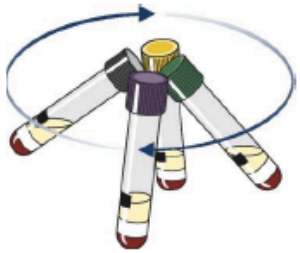
T SPOT®.*TB*

# Technical steps



Nil Control | ESAT-6 CFP-10 | Mitogen Control

1) 1mL of whole blood (x4) and incubation at +37°C for 16-24 h.

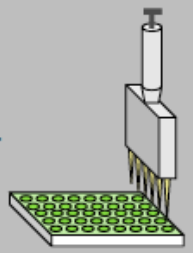


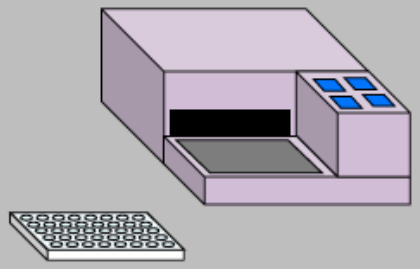
2) 15 minutes Centrifugation

IFN-γ is stable at 2-8° C for at least 4 weeks

**Step 2: INF-γ ELISA testing**

3) Add plasma and conjugate. Incubation 2H at room temperature

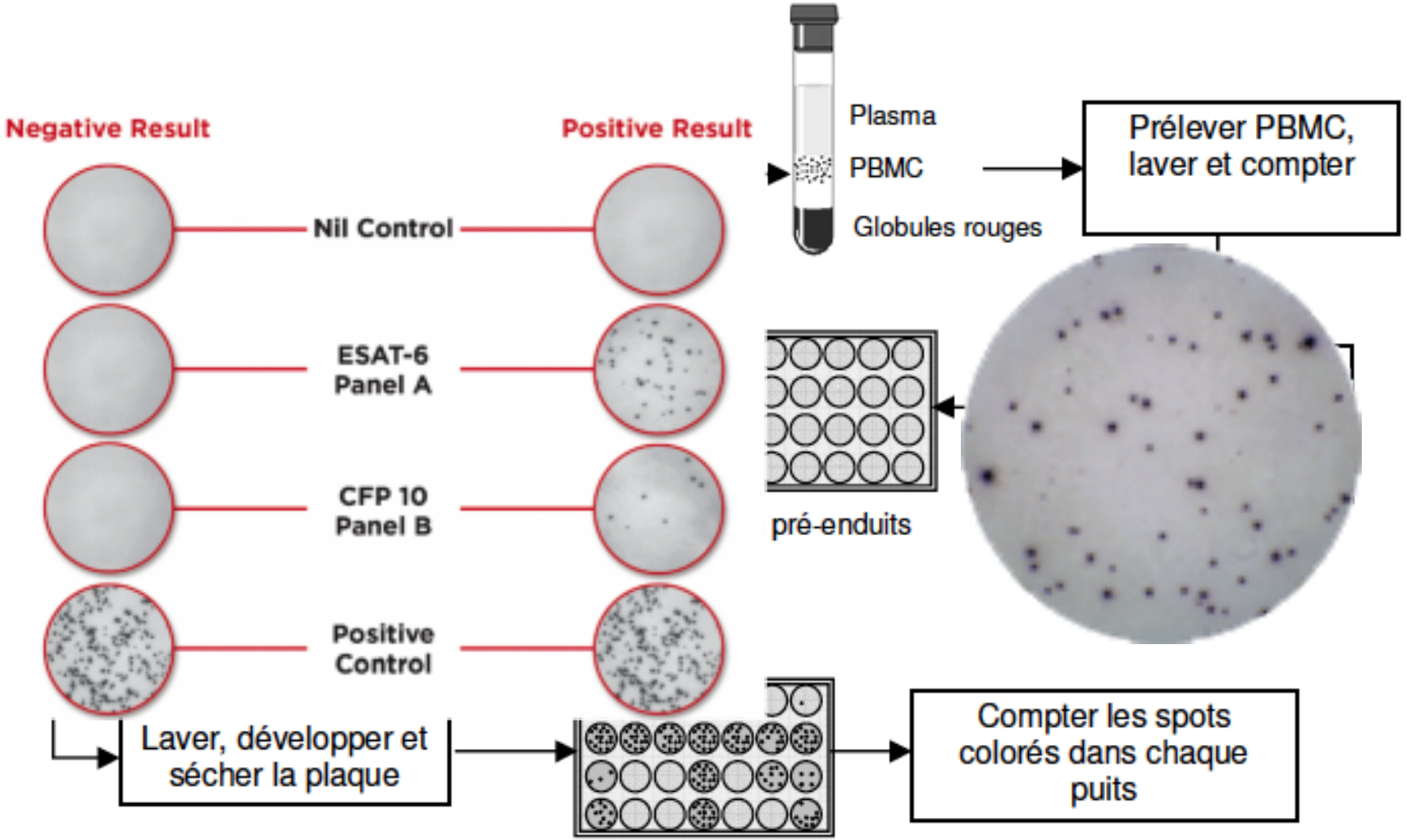


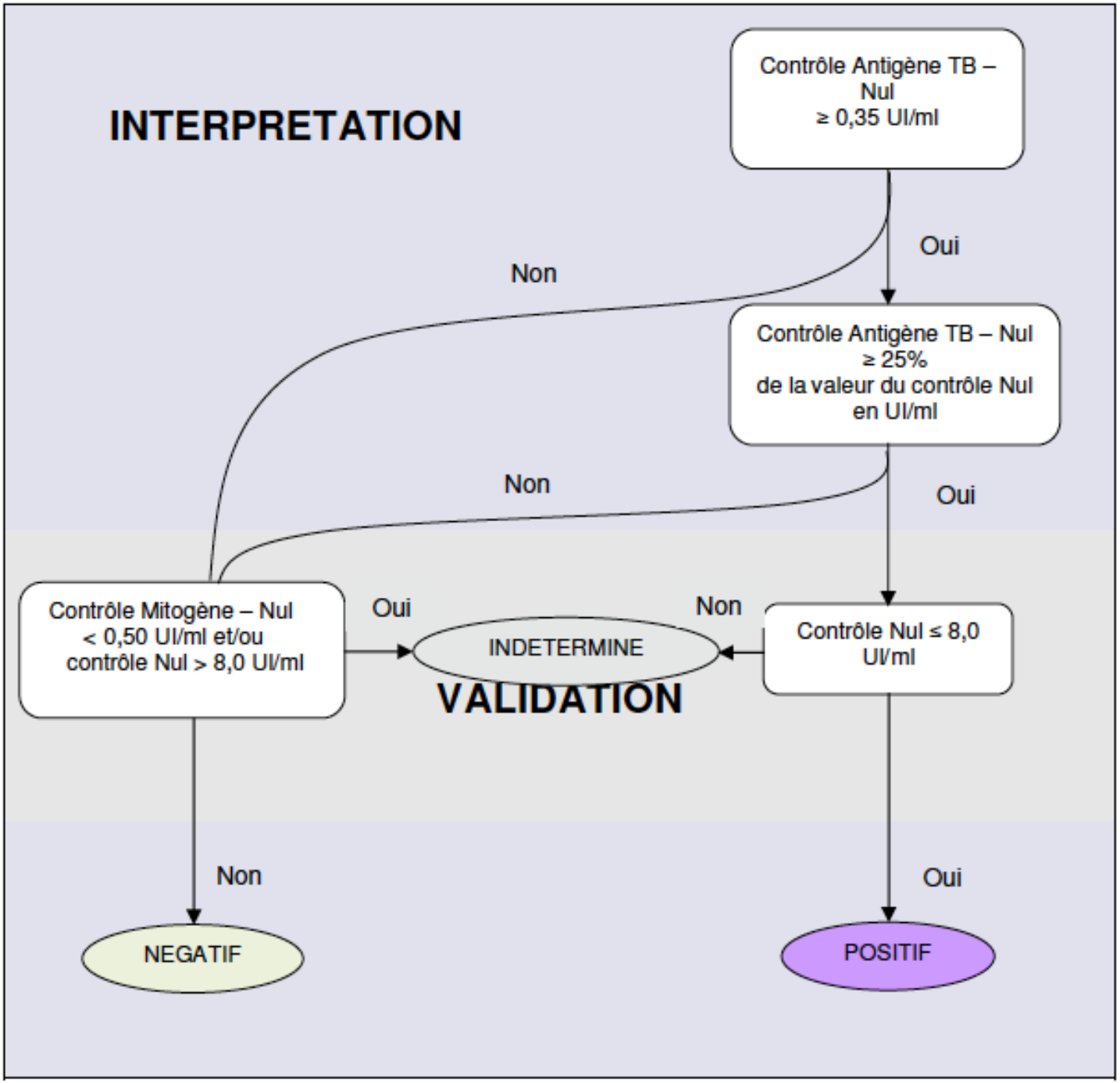


4) Wash then add Substrate. OD reading after 30 min.

