Rapid Diagnosis of Tuberculosis
An Overview

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埃及時代
西元前 3700-1000年

土偶
木乃伊（Nesperhan, priest of Amun）
South-East Asia Region had the largest number of new tuberculosis (TB) cases, which accounted for 35% of the global new and relapse cases.
**Trends of Tuberculosis in Taiwan**

**Incidence, 1979-2006**

Inappropriate current TB control infrastructure
Active reporting
Increased rates of MDRTB
Special hosts (HIV, DM, alcoholism)

Statistics of Communicable Diseases, CDC, Taiwan, 2006
**Drug-Resistant M. tuberculosis**

**2000-2006, NTUH**

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![Graph showing the percentage of resistance to different drugs from 2000 to 2006.](image)

P value


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**Drug-Resistant M. tuberculosis**

**2000-2006, NTUH**

**XDRTB (2005)**

Extensively drug-resistant TB: (MDR + one FG + one of capreomycin, amikacin, or kanamycin), 10 cases

![Graph showing the percentage of resistance to different drugs from 2000 to 2006.](image)

P value

Isolation of Mycobacteria
1996-2007, NTUH

Epidemiology of NTM
Environmental Sources

- Most NTM have been recovered from water and soil

<table>
<thead>
<tr>
<th>Mycobacteria</th>
<th>Sources of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAC, <em>M. kansasii</em></td>
<td>Tap water; airborne</td>
</tr>
<tr>
<td><em>M. marinum</em></td>
<td>Salt, fresh water, fish tanks, swimming pool</td>
</tr>
<tr>
<td><em>M. xenopi</em></td>
<td>Hot water; hospital heating tank (43-45°C)</td>
</tr>
<tr>
<td><em>M. simiae</em></td>
<td>Tap water</td>
</tr>
<tr>
<td><em>M. genavense</em></td>
<td>Dogs, pet bird (psittacine birds)</td>
</tr>
<tr>
<td>Rapid growers</td>
<td>Tap or distilled water, dialysate; nosocomial</td>
</tr>
</tbody>
</table>

- Environmental sources of infection are likely:
  - *M. ulcerans, M. haemophilum, M. szulgai, M. celatum, M. genavense, M. conspicumm*
Clinical Relevance of Mycobacteria Isolated from Respiratory Specimens

- M. tuberculosis
- M. kansasii
- M. avium-intracellare
- M. abscessus
- M. fortuitum
- M. celatum
- M. gordonae
- M. bovis, BCG?
- M. paratuberculosis

<table>
<thead>
<tr>
<th>Species pathogenicity</th>
<th>Patient risk factors</th>
<th>Test indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. gordonae, M. mucogenicum, M. terrae complex</td>
<td>NTM-associated syndrome</td>
<td>Severe immunocompromise</td>
</tr>
<tr>
<td>Contaminants</td>
<td>Non-specific symptoms</td>
<td>HIV</td>
</tr>
<tr>
<td></td>
<td>Incidental finding</td>
<td>Immunosuppressive drugs</td>
</tr>
</tbody>
</table>

Infections Caused by NTM
423 Patients, 1997-2003, NTUH

- M. avium complex
- M. abscessus
- M. chelonae
- M. fortuitum
- M. kansasii

Ting LW et al. Epidemiol Infect 2005
Tuberculosis

*M. celatum* pneumonia
NTUH, Acid-fast (+) 
Only 60% TB

9/12/2004, 3988628
Infected wound
AFS (+)

M. haemophilum
Multiple nodules mimicking tumor
## Level of Laboratory and Performance

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid-fast smears (I: &gt;15 specimens/week, within 24 h)</td>
<td>Yes</td>
</tr>
<tr>
<td>Culture (II: &gt;20 specimens/week)</td>
<td>No*</td>
</tr>
<tr>
<td>Identification of <em>M. tuberculosis</em> complex</td>
<td>No*</td>
</tr>
<tr>
<td>Identification of all mycobacteria</td>
<td>No*</td>
</tr>
<tr>
<td>Drug susceptibility of <em>M. tuberculosis</em> complex</td>
<td>No*</td>
</tr>
<tr>
<td>Drug susceptibility of nontuberculous mycobacteria (NTM)</td>
<td>No*</td>
</tr>
</tbody>
</table>

*Send to a qualified reference laboratory for analysis (within 24 h)*

| BSL-2 and BSL-3 | BSC Class I and Class II |

## Mycobacteriology Laboratory (I) NTUH

- **Safety**: Biosafety level 2
- **Decontamination and digestion**: NALC-NaOH
- **Acid-fast staining**: Fluorochrome, Kinyoun
- **Culture media**: LJ slant, 7H11, 7H9 broth (BACTEC-MGIT)
- **Culture condition**: 35-37°C; 5-10% CO₂; 6-8 w
- **Identification**: Biochemical, GLC, sequencing
- **Susceptibility**: Proportional, E test, disk diffusion
### Reporting the Average Number of AFB

