10th National Conference on Laboratory Aspects of Tuberculosis

April 18–19, 2017 / Atlanta, GA

Final Program

www.aphl.org/TBCon
### Schedule at a Glance:
**APHL 10th National Conference on Laboratory Aspects of Tuberculosis**

#### MONDAY April 17

<table>
<thead>
<tr>
<th>Time</th>
<th>Event Description</th>
<th>Location</th>
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<tbody>
<tr>
<td>4:00 pm – 7:00 pm</td>
<td>APHL Registration</td>
<td>Atrium Registration 3</td>
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<tr>
<td>4:00 pm – 7:00 pm</td>
<td>Exhibitor and Poster Set Up</td>
<td>Atrium A</td>
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#### TUESDAY April 18

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<thead>
<tr>
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<tr>
<td>7:00 am – 4:30 pm</td>
<td>APHL Registration</td>
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<td>7:00 am – 6:30 pm</td>
<td>APHL/NTCA Poster Viewing</td>
<td>Atrium A</td>
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<td>9:30 am – 6:30 pm</td>
<td>APHL/NTCA Exhibit Hall</td>
<td>Atrium A</td>
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<tr>
<td>8:00 am – 9:30 am</td>
<td>Opening Session and Keynote</td>
<td>Room A703/704</td>
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<tr>
<td>9:30 am – 10:00 am</td>
<td>Morning Break</td>
<td>Atrium A</td>
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<tr>
<td>10:00 am – 11:45 am</td>
<td>TB Laboratory Workflows</td>
<td>Room A703/704</td>
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<tr>
<td>11:45 am – 1:00 pm</td>
<td>Lunch (on your own)</td>
<td>Atrium A</td>
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<tr>
<td>1:00 pm – 2:30 pm</td>
<td>Updates in TB Drug Susceptibility Testing</td>
<td>Room A703/704</td>
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<tr>
<td>2:30 pm – 3:00 pm</td>
<td>Afternoon Break</td>
<td>Atrium A</td>
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<tr>
<td>3:00 pm – 4:00 pm</td>
<td>The Trends Manuscript and Aggregate Report</td>
<td>Room A703/704</td>
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<tr>
<td>4:00 pm – 5:00 pm</td>
<td>Highlighting the Work in Our PHLs</td>
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<tr>
<td>5:00 pm – 6:30 pm</td>
<td>Combined APHL and NTCA Poster Session</td>
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#### WEDNESDAY April 19

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<tr>
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<td>APHL/NTCA Exhibit Hall</td>
<td>Atrium A</td>
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<tr>
<td>7:00 am – 8:00 am</td>
<td>Roundtable: Dawn of the TB Laboratory in 2017</td>
<td>Room A706</td>
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<td>8:00 am – 10:00 am</td>
<td>Combined APHL and NTCA Opening Session</td>
<td>Atrium B</td>
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<tr>
<td>10:00 am – 10:30 am</td>
<td>Morning Break</td>
<td>Atrium A</td>
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<tr>
<td>10:30 am – 12:30 pm</td>
<td>Molecular Testing</td>
<td>Room A703/704</td>
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<tr>
<td>12:30 pm – 1:30 pm</td>
<td>Lunch (on your own)</td>
<td>Room A703/704</td>
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<td>1:30 pm – 3:00 pm</td>
<td>Exploring Developments in TB Research</td>
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<td>3:30 pm – 4:00 pm</td>
<td>Afternoon Break</td>
<td>Atrium A</td>
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<tr>
<td>4:00 pm – 5:15 pm</td>
<td>TB Case Studies: Lessons from the Field</td>
<td>Room A703/704</td>
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<tr>
<td>5:15 pm – 5:30 pm</td>
<td>Closing Remarks and Conference Conclusion</td>
<td>Room A703/704</td>
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*Events highlighted in red are combined sessions with NTCA.*
### Schedule at a Glance:
**NTCA National TB Conference**  
**Culture of Collaboration**

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<thead>
<tr>
<th><strong>MONDAY April 17</strong></th>
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<tr>
<td>3:00 pm – 6:00 pm</td>
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<td>Exhibitor and Poster Set Up</td>
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<td>Atrium Foyer</td>
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<td>9:30 am – 6:30 pm</td>
<td>NCTA/APHL Exhibit Hall</td>
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<td>8:00 am – 5:30 pm</td>
<td>NTCA Pre-meetings</td>
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<td>5:00 pm – 6:30 pm</td>
<td>Combined NTCA and APHL Poster Session</td>
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<td>8:00 am – 6:30 pm</td>
<td>NCTA/APHL Exhibit Hall</td>
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<tr>
<td>8:00 am – 10:00 am</td>
<td>Session I: NTCA Opening Session</td>
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<td>10:00 am – 10:30 am</td>
<td>Morning Break</td>
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<tr>
<td>10:30 am – 12:15 pm</td>
<td>Session II: TB in Motion</td>
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<tr>
<td>12:15 pm – 1:30 pm</td>
<td>Lunch (on your own)</td>
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<td>12:15 pm – 1:30 pm</td>
<td>TB Screening in US Healthcare Workers</td>
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<td>1:30 pm – 3:30 pm</td>
<td>Session III: TB infection</td>
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<td>3:30 pm – 4:00 pm</td>
<td>Afternoon Break</td>
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<td>4:00 pm – 5:30 pm</td>
<td>RTMCC Breakout Sessions</td>
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<td>5:30 pm – 6:30 pm</td>
<td>NTCA New Member Reception</td>
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<td>6:30 pm – 9:00 pm</td>
<td>NTCA Social</td>
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<tr>
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<td>NTCA Registration</td>
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<td>8:00 am – 6:30 pm</td>
<td>Exhibit Hall / Poster Viewing</td>
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<tr>
<td>8:30 am – 10:15 am</td>
<td>Session IV: Working with Lab Partners</td>
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<td>10:15 am – 10:45 am</td>
<td>Morning Break</td>
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<tr>
<td>10:45 am – 12:30 pm</td>
<td>Session V: Understanding Transmission</td>
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<tr>
<td>12:30 pm – 2:00 pm</td>
<td>Lunch (on your own)</td>
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<td>2:00 pm – 3:00 pm</td>
<td>Award Presentations</td>
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<td>3:00 pm – 3:45 pm</td>
<td>Afternoon Break</td>
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<td>3:45 pm – 5:15 pm</td>
<td>Breakout Sessions</td>
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<td>Atrium B / Atrium Lobby</td>
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<td>(see NTCA program)</td>
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<th><strong>FRIDAY April 21</strong></th>
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<tr>
<td>7:00 am – 12:30 pm</td>
<td>NTCA Registration</td>
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<tr>
<td>8:00 am – 9:30 am</td>
<td>Breakout Sessions (Repeat Breakouts)</td>
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<tr>
<td>9:30 am – 10:00 am</td>
<td>Morning Break</td>
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<tr>
<td>10:00 am – 12:30 pm</td>
<td>Session VI: Science and Tech in Treatment of TB</td>
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*(see NTCA program)*
Welcome Letter

Welcome to the 10th National Conference on the Laboratory Aspects of Tuberculosis, co-located with the 2017 National TB Conference in Atlanta!

We are happy to convene the 10th National Conference on Laboratory Aspects of TB. We are once again co-locating the conference with the 2017 National TB Conference based on the overwhelming support we received for this at the 9th National Conference in 2015. We have worked hard to find additional opportunities to collaborate and network with our fellow TB clinicians and TB control program officials, including a combined poster session on Tuesday April 18, a joint opening session on Wednesday April 19, as well as coordinated break and lunch schedules.

