Reference Standards for Serological Diagnostic Tests for TB

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Reference Standard for Serological Tests

A test that is currently accepted as a reasonably, but not necessarily, as 100% accurate. It is used as the reference method (gold standard) for assessing performance of other test methods.

Culture of M. tuberculosis is the gold standard for TB diagnosis

A serological assay should NOT usually be compared to an assay that detects the micro-organism directly.

How will using cultures as gold standard for SD test (immunological assay) work?
Specifications for a Rapid POC Test

- **Sensitivity:**
  - >95% for smear positive, culture positive patients
  - ~60-80% for smear negative, culture positive patients

- **Specificity:**
  - >95% compared to culture

Comparative to the performance of NAAT
- NAAT was FDA approved in 1995/1999
- No NAAT POC test yet available for any disease
- Even with culture, clinical judgment finally rules

Spectrum of TB

- **Primary TB**
  - Direct smear + 5-10x10^3 AFB/ml

- **Reactivation TB**
  - Culture + (10-100 AFB/ml)
  - NAAT (~85% pos)
  - CXR/CT

- **Conc. smear - culture -**
  - Subclinical

- **Conc. smear + culture -**
  - Months-2/5

- **Conc. smear + culture +**
  - High burden/low income
  - Low burden/high income

- **M. tb Infection**
- **Pulmonary Pathology**
Sub-clinical TB in HIV- High Risk Subjects

• A subset of asymptomatic close contacts of infectious TB patients progress to symptomatic TB within months of follow up. This asymptomatic subset must have active subclinical infection.

  Canada: In 6 months of follow up, 6% of adult contacts of smear-positive TB cases developed TB whereas <1 of adult contacts of smear negative TB cases developed TB. (Grzybowski et al.1975: Bull. Int. Union. Tuberc 50 (1): 90)

  Malawi: Seven percent of the PTB patients had HH members who were diagnosed with TB in the previous 12 months, 1% of controls had TB in the family during the same time period. (Claessens et al. 2002: Int. J. Tubercle. Lung Dis 6 (3): 266)

• Immunological changes are detected during the months prior to manifestation of TB in close contacts who progressed to TB.

  Pakistan: Six percent of HHC developed TB in 2 years of follow-up. Sharp decline in γIFN production prior to development of TB. (Hussain et al. 2007: Clin. Vaccine Immunol. 14 (12):1578)

  Portugal: 6/10 HCW who cared for TB patients developed TB in 5 years of follow-up; no HCW with no contact with TB patients developed TB. Increased TH2 responses during months prior to clinical TB. (Ordway et al. 2004: JID. 190:756-766).

Ethiopia: 29% of high responders progressed to TB??

Sub-clinical TB in HIV+ Subjects

• Active TB estimated to be present up to 1.3 years prior to diagnosis in HIV+ and up to 4.2 years in HIV- African Gold Miners.
  Corbett EL et al. Am J Respir Crit Care Med 170:673, 2004

• Intensified case finding studies have shown that ~ one fifth of HIV+ patients from Africa and Asia who have culture proven TB are asymptomatic (across a range of CD4 T cell counts)
  Kranzer K et al., Lancet Infect Dis 10, 93, 2010
  Mtei L et al., Clin Infect Dis 40, 1500, 2005

• Antibodies to MS, MPT51, PPE55 present in retrospective sera obtained up to 6 months before TB diagnosis in HIV+ patients from the US and India
  Singh KK et al., Infect Immun 73:5004, 2005
  Singh KK et al., Clin Diagn Lab Immunol 12:354, 2005

• Antibodies to MS, ESAT6, CFP10 present in retrospective sera obtained from HIV co-infected individuals who developed active TB during a multicenter prospective study on pulmonary complications of HIV/AIDS conducted among >1300 subjects in the USA in the 1980s.

• Abs were present up to 20 months before manifestation of TB
  Gennaro ML et al., Int J Tuberc Lung Dis 11:624, 2007
Ruling out TB

- Response to short course of broad spectrum antibiotics plays a major role in the clinical decision of ruling out TB.

- Studies from different settings have shown that between 8-50% of the SN TB patients with abnormal chest X rays who ultimately had positive \( M. \text{tb} \) cultures showed symptomatic response to a trial of antibiotics

- Symptomatic or clinical response to broad spectrum antibiotics has poor discriminatory power and does not reliably exclude TB.

Potential Targets for a Rapid Test


Malate Synthase (GlcB or \( M. \text{tb} \) 81)
TBF6+DPEP

73-75% sensitivity in smear positive TB (case control studies)
Combination of select antigens provide higher sensitivity
Specificity of anti-MS Abs

**TABLE 8. Specificity estimates by type of comparison**

<table>
<thead>
<tr>
<th>Antigen name</th>
<th>Specificity (%)^a</th>
<th>Patients with non-tuberculous respiratory disease</th>
<th>Healthy subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant 38 kDa</td>
<td>97 (90–99) (6)</td>
<td>90 (57–99) (6)</td>
<td></td>
</tr>
<tr>
<td>Recombinant malate synthase</td>
<td>97 (91–100) (4)</td>
<td>99 (81–100) (4)</td>
<td></td>
</tr>
<tr>
<td>Recombinant CFP-10</td>
<td>99 (92–100) (3)</td>
<td>90 (43–99) (3)</td>
<td></td>
</tr>
<tr>
<td>Native 38 kDa</td>
<td>96 (90–99) (6)</td>
<td>98 (92–100) (4)</td>
<td></td>
</tr>
<tr>
<td>DAT</td>
<td>55 (30–76) (4)</td>
<td>97 (88–100) (3)</td>
<td></td>
</tr>
</tbody>
</table>

^a The data represent the posterior means (95% credible intervals) (number of studies).

