Laboratory Diagnosis and Antimicrobial Susceptibility Testing of *Mycobacterium tuberculosis* Complex

Marie-Claire Rowlinson, PhD D(ABMM)
Calin Chiribau, PhD, MT(ASCP)
Florida Bureau of Public Health Laboratories
SNTC Comprehensive Clinical TB Course,
September 27, 2016

Objectives

At the end of this session participants will be able to:

• Discuss optimal specimens for detection of TB and the effect of poor quality specimens on test results to increase the providers’ knowledge of proper specimen collection and handling
• List the methods used in the laboratory to diagnose or rule-out TB that ensure the appropriate diagnosis and treatment of TB patients
• Describe challenges in the interpretation of diagnostic tests to improve providers’ understanding of the role of laboratory tests in the clinical diagnosis of TB
• Describe the process for reading and interpreting an acid-fast bacilli (AFB) smear to improve the provider’s knowledge of this important initial test for diagnosing TB
• Identify key laboratory equipment that is used in the identification and characterization of *Mycobacterium tuberculosis* complex to increase knowledge of laboratory tests for the diagnosis of TB
United Nations, Millennium Development Goals – TARGET: “Have halted by 2015 and begun to reverse the incidence of malaria and other major diseases.”

Global, STOP TB Partnership – TARGET: “A global movement to accelerate social and political action to stop the spread of TB around the world… and eliminate TB.” Global Plan to Stop TB 2006-2015. (New Diagnostics Working Group)

U.S., Healthy People 2020 (2010-2020) – TARGET: “Reduce TB. Increase treatment completion rate of all TB patients. Increase the treatment completion rate of contacts to sputum smear-positive cases who are diagnosed with latent TB infection. Reduce the average time for a laboratory to confirm and report TB cases.”

International Union Against TB and Lung Disease (IUATLD): “Effective laboratories are essential to tuberculosis control not only for diagnosing infectious cases, but also for detecting multidrug-resistant strains of TB.”

Eliminating TB – why is the laboratory important?

Pre-Analytic
Section 1: Specimen type, specimen collection, transport, test request

Analytic
Section 2: Methods to diagnose and rule-out tuberculosis

Post-Analytic
Section 3: Challenges in TB testing and interpreting results

Effective laboratories are essential to tuberculosis control not only for diagnosing infectious cases, but also for detecting multidrug-resistant strains of TB.
Section 1: Specimen type, specimen collection, transport, test request

Pre-analytical considerations!

➢ Healthcare providers play a crucial role
➢ The laboratory has a duty to ensure that healthcare providers understand the importance of/receive training on the pre-analytical step
➢ Communication is key
Specimen collection and transport

- Specimen collection, transport and test ordering are where it all begins for a laboratory test.
- A bad sample = a bad result! The result can be affected if:
  - A specimen is not collected, transported or preserved (e.g. media, temperature) correctly and not delivered in a timely manner.
  - An incorrect test order or if there is inadequate/inaccurate patient information on a requisition form.

Specimen type and specimen collection

Good sputum specimens!
- the best specimen for the laboratory diagnosis of pulmonary TB.

- Specimen type dependent on various factors:
  - Suspected location of disease.
  - Ability of patient to produce sputum – induced, bronchial alveolar lavage.
  - Age of patient e.g. pediatric patients, gastric lavage.

- Multiple specimens may be necessary to rule-out or confirm disease.
Collection of sputum specimens

Instruction should be provided by TB control programs/laboratories regarding specimen collection
- Note should be made if sputum is induced
- Don’t pool specimens
- 5-10 ml collected
- 3 early morning ideal

General specimen collection rules

Specimen collection:
• Try to collect a specimen before any antimicrobial treatment has been initiated
• Collect sufficient specimen for testing requested
• Swabs are not acceptable, stool is not recommended

Specimen shipping
• If you can’t ship a specimen straight away, you may need to refrigerate it (not necessary for normally-sterile specimen sources)
• Send specimens as soon as they are collected - receipt in lab within 24 hrs is ideal – don’t batch!
• Package correctly – protects staff
Test Ordering

Test requisition forms
- Paper or electronic
- Provide complete information
- Order the right test
  - In your location which tests are performed automatically or by reflex vs. which tests must be ordered?
  - Who can order tests? Do some require an approval?

