Competing Priorities for TB Laboratory Services

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Microbial Diseases Laboratory
October 2010
CTCA/CAPHLD joint session
Where to put the priority?

- Selective liquid culture
- First line drug susceptibility testing
- Second line drug susceptibility testing
- Confirmatory testing using agar or molecular
- Nucleic acid amplification (NAA) detection of TB complex directly in specimens
- Molecular detection of drug resistance
- Quantiferon or other IGRA test for latent TB
- Genotyping
Changing priorities

• New technologies (e.g. GeneXpert, pyroseq)
• New guidelines (e.g. NAA)
• New information about impact of using rapid technologies (e.g. beacons—Banerjee et al.)
• Dwindling resources
  – Recession means reduced funding for public health
  – Challenge of training and retention of qualified staff
Molecular detection of drug resistance has clinical/public health impact


Rapid drug susceptibility testing with a molecular beacon assay is associated with earlier diagnosis and treatment of MDRTB

“Use of the MB assay reduced time to detection and treatment of MDR TB”
Molecular detection of drug resistance has clinical/public health impact

Treatment characteristics of MDRTB patients with & without beacons testing

<table>
<thead>
<tr>
<th>Outcome</th>
<th>MB</th>
<th>No MB</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median days case report to MDR treatment initiation</td>
<td>38</td>
<td>79</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Median days to culture conversion</td>
<td>61.5</td>
<td>84</td>
<td>0.258</td>
</tr>
<tr>
<td>Median days to culture conversion, smear + patients only</td>
<td>63</td>
<td>90</td>
<td>0.17</td>
</tr>
<tr>
<td>Median days of treatment among patients that completed</td>
<td>732</td>
<td>751</td>
<td>0.61</td>
</tr>
<tr>
<td>treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Impact of MB testing

• The median time to beginning an MDR regimen for patients with MDR TB was 41.5 days earlier when MBs were obtained.

• Total treatment duration and time to culture conversion were both shorter when MBs were used.

• When MDR TB was NOT present, patients were spared from an expanded MDR regimen.

• Conclusion: when DRTB is suspected, ask for molecular detection of drug resistance
Rationale for increasing the use of NAA testing

The way it should work:
TB suspects are identified: prolonged cough (of > 2 weeks), +/- hemoptysis, weight loss, night sweats, fatigue, chest pain, fever

TB suspects should be put in isolation, and started on anti-TB drugs pending laboratory results.
Problem
Median time to initiation of therapy:
AF smear + patients: 1 day
AF smear neg patients: 22 days

NAA to detect TB in smear-negative patients is critical!
Updated Guidelines for the Use of Nucleic Acid Amplification Tests in the Diagnosis of Tuberculosis

MMWR 58(1): 7-10  January 16, 2009

“CDC recommends that NAA testing be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary tuberculosis 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.”
Looking more closely at CDC guideline

• NAA is recommended “from each patient with signs and symptoms of pulmonary tuberculosis”

• What about the sputum or bronchoscopy sample that arrives in the laboratory with a request for routine, fungal, and AFB culture?
  – Does that patient really have signs and symptoms of tuberculosis?
“patient with signs and symptoms of pulmonary tuberculosis”

For many hospitals, sputum specimens arrive in the lab with a request for “routine, fungal, and AFB culture”

– Are these from patients with signs and symptoms of pulmonary tuberculosis? Probably not

– Who sends sputum for AFB to your lab?
  • TB clinic?
  • Patients screened to be true TB suspects?
  • If so, NAA should be standard of practice
The NAA method with FDA clearance for testing smear-negative samples: Gen-Probe Mycobacterium tuberculosis Direct (MTD)

Transcription mediated amplification (not PCR)

Target is rRNA (present in multiple copies/cell)

FDA clearance for smear negative samples obtained in 1999
Better NAA techniques have been developed, but not developed commercially as yet

- **Real-time PCR developed by New York State laboratory (Dr. Kim Musser and others)**
- **Reported at August, 2008, APHL 5th National Conf on Lab Aspects of TB**
- **May be more sensitive than MTD, less labor intensive, quicker, with less potential for amplicon carryover or technical error**
Cepheid GeneXpert

- Grants for development from NIH and FIND
- Combines sample preparation (using microfluidics) with real-time PCR
- Detects *M. tb* and predicts drug resistance (to rifampin as now configured)
Rapid Molecular Detection of Tuberculosis and Rifampin Resistance (Cepheid Xpert MTB/Rif)

C. Boehme, et al NEJM Sept. 2010

Among culture-positive patients, a single, direct MTB/RIF test identified 551 of 561 patients with smear + TB (98.2%) and 124 of 171 with smear-neg TB (72.5%).