Specificity of Anti-MS Abs

<table>
<thead>
<tr>
<th>Reference</th>
<th>Protein</th>
<th>Sensitivity %</th>
<th>Specificity %^*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hendrickson, et. al (2000)</td>
<td>MS</td>
<td>57 (n=67)</td>
<td>96 (n=97)</td>
</tr>
<tr>
<td>Houghton, et. al (2002)</td>
<td>MS</td>
<td>58 (n=66)</td>
<td>97 (n=141)</td>
</tr>
<tr>
<td>Mukerjee, et. al. (2004)</td>
<td>MS</td>
<td>44 (n=83)</td>
<td>97 (n=54)</td>
</tr>
<tr>
<td>Singh, et. al. ** (2005)</td>
<td>MS</td>
<td>70 (n=40)</td>
<td>98 (n=59)</td>
</tr>
<tr>
<td>Wanchu, et. al. *** (2008)</td>
<td>MS</td>
<td>75 (n=136)</td>
<td>97 (n=90)</td>
</tr>
</tbody>
</table>

^* Specificity tested in PPD+, PPD- subjects from SE Asia, Latin America, Russia, China
Patients with Pneumonia, Asthma, NTM, HIV+ subjects etc.

No difference in anti-MS responses between subjects with no LTBI, recent LTBI and previous LTBI (Rabahi M.F. et al., 2007. BMC Infect Dis. 7: 148)
Absence of anti-MS Abs in LTBI

Absence of anti-MS Abs in Patients with NTBLD & NTBLI

<table>
<thead>
<tr>
<th>Patient Statusa</th>
<th>Source</th>
<th>n</th>
<th>Smear</th>
<th>TbF6 + DPEP</th>
<th>Mtb81</th>
<th>TbF6 + DPEP + Mtb81</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV+TB+</td>
<td>Sub-Saharan Africa</td>
<td>59</td>
<td>+</td>
<td>29</td>
<td>46</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>-</td>
<td>1</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>64</td>
<td>+</td>
<td>30 (46.9)</td>
<td>51 (79.7)</td>
<td>54 (84.4)</td>
</tr>
<tr>
<td>HIV-TB+</td>
<td>Sub-Saharan Africa</td>
<td>66</td>
<td>+</td>
<td>47 (71.2)</td>
<td>38 (57.6)</td>
<td>56 (84.8)</td>
</tr>
<tr>
<td>HIV+TB- PPD+b</td>
<td>United States</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PPD+b</td>
<td>Africa-Europe-Asia-Americas</td>
<td>57</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>PPD+b</td>
<td>Africa-Europe-Asia-Americas</td>
<td>29</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Lung Cancer</td>
<td>China</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Bone Cancer</td>
<td>China</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Non-TB lung infections</td>
<td>China/Caucasian</td>
<td>18</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>China/Caucasian</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

A subset of House-hold Contacts of SP TB Patients have Anti-MS Abs

Delta OD

PPD+/PPD- (n=52)
Endemic Controls (n=38)
HH Contacts (n=55)

SCTB or poor specificity?

Anti-MS Antibodies During SCTB in HIV+ TB Patients

Laal et al: 1998; JID
Anti-MS Abs in Asymptomatic HIV+ Subjects

SCTB or poor specificity?

Culture as reference standard for a SD?

- The immune system can respond to *in vivo* antigen at thresholds that are lower than are detected by culture.
- Does the presence of anti-MS antibodies indicate a high-risk for progression to TB or poor specificity?
- Studies with well characterized, long term followed cohorts of high-risk individuals may provide answers.
**Spectrum of TB**

- **Primary TB**
  - Direct smear +
  - 5-10x10^3 AFB/ml
- **Reactivation TB**
  - Direct smear -
- **Bacterial Burden**
  - Culture + (10-100 AFB/ml)
  - NAAT (~98% pos)
  - CXR/CT
- **Conc. smear -**
  - Culture +
  - NAAT (~60% pos)
  - CT
- **Conc. smear +**
  - 10^2-10^3 AFB/ml
  - CXR
- **Sub-clinical**
- **M. tb Infection**
- **Pulmonary Pathology**
- **LTBI**
- **High burden/low income**
- **Low burden/high income**

**Anti-MS Abs in HIV- TB Patients**

- Delta OD
  - Direct Smear
  - Concentrated Smear
- **SA HIV- Sm+**
- **Ind HIV- Sm+**
- **Ind HIV-Sm-**
- **US HIV- Sm+**
- **US HIV- Sm-**
- **HIV/TST+**
Sensitivity of a SD Test

What must the SD be as sensitive as?

- Direct sputum smear?
- Concentrated sputum smear?
- NAAT
- Solid culture, Liquid culture

“The sensitivity of the direct sputum smear is highly variable. Even a standardized POC test that can replace the direct smear will revolutionize TB control”.

(Max Salfinger)

Reference Standards for SD

Sensitivity: Culture (but rethink sensitivity of POC)

Specificity: Endemic subjects
           PPD+/IGRA+ subjects
           BCG Vaccinated subjects
           NTBLD Patients
           NTBLI Patients
           HIV+TB- Patients at low risk for TB
           Culture negative TB suspects