For the first time since 1992, the overall number of TB cases has increased to 9,557 cases in 2015, a 1.6% increase from 2014 (9,406 cases). Despite the progress since 1992 in decreasing TB cases, the increase in 2015 indicates that we must intensify our efforts by strengthening our existing systems to prevent transmission of infectious TB and increase efforts to detect and treat latent TB infection in high-risk populations. We, as laboratorians, cannot do this alone — we must work together within our own laboratories, within our own health departments and across our associations. This need was also recognized by our colleagues from the NTCA when they themed their 2017 National TB Conference the “Culture of Collaboration.”

TB Facts Today

- The TB incidence rate is 3.0 cases per 100,000 population and has remained stable from 2013–2015.
- After declines in TB cases every year from 1993–2014, 2015 saw an increase.
- The case rates among foreign born persons has continually decreased since 1996 and TB continues to disproportionately affect racial/ethnic minorities.
- TB drug resistance rates remain relatively stable, with a slight decrease of MDR TB in 2015 to 1.2% (89 cases) compared to 1.3% (94 cases) in 2014.
- More work is needed in order to reach the Healthy People 2020 goal of no more than 1 new case of TB per 100,000 population or to achieve the goal of TB elimination (1 case per 1,000,000) set in 1989.

The Laboratory Is Essential in the fight against TB; providing high quality results with newer technology allowing for faster delivery. The following highlights significant contributors to quality laboratory deliverables:

- CDC’s Division of TB Elimination Laboratory Branch continues to provide timely reference services, with the California Department of Health, Microbial Disease Laboratory also serving as a reference center for antimicrobial susceptibility testing for states with low TB incidence.
- Next generation sequencing methods are being increasingly utilized to assist our TB control colleagues in TB prevention and control, including piloting retrospective analysis of clusters and prospective universal sequencing to more rapidly determine clusters and prevent transmission of TB as well as identify and treat drug resistant TB.
- MALDI-TOF technology continues to be progressively implemented in TB Laboratories to help achieve cost savings.
- Continuous quality improvement efforts are being exercised to help assess laboratory workflow and algorithms to gain efficiencies in an environment of a decreasing skilled labor force.
- Increasing access to interferon gamma release assays as we push towards improved detection of latent TB infections.

At the 10th National Conference we will hear more about these advances. I hope you enjoy the presentations and take some time to interact with each other and the poster presenters, and visit the exhibits.

I would like to enthusiastically thank our planning committee for all their hard work in bringing us such an exciting agenda!

Paula M. Vagnone, MT (ASCP)
Chair, program planning committee, 10th National Conference on the Laboratory Aspects of Tuberculosis
About APHL

The Association of Public Health Laboratories (APHL) works to strengthen laboratory systems serving the public’s health in the United States and globally. APHL represents state and local governmental health laboratories in the United States. Its members, known as “public health laboratories,” monitor and detect health threats to protect the health and safety of Americans. Founded over 60 years ago as a forum for state public health laboratory directors, APHL brings together laboratory professionals from public health, environmental, agricultural and food safety laboratories.

APHL’s Mission

Shape national and global health outcomes by promoting the value and contributions of public health laboratories and continuously improving the public health laboratory system and practice.

APHL Board of Directors

A. Chris Whelen, PhD, D(ABMM), president
laboratory director, Hawaii Department of Health State Laboratories

Christopher G. Atchison, MPA, secretary-treasurer
laboratory director, State Hygienic Laboratory at the University of Iowa

Ewa King, PhD, president-elect
laboratory director, Rhode Island State Health Laboratories

Judith C. Lovchik, PhD, D(ABMM), past president
assistant commissioner, Public Health Protection and Laboratory Services, Indiana State Department of Health

Joanne Bartkus, PhD, D(ABMM) member-at-large
laboratory director, Minnesota Public Health Laboratory Division

Grace E. Kubin, PhD, member-at-large
laboratory director, Texas Department of State Health Services — Central Campus

Scott J. Zimmerman, DrPH, MPH, HCLD (ABB) member-at-large
laboratory director, North Carolina State Laboratory of Public Health

Maria Lucia Ishida, PhD, public health associate institutional member representative
laboratory director, New York State Department of Agriculture & Markets

Tamara Theisen, MT(ASCP) local institutional member representative
division director, Saginaw County Public Health Laboratory

Mark Wade, local institutional member representative
laboratory services director, San Antonio Metro Health District Laboratory
Conference Planning Committee

Mary Robin Connelly, MMSc, Georgia Public Health Laboratory
Tracy Dalton, PhD, Centers for Disease Control and Prevention
Denise Dunbar, Texas Department of State Health Services
Anne M. Gaynor, PhD, Association of Public Health Laboratories
Ryan Jepson, State Hygienic Laboratory at the University of Iowa
Stephanie P. Johnston, MS, Centers for Disease Control and Prevention
Beverly Metchock, DrPH, D(ABMM), Centers for Disease Control and Prevention
Ailyn Pérez-Osorio PhD, Washington State Department of Health
Jafar H. Razeq, PhD, HCLD (ABB), Katherine A. Kelley Public Health Laboratory (CT)
Cortney Stafford, MPH, Centers for Disease Control and Prevention
Angela Starks, PhD, Centers for Disease Control and Prevention
Frances Tyrrell, MT (ASCP), Centers for Disease Control and Prevention
Paula M. Vagnone, MT(ASCP), Minnesota Department of Public Health
Kelly Wroblewski, MPH, Association of Public Health Laboratories
Monica Youngblood, MPH, Centers for Disease Control and Prevention
Paul Zell, MPH, Association of Public Health Laboratories

Continuing Education Credits Available

APHL is an approved provider of continuing education programs in the clinical laboratory sciences through the American Society of Clinical Laboratory Science (ASCLS) P.A.C.E® program. Attendees have the opportunity to earn up to 13.5 contact hours by attending the entire conference. Attendance rosters must be signed at each attended session for credit, and the P.A.C.E® certificate must be signed and certified by APHL staff at the registration desk at the end of your time at the conference.
Conference Schedule

MONDAY, APRIL 17

APHL Registration: 4:00 pm – 7:00 pm • Atrium Registration 3
NTCA Registration: 3:00 pm – 6:00 pm • Atrium Foyer
Poster Setup: 4:00 pm – 7:00 pm • Atrium A

TUESDAY, APRIL 18

APHL Registration: 7:00 am – 4:30 • Atrium Registration 3
NTCA Registration: 7:00 am – 6:30 pm • Atrium Foyer
NTCA Pre-Meetings: 8:00 am – 5:30 pm • See NTCA Program
Exhibit Hall: 9:30 am – 6:30 pm • Atrium A
Poster Viewing: 7:00 am – 6:30 pm • Atrium A

DAY 1
588-832-15, 6.0 contact hours for the entire day
At the conclusion of this day, the participant will be able to:
• Discuss the technologies available to TB laboratories for the identification of Mycobacteria species from culture
• Identify different approaches to drug susceptibility testing that may lead to changes in laboratory practices
• Summarizing self-reported cooperative agreement public health laboratory TB workload, turnaround time and testing method data through trend and aggregate data analysis