5) Calculation and results printing

# T-SPOT®.TB


Negative Result
Positive Result
Nil Control
ESAT-6
Panel A
CFP 10
Panel B
Positive
Control
Plasma
PBMC
Globules rouges
Prélever PBMC,
laver et compter
pré-enduits
Laver, développer et
sécher la plaque
Compter les spots
colorés dans chaque
puits


INTERPRETATION
Contrôle Antigène TB – Nul
≥ 0,35 UI/ml
Oui
Non
Contrôle Antigène TB – Nul
≥ 25%
de la valeur du contrôle Nul
en UI/ml
Non
Oui
Contrôle Mitogène – Nul
< 0,50 UI/ml et/ou
contrôle Nul > 8,0 UI/ml
Oui
INDETERMINE
Non
Contrôle Nul ≤ 8,0
UI/ml
VALIDATION
Non
Oui
NEGATIF
POSITIF

# M.Tuberculosis antigens in IGRA

| complex | | | | | | | |
|---|---|---|---|---|---|---|---|
| *M. tuberculosis* | + | + | + | *M. Abcessus* | - | - | + |
| *M. africanum* | + | + | + | *M. avium* | - | - | + |
| *M. bovis* | + | + | + | *M. branderi* | - | - | + |
| | | | | *M. celatum* | - | - | + |
| BCG strains | ESAT-6 | CFP-10 | TST | *M. chelonae* | - | - | + |
| *Gothenberg* | - | - | + | *M. fortuitum* | - | - | + |
| *Moreau* | - | - | + | *M. gordonii* | - | - | + |
| *Tice* | - | - | + | *M. intracellulare* | - | - | + |
| *Tokyo* | - | - | + | ***M. kansasii*** | + | + | + |
| *Danish* | - | - | + | *M. malmoense* | - | - | + |
| *Glaxo* | - | - | + | ***M. marinum*** | + | + | + |
| *Montréal* | - | - | + | *M. oenavense* | - | - | + |
| *Pasteur* | - | - | + | *M. scrofulaceum* | - | - | + |
| | | | | *M. smegmatis* | - | - | + |
| | | | | ***M. szulgai*** | + | + | + |
| | | | | *M. terra* | - | - | + |

# Interpretation

- **Negative QFT**
  - Latent TB infection very unlikely
  - A negative result allows to eliminate LTBI with a probability close to 100% (NPV of 99.7%*)

- **Positive QFT**
  - Latent TB infection very likely
  - In addition to clinical examination and anamnesis, a positive result allows to orient diagnosis toward a recent or old Latent TB infection (Specificity of 98%)

- *An indeterminate QFT result means that the patient's immune system is weakened (immunocompromised). This kind of result is very informative and must orient toward a specific patient management. An indeterminate result can also be the consequence of sample mishandling which need to be verify before interpretation.*

# Immunological tests :TST /IGRA test

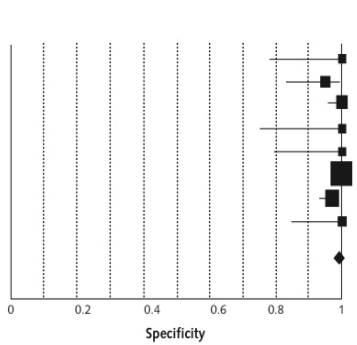
| | | | |
|---|---|---|---|
| Antigens used in the test | *Tuberculin: more than 200 antigens* | *ESAT6, CFP10* | • **Specific to *M. tuberculosis*** <br> • **No cross-reactivity with BCG** |
| Patient Management | *Day 1: Injection* <br> *Day 3: Reading* | *Only one sampling* <br> *Only one visit* | • **No loss of patients** |
| Type of test | *In vivo testing* <br> *Operator dependent* | *In vitro testing* <br> *Single blood sampling* <br> *Standardized reading* <br> *Quality controls included* | • **No booster effect** <br> • **Reproducibility** <br> • **Accuracy** <br> • **Possible automation** |
| Controls | *None* | *Internal negative and positive controls ( Nil, Mitogen)* | • **Avoid false negative results** |
| Positive threshold | *Different thresholds* | *Unique threshold defined at 0.35 IU/mL* | • **Better precision** <br> • **Specificity of 98%** |

Sources:

1 – Diel et al, Performance of IGRAs and the TST: *A new and up-to-date Meta-Analysis, Chest 2009*

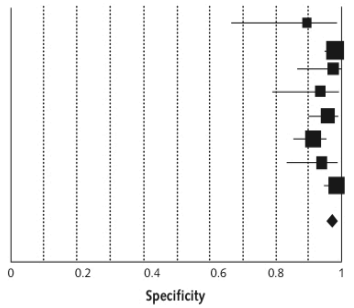
*2 – Cheallaigh 2013*

# Impact of BCG vaccination on Interferon-γ Assays



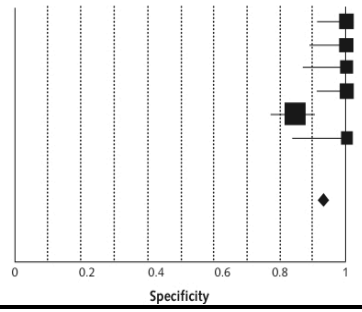

| Study, Year (Reference) | Specificity (95% CI) | Patients, *n/n* |
|---|---|---|
| Brock et al., 2001 (35) | 1.00 (0.78–1.00) | 15/15 |
| Brock et al., 2004 (36) | 0.95 (0.83–0.99) | 38/40 |
| Taggart et al., 2006 (37) | 1.00 (0.96–1.00) | 81/81 |
| Palazzo et al., 2008 (24) | 1.00 (0.75–1.00) | 13/13 |
| Bua et al., 2007 (18) | 1.00 (0.79–1.00) | 16/16 |
| Mazurek et al., 2007 (38) | 1.00 (0.99–1.00) | 543/544 |
| Franken et al., 2007 (39) | 0.97 (0.93–0.99) | 166/171 |
| Detjen et al., 2007 (25) | 1.00 (0.85–1.00) | 22/22 |

Pooled specificity = 0.99 (0.98–1.00)
Chi-square = 15.88; $P = 0.026$
Inconsistency $I^2 = 55.9\%$




| Study, Year (Reference) | Specificity (95% CI) | Patients, *n/n* |
|---|---|---|
| Brock et al., 2001 (35) | 0.89 (0.67–0.99) | 17/19 |
| Mori et al., 2004 (7) | 0.98 (0.95–0.99) | 209/213 |
| Ravn et al., 2005 (9) | 0.97 (0.87–1.00) | 38/39 |
| Brock et al., 2004 (36) | 0.94 (0.79–0.99) | 30/32 |
| Kang et al., 2005 (10) | 0.96 (0.90–0.99) | 95/99 |
| Lee et al., 2006 (11) | 0.92 (0.85–0.96) | 120/131 |
| Kobashi et al., 2006 (15) | 0.94 (0.83–0.99) | 47/50 |
| Soborg et al., 2007 (40) | 0.99 (0.95–1.00) | 137/139 |

Pooled specificity = 0.96 (0.94–0.98)
Chi-square = 13.81; $P = 0.055$
Inconsistency $I^2 = 49.3\%$




| Study, Year (Reference) | Specificity (95% CI) | Patients, *n/n* |
|---|---|---|
| Lalvani et al., 2001 (41) | 1.00 (0.91–1.00) | 40/40 |
| Pathan et al., 2001 (42) | 1.00 (0.89–1.00) | 32/32 |
| Lalvani et al., 2001 (43) | 1.00 (0.87–1.00) | 26/26 |
| Chapman et al., 2002 (44) | 1.00 (0.91–1.00) | 40/40 |
| Lee et al., 2006 (11) | 0.85 (0.77–0.90) | 111/131 |
| Detjen et al., 2007 (25) | 1.00 (0.84–1.00) | 21/21 |

Pooled specificity = 0.93 (0.86–1.00)
Chi-square = 33.60; $P < 0.001$
Inconsistency $I^2 = 85.1\%$

No BCG vaccination (8 studies)

Quantiferon TB-Gold ©

Quantiferon-TB Gold in tube©

**Specificity 99% (95-100%)**

BCG Vaccination (8 studies)

Quantiferon TB-Gold ©

Quantiferon-TB Gold in tube©

**Specificity 96% (89-99%)**

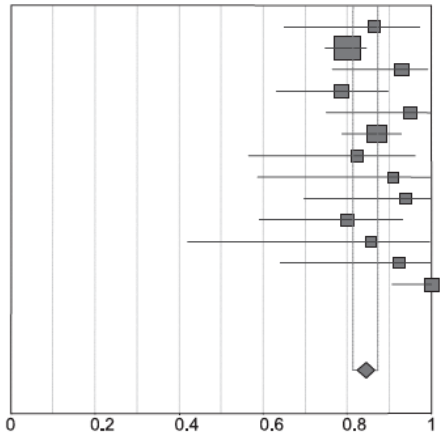
BCG or not (6 studies )

