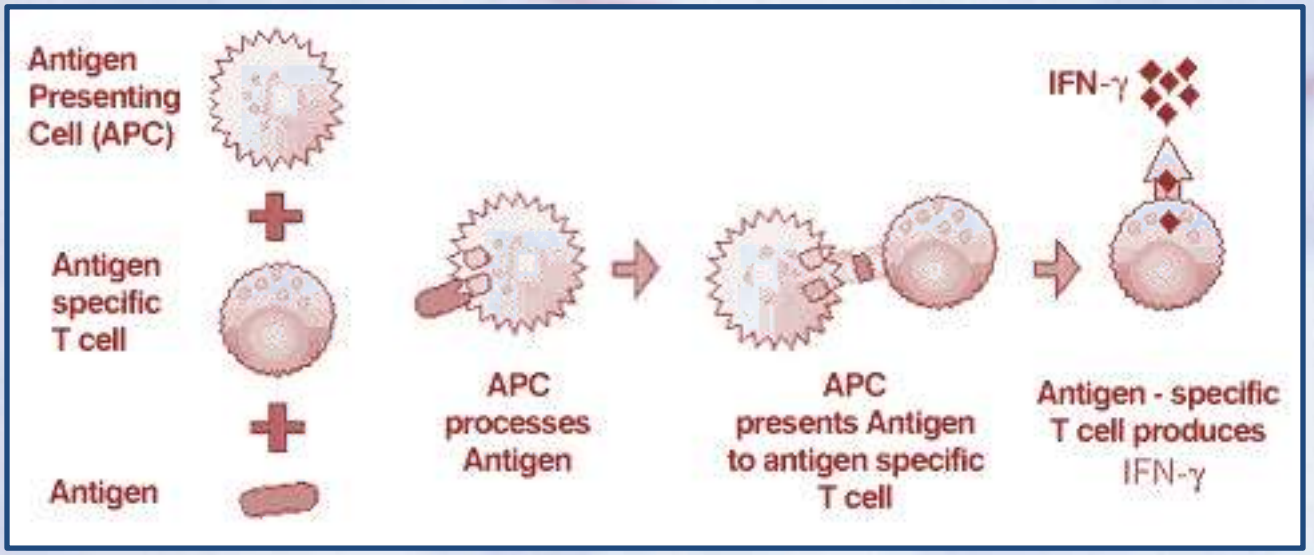
# IGRA guidelines




P. Van Bleyenbergh
and
Respiratory Infections Working Group

# Financial statement

- The speaker has no financial involvement with any organization or entity with a financial interest in the subject matter of materials discussed
- This presentation is given without any financial rewards

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# For whom are these IGRA guidelines intended?

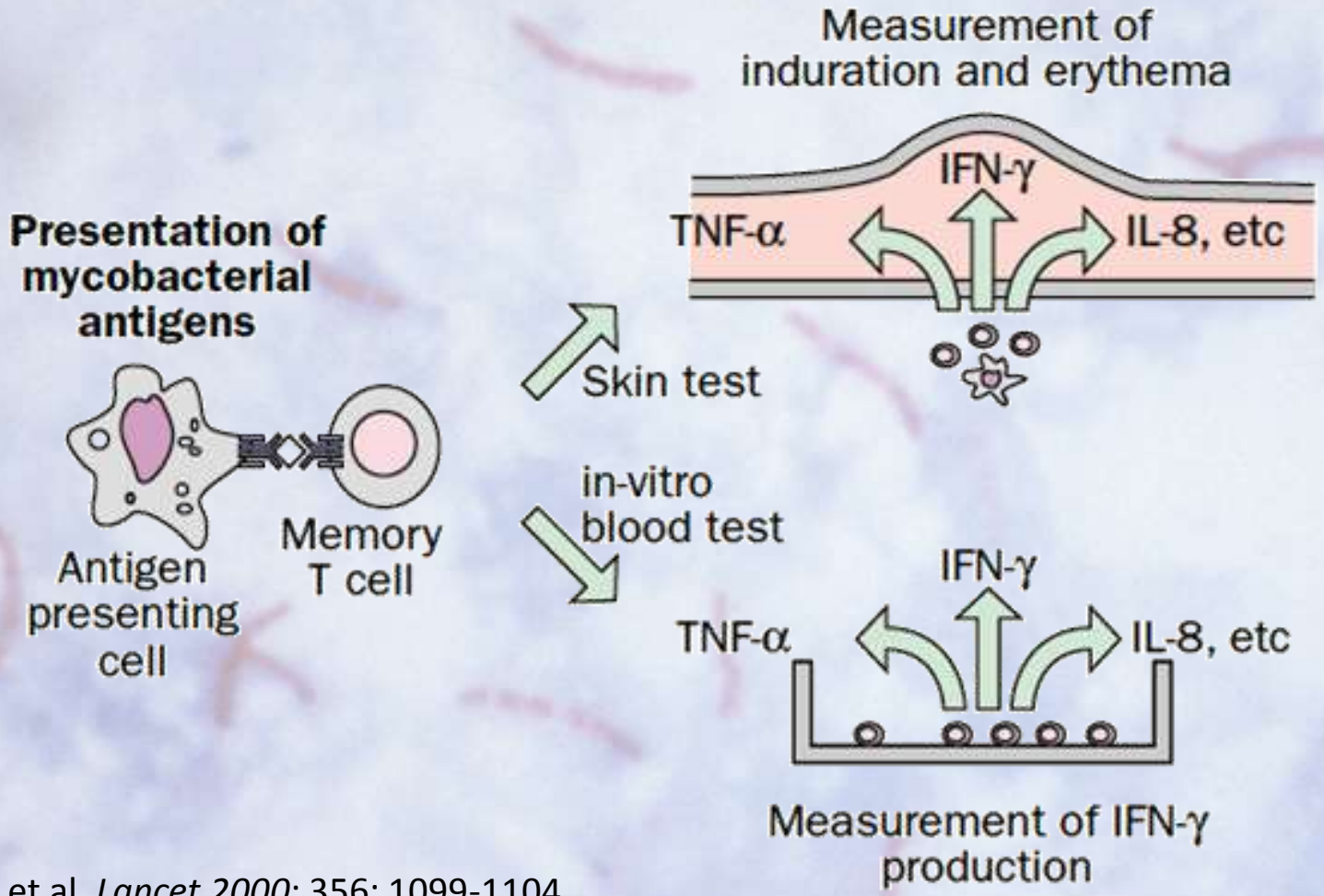
- These IGRA guidelines are intended for all healthcare workers concerned about *diagnosing latent tuberculosis infection* (LTBI).