8:00 am – 9:30 am • Room A703/704

Opening Session and Keynote
Welcome to the 10th National Conference on Laboratory Aspects of Tuberculosis! The Chair of the Conference Planning Committee will welcome everyone including our two featured speakers. We will first hear some opening remarks from the Director of the Division of Tuberculosis Elimination (DTBE) at the Centers for Disease Control and Prevention. The keynote address will be provided by Dr. Timothy Rodwell, Assistant Professor at UCSD and a Senior Scientific Officer at the Foundation for Innovative Diagnostics (FIND).
Welcome to the 10th National Conference on Laboratory Aspects of TB
Paula M. Vagnone, MT(ASCP), Minnesota Department of Health

Opening Remarks
Philip LoBue, MD, FACP, FCCP, Division of Tuberculosis Elimination, CDC

Diagnosing and Treating Drug Resistant TB in the 21st Century Using NGS and Intelligent Decision Support Tools
Timothy Rodwell, MD, PhD, MPH, University of California at San Diego and Foundation for Innovative Diagnostics (FIND)

9:30 am – 10:00 am • Atrium A
Morning Break

10:00 am – 11:45 am • Room A703/704
Is There a Better Way to Do This? A Snapshot of a Few TB Laboratory Workflows!
Do you wonder if there is a more efficient way to run your TB Lab? Perhaps you want to finally put your HPLC to rest and are wondering how to implement your new MALDI most effectively into your workflow or thinking about the line probe assay, but are not sure how it works. Come to this session to learn how a few states organize their workflow utilizing a variety of different methods, including PCR, MALDI and the line probe assay, and how one state went through a LEAN project to make improvements and reduce costs.

Moderator: Ryan Jepson, M(ASCP), State Hygienic Laboratory at the University of Iowa

Cart Off the Savings with Lean
Jennifer Eastman, PhD, HCLD/TS(ABB), Alaska State Public Health Laboratory

It’s the Massachusetts Way: Using the MALDI-TOF to Identify Mycobacteria Species
Tracy Stiles, M(ASCP), William A Hinton State Laboratory Institute (MA)

Is This Heaven?! No, It’s Identification of Mycobacterium sp. from Culture Using PCR!
Ryan Jepson, M(ASCP), State Hygienic Laboratory at the University of Iowa

Line It Up: Another Identification Option — the Line Probe Assay
Paula M. Vagnone, MT(ASCP), Minnesota Department of Health
11:45 am – 1:00 pm
Lunch (on your own)

1:00 pm – 2:30 pm • Room A703/704
The Times They Are-a-Changin’ — Updates in Drug Susceptibility Testing of Mycobacterium tuberculosis

Drug susceptibility testing for MTBC can be challenging for a laboratory. This session examines different approaches and aspects of providing reliable DST results, and highlights emerging concepts in improved service delivery.

Moderator: Frances Tyrrell, MT (ASCP), Centers for Disease Control and Prevention

Improving MGIT PZA Susceptibility Testing — A Multi-laboratory Evaluation of Alternative Inoculum Dilutions
Glenn Morlock, MS, Centers for Disease Control and Prevention

MIC = Many Inherent Challenges
Marie-Claire Rowlinson, PhD, D(ABMM), Florida Bureau of Public Health Laboratories

Updates to the CDC MDDR Service
Beverly Metchock, DrPH, D(ABMM), Centers for Disease Control and Prevention

A Review of the National TB DST Reference Center
Anne M. Gaynor, PhD, Association of Public Health Laboratories

2:30 pm – 3:00 pm • Atrium A
Afternoon Break

3:00 pm – 4:00 pm • Room A703/704
A Tale of Two Reports: The Trends Manuscript and Aggregate Report

CDC laboratory consultants will present public health laboratory TB testing volumes, methods and turnaround time aggregate data and trends based on TB cooperative agreement reports.

Moderator: Stephanie P Johnston, MS, Centers for Disease Control and Prevention

Frances Tyrrell, MT(ASCP), Centers for Disease Control and Prevention

CDC Aggregate Report 2012–2015
Monica Youngblood, MPH, Centers for Disease Control and Prevention
4:00 pm – 5:00 pm • Room A703/704

Highlighting the Work in Our PHLs

This session will highlight the findings of a few selected outstanding submitted poster abstracts that have broader lessons to be shared with the conference. We may also provide a short preview to the poster session with short presentations from the other poster presenters.

Moderator: Angela Starks, PhD, Centers for Disease Control Prevention

Evaluating the Significance of Equivocal Mycobacterial Smear Results
Jessica Gentry, Indiana State Department of Health

The Molecular ‘MDR Screen’ Is an Important Tool in the Diagnosis and Initiation of Appropriate Therapy in TB Patients in the State of Florida
Calin Chiribau, PhD, MLS(ASCP), Florida Department of Health- Bureau of Public Health Laboratories

Evaluation of a Multiplexed PCR and Melt Curve Analysis Method for Rapid Identification of Mycobacterium tuberculosis Complex (MTBC) from Positive MGIT Broth
Stacy White, PhD, Arizona Bureau of State Laboratory Services

Evaluation of a Two-Week 7H10 Agar Proportion Method for Isoniazid and Rifampin Drug Susceptibility Testing of Mycobacterium tuberculosis Complex
Jan Owen, BS, Texas Department of State Health Services

5:00 pm – 6:30 pm • Atrium A

Combined APHL and NTCA Poster Session

Thank you to our exhibitors for their support of the conference!

Please visit them in Atrium A during breaks.
WEDNESDAY, APRIL 19

APHL Registration: 7:00 am – 4:30 pm • Atrium Registration 3
NTCA Registration: 7:00 am – 5:30 pm • Atrium Foyer
NTCA Meetings: 8:00 am – 5:30 pm • See NTCA Program
Exhibit Hall: 8:00 am – 6:30 pm • Atrium A
Poster Viewing: 7:00 am – 6:30 pm • Atrium A

DAY 2
588-833-17, 7.25 contact hours for the entire day

At the conclusion of the day, the participant will be able to:

• Discuss approaches for dealing with the current challenges facing the public health TB laboratory
• Describe the processes for decision making and implementing new molecular methods in the mycobacteriology laboratory
• Summarize the newest trends in the areas of diagnostics, drug resistance and pathogenesis of tuberculosis
• Discuss several case presentations to highlight the complexity of the day-to-day diagnostic testing in the mycobacteriology laboratory

7:00 am – 8:00 am • Room A706

Roundtable: Batman vs. Superman: Dawn of the TB Laboratory in 2017

Both Batman and Superman fight crime but do it in different ways. There is no one algorithm that fits all approaches in TB testing. Factors such as personnel, workload volume, specimen transport, specimen type, instrumentation available and expertise all affect what algorithm you may currently use or are moving to in the future. Please join us for a discussion group on your TB experiences and can we learn a better way of providing quality information in the age of diminishing personnel and funds. We encourage all to come share their experiences.

Co-Moderator: Mary Robin Connelly, MMSc, Georgia Public Health Laboratory
Co-Moderator: Denise Dunbar, Texas Department of State Health Services

8:00 am – 10:00 am • Atrium B

2017 National TB Conference Opening Session

In the spirit of the 2017 National TB Conference Theme “Culture of Collaboration,” the attendees of the 10th National Conference on Laboratory Aspects of Tuberculosis will join our colleagues for their opening session.
10:00 am – 10:30 am • Atrium A
Morning Break

10:30 am – 12:30 pm • Room A703/704
Molecular Testing: Keeping Up with the Joneses or Performing Appropriate Testing?