T-SPOT.TB ©

**Specificity 93% (85-100%)**

Pai M. Ann Intern Med 2008; 149:177-184

# IGRA sensitivity in active tuberculosis




| Study, Year [Reference] | Sensitivity (95% CI) | | Patients (n/n) |
|---|---|---|---|
| Bartu et al. 2008[15] | 0.86 | (0.65 - 0.97) | 19/20 |
| Chee et al. 2008[35] | 0.80 | (0.75 - 0.85) | 224/280 |
| Detjen et al. 2007[10] | 0.93 | (0.76 - 0.99) | 26/28 |
| Dominguez et al. 2008[28] | 0.79 | (0.63 - 0.90) | 33/42 |
| Gerogianni et al. 2008[18] | 0.95 | (0.75 - 1.00) | 19/20 |
| Harada et al. 2008[36] | 0.87 | (0.79 - 0.93) | 87/100 |
| Palazzo et al. 2008[22] | 0.82 | (0.57 - 0.96) | 14/17 |
| Aichelburg et al. 2009[38] | 0.91 | (0.59 - 1.00) | 10/11 |
| Bianchi et al. 2009[16] | 0.94 | (0.70 - 1.00) | 15/16 |
| Kampmann et al. 2009[25] | 0.80 | (0.59 - 0.93) | 20/25 |
| Lighter et al. 2009[40] | 0.86 | (0.42 - 1.00) | 6/7 |
| Markova et al. 2009[41] | 0.92 | (0.64 - 1.00) | 12/13 |
| Sauzullo et al. 2009[42] | 1.00 | (0.91 - 1.00) | 38/38 |

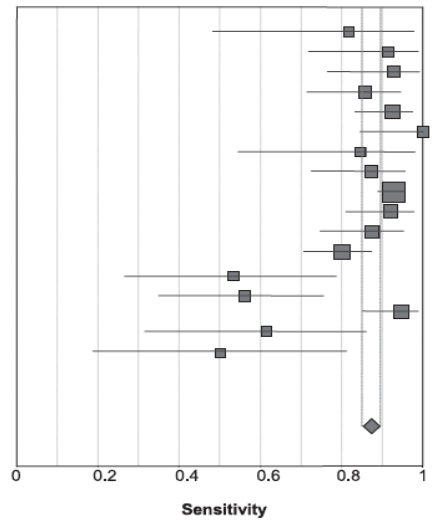
Pooled Sensitivity = 0.84 (0.81 to 0.87)
Chi-square = 25.25; d.f. = 12 (p = 0.0137)
Inconsistency (I-square) = 52.5 %

13 studies

Quantiferon-TB® Gold in tube

Sensitivity from 80 to 100%

**Sensitivity 84% (81-87%)**




| Study, Year (Reference) | Sensitivity (95% CI) | | Patients (n/n) |
|---|---|---|---|
| Ferrara et al. 2006[5] | 0.82 | (0.48 - 0.98) | 9/11 |
| Goletti et al. 2006[43] | 0.91 | (0.72 - 0.99) | 21/23 |
| Detjen et al. 2007[10] | 0.93 | (0.76 - 0.99) | 26/28 |
| Dominguez et al. 2008[28] | 0.86 | (0.71 - 0.95) | 36/42 |
| Kang et al. 2007[11] | 0.93 | (0.83 - 0.98) | 62/67 |
| Kim et al. 2007[12] | 1.00 | (0.85 - 1.00) | 22/22 |
| Vincenti et al. 2007[14] | 0.84 | (0.55 - 0.98) | 11/13 |
| Wang et al. 2007[44] | 0.87 | (0.73 - 0.96) | 34/39 |
| Chee et al. 2008[35] | 0.93 | (0.89 - 0.96) | 254/274 |
| Higuchi et al. 2009[45] | 0.92 | (0.81 - 0.98) | 47/51 |
| Kobashi et al. 2008[22] | 0.88 | (0.75 - 0.95) | 42/48 |
| Soysal et al. 2008[23] | 0.80 | (0.71 - 0.87) | 80/100 |
| Warier et al. 2009[46] | 0.53 | (0.27 - 0.79) | 8/15 |
| Kampmann et al. 2009[25] | 0.56 | (0.35 - 0.76) | 14/25 |
| Kim et al. 2009[47] | 0.95 | (0.85 - 0.99) | 53/56 |
| Markova et al. 2009[41] | 0.62 | (0.32 - 0.86) | 8/13 |
| Nicol et al. 2009[27] | 0.50 | (0.19 - 0.81) | 5/10 |

Pooled Sensitivity = 0.875 (0.85 to 0.90)
Chi-square = 65.59; d.f. = 16 (p = 0.0000)
Inconsistency (I-square) = 75.6 %

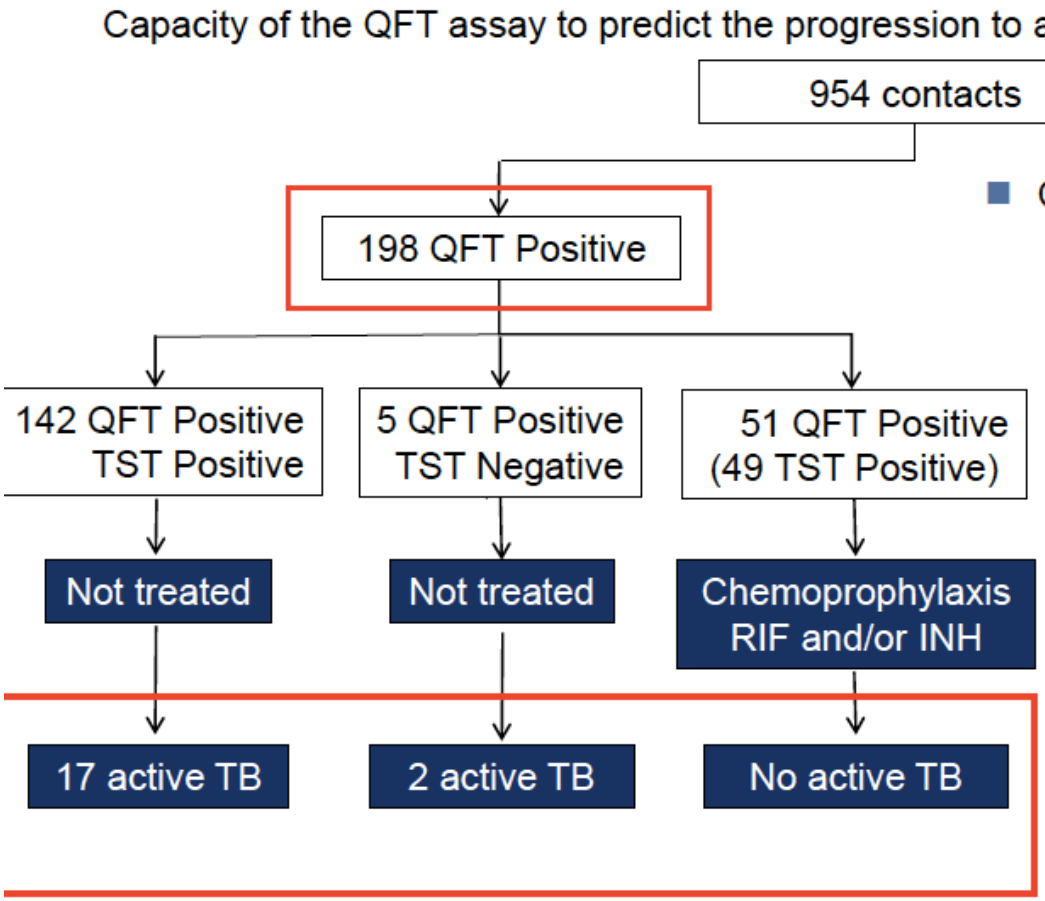
17 studies

T-SPOT.TB®

Sensitivity from 50 to 100% **Sensitivity 93% (85-100%)**

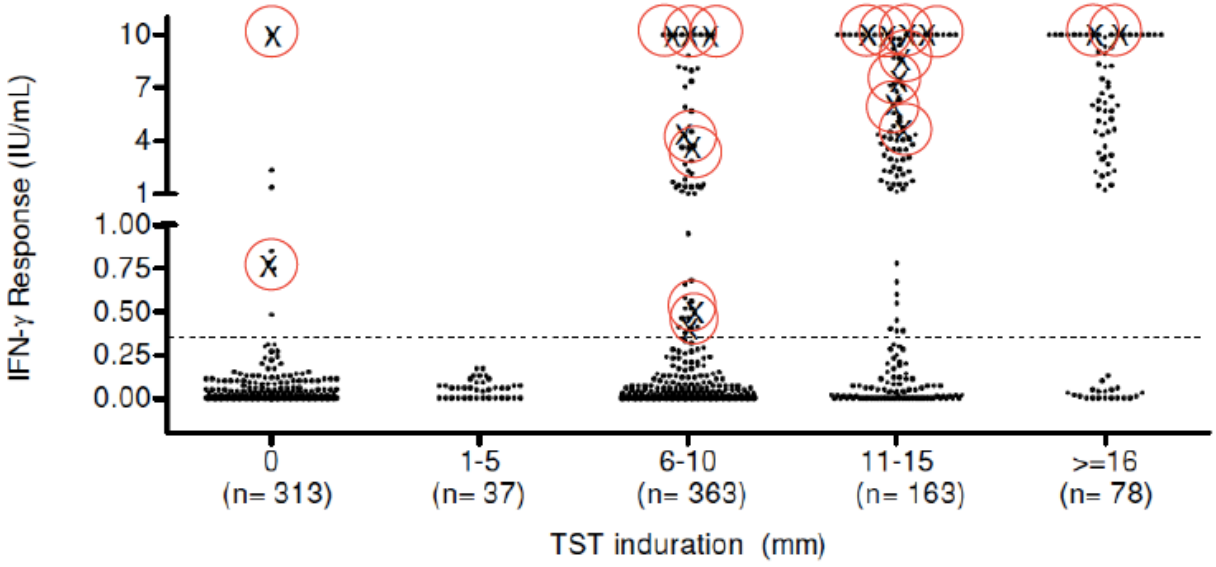
Diel R. Chest 2010

# IGRA Predictive value /TST ( Diel 2010 )




- Contacts with a positive QFT result
  - All 19 active TB were QFT positive
  - Active TB not detected by TST in relation to cut-off value:
    - **2** missed TB at ∅ >5 mm
    - **9** missed TB at ∅ >10 mm
    - **17** missed TB at ∅ >15 mm