Helps to triage specimens to correct place and get the correct test

Example requisition from Florida BPHL
Specimen collection and transport - FAQ

- Why is a swab not a good specimen type?
- Why not pool sputum specimens, if collecting more than one?
- Why is it important to note if a sputum is induced on the requisition?
- Why is it important to ship correctly?
- Why not batch send?

Specimen rejection policy

- What is the policy of the laboratory?
  - Specimens should be received within 7 days of collection
  - Specimen should be sufficient to run requested tests
  - Specimen should be collected in the appropriate container under appropriate conditions
  - Specimen should be labeled with at least 2 patient identifiers
  - A requisition should accompany the specimen (and complete information should be present)
Case 1: A Waiting Game

Patient history:
• A cruise ship employee from Indonesia presented to the ED with lower quadrant abdominal pain
• An abdominal biopsy specimen was sent for pathology testing...and routine microbiology
• The microbiology laboratory only received a small sample for smear and culture, NAAT was not requested

Laboratory results:
• Pathology results revealed caseating granulomas and histopathology revealed AFB
  - Unexpected result, given pt was admitted with a differential of lymphoma or colorectal cancer
• AFB smear was negative, specimen by this point had been discarded. The culture was incubating.... Waiting game!
• The patient had pleural effusions and nodules
• Additional sputa specimens were collected but were smear negative
Case 1: A Waiting Game

What action could be taken?

A) Perform a NAAT on a sample from the incubating culture
B) Collect a specimen from another source
C) Send the pathology specimen for TB testing at CDC’s Infectious Disease Pathology Branch (IDPB)
D) Begin treatment for TB – what regimen?
E) All of the above
Case 1: A Waiting Game

Outcome:

- Abdominal biopsy culture became positive for *Mycobacterium tuberculosis* complex (MTBC) 13 days from date of collection
- Isolate was forwarded to the state public health laboratory where molecular testing confirmed that it was MTBC and determined that it was resistant to isoniazid and rifampin
- A sputum collected a few days after this was positive...

Case 1: A Waiting Game

- What could have been done to improve the timely diagnosis of MDR-TB in this case?
  - If TB was on the differential more fresh specimen could have been provided to the microbiology lab
  - NAAT for TB could have been ordered
- What were the laboratory challenges:
  - Pathology specimens
  - Test methods for non-respiratory specimens
  - Availability of a molecular test for determining drug resistance
In summary, it is important to:

- Collect the appropriate specimen
- Request the appropriate test
- Consider options/resources e.g. state public health laboratory, CDC

Case 1: A Waiting Game

Patient history:
- The state public health laboratory received a sputum specimen from a commercial laboratory but there was insufficient sample in the tube to do all the testing ordered on the test requisition
- It appeared that the specimen had leaked from the primary container

Case 2: A Laboratory Dilemma
Case 2: A Laboratory Dilemma

What should the laboratory do in this situation?

A) Ask for additional specimens before proceeding with any testing
B) Reject specimen – quantity not sufficient for tests required
C) Dilute the sample and perform all testing as requested – making note on the report of the insufficient quantity

Case 2: A Laboratory Dilemma

- The laboratory often receives specimens which are not of an ideal quality
- In a snapshot of 1,000 specimens from two weeks in November 2015:
  - 68 specimens were leaking (5 had to be canceled)
  - 19 had insufficient quantity (less than 5ml)
  - 4 were received more than 7 days after the specimen was collected
  - 3 were fixed in formalin and cannot be processed in the diagnostic TB laboratory (all were canceled)
  - 1 specimen container had no name (canceled)
Case 2: A Laboratory Dilemma

• The laboratory tries its best not to reject specimens (technically, 9.5% of the 1,000 specimens mentioned could have been)
• Instead you may notice a disclaimer or notes on the report:
  e.g. “less than optimal amount of specimen received”
  e.g. “specimen leaked in transit, results may be unreliable”
  e.g. “specimen older than 7 days, results may be unreliable”

In summary, it is important to:

➤ Collect the appropriate specimen – which includes collecting sufficient quantity
➤ Request the appropriate test – ensure you have enough specimen for the tests being ordered
➤ Ensure the specimen is packaged and shipped appropriately and in a timely manner
➤ May need to send more than one specimen
Section 2: Methods to diagnose and rule-out tuberculosis

