Update on Tuberculosis Laboratory Investigation

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Al-Khatib Medical Laboratory
HISTORY of Tuberculosis

Tuberculosis Is an Ancient Disease Identified as Spinal Tuberculosis in Egyptian Mummies. History dates to 1550 – 1080 BC. Identified by PCR.
A tribute to Robert Koch

- World TB Day is March 24. This annual event commemorates the date in 1882 when Dr. Robert Koch announced his discovery of *Mycobacterium tuberculosis*, the bacteria that cause tuberculosis (TB).
Some scientific achievements through 125 years

1882
R. Koch, 
*M. tuberculosis*

1920
Löwenstein, 
Jensen

1882/1883
Ehrlich, 
Ziehl and 
Neelsen

1921
Calmette 
Guerin

1907/1909
von Pirquet, 
Mantoux 
TST

1943
Waksman, 
Streptomycin
Tuberculosis A Historical Disease Progress to Future

THE NEXT TO GO

FIGHT TUBERCULOSIS!
Red Cross Christmas Seal Campaign
THE RETURN OF THE WHITE PLAGUE

GLOBAL POVERTY AND THE 'NEW' TUBERCULOSIS

EDITED BY MATTHEW GANDY AND ALIMUDDIN ZUMLA
Global incidence of tuberculosis
Still rising as a result of the growing epidemic in Africa
Laboratory diagnosis

- Direct Microscopy
- Culture
- Nucleic acid technology
- Immunological methods
  - Skin test (TST)
  - Rapid test
  - IGRA test
Microscopy and Culturing still a top priority
Pulmonary Tuberculosis

Morning Sputum specimens

Sputum sample is obtained by coughing and is examined in the laboratory.
Laboratory Diagnosis

1- Sputum smears stained by Z-N stain

Two morning successive mucopurulent sputum samples are needed to diagnoses pulmonary TB.
Smear showing Acid Fast Bacilli.
What is Smear Positivity

WHO

All patients who have submitted two Specimens and found to be positive for identification of AFB
## Grading

<table>
<thead>
<tr>
<th>If the slide has</th>
<th>Result</th>
<th>Grading</th>
<th>No. of fields to be examined</th>
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<tr>
<td>More than 10 AFB /oil immersion field</td>
<td>pos</td>
<td>3+</td>
<td>20</td>
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<td>1-10 AFB/ oil immersion field</td>
<td>pos</td>
<td>2+</td>
<td>50</td>
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<tr>
<td>10-99 AFB/100 oil immersion field</td>
<td>pos</td>
<td>1+</td>
<td>100</td>
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<tr>
<td>1-9 AFB/100 oil immersion field</td>
<td>pos</td>
<td>Scanty*</td>
<td>100</td>
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<tr>
<td>No AFB in 100 oil immersion field</td>
<td>neg</td>
<td></td>
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</table>

*record actual number of bacilli seen in 100 fields
2- Detecting AFB by fluorochrome stain using fluorescence microscopy:
The smear may be stained by auramine-O dye.
Acid Fast Bacilli as seen under Fluorescent Microscope
Direct Microscopy

- Preferred to do two methods
- Make sure it is real sputum
- At least 3 specimen
Microscopic Exam

Smears:
Specificity 42%
Sensitivity:

46% in pulmonary TB
31% in extrapulmonary TB

Clinical and Diagnostic Laboratory Immunology, April 2005, p. 491-496, Vol. 12, No. 4
Culturing Mycobacterium

- Culturing for isolation of Mycobacterium spp continues to be a **Gold standard**, particularly in Developing countries.
- Need only 10 – 100 bacilli / 1 ml of sputum.
Growth of Acid fast bacilli on L J Medium.
Eight Week Growth of Mycobacterium tuberculosis on Lowenstein-Jensen Agar
June 2007: Liquid culture and DST in LMIC were endorsed by WHO’s Strategic and Technical Advisory Group for TB (STAG TB) and subsequently issued as WHO policy.

Culture

- Specificity 59%
- Sensitivity:
  - 63% in pulmonary TB
  - 42% in extrapulmonary

Clinical and Diagnostic Laboratory Immunology, April 2005, p. 491-496, Vol. 12, No. 4
Limitation in Culturing

- Mycobacterium spp are slow growing.
- Need 6 – 8 weeks for growing.
- Specimens can be contaminated while growing, needs repeated specimens, in turn patients lose confidence in Laboratories.
Molecular Methods in Diagnosis of Tuberculosis
Lipid-Rich Cell Wall of Mycobacterium

Mycolic acids

- Arabinogalactan mycolate layer
- Peptidoglycan layer

Polypeptides (e.g., PPD) Protein Derivative

Mycolic acids (free lipids, glycolipids, peptidoglycolipids)

Cytoplasm Cytoplasmic membranes
Advantages:

- Rapid procedure (3–4 hours)
- High sensitivity (1-10 bacilli / ml sputum)
Real Time PCR replacing older Methods
PERIPHERAL BLOOD-BASED POLYMERASE CHAIN REACTION IN DIAGNOSIS OF PULMONARY TUBERCULOSIS