# Predictive value of IGRA /TST



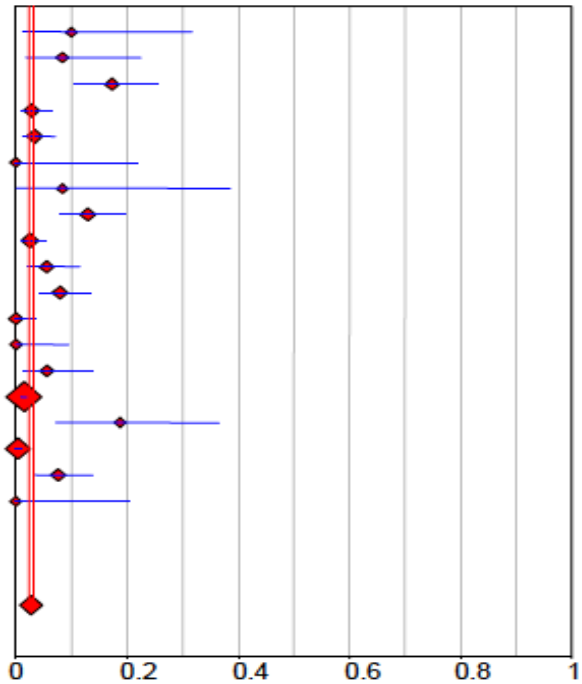

**NPV = 100%**

**Progression rate = 12.9%**

**Figure 2:** Comparison of the level of responses for QFT and TST for the 954 subjects with both results available. The QFT response is the level of IFN-γ (IU/mL) in the TB-Antigen stimulated plasma sample with that for the Nil control subtracted. The 19 individuals who developed TB disease are marked by X. Responses ≥ 10 IU/mL for the QFT assay are shown as 10 IU/mL. The dotted line represents the 0.35 IU/mL cutoff for the QFT test.

# PPV for progression commercial IGRA s

**19 studies**
**5194 persons**
**141 tuberculosis cases**
**VPP : 2,7% (IC 95% 2,3-3,2)**




PPV for progression commercial IGRAs

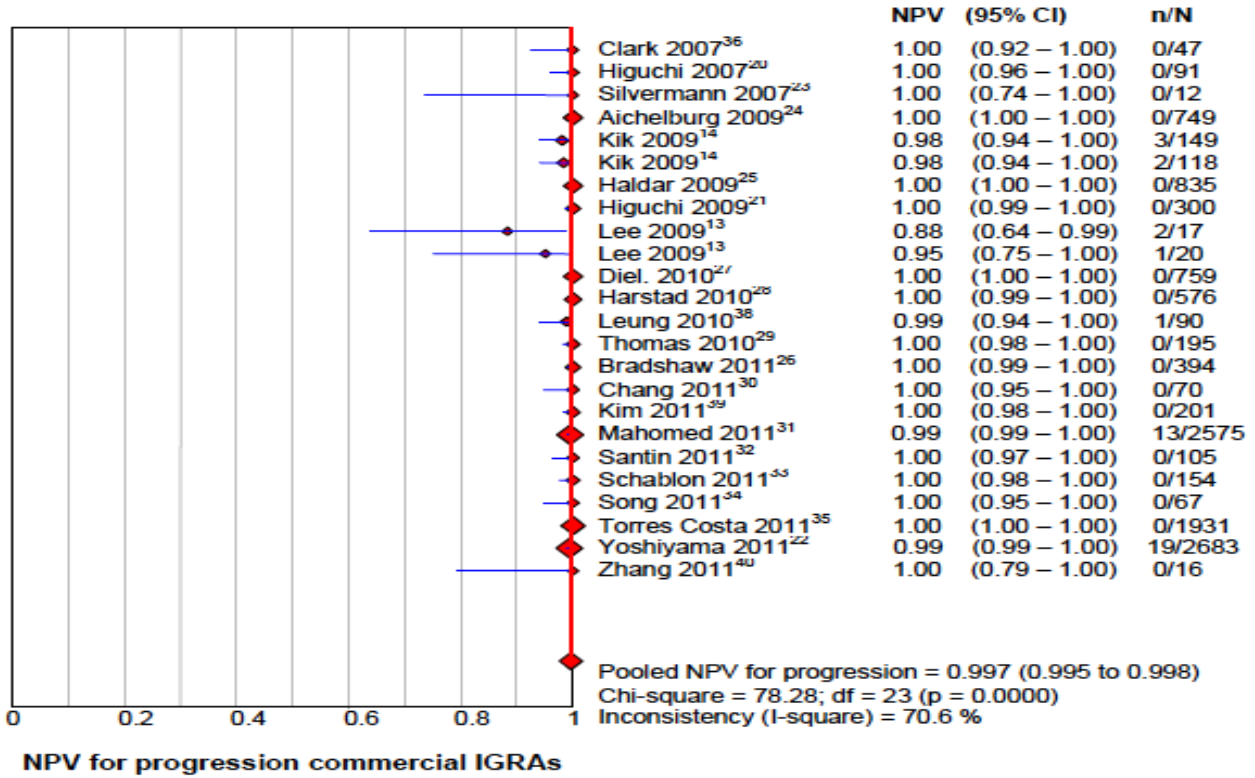
| | PPV | (95% CI) | n/N |
|---|---|---|---|
| Clark 2007[36] | 0.10 | (0.01 – 0.32) | 2/20 |
| Aichelburg 2009[24] | 0.08 | (0.02 – 0.22) | 3/36 |
| Haldar 2009[25] | 0.17 | (0.11 – 0.26) | 19/110 |
| Kik 2009[14] | 0.03 | (0.01 – 0.06) | 5/178 |
| Kik 2009[14] | 0.03 | (0.01 – 0.07) | 6/181 |
| Lee 2009[13] | 0.00 | (0.00 – 0.22) | 0/15 |
| Lee 2009[13] | 0.08 | (0.00 – 0.38) | 1/12 |
| Diel 2010[27] | 0.13 | (0.08 – 0.19) | 19/147 |
| Harstad 2010[28] | 0.03 | (0.01 – 0.05) | 6/238 |
| Jonnalaggada 2010[37] | 0.05 | (0.02 – 0.11) | 6/110 |
| Leung 2010[38] | 0.08 | (0.04 – 0.13) | 12/151 |
| Thomas 2010[29] | 0.00 | (0.00 – 0.03) | 0/107 |
| Bradshaw 2011[26] | 0.00 | (0.00 – 0.09) | 0/37 |
| Kim 2011[39] | 0.06 | (0.02 – 0.14) | 4/71 |
| Mahomed 2011[31] | 0.01 | (0.01 – 0.02) | 39/2669 |
| Song 2011[34] | 0.19 | (0.07 – 0.36) | 6/32 |
| Torres Costa 2011[35] | 0.00 | (0.00 – 0.01) | 4/945 |
| Yoshiyama 2011[22] | 0.08 | (0.04 – 0.14) | 9/119 |
| Zhang 2011[40] | 0.00 | (0.00 – 0.21) | 0/16 |

Pooled PPV for progression = 0.027 (0.023 to 0.032)
Chi-square = 171.38; df = 18 (p = 0.0000)
Inconsistency (I-square) = 89.5 %

Diel R. Chest 2012

# NPV for progression commercial IGRAs

**24 studies**
**12154 persons**
**41 tuberculosis cases**
**VPN : 99,7% (IC 95% 99,5-99,8)**




Diel R. Chest 2012

# Comparaison PPV IGRA &TST

Predictive positive value among high risk patients

IGRA
98 tb cases / 1436 IGRA +, 6,8%

TST
80 Tb cases / 3391 IDR +,2,4%

P<0,0001

Diel R. Chest 2012

# Prognostic value of interferon-γ release assays and tuberculin skin test in predicting the development of active tuberculosis (UK PREDICT TB): a prospective cohort study

- Between May 4, 2010, and June 1, 2015, 10 045 people were recruited, of whom 9610 were eligible for inclusion. Of this cohort, 4861 (50.6%) were contacts and 4749 (49.4%) were migrants.
- Participants were followed up for a median of 2.9 years (range 21 days to 5.9 years). 97 (1.0%) of 9610 participants developed active tuberculosis (77 [1.2%] of 6380 with results for all three tests).

