Biomarkers In tuberculosis
a physician point of view

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Haute Autorité de Santé
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

Inoculum (bacterial load) Host immunity

No infection

Tuberculosis infection (~ 50 millions per year)

Strong and protective immune response

Controlled TB growth

Bacterial growth stopped
Eradication of MTB (10%)
Sterilizing Immunity

Continuous TB growth

Weak and insufficient immune response

Controlled bacterial growth
Persistence of few viable bacillus (90%)
Latent Tuberculosis Infection
2.2 billions patients worldwide

8 to 10 weeks

Efficient immunity

Never symptomatic
Never contagious

Immunity weakness

Active Tuberculosis
Incidence = 8.8 Millions/year
Some conditions increase the risk to develop active Tb

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Estimation of relative risk*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS</td>
<td>110 - 170</td>
</tr>
<tr>
<td>Well controlled HIV infection</td>
<td>50 - 110</td>
</tr>
<tr>
<td>Solid Organ Transplantation</td>
<td>20 - 74</td>
</tr>
<tr>
<td>Chronic Hemodialysis</td>
<td>10 - 25</td>
</tr>
<tr>
<td>Head and neck cancer</td>
<td>16</td>
</tr>
<tr>
<td>Recent tuberculosis infection (&lt;2 years)</td>
<td>15</td>
</tr>
<tr>
<td>Systemic prolonged corticosteroids therapy</td>
<td>4.9</td>
</tr>
<tr>
<td>Anti-TNF α treatment</td>
<td>1.5 - 4</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2 - 3.6</td>
</tr>
<tr>
<td>Malnutrition (body mass index &lt; 20 kg/m²)</td>
<td>2 - 3</td>
</tr>
<tr>
<td>Passive smoking</td>
<td>2 - 3</td>
</tr>
</tbody>
</table>
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.

Optimize use of current & new tools emerging from pipeline, pursue universal health coverage and social protection

Introduce new tools: a vaccine, new drugs & treatment regimens for treatment of active TB disease and latent TB infection, and a point-of-care test

Current global trend: -1.5%/year

-10%/year by 2025

-5%/year

-17%/year
Which Strategy to end TB?

End TB Strategy: 90% by 2035
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

World 7 billion

Infected 2.3 billion

Disease 9 million/yr
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 develop 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

- Infection
- TIME
- Active TB
- Active Disease
- Antibody
- Mycobacterial Load
- Latent TB Infection
- Cell Mediated Immunity

Graph showing the immune response over time with stages of infection, latency, and disease progression.
Biomarkers?

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

- 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)

<table>
<thead>
<tr>
<th></th>
<th>TST</th>
<th>IGRA</th>
<th>Culture</th>
<th>Sputum smear</th>
<th>Infectious</th>
<th>Symptoms</th>
<th>Preferred treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection eliminated</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Usually positive</td>
<td>Positive</td>
<td>Positive or negative</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>Positive</td>
<td>Intermittently positive</td>
<td>Positive</td>
<td>Usually negative</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Latent TB infection</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Sporadically</td>
<td>Mild or none</td>
<td></td>
</tr>
<tr>
<td>Subclinical TB disease</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Yes</td>
<td>Mild to severe</td>
<td></td>
</tr>
<tr>
<td>Active TB disease</td>
<td>Usually positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>None</td>
<td>None</td>
<td>Preventive therapy</td>
</tr>
</tbody>
</table>
### Defining criteria for the five categorical states of tuberculosis

from Drain and al Clin Microbiol Rev 07/2018

<table>
<thead>
<tr>
<th>Categorical state of TB</th>
<th>Exposure to M. tub</th>
<th>Viable M. tub pathogen</th>
<th>M. Tub has metabolic activity: progression?</th>
<th>RX ab or microbi evidence of active MT</th>
<th>Symptoms of tuberculosis Microb+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eliminated TB</td>
<td>+</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Latent TB</td>
<td>+</td>
<td>+</td>
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<tr>
<td><strong>Incipient TB</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
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<tr>
<td>Subclinical TB</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Active TB disease</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
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


- 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

Principle of the immune reaction
Technical steps

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

- Nil Control
- ESAT-6 Panel A
- CFP 10 Panel B
- Positive Control

Positive Result

1. Prélever PBMC, laver et compter
2. Laver, développer et sécher la plaque
3. Compter les spots colorés dans chaque puits
INTERPRETATION