- IGRAs should not replace the standard diagnostic methods (microbiology, molecular tests, clinical and radiological assessment) for diagnosing active TB.

- A negative IGRA does not rule out active TB.

# What are IGRAs?

# IGRA = Interferon-γ Release Assay

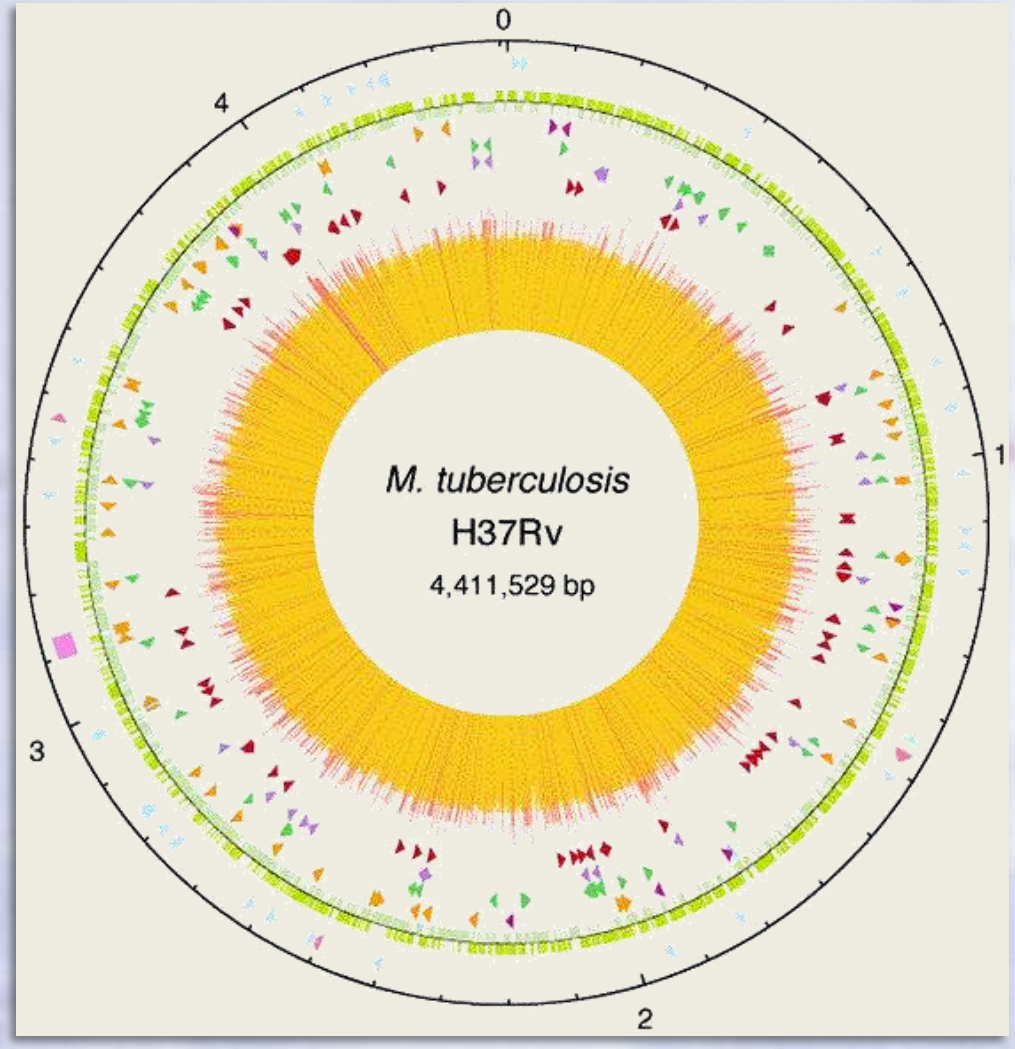


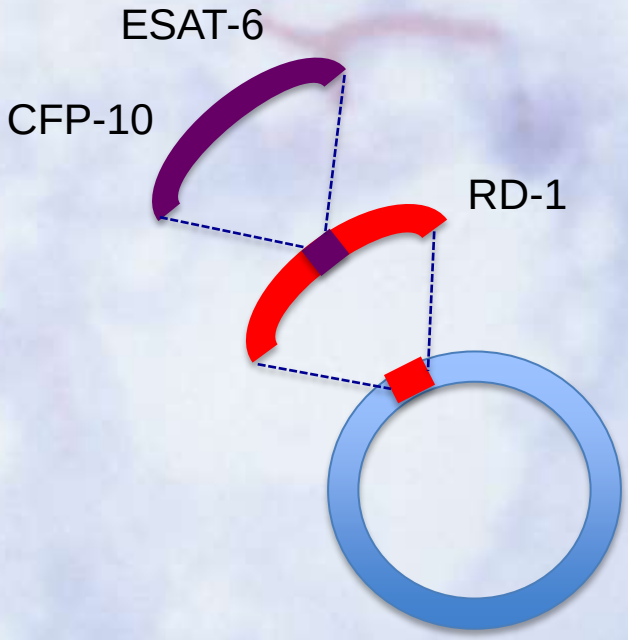


Andersen P et al. *Lancet 2000*; 356: 1099-1104

Pai M et al. *Lancet Infect Dis 2004*; 4: 761-776

# *M. tuberculosis* genome




Cole ST et al. *Nature 1998*; 393: 537-544
Behr MA et al. *Science 1999*; 284: 1520-1523