- In all tests, annual incidence of tuberculosis was very low in those who tested negatively (ranging from 1.2 per 1000 person-years, 95% CI 0.6–2.0 for TST-5 to 1.9 per 1000 person-years,95% CI 1.3–2.7, for QuantiFERON-TB Gold In-Tube).

- Annual incidence in participants who tested positively were highest for T-SPOT.TB (13.2 per 1000 person-years, 95% CI 9.9–17.4), TST-15 (11.1 per 1000 person-years, 8.3–14.6),and QuantiFERON-TB Gold In-Tube (10.1 per 1000 person-years, 7.4–13.4). Positive results for these tests were significantly better predictors of progression than TST-10 and TST-5 (eg, ratio of test positivity rates in those progressing to tuberculosis compared with those not progressing T-SPOT.TB vs TST-5: 1.99, 95% CI 1.68–2.34; p<0.0001).
- However, TST-5 identified a higher proportion of participants who progressed to active tuberculosis (64 [83%] of 77 tested) than all other tests and TST thresholds (≤75%).

Lancet Infect Dis 2018;
18: 1077–87

Ibrahim Abubakar, Francis Drobniewski, Jo Southern, Alice J Sitch, Charlotte Jackson, Marc Lipman, Jonathan J Deeks, Chris Griffiths,
Graham Bothamley, William Lynn, Helen Burgess, Bobby Mann, Ambreen Imran, Saranya Sridhar, Chuen-Yan Tsou, Vladyslav Nikolayevskyy,
Melanie Rees-Roberts, Hilary Whitworth, Onn Min Kon, Pranab Haldar, Heinke Kunst, Sarah Anderson, Andrew Hayward, John M Watson,
Heather Milburn, Ajit Lalvani on behalf of the PREDICT Study Team*UK predict Tb

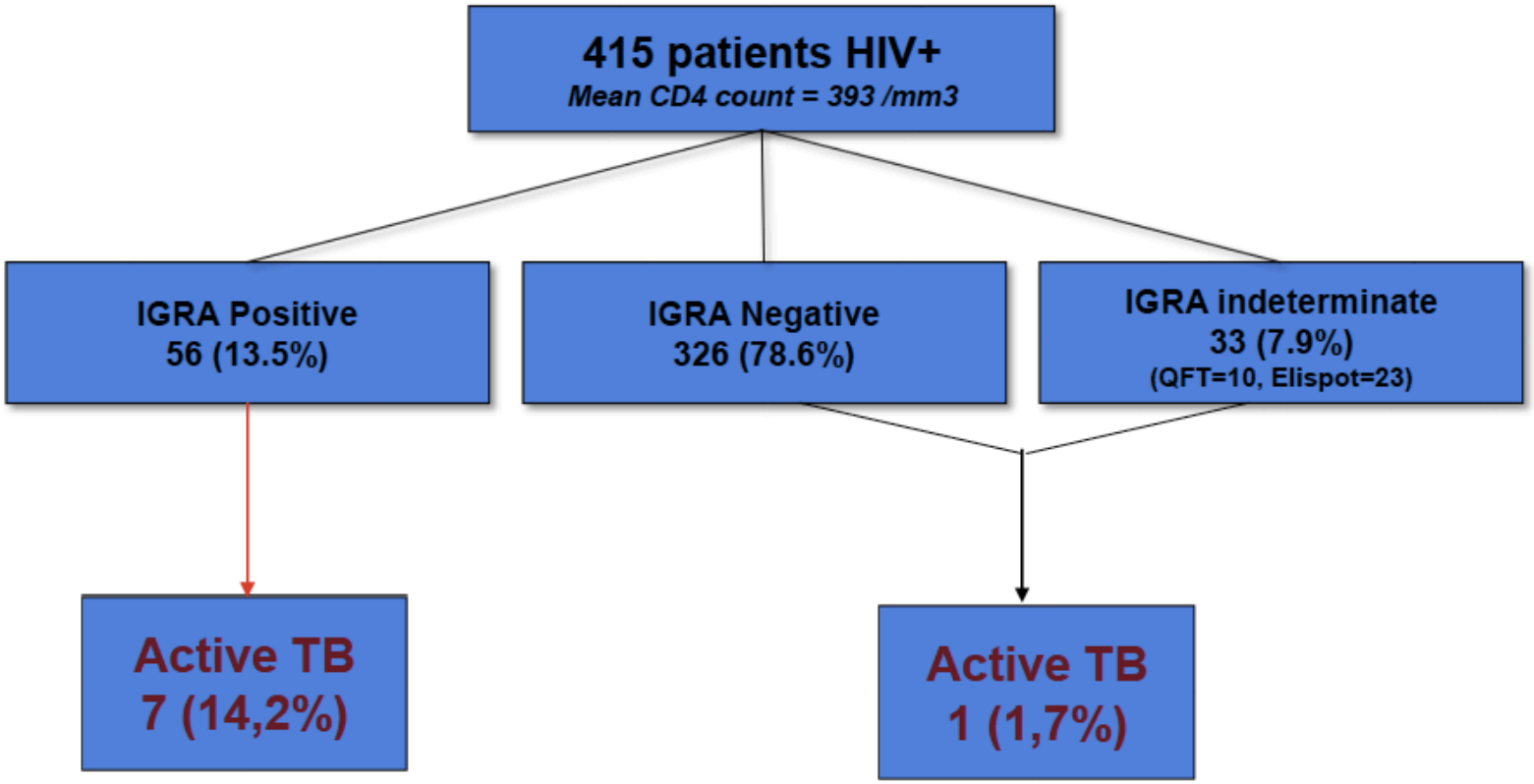
| | TST-5 | TST-10 | TST-15 | T-SPOT.TB | QuantiFERON-TB Gold In-Tube |
|---|---|---|---|---|---|
| TST-5 | -- | 1·25 (1·15–1·36; <0·0001) | 1·64 (1·44–1·87; <0·0001) | 1·99 (1·68–2·34; <0·0001) | 1·52 (1·26–1·83; <0·0001) |
| TST-10 | -- | -- | 1·31 (1·617–1·47; <0·0001) | 1·59 (1·34–1·88; <0·0001) | 1·21 (1·01–1·46; 0·041) |
| TST-15 | -- | -- | -- | 1·21 (1·01–1·43; 0·037) | 0·93 (0·76–1·13; 0·453) |
| T-SPOT.TB | -- | -- | -- | -- | 0·77 (0·66–0·89; 0·0003) |

Values indicate the ratio of test positivity rates (with 95% CI and p values) in participants who progressed to active tuberculosis compared with those who did not comparing test A (horizontally across table) with test B (vertically up table). A value above 1 indicates a positive result on test A is a stronger predictor of progression to tuberculosis than a positive result on test B. TST=tuberculin skin test. TST-5=TST with threshold ≥5 mm. TST-10=TST with threshold ≥10 mm. TST-15=BCG-dependent definition of TST: ≥15 mm for BCG-vaccinated participant and >5 mm non-vaccinated participant.

*Table 4:* **Predictive value of tests by pairwise comparisons for progression to tuberculosis**

# Performance of QFT for LTBI screening in HIV + patients ( Bourgarit)

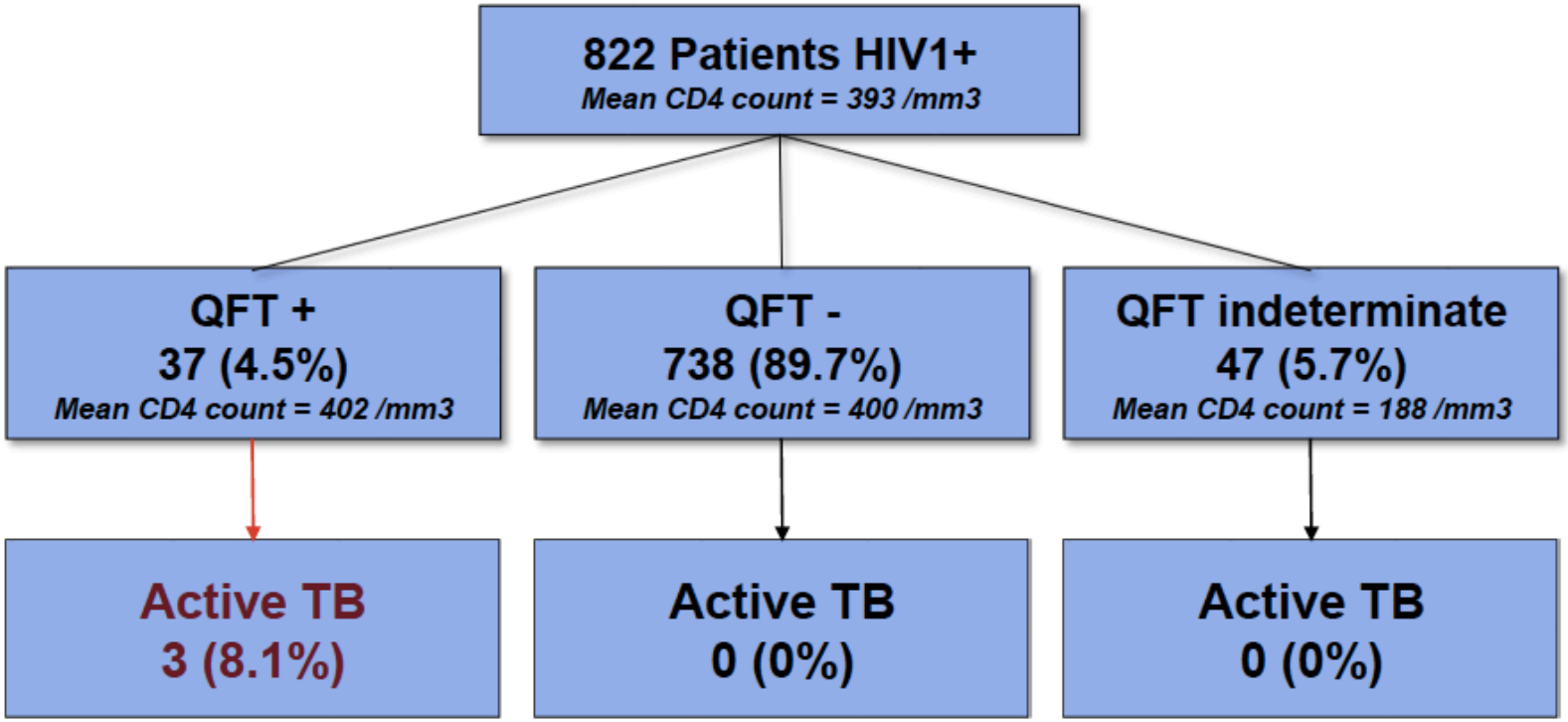
- Relative risk to develop an active TB is 44 *(IC 0,95% [5,5;351,0])*