- **PCR PB**
  - Sensitivity 20%
  - Specificity 95%

- **PCR Site of infection**
  - Sensitivity 90%
  - Specificity 95%
## Sensitivity for TB Detection

<table>
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<tr>
<th>Technique</th>
<th>No. Bacilli</th>
<th>Bacilli/ml</th>
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<td>Microscopy</td>
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<td>PCR</td>
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MDR Tuberculosis

**MDRTB** refers to strains of the bacterium which are proven in a laboratory to be resistant to the two most active anti-TB drugs, isoniazid and rifampicin. Treatment of MDRTB is extremely expensive, toxic, arduous, and often unsuccessful.
Genetics of Rifampin Resistance in *M. tuberculosis*
RIF resistance as surrogate marker for MDR TB

- RIF resistance as a mono resistance not very frequent (5-15% of them)
- 80-95% of RIF resistant strains are also resistant to INH

Cepheid®

Xpert™ MTB

GeneXpert

Automated Sample Prep, Amplification and Detection

<120 minutes
A new test could spark revolution in TB fight

The new test can diagnose the disease in 100 minutes, whereas current tests take 3 months.

MidEast Online
From Kit Kiland

TB strikes the poor in developing countries.
Rapid Molecular Detection of Tuberculosis and Rifampin Resistance

Catharina C. Boehme, M.D., Pamela Nbeta, M.D., Doris Hilleleman, Ph.D., Mark P. Nicol, Ph.D., Shubhada Shenai, Ph.D., Fiorella Krapp, M.D., Jenny Allen, B.Tech., Rasim Tahirli, M.D., Robert Blakemore, B.S., Roxana Rustomjee, M.D., Ph.D., Ana Milovic, M.S., Martin Jones, Ph.D., Sean M. O’Brien, Ph.D., David H. Persing, M.D., Ph.D., Sabine Ruesch-Gerdes, M.D., Eduardo Gotuzzo, M.D., Camilla Rodrigues, M.D., David Alland, M.D., and Mark D. Perkins, M.D.

sample processing in 1730 patients with suspected drug-sensitive or multidrug-resistant pulmonary tuberculosis. Eligible patients in Peru, Azerbaijan, South Africa, and

CONCLUSIONS

The MTB/RIF test provided sensitive detection of tuberculosis and rifampin resistance directly from untreated sputum in less than 2 hours with minimal hands-on time. (Funded by the Foundation for Innovative New Diagnostics.)

N ENGL J MED 363;11 NEJM.ORG SEPTEMBER 9, 2010
Immunological methods

- Tuberculin Skin test (TST) (Mantoux)
- Antibodies detections
- IGRA
Tuberculin skin test

1. Mantoux test
   . 0.1 ml of PPD containing 5 TU is injected intradermally on flexor aspect of forearm
   . Examine after 48 – 72 hrs

Color change without induration is not included in the measurement
Applying the tuberculin skin test
Applying the tuberculin skin test

Courtesy of Dr. Marc Steben
Tuberculin Test
( Mantoux Test )

- Diameter of induration
  - Negative: 0 - <5 mm
  - Positive: >5 –15 mm
  - Strong Positive: > 15 mm
- >10 mm children <4 years
Specific Detection of Tuberculosis Infection
Gerald H. Mazurek and Izuo Tsuyuguchi

Specificity: 35.4% (cutoff: 10-mm induration).
Sensitivity: 65.8%.
Antibodies Detection
Nisar Khan, Ihsanullah Mian,* Zia-Ullah,** Jan Muhammad

PERFORMANCE OF ICT-TB TEST IN THE DETECTION OF PULMONARY AND EXTRA-PULMONARY TUBERCULOSIS

JAMC 21(3): Jul-Sep 2009

The sensitivity, specificity, and negative predictive value of the ICT Tuberculosis test for pulmonary TB were 33.3%, 100%, and 52.9%
Currently marketed rapid serologic TB tests vary widely in performance but generally **perform poorly compared to** a combined reference standard using well-characterized archived serum specimens.
Interferon –γ Assay (IGRA)

- QuantiFERON TB gold Test
- T-Spot Test.

whole-blood assays
Mycobacterium DNA

RD1
Interferon –γ Relased
IGRA
Specificity: 98.1%
Sensitivity: 89%

Specific Detection of Tuberculosis Infection
An Interferon γ-based Assay Using New Antigens

Effective in children less than 6 months of age.

IGRA positive result cannot distinguish between active tuberculosis and LTBI.

Not effected by BCG.
Implementing policy recommendations
Patients with TB products

- Sputum
- Urine
- Pus
- Body fluid

- Direct Exam
- PCR + Drug Resistance
- Culture
Patients suspected TB but No TB products

- TST
- QuantiFERON TB gold Test
- T-Spot Test.
Patients with Non-TB products

- TST if positive go to blood test for IGRA - QuantiFERON TB gold Test - T-Spot Test.

### TB distribution in Syria 1/1-30/6/2010

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11/14/2010
Thank you for your attention