In a time when so many new technologies are emerging it can be hard to know what is right or necessary for your laboratory. Balancing the need to keep up with performing appropriate testing this session will focus on providing practical information related to the use of molecular testing methods in the TB laboratory. Each speaker will address the processes for decision making and/or implementing the given method to help other laboratories think through their own decision making and ensure it is appropriate to meet their own needs.

Moderator: Jafar H. Razeq PhD, HCLD (ABB), Katherine A. Kelley Public Health Laboratory, Connecticut Department of Public Health

Considerations for the Use of Next Generation Sequencing in the TB Laboratory
Lauren Cowan, PhD, Centers for Disease Control and Prevention
Kelly Wroblewski, MPH, Association of Public Health Laboratories

Implementing Whole-Genome Sequencing on Clinical Isolates of Mycobacterium tuberculosis Complex in New York
Kimberlee A. Musser, PhD, Wadsworth Center, New York State Department of Health

Perspective from an APHL/CDC TB Whole Genome Sequencing Pilot Lab
Kevin Sohner, BS, Ohio Department of Health Laboratory

Alternative Specimen Types for TB Identification Using Xpert MTB/RIF
Dorothy Baynham, MT(ASCP), Tennessee State Department of Health

12:30 pm – 1:30 pm
Lunch (on your own)
1:30 pm – 3:00 pm • Room A703/704

**Exploring Developments in TB Research**

This session will have speakers presenting on their latest research and findings related to TB diagnostics, drug resistance and the impact of updated treatment regimens on laboratory testing.

Moderator: Ailyn Pérez-Osorio PhD, Washington State Department of Health

- **Acquired Resistance to New TB Drugs (Delaminid and Bedaquiline)**
  Sébastien Gagneux, PhD, Swiss Tropical and Public Health Institute

- **A New Paradigm for the Pathogenesis of Pulmonary Tuberculosis**
  Robert Hunter, MD, PhD, University of Texas Health Sciences Center

- **Updates from the CDC Applied Research Team**
  James E Posey, PhD, Centers for Disease Control and Prevention

3:30 pm – 4:00 pm • Atrium A

**Afternoon Break**

4:00 pm – 5:15 pm • Room A703/704

**TB Case Studies: Lessons from the Field**

This session will have several interesting case presentations which highlight the complexity of TB testing. The presentations will walk you through the case with interactive questions throughout to enable you to see how you would do if you had the case in your laboratory.

Moderator: Jafar H. Razeq PhD, HCLD (ABB), Katherine A. Kelley Public Health Laboratory, Connecticut Department of Public Health

- **Approaches to Solving a False Positive Mystery**
  Denise Dunbar, Texas Department of State Health Services

- **All We Need Are 3 Colonies**
  Alla Ostash, BS, Washington State Department of Health

- **The Concentration That Kills: A Case Study Evaluating TB Recovery and the Potential Impact of an Increased Concentration of Sodium Hydroxide**
  Ryan Jepson, M(ASCP), State Hygienic Laboratory at the University of Iowa

5:15 pm – 5:30 pm • Room A703/704

**Closing Remarks and Conference Conclusion**

A short wrap-up of the major topics and new information covered in the conference by the Conference Planning Committee Chair.

Paula M. Vagnone MT(ASCP), Minnesota Department of Health
Poster Abstracts

Poster 1:
Testing Algorithms for *Mycobacterium tuberculosis* complex using Real Time-Polymerase Chain Reaction: When to implement GeneXpert assay?
S. Hall, N. Robinson. Alabama Bureau of Clinical Laboratories, Montgomery, Alabama

**Objective:** To determine the most effective and efficient algorithm to perform PCR analysis by GeneXpert assay on tuberculosis specimens.

**Study Design:** Traditionally, *Mycobacterium tuberculosis* complex (MTB-complex) is identified by conventional methods. However, molecular methods are now being used more often. The previous algorithm of acid fast bacilli (AFB) smear positive patients who were new to the Bureau of Clinical Laboratories (BCL) was run on the GeneXpert automatically. The Xpert® MTB/RIF assay, performed on the GeneXpert® Instrument Systems, is a qualitative, nested real-time polymerase chain reaction (PCR) in vitro diagnostic test for the detection of MTB-complex DNA in raw sputum or concentrated sputum sediment prepared from induced or expectorated sputum. In specimens where MTB-complex is detected, the Xpert MTB/RIF assay also detects the rifampin-resistance associated mutations of the rpoB gene. This assay was used to test 551 tuberculosis specimens between January 8, 2015 and October 12, 2016 using the new algorithm that is based on AFB smear results in addition to physician requests. If a patient is AFB smear positive and is not on file at the BCL, PCR by GeneXpert is performed. If a patient is smear negative and a physician requests PCR by GeneXpert based on clinical presentation of the patient, PCR is also performed.

**Results:** Of the 551 specimens, 108 were positive by GeneXpert yielding a positivity rate of nearly 20%. The 98 positives from the 304 AFB smear positive specimens had a positivity rate of 32% and the 10 positives from the 247 AFB smear negative specimens had a positivity rate of 4%. Of the 108 PCR positives, 105 grew MTB-complex. In addition, there were two cultures that were AFB smear negative and PCR negative that grew only a few colonies of MTB-complex which were below the limit of detection by the GeneXpert assay.

**Conclusion:** The new algorithm of utilizing the AFB smear result in addition to the physicians’ requests yielded an additional 10 positives. Using the old algorithm of AFB smear result only, MTB-complex would not have been identified in the 10 specimens until final culture results. Utilizing the AFB smear result and the physicians’ requests yielded a high rate of positive PCR by GeneXpert samples.
Poster 2:
Evaluation of a Multiplexed PCR and Melt Curve Analysis Method for Rapid Identification of *Mycobacterium tuberculosis* complex (MTBC) from Positive Broth


**Objective:** To evaluate the performance characteristics of a relatively inexpensive, simple, PCR/meltcurve method described in Kim et. al. in the rapid identification of MTBC from positive MGIT broths at the Arizona State Public Health Laboratory (ASPHL).

**Study Design:** The analytical sensitivity, specificity and reproducibility of the PCR/meltcurve method was performed by spiking negative MGIT broths with known concentrations of organisms and tested. In addition, clinical MGIT broths inoculated with patient samples were tested in parallel with the new method and current method in their ability to identify MTBC. From optimization experiments, a PCR/meltcurve reaction was considered positive for MTBC if the Ct value is ≤ 40 and the melting temperature was between 84-87 °C. All positive clinical MGIT broths were sub cultured onto solid media to confirm presence of MTBC. Samples from failed HPLC runs were reflexed to MALDI-TOF for identification.

**Results:** The limit of detection of the assay was determined to be 8.6x10^3 CFU/ml. The assay was highly specific, reproducible and reliably detected MTBC from different lineages. A total of 269 positive clinical MGIT samples were tested, 105 of which were positive for MTBC based on recovery on solid media and identification by MALDI-TOF. The PCR/meltcurve method detected MTBC in 103 of the 105 samples (98%), whereas HPLC could detect MTBC in 43 of the 105 samples (40%) successfully on the first attempt.

**Conclusions:** Here, we present a simple, rapid, cost effective PCR/meltcurve method for the detection of MTBC in positive MGIT broths. The HPLC method is technically demanding and labor intensive. A successful run of ten samples takes a full day for extraction and analysis on the instrument, with interpretation and reporting completed the next day. The meltcurve/PCR method, by comparison, takes less than 2 hours for results and is simple to perform, requiring very minimal hands on time. Furthermore, when comparing costs between the two methods, the PCR/meltcurve method is relatively inexpensive by an estimated four fold when based on consumables alone. Our study demonstrates that this method is a reliable replacement for the HPLC when detecting MTBC in positive MGIT broths.
Identification and validation of new mechanisms of isoniazid resistance in *Mycobacterium tuberculosis*

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1Division of Tuberculosis Elimination, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia, 2Critical Path Institute, Tucson, Arizona.

