TB Intensive
San Antonio, Texas
December 1-3, 2010

Diagnosis of TB: Laboratory
Max Salfinger, MD; Florida DOH
December 1, 2010
What’s HOT:

- Patient-oriented
- Shortest TAT possible
- Molecular assays
- Electronic reporting

“It is health which is real wealth not pieces of silver and gold.”

Mahatma Gandhi, 1869-1948
Promoting outcomes for TB patients


Adherence

Cure

Time to negativity?

RIF resistance?

Tuberculosis?

Chest x-ray

History/physical exam

SEES A DOCTOR

Adherence/Cure
Collection and Transport

- Quality testing requires quality specimen
- 5 to 10 ml sputum
- Proper declaration – shipper’s responsibility
- Diagnostic specimen

TUBERCULOSIS

Yes
or
No
?

Identification
Growth detection
Microscopy
Nucleic acid amplification
HEALTHY PEOPLE 2010

14-14

Reduce TAT for laboratory Dx

Target: 2 d for 75%

[21 d // ’96]

U.S. Department of Health and Human Services, January 2000
Nucleic Acid Amplification

- FDA approved for respiratory specimens
  - Smear-positive (December, 1995)
  - Smear-negative* (September, 1999)
- MMWR, January 16, 2009 [Universal]
  - Florida DOH Memo (June 19, 2009)
“NAA testing should be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management or TB control activities”.

MMWR Jan 16, 2009

Reduction in turnaround time for laboratory diagnosis of pulmonary TB by routine use of NAAT


Processing: 5 days; NAAT 4 days; broth medium monitored 7 days

NAAT (first specimen) – AFB and culture (3 specimens) - 797 pt [81 TB]

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sens</th>
<th>Spec</th>
<th>PPV</th>
<th>NPV</th>
<th>Mean TAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB-3</td>
<td>70</td>
<td>98</td>
<td>79</td>
<td>96.7</td>
<td>1</td>
</tr>
<tr>
<td>NAAT-1</td>
<td>90</td>
<td>100</td>
<td>100</td>
<td>98.9</td>
<td>2</td>
</tr>
<tr>
<td>Culture-3</td>
<td>96</td>
<td>100</td>
<td>100</td>
<td>99.6</td>
<td>18</td>
</tr>
</tbody>
</table>
Cumulative TAT


Microscopy

- Ziehl-Neelsen and fluorochrome
- 5,000 to 10,000 bacilli per ml for a positive smear
- Results within 24 hours
- Smear-positive / culture-negative
Culture

Demanding *instant* results!

20 Min 20 Hours
Processing Sputum

- Procedures kill all but 10-20% of the mycobacteria
- Contamination
  - 2-5% of sputum specimens on Lowenstein-Jensen medium (LJ)

Growth Detection

- 78% culture positive TB cases in 2007
- Solid and liquid medium
- Commercial broth system
- Smear-positive/ culture-negative
BACTEC 460TB and MGIT 960

- **BACTEC 460 TB**
  - shorter TAT
  - semi-automated
  - radioactive
  - requires needles
  - special gas mix

- **BACTEC MGIT 960**
  - fully automated, walk-away
  - non-radiometric
  - no need for needles (inoculation and testing)
  - no need for manual loading of vials
  - no need to establish reading schedules

Identification

- **152** species in genus *Mycobacterium* as of September 2010

- Clinical & Environmental Microbiology Branch @ CDC Division of Healthcare Quality Promotion

- **M. tuberculosis complex**
  *M. tuberculosis; M. bovis; M. bovis* BCG; etc.
**Identification**

- Quantum leap
  - Nucleic acid probe kits
- High Performance Liquid Chromatography
  - Cell wall analyses
- **PCR Restriction Analysis**
- DNA sequencing
- Biochemicals are second

**NAAT, AccuProbe, and 16S Sequencing**

- **Detect all members of M. tuberculosis complex**
  - *M. tuberculosis*
  - *M. bovis*
  - *M. bovis BCG*
  - *M. africanum*
    - *M. caprae*
    - *M. microti*
    - *M. canettii*
    - *M. pinnipedii*
    - *M. mungi*
Screening 1,685 Clinical Isolates Belonging to the TBC (2001-2004)