# IGRAs are more specific for *M. tuberculosis* infection

| Strain tested | Antigens | |
|---|---|---|
| | ESAT-6 | CFP 10 |
| **Tuberculosis complex** | | |
| *M tuberculosis* | + | + |
| *M africanum* | + | + |
| *M bovis* | + | + |
| BCG substrain | | |
| gothenburg | – | – |
| moreau | – | – |
| tice | – | – |
| tokyo | – | – |
| danish | – | – |
| glaxo | – | – |
| montreal | – | – |
| pasteur | – | – |

| Strain tested | Antigens | |
|---|---|---|
| | ESAT-6 | CFP 10 |
| **Environmental strains** | | |
| *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* | – | – |

Andersen P et al. *Lancet 2000*; 356: 1099-1104

# Which IGRAs are available?

- **Measure Δ IFN-γ concentration**
  - e.g. QuantiFERON®-TB Gold In-Tube
    - Whole Blood stimulated with TB antigens
    - Measure IFN-γ by ELISA

- **Measure Δ # of cells releasing IFN-γ**
  - e.g. T SPOT.*TB*® (ELISpot)
    - PBMCs stimulated with TB antigens
    - Count spots


Lalvani A et al. *Enferm Infecc Microbiol Clin. 2009*;10: 628-636

# Indeterminate results: test vs. host failure

- **High background IFN-γ**
  (abnormal negative control)
  - Concurrent illness
  - Mitogen put in wrong well (nil)
  - Defective tubes
- **Low mitogen**
  (abnormal positive control)
  - Transient or chronic immune suppression
  - GFT-G or T-SPOT: no mitogen in control well
  - QFT-GIT: defective tubes, overfilling, inadequate shaking

# IGRAs:
## time interval to conversion

- Interval for positive conversion following exposure to a patient with active TB is unclear
  - TST: 2-12 weeks → 8 weeks
  - IGRA:
    - NICE guidelines (UK): 6 weeks
    - CDC guidelines (USA): 8-10 weeks
    - ERS guidelines (EUR): 8 weeks

Erkens CGM et al. *ERJ 2010*; 36: 925-949

- Recent study:
  "IGRA conversion generally occurred 4-7 weeks after exposure, although it could be as late as 14-22 weeks!"

Lee S W et al. *Eur Respir J* 2011; 37: 1447-1452

# What are the (dis)advantages of IGRAs?

| | TST | IGRA |
|---|---|---|
| Cross-reactivity with BCG | Yes | No |
| Cross-reactivity with NTM | Yes | Unlikely |
| Negative/positive control | No | Yes |
| Reliability/reproducibility | Moderate & variable | High |
| Boost effect | Yes | No |
| Patient visits | Two | One |
| Trained personnel required | Yes | Yes |
| Laboratory infrastructure required | No | Yes |
| Time to obtain result | 3days | 1-2days |
| Material costs | Low | Moderate to high |

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

# Evaluation of IGRAs

**Lack of "gold standard" for LTBI!**

- Sensitivity → Compare to culture
  - Sensitivity: # positives/# culture (+) people tested
- Specificity → Subjects at low risk for LTBI
  - Specificity: # negative/# low-risk people tested

- ✓ Accuracy of IGRAs
- ✓ Agreement with TST
- ✓ Positive results vs. exposure
- ✓ Predicting TB disease

# Performance of IGRA test

- **Sensitivity**

| Series | Diagnostics | Subject | Studies n | Summary sensitivity (95% CI) |
|---|---|---|---|---|
| 1 | QFT-G | TB patients, adult | 21 | 0.80 (0.78–0.82) |
| 2 | QFT-G-IT | TB patients, adult | 6 | 0.74 (0.69–0.78) |
| 3 | QFT-G/G-IT | TB patients, child | 9 | 0.82 (0.75–0.87) |
| 4 | QFT-G/G-IT, T.SPOT | HIV-infected TB patients | 5 | 0.70 (0.60–0.79) |
| 7 | T.SPOT | TB patients | 13 | 0.90 (0.86–0.93) |
| 8 | TST | Healthy subjects | 20 | 0.77 (0.71–0.82) |