<table>
<thead>
<tr>
<th>Number of AFB found at 1000x</th>
<th>Report as:</th>
<th>Report as:</th>
<th>If count is made at 250x, then report as*:</th>
<th>If count is made at 450x, then report as:</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Negative for AFB</td>
<td>Negative for AFB</td>
<td>Negative for AFB</td>
<td>Negative for AFB</td>
</tr>
<tr>
<td>1-2 per 300 fields</td>
<td>(Order repeat specimen)**</td>
<td>Number seen. (Order repeat specimen)**</td>
<td>Number seen. (Order repeat specimen)**</td>
<td>Number seen. (Order repeat specimen)**</td>
</tr>
<tr>
<td>1-9 per 100 fields</td>
<td>1+</td>
<td>Number seen per 100 fields</td>
<td>Number seen/10 per 100 fields</td>
<td>Number seen/4 per 100 fields</td>
</tr>
<tr>
<td>1-9 per 10 fields</td>
<td>2+</td>
<td>Number seen per 10 fields</td>
<td>Number seen/10 per 10 fields</td>
<td>Number seen/4 per 10 fields</td>
</tr>
<tr>
<td>1-9 per field</td>
<td>3+</td>
<td>Number seen per field</td>
<td>Number seen/10 per field</td>
<td>Number seen/4 per field</td>
</tr>
<tr>
<td>&gt;9 per field</td>
<td>4+</td>
<td>&gt;Number seen per field</td>
<td>&gt;Number seen/10 per field</td>
<td>&gt;Number seen/4 per field</td>
</tr>
</tbody>
</table>

*i. e., divide the actual number seen by 10, for example: 30 AFB were observed in 100 fields at 250x, then 30/10=3 AFB/100 fields, or 1+*  

**Only 1 to 2 AFB per 300 fields is not considered positive, but does indicate that another specimen should be requested, and another smear made from the new specimen.
M. abscessus

M. abscessus (5th day)

M. chelonae

Single clone with different morphotypes
Acid-Fast Smear-Positive

TB or NTM?

- September 2005-June 2006
- 100 patients with positive AFS
  - 65 patients were culture-positive for TB
  - 35 patients were NTM (MAC 11, rapid growers 15)
- 16 were NTM infections

AFS (+) 35% NTM

Unnecessary isolation and anti-TB treatment

AFS-positive due to NTM more likely in
- Bronchiectasis, old pulmonary TB, pulmonary fibrotic change

Data submitted
### CDC Recommended Criteria

**Colony Enumeration**

<table>
<thead>
<tr>
<th>Quantitation</th>
<th>Report</th>
</tr>
</thead>
<tbody>
<tr>
<td>No colonies</td>
<td>No AFB growth</td>
</tr>
<tr>
<td>&lt;50 colonies</td>
<td>Actual count</td>
</tr>
<tr>
<td>50-100 colonies</td>
<td>1+</td>
</tr>
<tr>
<td>100-200 colonies</td>
<td>2+</td>
</tr>
<tr>
<td>200-500 colonies</td>
<td>3+</td>
</tr>
<tr>
<td>&gt;500 colonies</td>
<td>4+</td>
</tr>
</tbody>
</table>

**Extent of disease or response to therapy**

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![Images of colonies and bacteria](image-url)
Nucleic Acid Amplification (NAA)

- MTD (Gen-Probe), Roche Cobas Amplicor
- Available for *M. tuberculosis* complex, MAC, *M. gordonae*, and *M. kansasii*
- For culture confirmation only (poor sensitivity for direct detection from clinical specimens)
- Combined with commercial (BACTEC) systems
- Limitations
  - NOT differentiate between *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum*, and *M. microti*
  - False (+) with MTB probe: *M. terrae*, *M. celatum*