<table>
<thead>
<tr>
<th></th>
<th>NUMBER</th>
<th>PERCENT</th>
</tr>
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<tbody>
<tr>
<td>M. tuberculosis</td>
<td>1,594</td>
<td>94.6%</td>
</tr>
<tr>
<td>M. africanum</td>
<td>31</td>
<td>1.8%</td>
</tr>
<tr>
<td>M. bovis</td>
<td>36</td>
<td>2.1%</td>
</tr>
<tr>
<td>M. caprae</td>
<td>1</td>
<td>0.1%</td>
</tr>
<tr>
<td>M. bovis BCG</td>
<td>23</td>
<td>1.4%</td>
</tr>
</tbody>
</table>

Susceptibility testing or Detection of drug resistance
Susceptibility Testing

- On all initial M.tb isolates (2007: 88.7%, US)
  - SIRE + PZA
- Faster with radiometric
  - Fastest with $rpoB$ analysis
- Confirmation of drug resistance

RIFAMPIN resistance

- Yes
- or
- No
- ?

Clinical course

Egg-based AST

Agar-based AST

Radiometric/Non-r $rpoB$ analysis [line probes & RT-PCR]
Codon 526 (Cytosine-Adenine-Cytosine) encodes histidine in a susceptible strain; replaced with (Guanine-Adenine-Cytosine) aspartate in a resistant strain.

### Molecular testing:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Gene</th>
<th>% mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIF</td>
<td>rpoB</td>
<td>&gt;96%</td>
</tr>
<tr>
<td>INH</td>
<td>katG</td>
<td>40-60%</td>
</tr>
<tr>
<td>INH-ETH</td>
<td>inhA</td>
<td>15-43%</td>
</tr>
<tr>
<td>PZA</td>
<td>pncA</td>
<td>72-97%</td>
</tr>
<tr>
<td>F-quinolones</td>
<td>gyrA</td>
<td>75-94%</td>
</tr>
<tr>
<td>Capreomycin</td>
<td>tlyA</td>
<td>unknown</td>
</tr>
</tbody>
</table>

Curry Center: Drug-resistant tuberculosis - A survival guide for clinicians, 2nd Ed. 2008
Molecular Drug Resistance Detection (MDDR)

Isolates (specimens the exception)

Contact information:

Telephone 404-639-2455
Fax 404-639-5491
Email TBLab@cdc.gov

CDC Mycobacteriology Laboratory Branch (MLB)

CDC Performance Data:

<table>
<thead>
<tr>
<th>Drug Gene</th>
<th>Sensitivity Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIF rpoB</td>
<td>96.1% 97%</td>
</tr>
<tr>
<td>INH inhA + katG</td>
<td>88.6 98.7</td>
</tr>
<tr>
<td>FQ gyrA</td>
<td>82.2 97</td>
</tr>
<tr>
<td>KAN rrs + eis</td>
<td>86.8 96.9</td>
</tr>
<tr>
<td>AMK rrs</td>
<td>87.9 99</td>
</tr>
<tr>
<td>CAP rrs + tlyA</td>
<td>44.6 85.9</td>
</tr>
</tbody>
</table>
Limitations

- Potential to identify mutations that do not confer phenotypic resistance
- Not all genetic loci associated with resistance are known; therefore, ‘no mutation detected’ does not rule out resistance

INNO-LiPA Rif. TB
Innogenetics, Belgium
GenoType MTBDRplus
Hain Lifescience, Germany
GeneXpert
Cepheid, California
GenoType® Series Mycobacteria

- GenoType® Mycobacterium CM/AS
- GenoType® MTBC
- **GenoType® MTBDRplus**
- GenoType® Mycobacteria Direct

GenoType® MTBDRplus

1. DNA preparation
2. PCR
3. Hybridization
4. Evaluation
Reaction zones of the GenoType® MTBDRplus

Conjugate Control (CC)
A line must develop in this zone, documenting the efficiency of conjugate binding and substrate reaction.

Amplification Control (AC)
When the test is performed correctly, a control amplicon generated during amplification will bind to the Amplification Control zone on the strip. A missing band therefore indicates mistakes during amplification set-up or the carry-over of amplification inhibitors with the isolated DNA. In case of a positive test result, the signal of the Amplification Control zone can be weak. In this case, however, the amplification reaction was performed correctly and the test does not have to be repeated.