# Performance of QFT for LTBI screening in HIV + patients

Adapted from Aichelburg, Clinical Infectious Diseases, 2009

- 73.8% of QFT+ had a positive TST, and 26.2% has a negative TST, without difference of CD4 count.

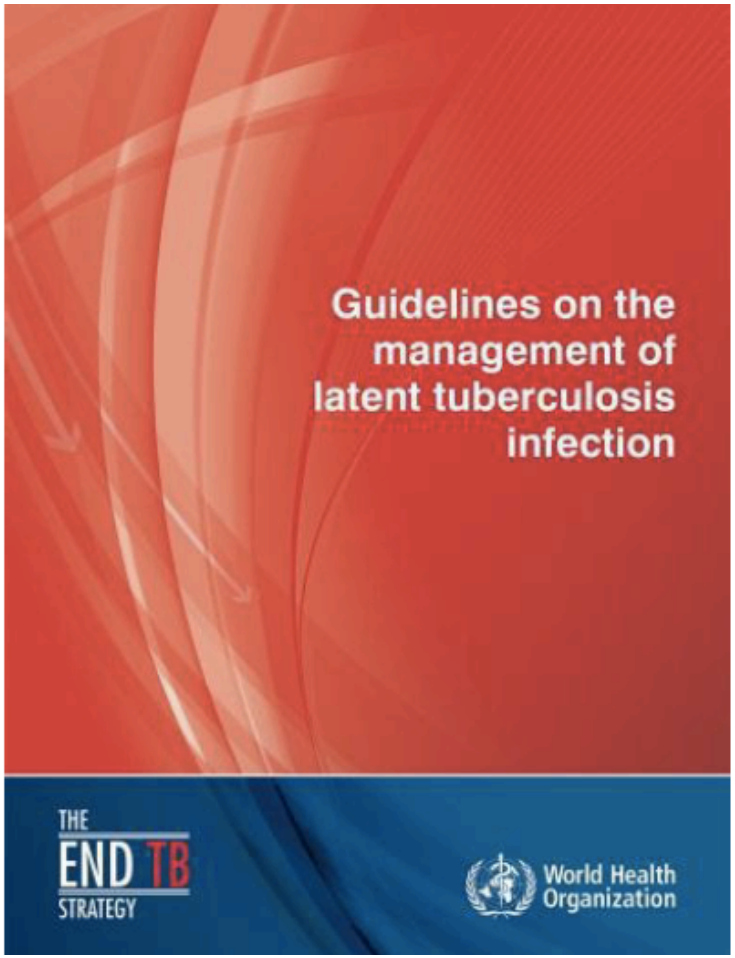



Median of follow-up [IQR]: 19 months [12-21]

# WHO guidelines on LTBI screening

| LTBI testing should be **performed** in | LTBI testing should be **considered** for | LTBI testing conditional |
|---|---|---|
| People living with HIV | ▪ Prisoners | ▪ People with harmful alcohol use and tobac smokers |
| Adult and child contacts of pulmonary TB cases | ▪ Health Care Workers | ▪ Underweight people |
| Patients initiating anti-TNFα treatment | ▪ Immigrants from high TB burden countries | ▪ People with diabetes |
| Patients receiving dialysis | ▪ Homeless persons | *UNLESS they fall into or of the other categories |
| SOT or HPSCT patients | ▪ Illicit drug users | |
| Patients with silicosis | | |

# WHO recommandations




## LTBI TESTING

**Should be performed** in people living with HIV, adult and child contacts of pulmonary TB cases, patients initiating anti-tumour necrosis factor (TNF) treatment, patients receiving dialysis, patients preparing for organ or hematologic transplantation, and patients with silicosis.

**Should be considered** for prisoners, health-care workers, immigrants from high TB burden countries, homeless persons and illicit drug users.

**Is not recommended** in people with diabetes, people with harmful alcohol use, tobacco smokers, and underweight people provided they are not already included in the above recommendations.

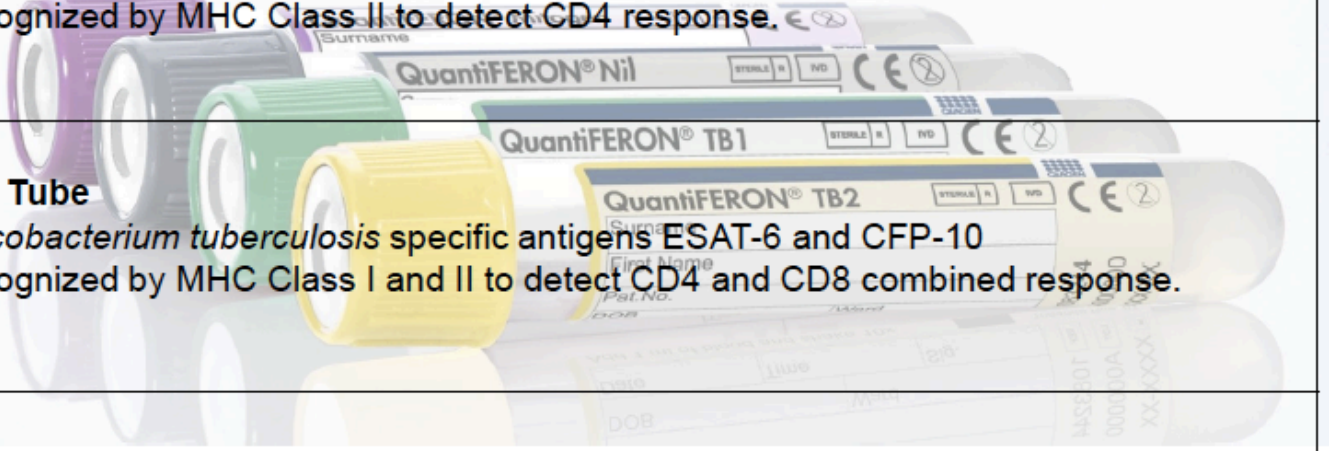
Is it possible to predict better which individuals infected with mycobacterium tuberculosis wil develop tuberculosis disease ?

# Quantiferon plus test

- Negative control
- Allows adjustment for background noise.

**TB1 ANTIGEN Tube**

- Includes *Mycobacterium tuberculosis* specific antigens ESAT-6 and CFP-10
- Peptides recognized by MHC Class II to detect CD4 response.




**TB2 ANTIGEN Tube**

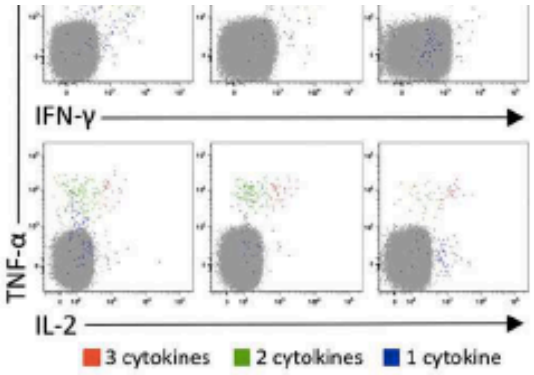
- Includes *Mycobacterium tuberculosis* specific antigens ESAT-6 and CFP-10
- Peptides recognized by MHC Class I and II to detect CD4 and CD8 combined response.