**Objective:** Globally, ~14% of all tuberculosis (TB) cases are isoniazid (INH)-resistant, and ~10-12% of INH resistance is unexplained. The validation of novel INH resistance mechanisms can enhance the accuracy of rapid molecular tests for INH resistance including those for detection of both INH and rifampin resistance (i.e., multidrug resistance).

**Study Design:** We identified 13 *M. tuberculosis* clinical isolates from archived samples that were previously classified as INH resistant (INHR) by phenotypic testing but lacked mutations commonly associated with INH resistance. The MIC for INH was determined using a microdilution assay and whole genome sequencing (WGS) was performed to identify mutations associated with INH resistance.

**Results:** Four strains were susceptible to INH while most exhibited low-level (4/13) or high-level (5/13) resistance. We identified numerous distinct mutations in the INH resistance-associated loci katG, ahpC, ahpD, fabG1, and fabG3 (but none in furA, inhA, or Rv1910c). One high-level INHR strain did not harbor mutations in any aforementioned loci. We performed functional genetics to determine if the katG V1A mutation confers resistance to INH. We confirmed the mutation was successfully introduced into the pansusceptible strain H37Rv by Sanger sequencing and found that it does indeed confer INH resistance.

**Conclusion:** A mutation discovered by WGS (katG V1A) was shown by functional genetics to confer INH resistance. Additional analyses will be conducted to determine the contribution of other mutations identified for INH resistance. Examination of isolates with unknown mechanisms of resistance by WGS could aid assay development by identifying novel resistance markers.
Poster 4:
The Molecular ‘MDR Screen’ is an Important Tool in the Diagnosis and Initiation of Appropriate Therapy in TB Patients in the State of Florida

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¹Florida Department of Health, Tallahassee, Florida, ²University of Florida, Department of Medicine, Division of Infectious Diseases and Global Medicine, Gainesville, Florida

Objective: The Florida Department of Health Bureau of Public Health Laboratories (BPHL) algorithm for the diagnosis and anti-mycobacterial susceptibility testing (AST) of tuberculosis (TB) includes a molecular ‘MDR (multi drug resistance) screen’ for the rapid and accurate detection of drug resistance in TB. This study reviews the performance of the MDR screen and initiation of appropriate TB patient therapy.

Study Design: Analysis of all AST data over a 2-year period was performed, followed by review of patient treatment records for MDR cases. The following data were analyzed: date of MDR screen result by Hain Genotype® MTBDRplus assay, version 2 (Hain LifeScience); date of phenotypic AST result by Sensititre MIC assay (TREK Diagnostic Systems, Thermo Fisher); and date of initiation of appropriate MDR treatment regimen.

Results: From January 1, 2014 to December 31, 2015, MDR screen was performed on n=618 primary clinical specimens. Of 618 primary specimens a result could not be determined for n=84 (indeterminate or positive control band for TB missing). Of the 534 primary specimens for which a result was known, n=55 showed resistance to one or both rifampin (RIF) and isoniazid (INH): 14 were MDR (resistant to both RIF and INH), 5 were RIF mono-resistant, and 36 were INH resistant. In-depth review of the MDR cases showed that 9 were Florida patients, for which treatment data was available. Of these patients, the average number of days from date of MDR screen result to change to appropriate therapy was n=6.9 days (range 1-16 days). The number of days from change in appropriate therapy to when the phenotypic AST was available was n=30.2 days (range 11-77 days).

Conclusions: From 2014-2015, 10.3% of primary clinical specimens tested by the MDR screen showed resistance to one or both RIF and INH, MDR-TB was detected in 2.6%. The MDR screen provided results for change to appropriate therapy for MDR patients that would not have been available for several days, and in most cases weeks, if clinicians had waited for phenotypic AST results. Ultimately placing patients on appropriate therapy in a timely manner has a positive impact on patient and public health outcomes.
Poster 5:
Evaluation of Gene Xpert MTB/RIF in 70 culture positive cases of tuberculous meningitis
K. Sharma, M. Modi, A. Sharma, P. Ray, S. Verma

1Department of Medical Microbiology, 2Department of Neurology, and 3Department of Internal Medicine, Post Graduate Institute of Medical Education and Research, Chandigarh, India

Objectives: Rapid and specific diagnosis of tubercular meningitis (TBM) is of paramount importance to decrease associated morbidity and mortality. Therefore, the present study was undertaken to compare Gene Xpert MTB/RIF (GX) assay and Multiplex PCR (MPCR) using three targets specific for Mycobacterium tuberculosis including IS6110, MPB64 and Protein b for diagnosing TBM as described previously from our center.

Study Design: GX and Multiplex PCR using three targets specific for Mycobacterium tuberculosis like IS6110, MPB64 and Protein B as been described previously from our center for diagnosis of TBM, published in Journal of neurology 2011, were performed on total 90 cerebrospinal fluid samples. These included CSF samples from 70 patients with culture positive TBM, CSF samples from 10 Non-TB infectious meningitis and CSF samples from 10 Non-infectious neurological disorder samples. These 20 non TB CSF samples were included in control group. Phenotypic testing by 1% proportion method was carried out for rifampicin and isoniazid from the 70 CSF culture isolates and the rpoB gene sequencing was also done for diagnosing rifampicin resistance. We have taken culture as gold standard and all results were compared to culture to calculate sensitivity. For specificity we have taken 20 non TB control group CSF samples as described previously.

Results: GX and MPCR were positive in 58/70 (82.85%) and 65/70(92.85%) patients respectively. Both tests were negative in all controls (20 control CSF samples). Rif resistance was detected in 8 of 70 (11.42%) by GX, and in 7 MPCR positive with rpoB gene sequencing. Out of the 70 isolates subjected to phenotypic drug susceptibility testing, 63 were sensitive to Rif and INH and 7 were found to be resistant to both Rif and INH. Thus, there was one case of false Rif resistance detected by GX, which was Rif sensitive on rpoB gene sequencing and phenotypic drug susceptibility testing. False Rif resistance was observed with probe B of Gene Xpert. Resistance mutations detected on rpoB gene sequencing were at 531 codon (3 cases), 533(2 cases) and 516 codon (1 case) and 526 codon (1 case) respectively. These mutations are picked up by GX as well. Cost of doing MPCR is less than 1 dollar where as GX is 10$.

Conclusion: MPCR had a higher sensitivity than GX for diagnosing TBM. MPCR is robust and cost effective method for diagnosis of TBM in low resource and high endemic country.
Poster 6:

Evaluation of Multi targeted Loop-Mediated Isothermal Amplification (LAMP) for Rapid Diagnosis of Extra-pulmonary tuberculosis

K. Sharma¹, N. Batra¹, M. Modi², A. Sharma³, P. Ray¹, S. Verma³

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Objectives: Rapid and accurate diagnosis of extra-pulmonary tuberculosis (EPTB) is imperative for early treatment and better patient outcome. LAMP assay can be carried out in simple water bath under isothermal conditions in 60 minutes in any laboratory, even at rural setting in resource poor, endemic countries. We aimed to evaluate the LAMP test using IS6110 and MPB64 targets for M. tuberculosis complex diagnosis. Comparison of results of IS6110 LAMP, MPB64 LAMP with IS6110 PCR, culture and ZN (Ziehl-Neelsen) smear examination.