M. tuberculosis complex (TUB)
This zone hybridizes, as known, with amplicons generated from all known members of the Mycobacterium tuberculosis complex. If the TUB zone is negative, the tested bacterium does not belong to the M. tuberculosis complex and cannot be evaluated by this test system.

rpoB, katG and inhA probes
These reaction zones detect the respective gene regions (Uni probes) or they document, depending on their character, either by their absence (wild type probes) or their appearance (mutation probes) a resistance to rifampicin and/or isoniazid.

Each pattern that deviates from the wild type pattern indicates resistance of the tested strain!

Rifampicin resistance region of the rpoB gene

rpoB-Wildtype-probes: WT 1 to WT 8
rpoB-Mutation-probes: MUT D516V, H526Y, H526D, S531L

Detection of mutations through missing of wildtype signals
Detection of mutations through presence of mutation signals
### Implementation of rapid molecular screening for MDR TB in a high volume PHL in South Africa

Barnard M et al - AJRCCM April 1, 2008

Western Cape: TB incidence of 932; TB-HIV 28.2%; MDR rate in new (0.9%) and in previously treated pt (3.9%)  

**MTBDR\textit{plus} [Hain test, Germany] on AFB+ - 536 specimens**

<table>
<thead>
<tr>
<th></th>
<th>Sens</th>
<th>Spec</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIF-r</td>
<td>98.9</td>
<td>99.4</td>
<td>97.9</td>
<td>99.7</td>
</tr>
<tr>
<td>INH-r</td>
<td>94.2</td>
<td>99.7</td>
<td>99.1</td>
<td>97.9</td>
</tr>
<tr>
<td>MDR</td>
<td>98.8</td>
<td>100</td>
<td>100</td>
<td>99.7</td>
</tr>
</tbody>
</table>
Follow-Up Specimens I

- Follow-up specimens until **two** consecutive specimens are culture negative
- AFB smear negative
  - At least once a month
- AFB smear positive
  - Bi-weekly

1 sputum specimens per event, if contaminated - repeat
Follow-Up Specimens II

- Follow-up specimens until two consecutive specimens are culture negative...

- Initial cavitation and mo 2 culture positive
  - Extend INH/Rif from four to seven months

- Repeat susceptibility testing after 3 months

- Positive culture at month 4
  - Treatment failure

From a global perspective....
GeneXpert System
Cepheid, Sunnyvale, CA
RT-PCR, < 2 hours

Potential for point of care testing


- Limit of detection – 131 CFU/ml
- \textit{M. tuberculosis} viability – minus 8 log
- 107 clinical specimens/suspicion of TB – Vietnam
  - 100\% - 29/29 AFB + / Culture +
  - 84.6\% - 33/39 AFB- /solid Culture +
  - 71.7\% - 38/53 AFB- / solid/broth Culture
Passenger arrived from Spain on a Friday afternoon
Greeted by Public Health Authorities...
TB suspicion
Friday afternoon: conference call
Saturday afternoon: conference call
Lab results... 4:05 PM

The Molecular Lab in a Cartridge

• All Testing Done Within Cartridge
  – Sample Prep
  – Amplification
  – Detection
Tuberculosis - TB

Sanatorium Care

Current Care

Point of care...?

Reflections
Warning: If technology is master, we shall reach disaster - faster!

Martin von Planta
Discrepant Results

- **Pre-analytic**
  - Mislabling (ward, lab), bronchoscopy, sputum induction, etc.

- **Analytic**
  - Lab testing error, interpretation misjudgments

- **Post-analytic**
  - Lab reporting error, transcription error (patient chart)

"Every test performed without a strict indication, is not only spinning the wheels, it also means that the quality of a more meaningful test is jeopardized."

- **Over-use** of a test ...
- **Under-use** of a test ...
- **Mis-use** of a test  =  ERRORs [IOM]
Effective Laboratory/Program Partnership:

1 + 1 = 3

Program & Laboratory in action...

...a winning team!
Decisions warranted:

- At specimen collection:
  - AFB microscopy
  - Growth detection
  - NAAT-D... detection?
  - NAAT-R... resistance markers?
  - First-line DST - agar based?

- At growth detection:
  - Identification of *M. tuberculosis* complex
  - First-line DST - broth based
  - NAAT-R... resistance markers? [CDC]
  - Second-line and novel compound DST (broth or agar)?

Take Home Messages

- Limitations of tests
- Discrepant results, call lab
- Use public health lab
Never Give Up!

- Fighting TB
- Fighting poverty
- Standing up for PEACE on Earth!