Analytical considerations:
- The laboratory performs appropriate testing
- Laboratory ensures that quality testing is performed within recommended turnaround times
- Customer service, quality, timeliness
Mycobacteriology Laboratory - Capabilities

- The capability and capacity of laboratories to perform mycobacteriology testing varies greatly between laboratories
- It’s important to know the tests that are offered and the testing algorithm in place
- State and local public health laboratories tend to have a greater capacity for mycobacteriology testing and can also forward appropriate specimens and isolates to CDC

FL State Public Health Laboratory
Florida Department of Health, Bureau of Public Health Laboratories (FDOH BPHL)
Annual Testing Volumes - 2015

Total clinical specimens: 21,071
- # specimens (individual pts) tested by NAAT: 5,101
- # positive AFB cultures: 4,105
- # positive TB cultures: 1,573/4,105 (38%)
- # TB isolates (individual pts) set up for growth-based susceptibility testing for TB: 571
Ideal algorithm

- At a minimum, mycobacteriology laboratories should perform AFB smear and culture
- Labs should also *ideally* be able to perform the following molecular tests:
  - NAAT on the clinical specimen – or have the ability to refer this out in a timely manner (*ideally* on smear negative and/or smear positive)
  - a molecular susceptibility test on the clinical specimen – or have the ability to refer out in a timely manner
- Identification of mycobacteria and conventional, growth-based susceptibility testing
These should be performed on every specimen submitted for mycobacteriology testing, even if NAAT is ordered!

**AFB smear**
- Quantitative smear – fluorescent method recommended as more sensitive
- Reported as AFB seen with quantitation

<table>
<thead>
<tr>
<th>AFB seen</th>
<th>Quantitation and Report</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No AFB seen</td>
</tr>
<tr>
<td>1-2/300 fields</td>
<td>+/- Doubtful/Scanty</td>
</tr>
<tr>
<td>1-9/100 fields</td>
<td>1+ Rare</td>
</tr>
<tr>
<td>1-9/10 fields</td>
<td>2+ Few</td>
</tr>
<tr>
<td>1-9/field</td>
<td>3+ Moderate</td>
</tr>
<tr>
<td>&gt;9/field</td>
<td>4+ Many</td>
</tr>
</tbody>
</table>

- **Turnaround time:** 24 hrs from receipt in the lab
- **Report:** Positive/Negative and Quantitation
  - Why quantify?
  - It gives healthcare providers an idea of how infectious a person is
- **Challenges:** The AFB smear is a very important test because it can be performed cheaply and easily but what are some of the challenges?
  - Does not tell you if organism is viable
  - Does not tell you what type of mycobacteria
  - Insensitive test
AFB culture

AFB culture – gold standard in diagnostics

- Culture should be performed on solid and liquid media – enhancing the ability to detect and isolate AFB quickly (mixed cultures/contamination)
- Turnaround time: 7-10 days average for positive TB culture. Cultures are usually called negative at 6 weeks/42 days
- Report: Positive - Identification of mycobacterial species, Negative, or Contaminated
- Challenges:
  - Contamination
  - Slow growth

Identification of mycobacteria

MALDI-TOF: Many mycobacteria (MTBC and NTMs)

Accuprobe: MTBC, MAC, *M. kansasii*, *M. gordonae*

HPLC: Most mycobacteria – no longer recommended

DNA Sequencing: Most mycobacteria

PRA: Most mycobacteria (MTBC and non-tuberculous mycobacteria, NTMs)
Molecular methods

- Molecular methods are sensitive and specific and provide rapid results
- There are molecular methods that can be performed directly on the specimen for:
  - Diagnosis of MTBC
  - Decision regarding Airborne Infection Isolation (AII)
  - Antimicrobial susceptibility testing (AST) of MTBC
- If molecular methods are not available in the laboratory you send specimens to, that laboratory should have the ability to refer out

Molecular methods - NAAT

Nucleic Acid Amplification Test (NAAT)

- This may be referred to as NAAT, NAT, or PCR.
- Most NAATs use a real-time PCR method, CT values may be reported – what is that?