Study Design: LAMP assay using 6 primers was performed on various EPTB samples (CSF, Synovial fluid, Lymph node and tissue biopsies and various other samples like gastric aspirate, pus samples and urine samples) from patients suspected of EPTB. 200 patients, clinically suspected of EPTB (75 culture positive, 125 culture negative) and 100 non-TB control subjects were included in the study. Suspicion of EPTB was based on the clinical presentation, radiological examination and histopathological examination in all 200 patients. On evaluation 75 were found to be culture positive while the 125 culture negative patients included based on the composite reference standard (CRS) and response to ATT. Culture was used as reference standard in culture positive cases and CRS with response to ATT was used as reference standard in 125 clinically suspected cases of EPTB. For specificity calculations 100 non TB control were used and specificity was calculated with respect to that.

Results: Overall LAMP test (using either IS6110 or MPB64 targets) had sensitivity and specificity of 96% and 100%, respectively, for culture confirmed EPTB cases and 84% and 100%, respectively for culture negative, clinically suspected EPTB cases. Sensitivity of IS6110 LAMP, MPB64 LAMP, any target LAMP is used (either IS6110 or MPB64) and IS6110 PCR for culture negative EPTB (125) cases was 78.4%(98), 82%(103), 84%(105), and 70.4% (88 ) respectively. There were 7 cases which were missed by IS6110 LAMP and 2 cases by MPB64 LAMP. This LAMP assay is evaluated earlier by IS6110 LAMP primers in various pulmonary conditions and few EPTB cases. LAMP assay using two targets like IS6110 and MPB64 together at the same time for large number of EPTB samples have not been evaluated earlier.

Conclusion: LAMP assay using two targets at the same time is a promising technique for rapid diagnosis of EPTB in 60 minutes especially in a resource poor setting who are still battling with this deadly disease.
Poster 7:

Evaluating the Significance of Equivocal Mycobacterial Smear Results

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Objective: To determine the significance of mycobacterial smear results when very few acid fast bacilli are observed in the concentrated smear.

Study Design: All sputum specimens were digested and decontaminated using standard protocol. Concentrated smears were prepared, fixed using 5% phenol in 70% ethanol, and stained using Auramine O-phenol stain. Using a fluorescent microscope, smears were examined for AFB at 200x magnification for a minimum of 30 fields. When AFB were observed, they were counted at 500x magnification, and the results were reported qualitatively. All results were blindly verified by a second analyst. When reading discrepancies occurred, a third analyst served as a tie-breaker. A smear was reported as equivocal if only one, two, or three AFB were observed on the entire slide, and as positive if greater than three AFB were observed. Smear and culture data was collected for all specimens tested during a twelve month period and the smear-to-culture correlation was calculated separately for smear positives, smear negatives, and equivocal smears. Equivocal results were counted as smear positives for the purpose of calculating the culture correlation rate.

Results: From September, 2015 through August, 2016, a total of 2097 sputum specimens were processed for smear and culture. Of these, 24.6% (n=516) were smear positive, 69.7% (n=1462) were smear negative, and 5.7% were equivocal (n=119). 68.2% of the smear positive sputa resulted in a culture that was positive for one or more mycobacteria. 13.7% of the smear negative sputa resulted in a culture that was positive, and 36.2% of the equivocal sputa resulted in a positive culture. Of the 77 equivocal smears that did not result in a positive culture, 71.6% (n=53) were collected from a previously diagnosed MTBC patient or were accompanied by one or more positive NTM cultures.

Conclusion: In this data set, equivocal smears resulted in positive cultures approximately three times as often as negative smears. Therefore, even smears with very few visible AFB should be considered significant, as they are more likely to indicate an infectious patient status than a negative smear. For equivocal smear results collected from patients with an unknown TB status, reflex PCR testing should be performed to rule out MTBC, increasing the accuracy and speed of a TB diagnosis.
Poster 8:

*Mycobacterium canetti* Case Identified in Southeastern Michigan

A. Schooley¹, D. Kissner², J. Vanneste¹, S. Church¹, H. Seymour¹, M. Soehnlen¹

¹Michigan Department of Health and Human Services, Bureau of Laboratories, Lansing, Michigan, ²Wayne State University School of Medicine, Division of Pulmonary, Critical Care, and Sleep Medicine

**Objective:** The first reported case of *Mycobacterium canetti* was in 1969. There have been rare reports since of *M. canetti*, which is a progenitor species to *M. tuberculosis*, originating from the Horn of Africa. The Michigan Department of Health and Human Services identified a case of *M. canetti* in 2016 from a patient seen at a Michigan hospital.

**Study Design:** A 14 month old male from Yemen presented to a large southeastern hospital system in Michigan with lymphadenitis. The patient lived in a crowded house in Djibouti prior to arrival in Michigan. The family noticed swelling in the left submandibular region a month after his arrival. His primary care physician prescribed Augmentin, but another area of swelling appeared. His purified protein derivative TB skin test (PPD) and QuantiFERON-TB Gold test were positive.

**Results:** Growth characteristics, High Performance Liquid Chromatography (HPLC) mycolic acid testing, and Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) testing, indicated *Mycobacterium tuberculosis* complex (MTBC). Antimicrobial susceptibility testing was performed by Mycobacterium growth indicator tube (MGIT). Pyrazinamide results indicated resistance. Per Michigan Department of Health and Human Services (MDHHS) protocol, the sample was forwarded to CDC for additional testing. CDC returned results of a silent mutation, Ala⁴⁶Ala, in the pncA region. MIRU genotyping, performed at MDHHS, indicated *M. canetti*.

**Conclusions:** *Mycobacterium canetti*, which is intrinsically resistant to pyrazinamide and pyrazinoic acid, was identified in a Michigan patient originating from Yemen. Although *M. canetti* is part of the MTBC, there are differences in growth characteristics, intrinsic resistance, and molecular markers that are useful in the identification. The patient in this case recovered following appropriate organism identification and treatment of nine months with Rifampin and Isoniazid.
**Poster 9:**

**Frequency of mixed *Mycobacterium* species infections detected by the INNO-LiPA MYCOBACTERIA v2 hybridization line probe assay from 2015 to 2016**

S.D. Fisher, G. Kupferschmidt, J.T. Wotton, P.M. Vagnone, Minnesota Department of Health, Public Health Laboratory, Infectious Disease Laboratory, St. Paul, Minnesota

**Objective:** To evaluate the frequency of mixed mycobacteria infections detected from 2015 to 2016 using the Fujirebio (Ghent, Belgium) manufactured INNO-LiPA MYCOBACTERIA v2 line probe hybridization assay (LPA), which identifies 18 clinically relevant *Mycobacterium* sp.

**Study Design:** PCR is used to amplify the 16s-23s internal transcribed spacer region of Mycobacteria spp. This biotinylated amplified material is reverse hybridized with specific oligonucleotide probes immobilized on membrane strips. A final colorimetric step produces characteristic bands that are associated with specific *Mycobacterium* sp. All mycobacteria in specimens should be represented on the strip, even mixed infections.

Two years of data (2015 to 2016) was assessed for the number of specimens where more than one species of mycobacteria was detected by the LPA, compared to the total number of LPA positive specimens.