- **Specificity**

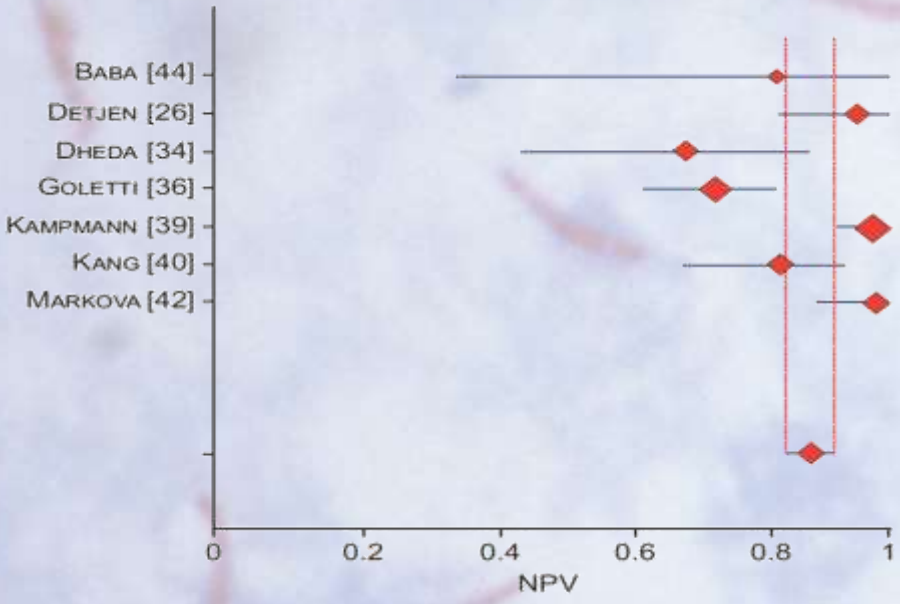
| Series | Diagnostics | Subject | Studies n | y specificity (96% CI) |
|---|---|---|---|---|
| 1 | QFT-G/G-IT | Healthy young adults | 12 | 0.98 (0.97–0.99) |
| 2 | QFT-G/G-IT | Healthy young adults, $BCG^-$ | 8 | 0.99 (0.98–1.00) |
| 3 | QFT-G/G-IT | Healthy young adults, $BCG^+$ | 8 | .96 (0.94–0.98) |
| 4 | T.SPOT | Predominantly BCG vaccinated | 8 | .93 (0.86–1.00) |
| 5 | TST | BCG not vaccinated | 6 | 0.97 (0.95–0.99) |
| 6 | TST | BCG vaccinated | 6 | 0.59 (0.46–0.73) |