- Available NAATs used include:
  - Cepheid GeneXpert MTB/RIF (FDA-clear. sputum only)
  - GenProbe Hologic MTD (FDA-approv. respiratory only)
  - Laboratory-developed tests
Molecular methods - NAAT

**Nucleic Acid Amplification Test (NAAT)**

- **Turnaround time:** 48 hrs from receipt in the lab
- **Report:** Positive, Negative, Indeterminate
  - What should you do if you get an indeterminate?
  - Submit another specimen
- **Challenges:** NAAT can be performed on AFB smear negative or positive samples, but challenges exist
  - May detect non-viable organism
  - Can be affected by patient on TB treatment
  - Sensitivity of smear-negative, extra-pulmonary specimens
  - Technical expertise required to perform
  - Expense

---

**When to perform NAAT for diagnosis of MTBC**

"**CDC recommends that NAA testing 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, such as contact investigations...**"

Molecular methods - NAAT

Cepheid GeneXpert
MTB/RIF assay

BD MAX
Laboratory-developed real
time-PCR test for MTBC

Molecular methods – “MDR Screen”

Molecular susceptibility testing (AST)

- Molecular detection of mutations associated with resistance in MTBC – ideally should be performed on all TB-positive specimens, by reflex (?)
- Tests can be performed directly on the specimen
- Commonly targeted genes for detection of resistance are listed below:

<table>
<thead>
<tr>
<th>Gene Locus</th>
<th>Mutation associated with resistance in:</th>
</tr>
</thead>
<tbody>
<tr>
<td>rpoB</td>
<td>Rifampin (and rifabutin)</td>
</tr>
<tr>
<td>inhA, katG</td>
<td>Isoniazid (and ethionamide)</td>
</tr>
<tr>
<td>embB</td>
<td>Ethambutol</td>
</tr>
<tr>
<td>pncA</td>
<td>Pyrazinamide</td>
</tr>
<tr>
<td>gyrA</td>
<td>Fluoroquinolones</td>
</tr>
<tr>
<td>rrs, eis, tlyA</td>
<td>Aminoglycosides and capreomycin</td>
</tr>
</tbody>
</table>
Molecular susceptibility testing
Available tests used include:
- Cepheid GeneXpert MTB/RIF - rifampin
- Hain GenoType MTBDRplus and MTBDRsl assays – rifampin, isoniazid and second line drugs
- DNA sequencing – all/any
- Next generation sequencing - all/any
Molecular methods – ‘MDR Screen’

Nucleic Acid Amplification Test (NAAT)

- **Turnaround time:** Varies from 48 hrs from receipt in the lab to several days (lab may need to batch test)
- **Report:** Resistance detected/not detected OR Mutation detected/not detected
- **Challenges:** MDR screen important for providing rapid result, but challenges exist
  - Availability of the test
  - Capability of laboratory staff to perform complex test – if not performed in-house ability to REFER out?
  - No result if there is low bacterial load (DNA) in sample
  - Challenge to validate in low R population
  - Expense

Conventional methods – DST

What do we mean by ‘conventional’ methods or DST – drug susceptibility testing?

- Term used interchangeably with ‘phenotypic’, ‘growth-based’, ‘culture-based’ susceptibility testing of anti-mycobacterial drugs

Conventional susceptibility testing

- Perform on at least one isolate from each MTBC-confirmed case - can be repeated depending on your program’s policy
  - Patient is still positive (after 60-90 days)
  - Positive TB from a different specimen source
Conventional methods – DST

• Available methods include:
  - BACTEC MGIT
  - VersaTREK
  - Sensititre MIC
  - Agar Proportion method – ‘gold standard’

Critical Concentration: the lowest concentration of drug that inhibits 95% of wild-type strains that have never been exposed to anti-TB drugs, and does not inhibit patient strains that are considered resistant.

Minimal Inhibitory Concentration (MIC): the lowest concentration of an antimicrobial, in a series of dilutions of a drug that will inhibit the visible growth of a microorganism.

• Turnaround time: Varies depending on method
  APM >21 days, MGIT 4-14 days, VersaTREK 3-13 days, Sensititre 10-21 days.