**Results:** It was determined that for years 2015 and 2016, the MDH TB Laboratory LPA detected approximately 5% mixed mycobacterial infections. Most of the mixed populations were *M. avium* complex with *M. intracellulare* infections followed by *M. avium* complex with *M. abscessus* infections. Other clinically relevant mixed infections were also detected in this study such as *M. tuberculosis* complex with *M. avium* complex but in far lower numbers but nonetheless considered significant for patient care.

**Conclusions:** In Minnesota, mixed mycobacteria infections do occur with low frequency, which can be easily and rapidly detected using the INNO-LiPA MYCOBACTERIA v2 hybridization line probe assay. Accurate mycobacterial identification and detection of mixed infections is essential for not only epidemiological investigations but for correct diagnosis and appropriate patient treatment.
Poster 10:
T-SPOT.TB Screening in High Risk School Children in Clark County, Nevada: Evaluation of Logistics and Cost Effectiveness Relative to an Onsite QuantiFERON-TB Gold Test


Objective: To assess whether conducting latent tuberculosis infection (LTBI) screening using a contracted service for the T-SPOT.TB test versus in-house utilization of a QuantiFERON-TB Gold (QFT-G) test is a logistic and cost-effective method in testing and identifying high risk school children.

Study Design: From February-August 2016, 604 high risk school children were screened for LTBI using a brief survey assessing TB risk factors. Participants were recruited for the study by outreach efforts conducted by a community agency at designated venues targeting primarily high risk immigrant children. Questionnaires were assessed to determine participant eligibility for T-SPOT.TB testing. Specimen collection was conducted in-house by the SNHD TB clinic. Specimen testing was conducted by a contracting vendor located 1,500 miles away. Following LTBI screening using the T-SPOT.TB test, the cost-effectiveness and feasibility of using this method versus future use of the QFT-G test was evaluated. A quantiferon analyzer was purchased and validated on a total of 44 test samples following the culmination of the T-SPOT.TB screening. The Nevada Bureau of Health Care Quality and Compliance is in the process of inspecting and reviewing QFT-G protocols prior to conducting patient testing.

Results: Of the 604 participants screened, 253 were assessed for eligibility and 88 (35%) received the T-SPOT.TB test. Evaluation of this screening strategy found that the limited testing results using the T-SPOT.TB method were due to logistic barriers involving restrictive specimen collection and processing turn-around times, difficulty in organizing schedules for the target population, and obstacles in training staff to adhere to newly developed protocols. Specimen processing time utilizing the T-SPOT.TB method for this study was 48-72 hours versus 24 hours attainable by implementation of the QFT-G. The costs of screening using the single T-SPOT.TB test was $107 per client, while the estimated cost of using an in-house QFT-G screening is $44 per client. The contracted T-SPOT.TB test was a labor intensive screening method which limited the number of tests performed per day, while the QFT-G, performed locally appears to offer increased efficiency and versatility.

Conclusions: Successful detection of LTBI in high risk groups during a limited time span requires implementing a targeted screening strategy utilizing a logistic and cost-effective method to impact overall TB incidence. Based upon these findings, instrumentation has been purchased by the SNHD laboratory for performance of the QFT-G testing, enabling the SNHD TB Clinic to comfortably expand the capability for LTBI screening.

Note: This research was supported by a grant award, a “Proposal for the Expanded Access to Interferon Gamma Release Assays (IGRA)” from the Association of Public Health Laboratories.
Objective: A study was undertaken to compare recovery rates of *Mycobacterium tuberculosis* in non-sputum sources. Cepheid (the manufacturer of the GeneXpert MTB/RIF kit) only has FDA approval for testing sputum sources. TDH Laboratory Services wanted to evaluate non-sputum sources for the following reasons:

*Mycobacterium can disseminate throughout the body and to various body fluids. It may present at detectable levels prior to the development of respiratory symptoms.*

Frequently, a non-sputum source may be the only specimen available for testing and therefore may be the only chance for laboratory confirmation of infection.

Study Design: Non-sputum sources received between July 2014- September 2015 were tested on GeneXpert. Sources varied from: bronchial washings, lymph nodes, urine, stool, blood, CSF, bone marrow and endotracheal aspirate. Twenty-five non-sputum specimens were received from various hospitals and health departments throughout the Tennessee. Specimens tested included both positive and negative specimens by fluorochrome smear. GeneXpert results were compared to reported culture results.

Results: Specimens tested for GeneXpert MTB/RIF had smear results ranging from negative to 10+. Seventeen specimens were reported as smear negative, GeneXpert negative and no growth by culture. Eight specimens had smear positive results with 6 positive GeneXpert results (2 negative). Five out of six positive GeneXpert results had culture confirmation of *Mycobacterium tuberculosis* complex; 1 was contaminated and unable to identify further. The contaminated culture had a previous sample test positive for MTBC. Two GeneXpert negative results were confirmed as *M. intracellulare*. 100% correlation between the Positive GeneXpert and culture results.

Conclusions: Reliable results can be obtained from non-sputum sources utilizing GeneXpert MTB/RIF. Therefore, GeneXpert testing will be routinely performed on acceptable respiratory and non-respiratory specimens received for Mycobacteriology Smear and Culture testing. Non-sputum sources will also have the following disclaimer added:

*This test has been approved by U.S. Food and Drug Administration for analysis of sputum specimens. Performance characteristics from specimen types other than sputum have been determined by TDH Laboratories Services.*
**Poster 12:**

**Evaluation of a Two Week 7H10 Agar Proportion Method for Isoniazid and Rifampin Drug Susceptibility Testing of *Mycobacterium tuberculosis* complex**

K. Jost Jr., D. Dunbar, J. Owen, Texas Department of State Health Services, Austin, Texas

**Objective:** The Centers for Disease Control and Prevention recommend that first-line drug susceptibility test (DST) results be reported within 17 days from the identification of *Mycobacterium tuberculosis* complex (MTBC) from culture by the use of a rapid growth-based DST method. 7H10 agar proportion (AP) DST is not considered a rapid DST method since conventionally, results are not reported until 3 weeks. The objective of this study was to determine the accuracy of INH and rifampin (RMP) AP results reported at 2 weeks.

**Study Design:** From 10/6/2015 through 3/30/2016, MTBC isolates were tested by examining 7H10 AP DST growth at 2 and 3 weeks at high power magnification (up to 100X). The data represented 26 consecutive weeks (6 months) of routine testing. The total number of AP tests for 0.2 mcg/ml INH, 1.0 mcg/ml INH, and RMP was 553, 550, and 552, respectively.

**Results:** Of the total tests, the number (%) of tests with conclusive (susceptible or resistant) 3 week results for 0.2 mcg/ml INH, 1.0 mcg/ml INH, and RMP was 529 (95.7%), 526 (95.6%), and 531 (96.2%), respectively. The number (%) of conclusive 3 week tests with conclusive 2 week results for 0.2 mcg/ml INH, 1.0 mcg/ml INH, and RMP was 521 (98.5%), 525 (99.8%), and 529 (99.6%), respectively. Compared to the 3 week AP result, the sensitivity of the 2 week result for 0.2 mcg/ml INH, 1.0 mcg/ml INH, and RMP to detect resistance was 88.1%, 93.3%, and 90.9%, respectively. Compared to the 3 week AP result, the specificity of the 2 week result for all three drug concentrations was 100%. The prevalence of resistance to 0.2 mcg/ml INH, 1.0 mcg/ml INH, and RMP in the study was 10.0%, 5.4%, and 3.8%, respectively.