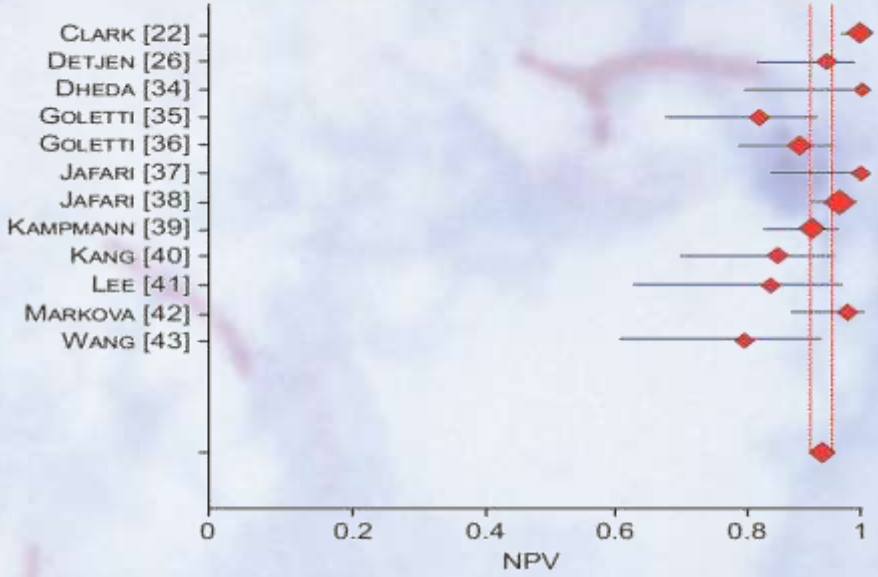
pooled 98-100%

pooled 88.7%

Lange C et al. *Respirology 2010*; 15: 220-240

# IGRAs:
# negative predictive value



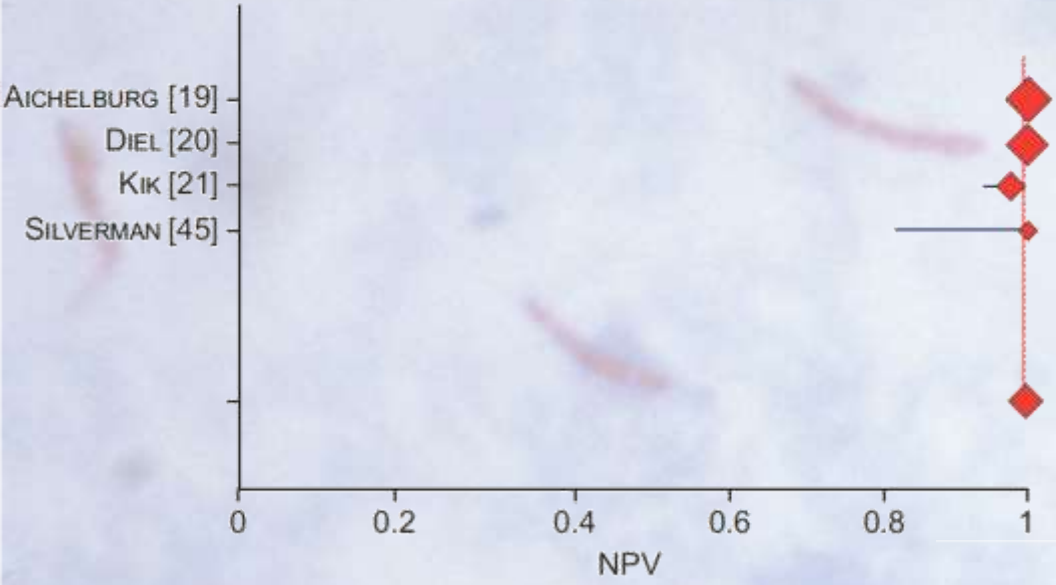




QFT-GIT
pooled: 0.88

T-SPOT.TB
pooled: 0.94

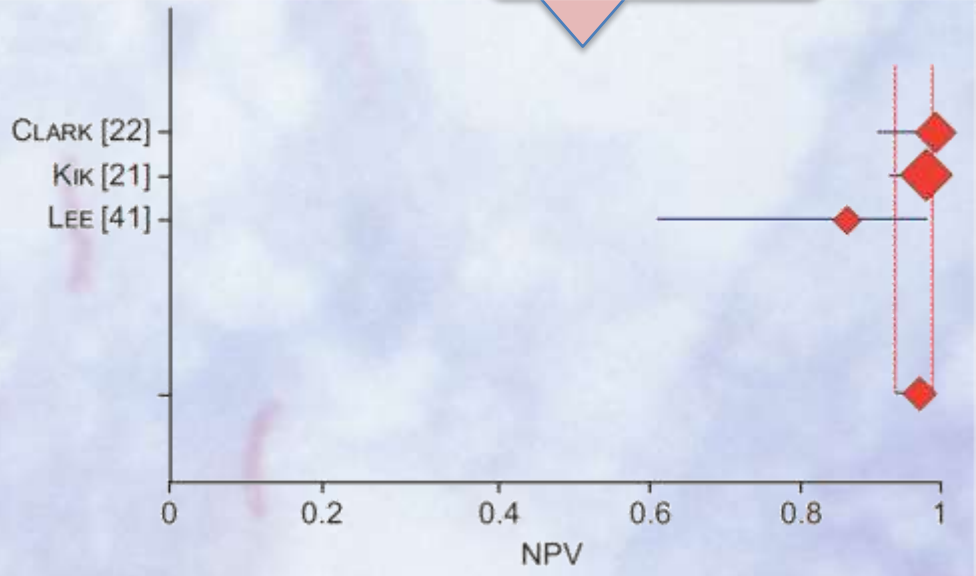
Diel R et al. *Eur Respir J* 2011; 37; 88-99

# IGRAs:
## NPV for progression to active TB




QFT-GIT
pooled: 0.998

T-SPOT.TB
pooled: 0.98

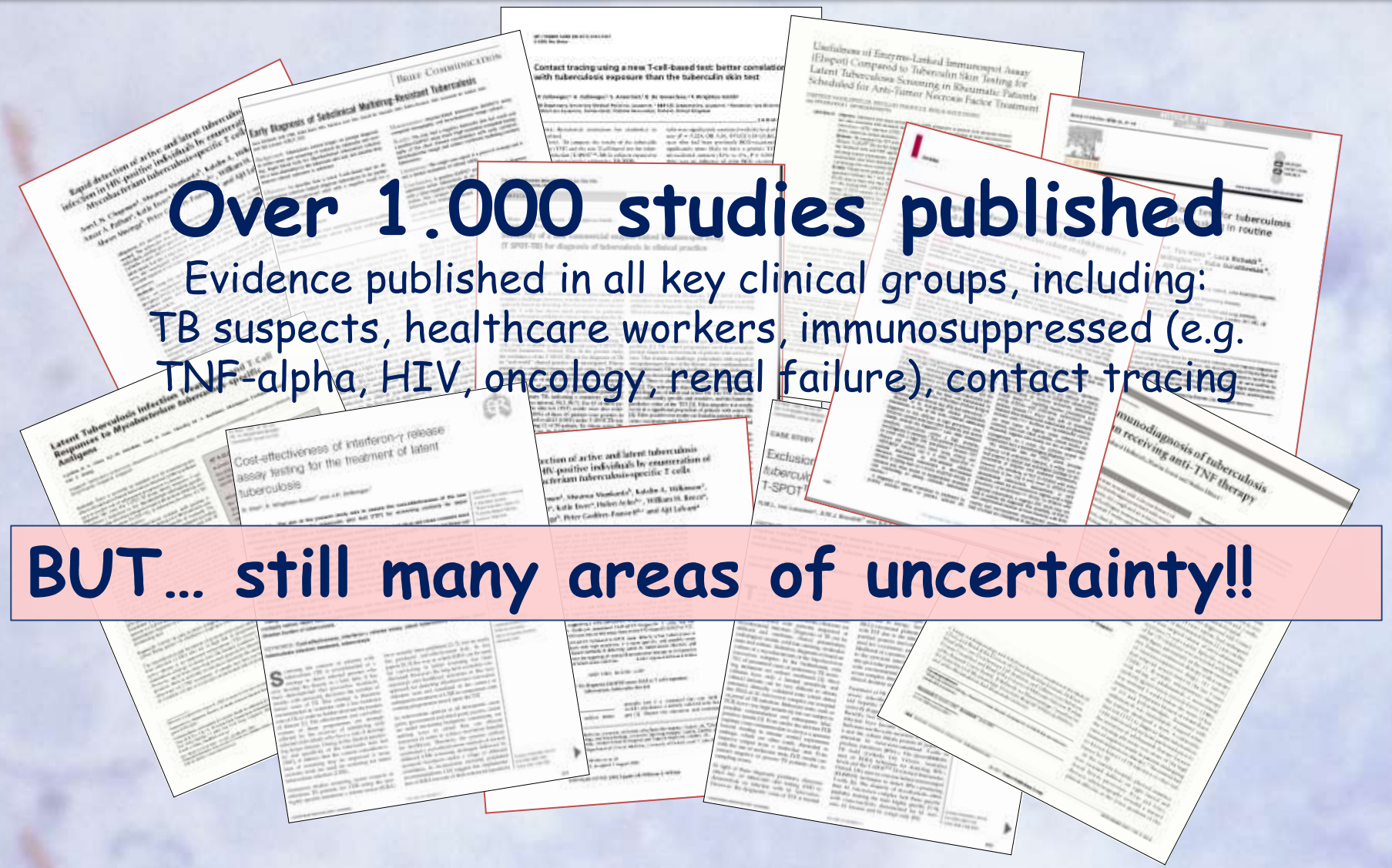



Diel R et al. *Eur Respir J* 2011; 37; 88-99

# How should IGRAs be used in different population groups and settings?

1. Children
2. Immunocompromised patients
3. HIV-infected patients
4. Anti-TNF therapy patients
5. Contact tracing
6. Screening of occupational healthcare workers
7. High-incidence TB settings/populations