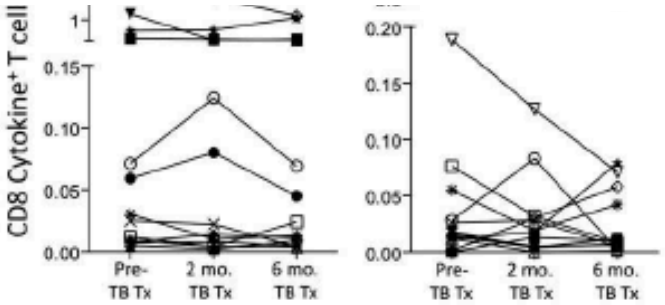
**Mitogen Tube**

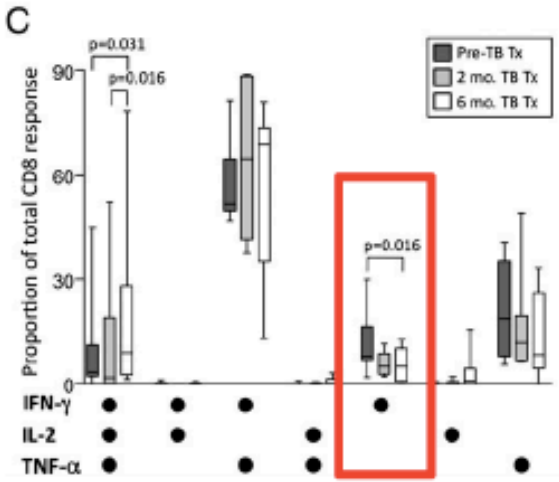
- Positive control
- Includes PHA and allows to check the functionality of the immune system
- Objectives:
  - To identify individuals with weakened immune system

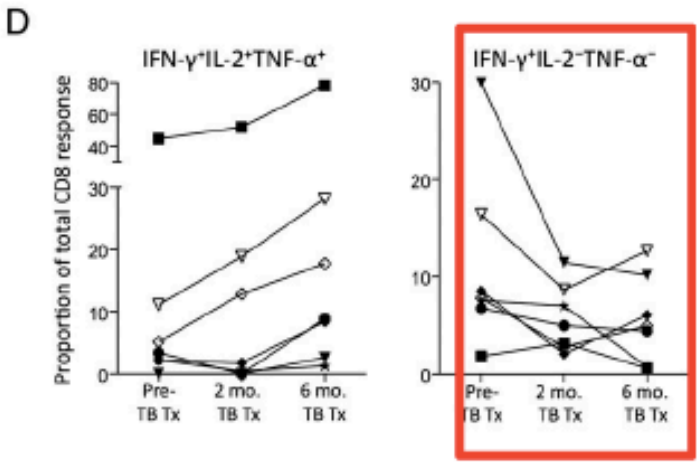
# CD8 + rate decreases with specific treatment in active tuberculosis

*Day et al, J Immunol 2011*



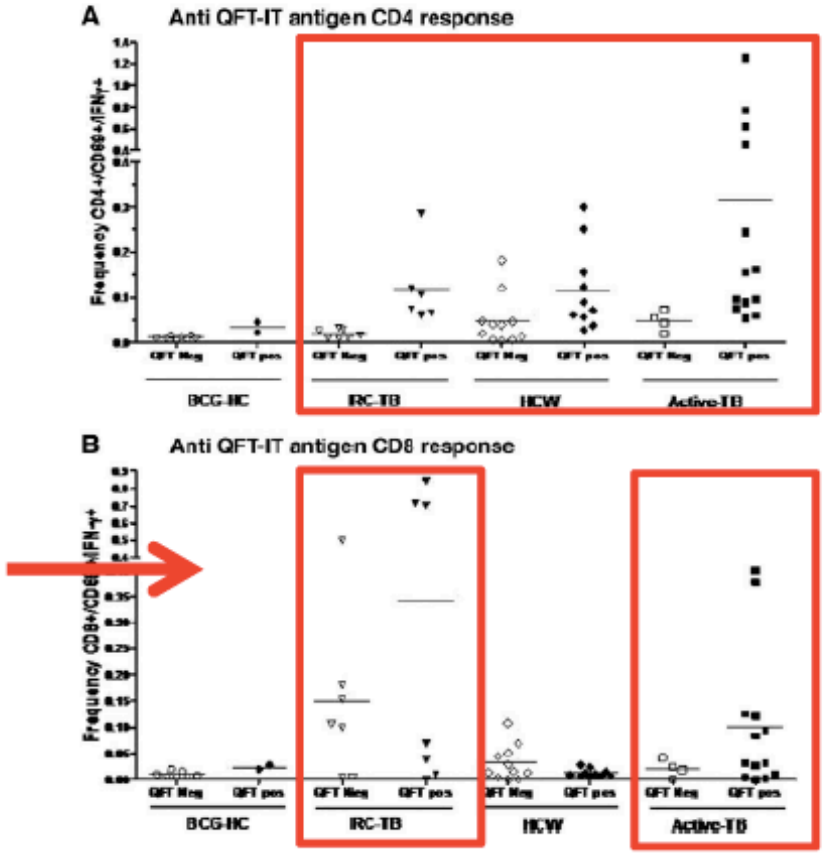



# CD8 reponse association with Recent exposure to active Tb

*Nikolova et al, Diagn Microb Inf Dis, 2013*

CD4

CD8



# Difference between TB2-TB1 > 0,6 UI/ml a marker for recent infection

*Pieterman et al, Tuberculosis, 2018*

| Test indication | IFN-γ > 0.6 IU/mL (% within positive results) |
|---|---|
| Tuberculosis infection in differential diagnosis | 7 (17%) |
| Contact investigation | 18 (33%) |
| Screening before immunotherapy | 2 (11%) |
| Periodic check by occupational health services | 3 (33%) |
| Other[a] | 2 (15%) |
| Unknown | 4 (33%) |
| Total | 36 |

[a] Screening of immigrants, screening of homeless, employment medical examination, other.

# Antigen specific CD8 + T cells in tuberculosis

Clear evidence for role of CD8+ T cells in *M. tuberculosis* (MTB) immunity

MTB-specific $CD8^+$ T cells secrete IFN-γ and other soluble factors to (1–3):

- Suppress MTB growth
- Kill infected cells
- Directly lyse intracellular MTB

TB-specific $CD8^+$ T cells that produce IFN-γ have been:

- More frequently detected in those with active TB disease vs. latent infection (4, 5)
- Associated with recent exposure to TB (6)
- Detectable in active TB subjects with HIV co-infection and young children (7, 8)
- Observed to decline when patients are exposed to anti-tuberculosis treatment (9)

# Evidence to suggest differential detection of CD4+ and CD8+ T cells during phases of MTB infection

| | |
|---|---|
| *"Antigen-specific CD4- and CD8-positive signatures in different phases of Mycobacterium tuberculosis infection"* | • Study demonstrates CD8+ T cell responses to QFT antigens are associated with recent infection<br>• Nikolova M. et al, 2013 |
| *"Mycobacterium tuberculosis-specific CD8+ T cells are functionally and phenotypically different between latent infection and active disease"* | • A study of 326 subjects with LTBI or active TB disease indicated CD8+ T cells in 60% active disease vs. 15% LTBI<br>• Rozot V. et al, 2013 |
| *"Mycobacterium tuberculosis specific CD8(+) T cells rapidly decline with anti-tuberculosis treatment"* | • Mtb specific CD8(+) T cell response declines with anti-tuberculosis treatment and could be a surrogate marker of response to therapy<br>• Nyendak M. et al, 2011 |

# QF Plus

**Enhanced performance**

- Increased sensitivity
- Sustained high specificity

**Improved performance in high-risk groups**

- People who are immunocompromised
- People living with HIV/AIDS

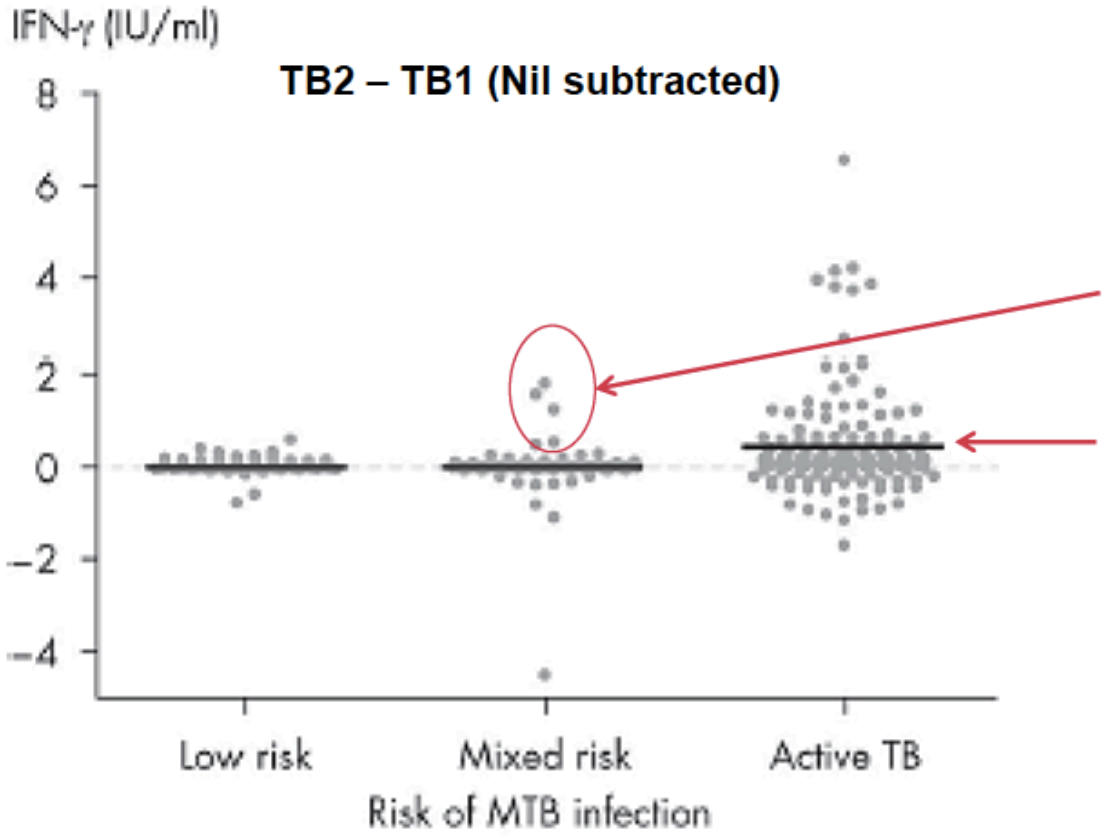
**Potential to provide additional clinical information**