• Report: Results may be reported two different ways:
  1. As a categorical result – susceptible or resistant (to a drug at a specific concentration)
  2. As a minimal inhibitory concentration (MIC) – the minimal level of drug at which the organism is inhibited - with an interpretation of susceptible or resistant at that level
Conventional methods – DST

- **Challenges:** Dependent on method used/available and complexity of TB case
  - Multi-drug treatment – challenge with comparing clinical data, treatment efficacy based on susceptibility results
  - Interpretation of susceptibility results
    - Historically has been based on critical concentration – interpretation of MICs is new to many TB healthcare providers
    - Discordance with other susceptibility results – molecular or additional conventional results
- **Curry International TB Center Reference**
  - “Drug-Resistant TB – A Survival Guide for Clinicians” 3rd Ed. An excellent resource on this topic

Conventional methods – DST

BACTEC MGIT

TREK Sensititre
Case 3: Performing the Right Test – Molecular is Best?

Patient history:

- A young, previously healthy male presents to the PMD with one week history of testicular swelling. He was diagnosed with epididymitis but despite treatment swelling returned
- Patient presented again, and over 3 month period had noted a 50lb weight loss, night sweats and non-productive cough
- Due to weight loss and dyspnea a chest x-ray was performed
Case 3: Performing the Right Test – Molecular is Best?

Specimen Collection:
→ based on chest x-ray, sputum collected to test for TB

Laboratory results:
• AFB smear positive, NAAT positive
• Specimens sent to state public health laboratory for molecular susceptibility testing
  - Hain MTBDRplus – rifampin result indeterminate
  - Xpert MTB/RIF: rifampin resistance detected
• Specimens referred to CDC’s Molecular Detection of Drug Resistant (MDDR) program
Case 3: Performing the Right Test – Molecular is Best?

CDC’s Molecular Detection of Drug Resistant (MDDR) results:

• MDDR report stated - CAC>AAC, His526Asn mutation associated with reported ‘low level’ rifampin resistance

• Explains ‘indeterminate’ Hain result, where the analysis of the rpoB gene showed a wild type band missing but no mutant band present

• The Xpert will result in rifampin resistance detected as any mutation will could cause this result, even a silent mutation

Case 3: Performing the Right Test – Molecular is Best?

What does ‘low-level’ rifampin resistance mean?

A) The organism is resistant to rifampin and should be treated as such

B) The organism is not resistant although there is a mutation in the rpoB gene

C) Some of the bacteria are resistant and some of them are susceptible

D) The significance of the mutation is unknown and whether the organism will respond to treatment with rifampin or not – consult an expert!
Case 3: Performing the Right Test – Molecular is Best?

Outcome:

- Multiple specimens were processed and were positive for TB in different sites
- Potentially drug resistant TB was identified as quickly as possible by molecular methods – excellent communication between the hospital, TB control, state laboratory and CDC
- Patient received the appropriate treatment based on laboratory results and completed 18 months of therapy

Case 3: Performing the Right Test – Molecular is Best?

- What additional testing could have been performed to improve the timely diagnosis?
  - Nothing – this was a very challenging case
  - Communication is important so that specimens can be coordinated and referred when needed
- What were the laboratory challenges:
  - Multiple specimen types
  - Negative results
  - Unusual molecular results/‘discordant’ results
In summary, it is important to:

- Order molecular tests – they are rapid and accurate
- Communicate often with the laboratory and clinicians
- Collect multiple specimen types if necessary and notify the laboratory
- Discuss any discordant results with the laboratory and consult experts if necessary

Case 3: Performing the Right Test – Molecular is Best?

Patient history:

- 25 year old patient, emigrated from Haiti 4 years ago
- Seen at CHD for pre-natal care at 8.5 weeks gestation – HIV negative, syphilis positive (treated)
- IGRA positive but patient was asymptomatic
- Seen at CHD 3 months later, still asymptomatic, chest X-ray performed

Case 4: Is Culture Redundant?
Case 4: Is Culture Redundant?

Left upper lobe infiltrate and nodules

Patient history:

• Positive IGRA, suspicious chest X-ray – patient was started on INH, RIF and EMB
• Sputum specimens collected
• Sputum AFB smear negative, NAAT negative. Liquid culture was negative. After six weeks ONE single colony seen on solid culture media
• By the time all these results are available the patient is 8 months pregnant...
Case 4: Is Culture Redundant?