# IGRAs:
# clinical evidence base



## Over 1.000 studies published

Evidence published in all key clinical groups, including:
TB suspects, healthcare workers, immunosuppressed (e.g. TNF-alpha, HIV, oncology, renal failure), contact tracing

**BUT… still many areas of uncertainty!!**

# !! REMINDER !!

**IGRAs should be used to detect/screen for latent tuberculosis (LTBI)**

**Always rule out active disease!!**

(microbiology, molecular tests, clinical and radiological assessment)

# Use of IGRAs in children

- Children <5 years: increased risk of infection and of developing active disease after exposure to contagious case
  Children >5 years: same immune response to TB infection as in healthy adults
- Available evidence is too scant to change current recommendations
- Essential to achieve highest sensitivity of detection when diagnosing LTBI, especially in children <5 years old

**TST** remains preferred test for detection of LTBI

**TST + IGRA** can increase sensitivity

When both tests are performed, treatment should be given in case of a positive result for either one of tests

# Use of IGRAs in immunocompromised patients

- Primary vs. secondary immunodeficiency
  → **heterogeneous group** of patients

- TST: low sensitivity (→ cut-off 5mm should be used)

- **IGRAs have higher sensitivity** but is this high enough to rule out TB infection?
  → 'probably' *YES* in low-incidence settings/populations

Two-step approach:

IGRA max 72h after TST

**1/ TST**

↳ *positive* → IGRA only if BCG vaccinated, otherwise LTBI

↳ *negative* → IGRA

**2/ IGRA**

↳ *positive* → LTBI

↳ *negative* → most probably no LTBI (low-incidence setting)

# Use of IGRAs in HIV-infected patients

- Upon diagnosis, all HIV-infected patients should undergo screening for latent TB!
- TST low sensitivity (and specificity) in HIV-patients!
- IGRA
  - High specificity
  - Sensitivity considerably higher compared to TST ***but***…
    - False-negativeresults!
    - More indeterminate results!! ~ CD4-cell count

| CD4 count (cells/ µL) | Indeterminate results (%) | Total number of subjects tested |
|---|---|---|
| >200 | 14 | 43 |
| 51-200 | 25 | 60 |
| ≤50 | 30 | 109 |

ECDC Guidance, Stockholm. www.ecdc.europa.eu 2011

# Use of IGRAs in HIV-infected patients

- Upon diagnosis, all HIV-infected patients should undergo screening for latent TB!

- TST low sensitivity (and specificity) in HIV-patients!

1/ CD4 cells ≤500/ml → **IGRA**
- IGRA positive: LTBI
- IGRA negative: *probably* no LTBI

2/ CD4 cells >500/ml → **TST** (cut-off 5mm)
- TST positive: LTBI (IGRA if BCG vaccinated)
- TST negative → IGRA

# Use of IGRAs in anti-TNF therapy patients

- Always **rule out active TB** (history, chest X-ray, sputum exam)!

- **TST negative** (<5 mm): no LTBI
  only if no immunocompromising conditions present and/or if no high-risk contact!

- **TST positive** (≥10 mm): LTBI (BCG vaccinated → IGRA)

- **TST intermediate** (5-9 mm) or **negative with immunocompromising condition present and/or high-risk contact** → IGRA

- **IGRA**
  ↳ *positive* → LTBI
  ↳ *negative* → most probably no LTBI (low-incidence setting)

# Use of IGRAs in contact tracing

- **To test = to treat!**

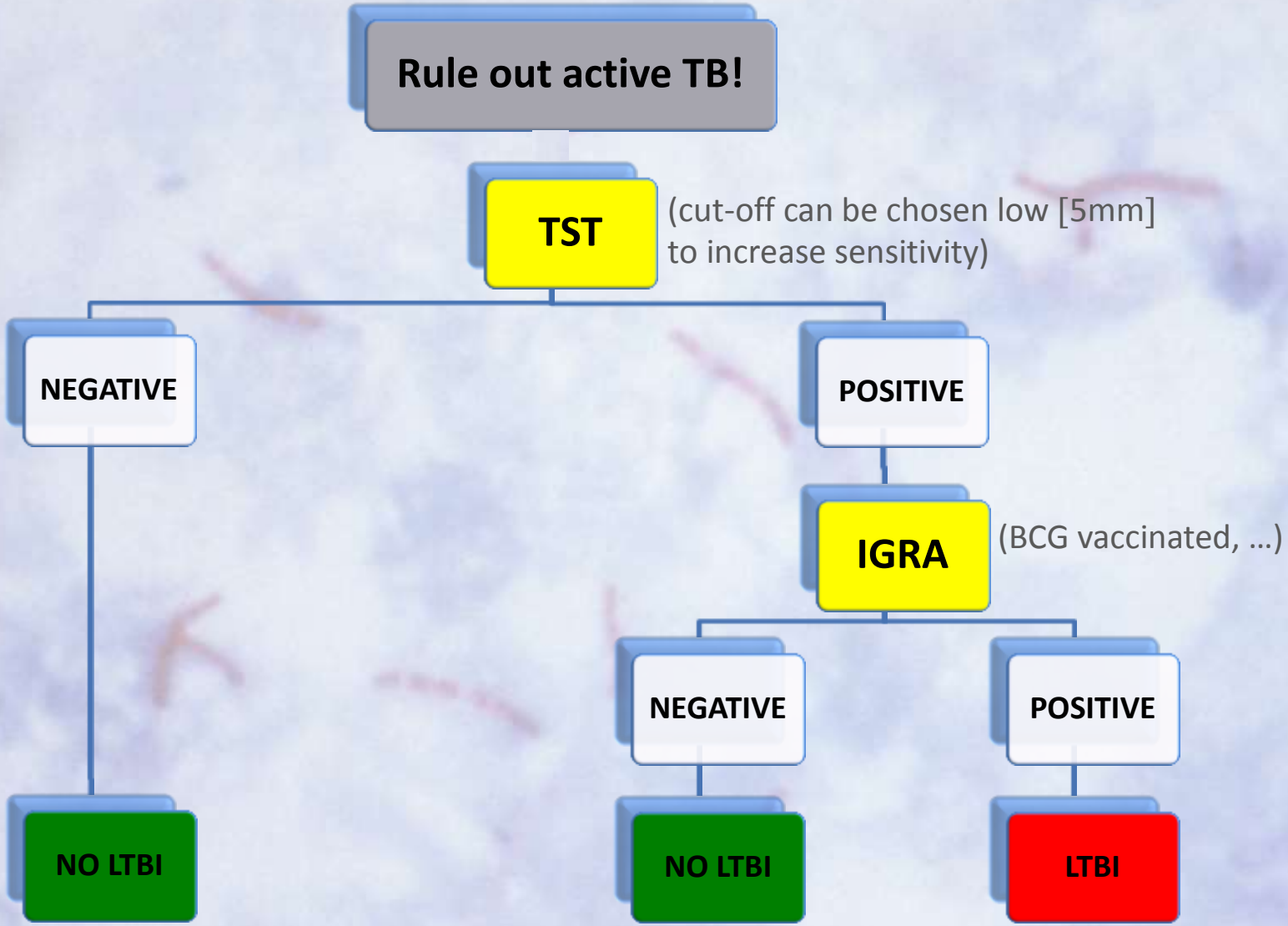
- A negative test, performed within the pre-allergic phase (6-8 weeks, range 2-12 weeks), should be repeated 8-12 weeks after last potential contact.
  **Pre-allergic time period: IGRA = TST**