### Commercial Direct Amplification Tests for *M. tuberculosis* Complex

<table>
<thead>
<tr>
<th>Feature</th>
<th>Transcription-Mediated Amplification (TMA)</th>
<th>Polymerase Chain Reaction (PCR)</th>
<th>Strand Displacement Amplification (SDA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplification method</td>
<td>Transcription-mediated</td>
<td>Polymerase chain reaction</td>
<td>Homogeneous Strand Displacement</td>
</tr>
<tr>
<td>Target</td>
<td>RNA</td>
<td>DNA</td>
<td>DNA</td>
</tr>
<tr>
<td>Probe</td>
<td>16S ribosomal RNA</td>
<td>16S ribosomal RNA gene</td>
<td>IS6110</td>
</tr>
<tr>
<td>Enzymes</td>
<td>Reverse transcriptase, T7 RNA polymerase</td>
<td>Taq DNA polymerase</td>
<td>BSOB1, EXO-BST</td>
</tr>
<tr>
<td>Amplicon containment</td>
<td>Procedural</td>
<td>Uracil-N-glycosylase (UNG)</td>
<td>Closed microwell</td>
</tr>
<tr>
<td>Assay time (hours)</td>
<td>3</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td># Samples per run</td>
<td>50</td>
<td>48</td>
<td>96</td>
</tr>
<tr>
<td>Format</td>
<td>Tube</td>
<td>Microwell plate</td>
<td>Microwell plate</td>
</tr>
<tr>
<td>Sample volume (µL)</td>
<td>450</td>
<td>100</td>
<td>500</td>
</tr>
<tr>
<td>Sample lysis</td>
<td>Sonication &amp; beads</td>
<td>60°C, NaOH, Triton X</td>
<td>Heat and sonication</td>
</tr>
<tr>
<td>Detection</td>
<td>Hybridization protection and chemiluminescence</td>
<td>Colorimetric</td>
<td>Fluorescent signal</td>
</tr>
</tbody>
</table>
Molecular Diagnosis

Performance Assessment of a Nested-PCR Assay (the RAPID BAP-MTB) and the BD ProbeTec ET System for Detection of Mycobacterium tuberculosis in Clinical Specimens


Department of Internal Medicine and Laboratory Medicine, National Taiwan University Hospital, and School of Medical Technology, National Taiwan University College of Medicine, Taipei, Taiwan.

Sensitivity 66.7% (88.9% vs. 57.1%) Specitivity 84.7%

Performance Assessment of the DR MTBC Screen Assay and the BD ProbeTec ET System for Direct Detection of Mycobacterium tuberculosis in Respiratory Specimens

Jann-Yuan Wang, Li-Na Lee, Hsiao-Lung Hsu, Po-Ren Hsueh, and Kwon-Tay Luh

Department of Internal Medicine and Laboratory Medicine, National Taiwan University Hospital, Taipei, Taiwan.

Sensitivity 56.6% (83.9% vs. 45.3%) Specitivity 98.9%
抗原偵測

檢體：MGIT or LJ elution

- **BD Capilia TB**
  - Antigen: MPB64 (MTB64) immunochromatographic assay (ICA)

檢體：Serum, Plasma, CSF, PE,…..

- **台塑結核菌抗原快速檢驗試劑**
  - Antigen: CFP10-ESAT
**Capilia TB Assay and the BD ProbeTec ET**

**Rapid Culture Confirmation of MTB**

<table>
<thead>
<tr>
<th>Assay (no. of samples)</th>
<th>Culture results</th>
<th>Predictive values (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M. tuberculosis</td>
<td>Sensitivity</td>
</tr>
<tr>
<td>All Assay (+)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All Assay (-)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This study demonstrated that the Capilia TB assay has a similar diagnostic value to the BD ProbeTec ET assay. With the immunochromatographic method, it is less time-consuming and does not require other laboratory equipment.


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**Correlation between Two Culture Confirmation Assays**

<table>
<thead>
<tr>
<th>Capilia TB result</th>
<th>CTB result</th>
<th>Culture result</th>
<th>M. tuberculosis</th>
<th>Nontuberculous mycobacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>109</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>66</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Collected from 2 patients after 10 and 27 days of antiTB treatment, respectively.

<sup>b</sup> *M. avium-intracellulare* complex in 1 and *M. chelonae* in the other.

<sup>c</sup> The 2 samples were collected from 2 patients after antiTB treatment.

<sup>d</sup> *M. fortuitum* in 1 and *M. triviale* in the other.

**Specific Antigens of M. tuberculosis**

<table>
<thead>
<tr>
<th>Mycobacterial strain</th>
<th>Antigen</th>
<th>ESAT-6</th>
<th>CFP 10</th>
<th>MPT 64</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tuberculosis complex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. tuberculosis</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>M. africanum</em></td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td><em>M. bovis</em></td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>BCG substrains</td>
<td>-</td>
<td>-</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td><strong>Environmental strains</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. abscessus</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>M. avium</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>M. cheloneae</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>M. intracellulare</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>M. kansasii</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>M. marinum</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>M. szulgai</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
**ELISPOT Test**

**ELISPOT: An In-Vitro Enzyme-Linked Immunosorbent Spot Assay Counting Sensitized T-Cells**

- **Presentation of mycobacterial antigens**
- **In-vitro blood test**
- **Antigen: PPD or TB specific antigens**
- **Measurement of IFN-γ Secreting T-Cells**

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**Diagnosis of Tuberculosis in the Suspects in Endemic Area by Using Enzyme-Linked Immunospot Assay for Gamma-Interferon**