The test was specific in 604 of 609 patients without tuberculosis (99.2%).

Cepheid may get FDA clearance initially for TB detection only.
Which labs should use Cepheid Xpert?

Using the instrument for multiple assays could improve cost-effectiveness

**IVD:** Staph aureus, MRSA, enteroviral meningitis, group B Strep, Vanco-resist enterococci

**ASR:** Flu A/B, norovirus, pertussis, Herpes simplex, resp. syncytial, anthrax

**RUO:** C. difficile, TB
Assessing your lab questions

91. Does your Mycobacteriology Laboratory perform or ensure access (via a reference laboratory) to nucleic acid amplification (NAA) testing for direct detection of *M. tuberculosis* complex in AFB smear-positive respiratory initial diagnostic specimen?

92. Does your Mycobacteriology Laboratory perform or ensure access (via a reference laboratory) to NAA testing for direct detection of *M. tuberculosis* complex in AFB smear-negative respiratory specimens from patients at high risk for TB?

93. Does your Mycobacteriology Laboratory perform or provide access (via a reference laboratory) to a *M. tuberculosis* complex NAA test that allows detection of inhibitors?

94. Does your Mycobacteriology Laboratory telephone, fax, or electronically report result of *M. tuberculosis* complex NAA testing results within 48 hours of receipt for 75% of specimens tested in the laboratory?

95. Does your Mycobacteriology Laboratory perform or ensure access (via a reference laboratory) to molecular detection of drug resistance, especially to rifampin and isoniazid?
Molecular Beacon Assay
(at MDL)

• Target: DNA

• Realtime PCR
  – PCR to amplify target sequences
  – At the same time, Molecular beacon probes are used to detect INH and RIF resistance mutations.
    • 2 MBs for INH (targeting katG & inhA)
    • 3 MBs for RIF (targeting core of rpoB)
Real-Time PCR

• 2 components
  – PCR to amplify target sequences.
  – A system to monitor PCR product.
    • Fluorophore-labeled probes
    • An optical device to detect fluorescence
    • Software to record data
• No post-PCR manipulations
  – Fast
    • when PCR is done, results are ready for interpretation.
  – No amplicon contaminations
What is a Molecular Beacon?

Hair-pin structure

← Loop (15-30 nt)
← Stem (5-7 nt)

Fluorophore →

← Quencher
Detection of Mutations with a Molecular Beacon
(Loop portion containing wildtype SQ)

Mutant Sequence

Wildtype Sequence

Molecular Beacon (off)

Hybrid (Molecular Beacon - On)

Fluorophore

Heat

Fluorophore

Light

Quencher

Amplicon

Courtesy of Dr. Probert
### Data for INH
3 years after implementation

<table>
<thead>
<tr>
<th>Cultures &amp; sediments Combined data (186)</th>
<th>INH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phenotypic results</td>
</tr>
<tr>
<td></td>
<td>R</td>
</tr>
<tr>
<td>MB</td>
<td>Mutation detected</td>
</tr>
<tr>
<td></td>
<td>No mutations</td>
</tr>
<tr>
<td></td>
<td>Inconclusive</td>
</tr>
</tbody>
</table>

R: Resistance

S: Sensitivity
Data for RIF
3 years after implementation

<table>
<thead>
<tr>
<th>Cultures &amp; sediments Combined data (186)</th>
<th>RIF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phenotypic results</td>
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MB Performance (3-year data)
(Agreement between MB and phenotypic drug results)

<table>
<thead>
<tr>
<th></th>
<th>INH</th>
<th>RIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultures</td>
<td>98.4%</td>
<td>99%</td>
</tr>
<tr>
<td>Sediments</td>
<td>93.6%</td>
<td>94.9%</td>
</tr>
<tr>
<td>Overall</td>
<td>96.1%</td>
<td>97.2%</td>
</tr>
</tbody>
</table>
Suggestion for requesting molecular beacons testing

• Acid-fast smear-positive specimen
• Some of the specimen sediment is available for sending to the reference lab (State Microbial Diseases Lab)
• Drug resistance is suspected, or
• A susceptible population has been exposed, or
• The culture is mixed or non-viable, so regular drug susceptibility testing can’t be done
Who Is At Higher Risk of MDR-TB?