- Most country guidelines favor a **two-step approach** (positive TST → IGRA) to increase specificity

- Belgium:
  - low-incidence setting (<20/100.000)
  - low BCG vaccination status
  - IGRA: higher price, no reimbursement, not readily available everywhere

# Use of TST and IGRA in contact tracing

(adults, children ≥5 yrs)



# Use of IGRAs in occupational HCW screening

- IGRAs have **some advantages**
  - higher specificity
  - no induction of booster effect
- **Lack of data on optimal cut-offs** for serial testing by IGRA
- **Unclear** interpretation and prognosis of IGRA conversions and reversions

1/ Pre-employment: **TST** (cf. contact tracing)

2/ No change in the strategy based on **TST** serial testing seems to be justified

# Use of IGRAs in high-incidence populations

- Many people have LTBI
- High level of BCG-vaccination
- Increased exposure to NTM
- Increased exposure to *M. leprae* homologues or IGRA-antigens

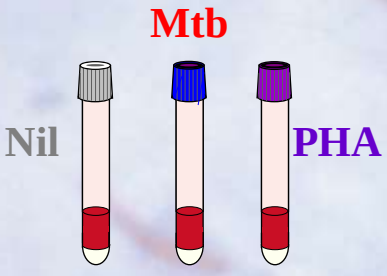
IGRAs have no added value to diagnose LTBI

Focus of prevention and control is to identify and treat active cases

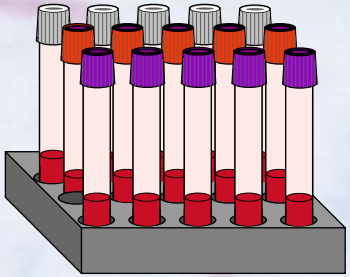
# What are the practical considerations of IGRAs in Belgium?

# QuantiFERON®-TB Gold In-Tube (QFT-GIT)

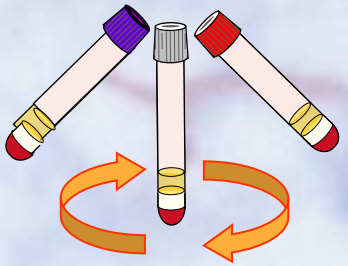
## Stage 1: Whole Blood Culture




Collect 1mL of blood in 3 tubes

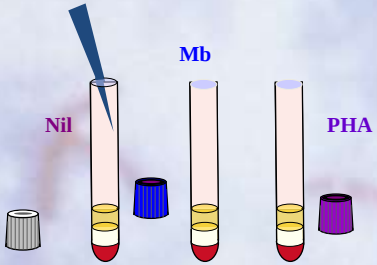


Incubate at 37ºC for 16-24 hours.

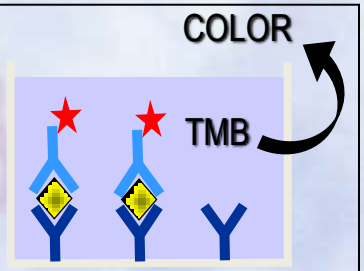


Centrifuge 5 minutes to separate plasma above gel

## Stage 2: Measure [IFN-γ] & Interpret




Collect 50 µL of plasma for ELISA




Measure [ IFN-γ ] in 'Sandwich' ELISA

Software calculates results and prints report

Cellestis. *www.cellestis.com*

# QuantiFERON®-TB Gold In-Tube Interpretation

| Interpretation | TB Response | Nil | Mitogen – Nil |
|---|---|---|---|
| **Positive** | ≥ 0.35 IU/ml and ≥ 25% of Nil | ≤ 8.0 | Any |
| **Negative** | < 0.35 IU/ml or < 25% of Nil | ≤ 8.0 | ≥ 0.5 |
| **Indeterminate** | < 0.35 IU/ml or < 25% of Nil | ≤ 8.0 | < 0.5 |
| | Any | > 8.0 | Any |