Contrôle Antigène TB – Nul $\geq 0,35$ UI/ml

- Oui

Contrôle Antigène TB – Nul $\geq 25\%$ de la valeur du contrôle Nul en UI/ml

- Non

Contrôle Mitogène – Nul $< 0,50$ UI/ml et/ou contrôle Nul $> 8,0$ UI/ml

- Oui

INDÉTERMINÉ

- Non

Contrôle Nul $\leq 8,0$ UI/ml

- Oui

POSITIF

- Non

NEGATIF
M. Tuberculosis antigens in IGRA

<table>
<thead>
<tr>
<th>Complex</th>
<th>+</th>
<th>+</th>
<th>+</th>
<th>M. Abcessus</th>
<th>-</th>
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</tr>
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<tbody>
<tr>
<td>M. tuberculosis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>M. avium</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>M. africanum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>M. branderi</td>
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<td>M. bovis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>M. celatum</td>
<td>-</td>
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<td>+</td>
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</table>

<table>
<thead>
<tr>
<th>BCG strains</th>
<th>ESAT-6</th>
<th>CFP-10</th>
<th>TST</th>
<th>M. cheloneae</th>
<th>-</th>
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<tbody>
<tr>
<td>Gothenberg</td>
<td>-</td>
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<td>+</td>
<td>M. fortuitum</td>
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<td>+</td>
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<tr>
<td>Moreau</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>M. gordonii</td>
<td>-</td>
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<td>+</td>
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<tr>
<td>Tice</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>M. intracellulare</td>
<td>-</td>
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<td>+</td>
</tr>
<tr>
<td>Tokyo</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>M. kansasii</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Danish</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>M. malmoense</td>
<td>-</td>
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<td>+</td>
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<tr>
<td>Glaxo</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>M. marinum</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Montréal</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>M. oenavense</td>
<td>-</td>
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<td>+</td>
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<tr>
<td>Pasteur</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>M. scrofulaceum</td>
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<td>+</td>
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<tr>
<td>M. smegmatis</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<td>+</td>
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<tr>
<td>M. szulgai</td>
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<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>M. terrae</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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</table>
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*  
|                          |                                 |              | No cross-reactivity with BCG |
| Patient Management       | Day 1: Injection  
                          | Day 3: Reading | Only one sampling  
                          | Only one visit | No loss of patients |
| Type of test             | In vivo testing  
                          | Operator dependent | In vitro testing  
                          | Single blood sampling  
                          | Standardized reading  
                          | Quality controls included | No booster effect  
                          | Reproducibility  
                          | Accuracy  
                          | 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  
                          | Specificity of 98% |

Sources:
2 – Cheallaigh 2013
Impact of BCG vaccination on Interferon-γ Assays

- **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

13 studies
Quantiferon-TB® Gold in tube
Sensitivity from 80 to 100% Sensitivity 84% (81-87%)

17 studies
T-SPOT.TB®
Sensitivity from 50 to 100% Sensitivity 93% (85-100%)
IGRA Predictive value / TST (Diel 2010)

Capacity of the QFT assay to predict the progression to active TB

- 954 contacts

- 198 QFT Positive

- 142 QFT Positive, TST Positive
  - Not treated
  - 17 active TB

- 5 QFT Positive, TST Negative
  - Not treated
  - 2 active TB

- 51 QFT Positive (49 TST Positive)
  - Chemoprophylaxis RIF and/or INH
  - No active TB

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 Ø > 5 mm
  - 9 missed TB at Ø > 10 mm
  - 17 missed TB at Ø > 15 mm
Predictive value of IGRA / TST

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)

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 %
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
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
<table>
<thead>
<tr>
<th></th>
<th>TST-5</th>
<th>TST-10</th>
<th>TST-15</th>
<th>T-SPOT.TB</th>
<th>QuantiFERON-TB Gold In-Tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>TST-5</td>
<td>--</td>
<td>1.25 (1.15–1.36; &lt;0.0001)</td>
<td>1.64 (1.44–1.87; &lt;0.0001)</td>
<td>1.99 (1.68–2.34; &lt;0.0001)</td>
<td>1.52 (1.26–1.83; &lt;0.0001)</td>
</tr>
<tr>
<td>TST-10</td>
<td>--</td>
<td>--</td>
<td>1.31 (1.61–1.47; &lt;0.0001)</td>
<td>1.59 (1.34–1.88; &lt;0.0001)</td>
<td>1.21 (1.01–1.46; 0.041)</td>
</tr>
<tr>
<td>TST-15</td>
<td>--</td>
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<td>--</td>
<td>1.21 (1.01–1.43; 0.037)</td>
<td>0.93 (0.76–1.13; 0.453)</td>
</tr>
<tr>
<td>T-SPOT.TB</td>
<td>--</td>
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<td>--</td>
<td>--</td>
<td>0.77 (0.66–0.89; 0.0003)</td>
</tr>
</tbody>
</table>

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])

415 patients HIV+
Mean CD4 count = 393/mm3

- IGRA Positive 56 (13.5%)
- IGRA Negative 326 (78.6%)
- IGRA indeterminate 33 (7.9%)
  (QFT=10, Elispot=23)

- Active TB 7 (14.2%)
- Active TB 1 (1.7%)
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.