**T SPOT-TB Assay**

- January 1 to June 30, 2005
- 65 suspects: fever or respiratory symptoms ≥2 weeks with compatible radiographic findings and/or histopathology
  - 39 active TB: 37 culture (+), 31 pulmonary
  - 26 non-TB: 8 (12.3%) NTM
- ELISPOT assay (enzyme-linked immunospot)
  - ESAT-6 (early secretory antigenic target 6) and CFP-10 (Culture filtrate protein 10)
  - IFN-γ by activated T cell

*Wang CY et al. Emerg Infect Dis 2007*
Diagnosis of Tuberculosis in the Suspects in Endemic Area

ELISPOT-TB Assay

<table>
<thead>
<tr>
<th>AFS (no.)</th>
<th>TB (n=39)</th>
<th>Non-TB (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SPOT (+)</td>
<td>SPOT (-)</td>
</tr>
<tr>
<td>Positive (33)</td>
<td>19</td>
<td>3</td>
</tr>
<tr>
<td>Negative (32)</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>Total (65)</td>
<td>34</td>
<td>5</td>
</tr>
</tbody>
</table>


ELISPOT and acid-fast stain results

- ELISPOT and acid-fast stain results

High accuracy (>80%) of the T SPOT-TB assay for active tuberculosis in clinical suspects, even in an area of high incidence of nontuberculous mycobacterial diseases

- M. marinum (1), M. chelonae (1), and MAC (1)

Quantiferon 與 T-spot
體外免疫診斷試劑敏感度與特異度比較

<table>
<thead>
<tr>
<th>TB</th>
<th>Non-TB</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Predictive Value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=109)</td>
<td>(n=45)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Interferon gamma assays provided more rapid diagnosis of tuberculosis (mean difference 8.23 ± 12.86 days, p < 0.001) than the conventional culture method.
- Use of interferon gamma assays may shorten the duration before diagnosis of tuberculosis, especially in smear negative patients and those with extrapulmonary disease

Chou CH data submitted

Enzyme-linked Immunospot Assay for Interferon-gamma in the Diagnosis of Tuberculous Pleurisy

Pleural effusion of undetermined etiology: 40

- Pleural fluid ELISPOT positive: 21
  - TB: 18
    - Biopsy not done: 6
    - Fluid PCR: (-) x2
    - Sputum PCR: (-) x2
    - Histology diagnostic: 9
    - Histology non-diagnostic: 3
  - Non-TB: 3
    - Biopsy: not done
    - Mesothelioma: 1
    - Adenocarcinoma: 1
    - MAC*: 1
- Pleural fluid ELISPOT negative: 19
  - TB: 1
    - Biopsy: not done
  - Non-TB: 18
    - Cancer: 8
      - Lymphoma: 1
      - Infarction: 1
      - Parapneumonic: 3
      - Heart failure: 3
      - MAC*: 1
      - Cytomegalovirus: 1

Enzyme-linked Immunospot Assay for Interferon-gamma in the Diagnosis of Tuberculous Pleurisy

- The sensitivity, specificity, positive, and negative predictive values of the assay on pleural fluid (blood) were 94.7 (77.8), 85.7 (90.5), 85.7 (87.5) and 94.7% (82.6%), respectively.
- Antigen-specific, interferon-gamma secreting T cells were concentrated 8 to 10 times in pleural fluid as compared with blood.
- ELISPOT assay for interferon-gamma can accurately diagnose tuberculous pleurisy and is helpful for patients not suitable for pleural biopsy and those whose biopsy results are non-diagnostic.


Diagnosis of Active Tuberculous Serositis by Antigen-Specific Interferon-γ Response of Cavity Fluid Cells

The cavity fluid IFN-γ assay could be a method for accurately and promptly diagnosing active tuberculous serositis.

82 HCWs: TST positive in 36 (42.7%), IFN-γ assay positive in 16 (19.5%) The overall agreement between the two tests was 67.5% TST-positive group: the IFN-γ levels increased significantly from 0.05 to 0.19 ($p = 0.011$), and 3 of 18 participants (16.7%) had conversion of their IFN-γ assay TST-negative group, the IFN-γ levels did not change after the TST IFN-γ level could be influenced by the TST, in the TST-positive population, when a follow-up IFN-γ assay is performed 2 to 4 weeks later Choi JC et al. Chest 2008;133:1415-20.
Tuberculous Lymphadenitis

Disseminated M. kansassii Infection
Subcutaneous lesions-Recurrence
Granuloma (+)
Caseating necrosis (+)
NTM tenosynovitis
TB Elispot (-)

Ciprofloxacin
+ Clarithromycin
2 months
M. marinum Infection

NTM tenosynovitis
TB Elispot (+)

T Spot (+)
Tuberculous Lymphadenitis?

11th WPCCID
Nov. 29-Dec. 3, 2008

www.wpccid2008.tw

Abstract submission
Deadline: July 31