• History of previous TB treatment, particularly if recent

• Foreign-born patients from countries or ethnicities with high prevalence of MDR
  ▪ Hmong refugees
  ▪ Tibetan ancestry
  ▪ Cases from former USSR, China, Korea, Peru, Honduras are disproportionately MDR
Who Is at Higher Risk of MDR-TB?(2)

- Poor response to standard 4-drug treatment
  - Culture remains (+) after 2 months treatment
- Known exposure to MDR-TB case
- Recent arriver (<1 year in US)
- HIV (+)
  - Higher incidence of Rifampin mono resistance
CDC MDDR
(Molecular Detection of Drug Resistance)

- DNA sequencing
  - Amplify target SQ by PCR
  - Cycle sequencing
  - Line up SQ by sequencer

- Loci examined are:
  - For INH: katG, inhA promoter—same as for MB.
  - For RIF: rpoB—same as for MB
  - For quinolone: gyrA
  - For aminoglycosides & Capreomycin: rrs, tylA, eis promoter

- CDC accepts cultures only
  - Growth from liquid or solid media.
MDDR

• Criteria for submission:
  – Known MDR, screen for XDR.
  – Contact of MDR.

• Advantages
  – Detect mutations associated with:
    • INH, RIF, quinolone and aminoglycoside/cyclopeptide drugs
  – Results show specific mutations
    • MB detects “presence of mutations”, does not show specific mutations.

• Disadvantages
  – Need to wait till culture grows, which may take weeks.
**CLIA ID #: 1100666319**

**Original Submitter:**

**Submitter to CDC:**
- CA Dept. of Public Health
- Microbial Diseases Laboratory Branch
- Abigail Duque/Lab
- Lisa True/Program

**CDC Specimen ID:** 20100015178  
**Specimen:** Mycobacterium tuberculosis complex isolate  
**Medium:** Ur  
**Date Collected:** 2/11/2010  
**Date Received:** 3/4/2010  
**Date Reported:** 3/5/2010  
**Patient:**  
**Submitter Specimen Identifiers:** 10A00250

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## Results for Molecular Detection of Drug Resistance; Conventional Drug Susceptibility Test in progress.

<table>
<thead>
<tr>
<th>Locus (region) examined</th>
<th>Result</th>
<th>Interpretation (based on in-house evaluation of 254 clinical isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rpoB (RRDR)</td>
<td></td>
<td>Rifampin resistant. (100% of isolates in our in-house evaluation of 254 clinical isolates with this mutation are RIF-R.)</td>
</tr>
<tr>
<td>inhA (promoter)</td>
<td>No mutation</td>
<td>Insoxamid resistant. (100% of isolates in our in-house evaluation of 254 clinical isolates with these mutations are INH-R.)</td>
</tr>
<tr>
<td>katG (ser 74 codon)</td>
<td>Mutation: AGC→AAG; Ser315Thr</td>
<td>Cannot rule out fluoroquinolone resistance. (86% of FQ-R isolates in our in-house evaluation of 254 clinical isolates have a mutation at this locus.)</td>
</tr>
<tr>
<td>gyrA (QRDR)</td>
<td>No mutation</td>
<td>Cannot rule out resistance to injectable drugs (kanamycin, capreomycin, amikacin). (In our in-house evaluation of 254 clinical isolates: 88% of AMK-R isolates have a mutation in the rrs locus; 58% of KAN-R isolates have a mutation in the rrs locus; 29% of KAN-R isolates have a mutation in the eis locus; 46% of CAP-R isolates have a mutation in the rrs locus; an additional 6% of CAP-R isolates have a mutation in the tlyA locus.)</td>
</tr>
</tbody>
</table>

* A negative result (e.g., no mutation) does not rule out contributory mutations present elsewhere in the genome.

Testing performed using in-house developed assays.

Reviewed by: Beverly Metchock  
Phone: 404 639-2455  
Fax: 404 639-5491  
TBLab@cdc.gov  
**Address:** 1600 Clifton Road, MS F08, Atlanta, GA 30333

Confidentiality, security, and integrity of patient data should be maintained in accordance with CLIA and HIPAA.
CDC MDDR Report

• Interpretation
  – The report states the % of R-strains studied at CDC having mutations in each locus.

• Examples:
  – gyrA, no mutation,
    • Cannot rule out fQ-R (86% of fQ-R isolates have a mutation at this locus).
  – rrs, no mutation,
    • Cannot rule out R to injectable drugs. (58% of KM-R and 88% of AK-R have a mutation in rrs locus).
  – rpoB, mutation: TCG>TTG Ser531Leu
    • RIF-R (100% of 254 isolates with this mutation are RIF-R)
Expanding Nucleic Acid Amplification Testing for TB in Public Health Laboratories

The Centers for Disease Control and Prevention (CDC) has awarded funds to the Association of Public Health Laboratories (APHL) for the purpose of expanding the use of Nucleic Acid Amplification Testing (NAAT) in public health laboratories. In the upcoming weeks, APHL will distribute a Request for Proposal (RFP) to member laboratories currently supported by the Laboratory Upgrade Component of the CDC TB Cooperative Agreement.