822 Patients HIV1+
Mean CD4 count = 393/mm³

QFT +
37 (4.5%)
Mean CD4 count = 402/mm³

- Active TB
  3 (8.1%)

QFT -
738 (89.7%)
Mean CD4 count = 400/mm³

- Active TB
  0 (0%)

QFT indeterminate
47 (5.7%)
Mean CD4 count = 188/mm³

- Active TB
  0 (0%)

Median of follow-up [IQR]: 19 months [12-21]
WHO guidelines on LTBI screening

<table>
<thead>
<tr>
<th>LTBI testing should be performed in</th>
<th>LTBI testing should be considered for</th>
<th>LTBI testing conditional</th>
</tr>
</thead>
<tbody>
<tr>
<td>People living with HIV</td>
<td>prisoners</td>
<td>People with harmful alcohol use and tobacco smokers</td>
</tr>
<tr>
<td>Adult and child contacts of pulmonary TB cases</td>
<td>health care workers</td>
<td>underweight people</td>
</tr>
<tr>
<td>Patients initiating anti-TNFα treatment</td>
<td>immigrants from high TB burden countries</td>
<td>people with diabetes</td>
</tr>
<tr>
<td>Patients receiving dialysis</td>
<td>homeless persons</td>
<td></td>
</tr>
<tr>
<td>SOT or HPSCT patients</td>
<td>illicit drug users</td>
<td>*UNLESS they fall into one of the other categories</td>
</tr>
<tr>
<td>Patients with silicosis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
WHO recommendations

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 will 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 response association with Recent exposure to active Tb

Nikolova et al, Diagn Microb Inf Dis, 2013
Difference between TB2-TB1 > 0.6 UI/ml a marker for recent infection

<table>
<thead>
<tr>
<th>Test indication</th>
<th>IFN-γ &gt; 0.6 IU/mL (% within positive results)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculosis infection in differential diagnosis</td>
<td>7 (17%)</td>
</tr>
<tr>
<td>Contact investigation</td>
<td>18 (33%)</td>
</tr>
<tr>
<td>Screening before immunotherapy</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>Periodic check by occupational health services</td>
<td>3 (33%)</td>
</tr>
<tr>
<td>Other&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2 (15%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (33%)</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
</tr>
</tbody>
</table>

<sup>a</sup> 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

"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
  - 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
# 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?
3arcellini et al. (July 2016) report the differential between antigen tubes TB2 and TB1 as a functional marker for CD8 stimulation.

5% 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, immunosuppressive therapies (anti TNF alpha)
• => In order to identify people to treat for LTBI

• HCWs before employment (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

<table>
<thead>
<tr>
<th>Time Period</th>
<th>ROC AUC (95% CI)</th>
<th>Sensitivity (95% CI)</th>
<th>Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>By 6 month period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-180</td>
<td>0.79 (0.76-0.82)</td>
<td>71.2% (66.6-75.2)</td>
<td>61%</td>
</tr>
<tr>
<td>181-360</td>
<td>0.771 (0.75-0.79)</td>
<td>62.9% (59.0-66.4)</td>
<td>61%</td>
</tr>
<tr>
<td>361-540</td>
<td>0.726 (0.70-0.76)</td>
<td>47.7% (42.9-52.5)</td>
<td>61%</td>
</tr>
<tr>
<td>541-720</td>
<td>0.540 (0.49-0.59)</td>
<td>29.1% (23.1-35.9)</td>
<td>61%</td>
</tr>
<tr>
<td>&gt;720</td>
<td>0.496 (0.43-0.56)</td>
<td>5.4% (2.4-13.0)</td>
<td>61%</td>
</tr>
<tr>
<td>By 12 month period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-360</td>
<td>0.779 (0.76-0.80)</td>
<td>66.1% (63.2-68.9)</td>
<td>61%</td>
</tr>
<tr>
<td>360-720</td>
<td>0.647 (0.62-0.673)</td>
<td>37.5% (33.9-41.2)</td>
<td>61%</td>
</tr>
<tr>
<td>Total time period</td>
<td>0.743 (0.73-0.76)</td>
<td>58.4% (56.1-60.7)</td>
<td>61%</td>
</tr>
</tbody>
</table>

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, the increase in positive predictive value of these tests compared with IGRAs appears small because of low specificity. 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 available: IGRA, serological markers,
• New markers in progress: RNA signature