- Risk-based algorithms
- Better assist patient assessment and management

**Harmonization of workflow options globally**

- 1-tube blood collection
- 4-point standard curve

## TB2 Minus TB1 (i.e. CD8/CD4 – CD4)




We suspect this is recent infection

Are they more likely to progress?

Does this information help inform clinical practice?

# TB2:TB1 differential as a surrogate measure for CD8 stimulation

Barcellini et al. (July 2016) report the differential between antigen tubes TB2 and TB1 as an functional marker for CD8 stimulation.

- 15% of 119 contacts had TB2-TB1 values >0.6 IU/mL
  - Significantly associated with proximity to the index case
    - p = 0.0029
  - Significantly associated with European origin
    - p = 0.043
    - Not significant for QFT-Plus overall results

"[QFT-Plus performance] suggests a role for the differential value between the two tubes **as a proxy for recent infection**."

# When to use IGRA tests

- Among contact persons ( recent exposure )
- Immunosuppressed / HIV, pre transplant, immmunosuppressive therapies ( anti TNF alpha)
- =>In order to identify people to treat for LTBI

- HCW s before employement ( as a reference)

# Who to treat to stop Tb ?

- Individuals with active Tb ( microbiological markers)
- Immunosuppressed individuals with Positive Igra test . QF + is more sensitive
- Recent IGRA test positive within 2 years ( 5 %)
- Children with positive IGRA test

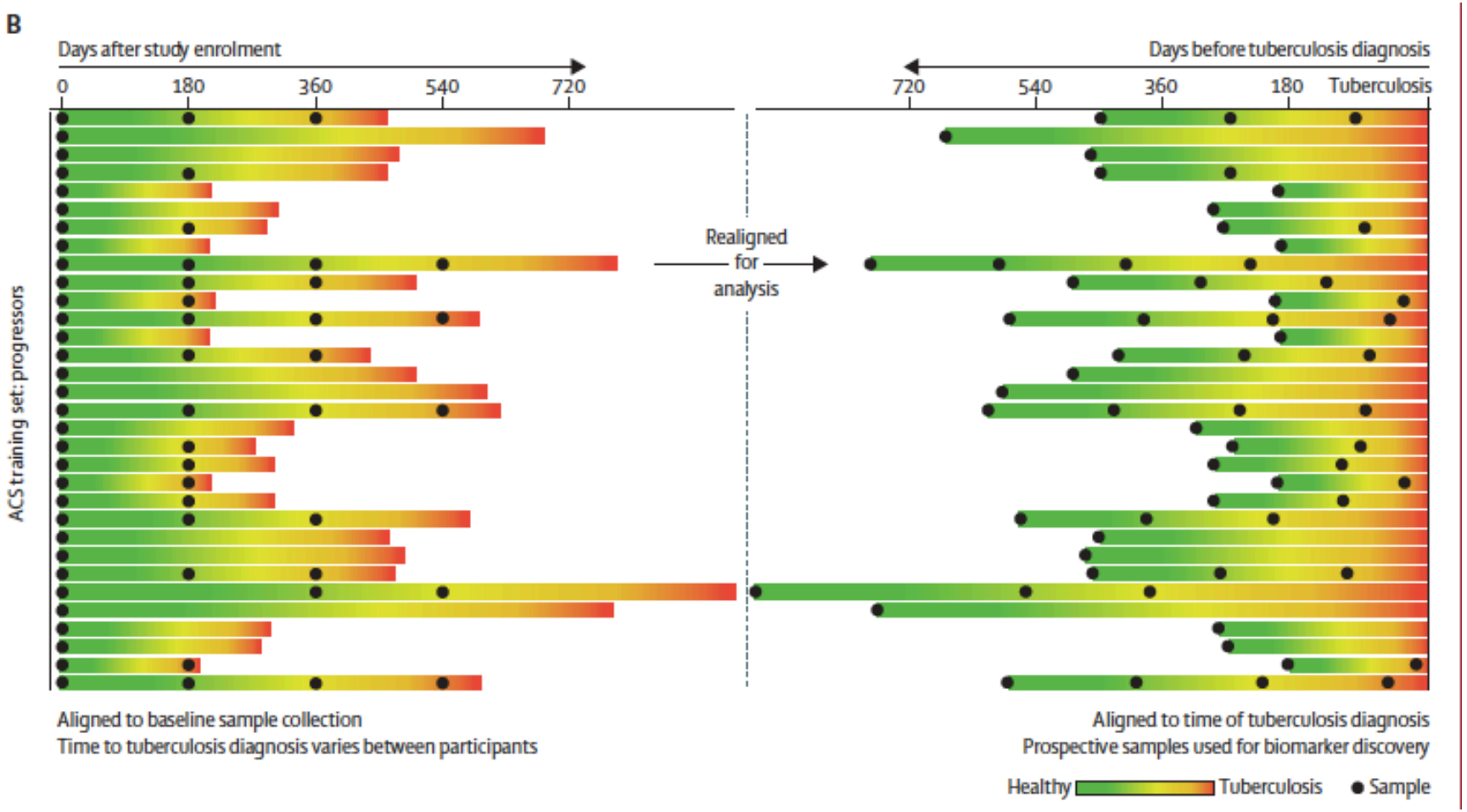
# New assays
# Transcriptional profiling

# Adolescent cohort study in South African adolescents with LTBI

- 16 gene signature identifying subjects as early as 18 months prior to the development of active TB
- CORTIS study , a prospective validation trial

- Further improvements in simplifying the host RNA signature to a 6-gene set is possible …

# RNA signature



# RNA signature sensitivity by days before tuberculosis diagnosis

| | ROC AUC (95% CI) | Sensitivity (95% CI) | Threshold |
|---|---|---|---|
| By 6 month period | | | |
| 1–180 | 0·79 (0·76–0·82) | 71·2% (66·6–75·2) | 61% |
| 181–360 | 0·771 (0·75–0·79) | 62·9% (59·0–66·4) | 61% |
| 361–540 | 0·726 (0·70–0·76) | 47·7% (42·9–52·5) | 61% |
| 541–720 | 0·540 (0·49–0·59) | 29·1% (23·1–35·9) | 61% |
| >720 | 0·496 (0·43–0·56) | 5·4% (2·4–13·0) | 61% |
| By 12 month period | | | |
| 1–360 | 0·779 (0·76–0·80) | 66·1% (63·2–68·9) | 61% |
| 360–720 | 0·647 (0·62–0·673) | 37·5% (33·9–41·2) | 61% |
| Total time period | 0·743 (0·73–0·76) | 58·4% (56·1–60·7) | 61% |

Sensitivity values are reported at a specificity of 80·0% (95% CI 78·6–81·4). ROC AUC=area under receiver operating characteristic curve. ACS=adolescent cohort study.

***Table 1*: Cross-validation performance of the tuberculosis risk signature in the ACS training set by days before tuberculosis diagnosis**

A new version of QuantiFERON-TB Gold In-Tube™ has been developed subsequent to the end of the study period that could have greater sensitivity for detecting latent tuberculosis infection; assessment of its ability to predict progression to active tuberculosis will be important in future studies. Although other new assays, such as transcriptional profiling, could improve the detection of incipient tuberculosis,[27] the increase in positive predictive value of these tests compared with IGRAs appears small because of low specificity.[28] Better use of existing assays remains crucial until a more specific and highly predictive commercial test is developed.

# Conclusion

- In an elimination TB perspective, Biomarkers are needed to identify individuals with TB infection and among them those who are at risk to develop active disease in order to provide preventive therapy
- Those biomarkers usually identify immune correlates of incipient and subclinical tuberculosis
- Some of them are yet avalaible : IGRA , serological markers,
- New markers in progress : RNA signature