Is this patient positive for TB?

A) Yes. Every TB colony on culture should be considered significant, even if there is only a single colony

B) No. The single colony is most likely contamination – it took so long to grow and the other tests were negative (e.g. NAAT, smear)

C) Maybe. Need to look at clinical picture

D) Maybe. Need to see if there is any chance of laboratory contamination

Case 4: Is Culture Redundant?

Laboratory Results:

- There was another positive TB that was cultured next to this positive culture – but the susceptibility patterns (and genotype) were different
- ‘MDR screen’ showed \textit{katG} and \textit{rpoB} mutations – MDR-TB!
- Isolate was sent to CDC’s MDDR program for additional molecular susceptibility testing

Clinical information:

- There was some clinical evidence that the patient had active disease
Case 4: Is Culture Redundant?

Case continued!

- CDC’s MDDR program identified the following mutations:
  - $rpoB$ : Ser531Leu
  - $katG$ : Ser315Thr
  - $embB$ : Met306Val
  - $pncA$ : Trp119Arg

- Patient’s regimen was changed
- Patient had induced delivery, baby was healthy with no evidence of disease

In summary, remember:

- Culture is the gold standard for diagnosis of TB
- Even ONE colony can be significant
- Collect additional specimens if necessary
- Compare laboratory results with clinical picture to enable decision-making
Section 3: Challenges in TB testing and interpreting results

Post-analytical considerations:

- Results are reported in a timely manner
- The laboratory is available to provide result interpretation when needed
- Communication between healthcare providers, TB control programs and the laboratory
Result – Turnaround Times (TAT)

<table>
<thead>
<tr>
<th>Test</th>
<th>Expected TAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB Smear</td>
<td>24 hours</td>
</tr>
<tr>
<td>NAAT for MTBC</td>
<td>48 hours</td>
</tr>
<tr>
<td>‘MDR Screen’</td>
<td>3-5 days</td>
</tr>
<tr>
<td>Culture (- Negative)</td>
<td>7-14 days for MTBC (-42 days/6 weeks)</td>
</tr>
<tr>
<td>Conventional DST</td>
<td>3-6 weeks</td>
</tr>
<tr>
<td>Genotyping</td>
<td>1 week from submission (can vary based on whether expedited or additional testing at CDC required)</td>
</tr>
</tbody>
</table>

Result Interpretations

<table>
<thead>
<tr>
<th>Test</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB Smear</td>
<td>Positive, AFB seen Quantification (1+, rare)</td>
</tr>
<tr>
<td>NAAT for MTBC</td>
<td>Positive, Positive for TB DNA, TB DNA Detected CT value?</td>
</tr>
<tr>
<td>‘MDR Screen’</td>
<td>- TB detected/RIF resistance detected - rpoB gene mutation detected - Mutation detected, CAC&gt;AAC, His526Asn</td>
</tr>
<tr>
<td>Culture</td>
<td>AFB cultured Identification (Mycobacterium abscessus)</td>
</tr>
<tr>
<td>Conventional DST</td>
<td>Susceptible, Intermediate, Resistant Critical Concentration/MIC Value</td>
</tr>
<tr>
<td>Genotyping</td>
<td>1 week from submission (varies if expedited or additional testing at CDC required)</td>
</tr>
</tbody>
</table>

• Don’t forget - laboratory staff are available for consultation!
Example result format

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Reference Range</th>
<th>Date Approved</th>
</tr>
</thead>
<tbody>
<tr>
<td>3110 AFB Smear (Cont., Fluorechrome)</td>
<td>Acid Fast Bacilli seen: 2+</td>
<td>1 per 10 fields</td>
<td>05/25/2013</td>
</tr>
<tr>
<td>3156 Nucleic Acid Amplification Test by Real-time PCR Note: Patient had previous positive RTPCRs within the last 6 months.</td>
<td>Cancelled</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3100 AFB Culture</td>
<td>Pending</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3200 Organism ID by FPA</td>
<td>Mycobacterium tuberculosis complex</td>
<td></td>
<td>01/23/2013</td>
</tr>
<tr>
<td>3315 Streptomycin MIC Streptomycin Interpretation</td>
<td>Resistant</td>
<td>Susceptible &lt;2.0 Intermediate 2.0-4.0 Resistant &gt;4.0</td>
<td>02/20/2013</td>
</tr>
<tr>
<td>Isoniazid MIC</td>
<td>2 µg/mL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Example result format