Laboratories will be asked to prepare proposals outlining ways in which their laboratory would approach increasing access to NAAT for either identification of *M. tuberculosis* complex or the molecular detection of drug resistance in their jurisdiction. This could be accomplished either by implementing or expanding NAAT access in the laboratory or contracting with another laboratory to perform the work.
The one-time funding will be awarded through 64 sub-grants administered by APHL. APHL will work with CDC to determine grant allocations to recipients based principally on workload and to assist recipients in determining the appropriate application of funding received. Potential applications of the funding include but are not limited to:

- Purchasing instrumentation for the implementation NAAT (e.g. Cephied GeneXpert System)
- Purchasing reagents to begin or expand a testing program
- Performing validation/verification studies to establish us of new technologies or techniques
- Expanding the patient populations or specimen types to which testing is currently offered (e.g. expand testing to include smear negative sputum specimens according to 2009 CDC Guidelines)
- Establishing relationship with reference laboratory for provision of NAAT
- Establishing or improving jurisdictional courier services to ensure the rapid transport of specimens
Pyrosequencing for rapid detection of M. tb complex and rapid detection of mutations associated with drug resistance

- Being evaluated at MDL
- Under development at CDC for use by “flagship laboratories”
DNA sequencing by pyrosequencing

- Detects DNA sequence during synthesis
- When a base is added, pyrophosphate is released
- Pyrophosphate is detected as light by first making ATP, then using luciferase
- Can sequence shorter segments than classical Sanger sequencing
- Technology license held by Qiagan
CDC “flagship laboratory” project

- CDC plans to solicit applications in late 2011 to become one of two regional testing centers for rapid detection of mutations associated with drug-resistant *M. tuberculosis*.
- Funding to begin 2012
- Pyrosequencing tentatively chosen for mutation detection
- Participating labs will also need to do culture-based drug suscept. testing for 1st and 2nd line drugs
How “flagship” labs might work

Public health laboratory has specimen or culture from patient known or suspected to have drug-resistant TB

Specimen or culture is submitted to flagship lab

Pyrosequencing is done immediately to detect mutations associated with drug resistance

If submitted sample was a culture, flagship lab follows up with drug sus testing in MGIT

If submitted sample was a specimen, submitting lab would follow up later by submitting a culture for drug sus testing
Tuberculosis
Tip of the Iceberg

Active cases

TB infection

Total Population
Advantages of blood IGRA

• Provides high specificity because antigens used are in the RD-1 locus of M. tuberculosis and are not found in the BCG vaccine --> BCG vaccinated patients do not test positive
• Is more sensitive than TST in testing immuno-compromised patients
• Only a single clinic visit is required
• No problem of “booster effect”—risk that repeated injection of PPD could → false +
• Reproducibility of IGRA, variability of TST readings
Provisional CDC recommendations 2010—IGRA or TST?

• IGRA preferred for testing people from groups that historically have poor rates of returning for TST reading
• IGRA preferred for people who have received BCG
• TST preferred for testing children younger than 5
CDC provisional recomm, cont’d

**BOTH** IGRA and TST may be recommended if:

- **Initial test is negative** and risk of infection or progression to active disease is high, or if signs and symptoms of TB are present.
- If the **initial test is positive** and additional evidence is required to encourage compliance with preventive therapy, or in healthy persons with low risk of infection and progression.
If your lab is going to do TB lab work, there are benchmarks

- Selective liquid medium, ID by rapid method, fluorescent acid-fast microscopy
- Do NAA or provide access to it
- Take advantage of molecular detection of drug resistance if the situation is right
Opportunities for help in meeting goals

- Molecular beacons testing available on smear pos sediments or cultures at MDL
- MGITs by mail for smaller volume labs (< 100 per month)
- MDL does 2\textsuperscript{nd} line drugs in MGIT
- Likely to be an NAA pilot project at State lab, funded by CDC/APHL
- CDC’s MDDR service excellent for detecting XDRTB (will be replaced by “Flagship laboratories”)
MGITs by mail

• A way to offer selective broth culture with low test volume
• Local public health lab decontam, concentration, smear, inoculate MGIT
• MGIT sent unincubated to MDL
• MDL incubates, does molecular beacons and drug sus testing on positives
• MDL could accommodate more (more cultures $\rightarrow$ increasing CDC funding)
Thank you