**Conclusions:** This study demonstrated that 0.2 mcg/ml INH, 1.0 mcg/ml INH, and RMP AP results can be determined at 2 weeks with 100% specificity and at least 88.1% sensitivity for at least 98.5% of tests reported at 3 weeks. A limitation of this study is that agar proportion is performed at relatively few U.S. Public Health Laboratories; most labs use MGIT 960 or VersaTREK systems for rapid growth-based first-line DST. However, these broth systems are known to miss clinically significant rifampin and ethambutol resistance detected by solid medium proportion methods. Compared to broth-based DST, 2 week AP DST for INH and RMP may allow more accurate detection of drug resistance and susceptibility in a similar timeframe.
Poster 13:
Optimizing the Public-Private Partnership for Rapid and Accurate TB Laboratory Testing

K. Pak1, 3, E. Desmond2, P. Barry3, Grace Lin2

1 Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, 2 Microbial Disease Laboratory, California Department of Public Health, Richmond, California, 3 Tuberculosis Control Branch, California Department of Public Health, Richmond, California

Background: In California, specimens from patients with suspected tuberculosis (TB) are tested by both public health and private sector laboratories for the presence of mycobacteria and undergo drug susceptibility testing (DST) as appropriate. TB control programs have noted delays with some private sector laboratories. Delays can impact public health because drug-resistant organisms may be ineffectively treated, acquire further resistance, and be transmitted within the community for prolonged periods of time.

Study Design: We analyzed California TB Registry data to describe the proportion of cases with sputum cultures performed outside a public health laboratory. We solicited reports of substantial delays in private sector laboratory results from the six regions served by the California TB Control Program. Reports were reviewed by program and laboratory staff to identify common contributors to delays. Several actions were taken to address these contributors.

Results: Among culture positive cases reported during 2010–2015, an increasing proportion of cultures were performed outside of public health laboratories (52% in 2010 vs 60% in 2015; Cochran-Armitage trend: p<.0001 ). Six of six regions reported instances of substantial delays. Recurring contributors were: transport, subculturing among multiple labs, underutilization of rapid tests, not reporting preliminary results, complex communication procedures, difficulty obtaining an isolate for molecular testing, and lack of reporting to public health. In response, we initiated a conference call with a large commercial laboratory to discuss instances of delay, better understand work distribution, and establish communication. Additionally, written notification and a webinar were presented to laboratories performing mycobacteriology testing in California which included a review of California regulations, an explanation of benefits of rapid TB laboratory results, and the availability of molecular testing in public health laboratories. Over 150 laboratorians attended from over 55 laboratories, and 72 of 72 (100%) attendees who evaluated the webinar indicated the presentation increased their knowledge of the subject matter.

Conclusion: Private sector laboratories play a critical role in the identification and diagnosis of TB in California. Through future review and continued efforts to build communication and partnerships between private and public laboratory networks, we aim to improve timeliness in TB specimen flow, processing, and reporting.
Objective: In 2009, CDC’s National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention (NCHHSTP) introduced a service mechanism called “Program Collaboration and Service Integration” (PSCI). Public health programs provide categorical services to persons who have multiple related disease risks, but often miss significant opportunities. PCSI’s strategic priority strengthens collaboration across disease program areas at the client level. Incarcerated populations have higher risk for HIV/AIDS, STDs and TB due to increased risk of disease transmission. Incarceration provides important opportunities for disease surveillance, diagnosis, treatment and prevention. In October 2015, the TB Elimination Program, HIV/STD & Viral Hepatitis Program, Information Technology Services Division, and Laboratory Services of Tennessee Department of Health (TDH) collaborated with the Tennessee Department of Correction (TDOC) to screen all new inmates for TB infection, HIV, syphilis, gonorrhea and chlamydia. The collaborative efforts and results of TDH’s and TDOC’s implementation of intake screening for TB, HIV, and STDs for all inmates entering the TDOC system are described.

Study Design: Multidisciplinary planning included feasibility, resource assessment, protocol development, intake flow analysis, information technology installation, staff training and data analysis at two intake prisons in Tennessee – The Tennessee Prison for Women and Bledsoe County Correctional Complex.

Results: Following pilot testing at both facilities, from December 2015 through May 2016 a total of 16,166 tests for five diseases were obtained. The aggregate test positivity rates were: TB infection – 5.0%; HIV infection – 0.8%; syphilis infection – 1.0%; gonorrhea infection – 2.2%; and chlamydia infection – 0.2%; positivity rates differed by gender.

Conclusion: A unique multi-program and agency collaboration successfully implemented integrated prison intake screening for five (5) diseases of public health importance in Tennessee – including *Mycobacterium tuberculosis*. Multi agency collaboration continues and testing for additional diseases is being explored.
Poster 15:

Adaptation of the use of the GeneXpert Assay for the detection of Rifampin-resistant *Mycobacterium tuberculosis* Complex in culture

A. Abuarqoub, Illinois Department of Public Health, Chicago, Illinois

**Objective:** To detect Rifampin-resistant *Mycobacterium tuberculosis* in culture using the Cepheid GeneXpert assay. This approach provides a rapid and cost-effective means for the diagnosis and treatment of patients.

**Study Design:**

**Sample Preparation:** A multi-step sample preparation was developed to ensure that no cultures were viable when tested using the GeneXpert assay. Cultures were dispersed in TE buffer and heated for 30 minutes at 100°C and then quickly frozen at -20°C for 30 minutes. Finally, 2ML of the sample reagent provided in the GeneXpert assay kit was added to 20ul from the specimen and incubated at room temperature for 15 minutes. To test if any organisms remained viable following the sample preparation, 200µl of each sample was inoculated on solid media and monitored for growth for 6 weeks. None of the cultures grew (see Table 1).

**GeneXpert Assay:** A total of 60 cultures were prepared as described above and analyzed using the GeneXpert assay following methods described by the manufacturer. The cultures were previously tested by conventional methods which verified that 26 were rifampin-sensitive *Mycobacterium tuberculosis*, 5 were rifampin-resistant *Mycobacterium tuberculosis*, and 29 were non-mycobacterium (NTM) cultures.

**Results:** The results of this study are shown in Table 1. A total of 31 *Mycobacterium tuberculosis* identified using conventional means were correctly identified using the GeneXpert assay. Of these cultures, 26 were rifampin-sensitive and 5 were rifampin-resistant. The GeneXpert assay correctly identified all of these cultures. Finally, the GeneXpert assay failed to detect *Mycobacterium tuberculosis* in all (29/29) of the NTM cultures.

**Conclusion:** The results of this study show that we have successfully adapted the use of the Cepheid GeneXpert assay for the detection of rifampin-sensitive and rifampin-resistant *Mycobacterium tuberculosis* in culture. This test algorithm reduces the time for culture identification from 48hr to 3:00hr and saves money that would otherwise be used for test employing additional assays and the cost to maintain other instrumentation other than the GeneXpert system. Also this approach reduces staff time and it does not require molecular expertise for the detection of rifampin resistance. The benefit from testing solid not liquid is because on the solid culture you can confirm the colony morphology with the result obtained although the procedure could be easily adapted to test liquid cultures.
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The newly added module, *Landscape and Language of Molecular Diagnostics for TB Drug Resistance*, is specifically designed to provide TB control staff, clinicians and nurses a brief overview of basic principles of molecular technology and test platforms used for molecular testing of *Mycobacterium tuberculosis* as a basis for understanding how mutations are associated with drug resistance and what reporting language might be used to describe resistance results.

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