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Reference Range</th>
<th>Date Approved</th>
</tr>
</thead>
<tbody>
<tr>
<td>3145 HAIN Test GenoType MTBDRplus</td>
<td>rpoB point mutation detected No katG and No inhA point mutation detected</td>
<td></td>
<td>04/20/2012</td>
</tr>
<tr>
<td>3315 Streptomycin MIC Streptomycin Interpretation</td>
<td>1 µg/mL Susceptible</td>
<td>Susceptible &lt;2.0 Intermediate 2.0-4.0 Resistant &gt;4.0</td>
<td>09/17/2015</td>
</tr>
<tr>
<td>Isoniazid MIC</td>
<td>5.06 µg/mL Susceptible</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rifampin MIC</td>
<td>15 µg/mL Resistant</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Susceptible 0.1 Intermediate 0.25-1.0 Resistant >1.0
Example result format

CDC’s Molecular Detection of Drug Resistance Program

<table>
<thead>
<tr>
<th>Locus (region) examined</th>
<th>Result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>rpoB (RRDR)</td>
<td>Mutation: CT(UPCUUU, Lys911Arg)</td>
<td>Low level but probably clinically relevant mutation.</td>
</tr>
<tr>
<td>inH (promoter)</td>
<td>No mutation</td>
<td></td>
</tr>
<tr>
<td>katG (sub16 codon)</td>
<td>Mutation: ACC→TCC, Ser315Thr</td>
<td>Lisinamide resistant (100% of isolates in our in-house evaluation of 550 clinical isolates with this mutation are INHr.)</td>
</tr>
</tbody>
</table>

Case 5: Discordant Results: Which Result is Correct?

• A 26-year old female patient, originally from Nepal – TB positive specimens in Kentucky
• Specimens were referred to BPHL in Florida for additional susceptibility testing
• BPHL performed smear on all three specimens which were positive, 2+
• NAAT was positive, specimen was reflexed for ‘MDR Screen’ by Hain test, which showed an rpoB mutation indicative of rifampin resistance, no katG or inhA mutation
• Specimen was referred to CDC’s MDDR program
Case 5: Discordant Results: Which Result is Correct?

- CDC's MDDR results showed the following:
  - Molecular susceptibility results: $rpoB$ – could not be amplified, $embB$ - mutation, $pncA$ - mutation, $katG$ mutation (Asp329Ala) – effect of mutation unknown
  - Phenotypic susceptibility results: Resistant to rifampin, rifabutin, isoniazid, ethambutol, streptomycin, pyrazinamide
- FL BPHL phenotypic susceptibility results:
  - Resistant to rifampin, rifabutin, isoniazid, ethionamide
  - Intermediate resistance to ethambutol and streptomycin

Is this patient resistant to isoniazid?
A) No, the Hain showed no mutations in the $katG$ gene
B) Yes, the MDDR molecular testing showed a mutation in the $katG$ gene
C) No, the MDDR molecular testing showed a mutation in the $katG$ gene but the significance of it is unknown
D) Yes, all phenotypic testing showed resistance to isoniazid
This case highlights the following:

- Molecular results are accurate and rapid but phenotypic results are still important
- Discordant results happen – especially the more tests that are performed, using different methodologies, in different laboratories
- When you get discordant results, recommend discussing with the laboratory/TB expert
- Compare laboratory results with clinical picture (e.g. response to treatment)

Your public health laboratory

- Provides valuable expertise
- Provides education, training and outreach
- Coordinates and communicates laboratory testing and reporting of results
- Refers specimens and isolates to CDC when appropriate
- Forwards TB isolates for genotyping – goal of 100% genotyping of TB cases
Thank you!

Acknowledgements

Susanne Crowe, Acting BPHL Bureau Chief/Jacksonville Lab Director
Betsy Jones/Calin Chiribau and BPHL Mycobacteriology and Molecular Sections

Southeastern National Tuberculosis Center

University of Florida