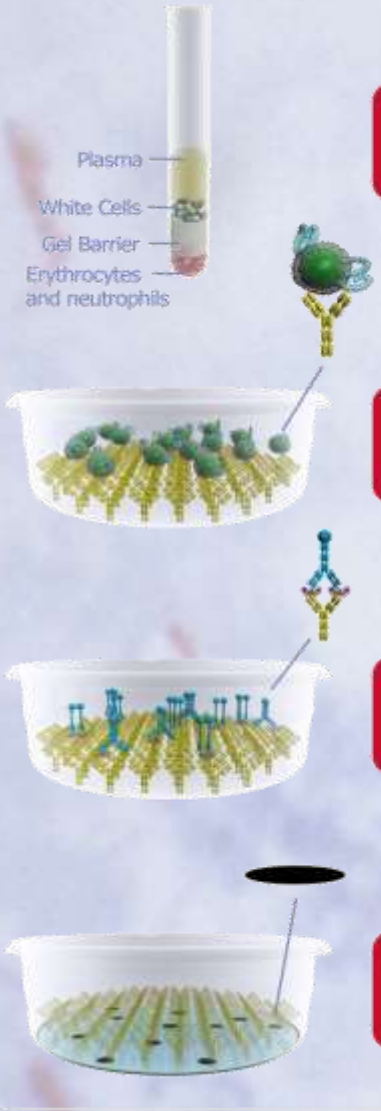
TB response is the IFN-γ concentration in plasma from blood stimulated with a single cocktail representing ESAT-6, CFP-10, and part of TB7.7, minus the IFN-γ concentration in plasma from unstimulated blood.

Cellestis. *www.cellestis.com*

# QuantiFERON®-TB Gold In-Tube Practical considerations

- Turn-around time (TAT): 26h for single test (lab hands-on 50min)
- Cost: ~45€ (not reimbursed at the moment)
- Advantages:
  - Can be automated
  - Can be ‘batched’
  - No interobserver differences
  - More broadly available in Belgium
- Disadvantages:
  - Amount of Tcells tested is variable
  - Short time before incubation (16h)
  - Slightly less sensitive than T-SPOT

# T-SPOT. TB

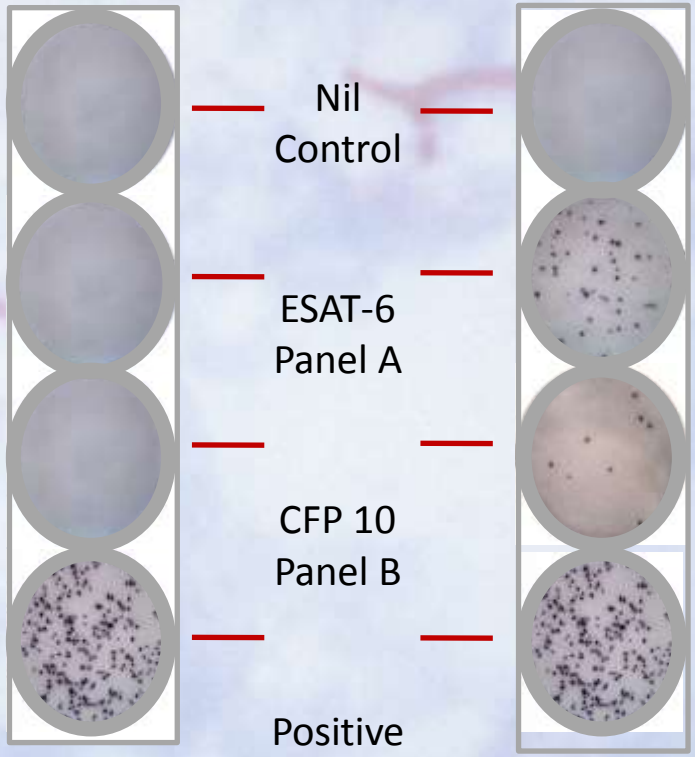



**1.** Collect blood sample, centrifuge to separate white blood cells which are washed and counted to maximise sensitivity.

**2.** Add WBCs [ ● ] & specific TB antigens [ ⬭ ] to wells pre-coated with antibodies to IFN-γ [ Y ] and incubate overnight (37°C, $CO_2$).

**3.** IFN-γ [ ▬ ] is released from activated T cells. Wash wells, add secondary conjugated antibody [ Y ]. Incubate for 1 hour

**4.** Wash wells, add substrate and incubate for 7 minutes. Stop reaction with water. One spot [ ▬ ] is the footprint of one activated T cell.




OxfordImmunotec. *www.oxfordimmunotec.com*

# T-SPOT. TB Interpretation

| Interpretation | TB Response | Nil | Mitogen |
|---|---|---|---|
| Positive | ≥ 6 spots | ≤ 10 spots | any |
| Negative | ≤ 5 spots | ≤ 10 spots | ≥ 20 spots |
| Borderline | 5, 6, or 7 spots | ≤ 10 spots | ≥ 20 spots |
| Indeterminate | ≤ 5 spots | ≤ 10 spots | < 20 spots |
| | any | > 10 spots | any |

TB Response is the higher number of spots resulting from stimulation of PBMCs with two separate cocktails of peptides representing ESAT-6 or CFP-10, minus the number of spots resulting from incubation of PBMCs with saline.

OxfordImmunotec. *www.oxfordimmunotec.com*

# T-SPOT. TB
# Practical considerations

- Turn-around time (TAT): 24h for single test (lab hands-on 3-4h)
- Cost: ~60€ (not reimbursed at the moment)
- Advantages:
  - Amount of Tcells standardised (250.000/well)
  - Slightly more sensitive than QFT, especially in immunocompromised pts
  - Data about non-sanguinous fluids… but not licenced!
- Disadvantages:
  - Cannot be automated, longer hands-on time
  - Cannot be ‘batched’
  - Inter-observer differences possible (in counting spots…)
  - Not available everywhere

# Still an open question…



**TST**

**IGRA**