

9th National Conference on Laboratory Aspects of Tuberculosis

Conference Program



June 8–9, 2015



APHL ASSOCIATION OF
PUBLIC HEALTH LABORATORIES®

Posters..... p. xx

Attendees p. xx

Meeting at a Glance

TIME	SUNDAY, JUNE 7	MONDAY, JUNE 8		TUESDAY, JUNE 9				
7:00am		Registration		Registration				
7:30am								
8:00am			Posters		Opening Session/ Keynote	Perspectives on TB Molecular Diagnostics		
8:30am								
9:00am								
9:30am						Break		
10:00am						Next Generation Se- quencing and Applications for TB	Break	
10:30am							Setting the Standard: The TB Lab System in the US	
11:00am							Highlighting the Work of Our Laboratories	
11:30am								
12:00pm						Lunch (on own)	Lunch (on own)	
12:30pm								
1:00pm						Navigating the World of DST	Developments in TB Research	
1:30pm								
2:00pm								
2:30pm						Break	Break	
3:00pm						Practical Approaches to Success in the TB Laboratory	TB Case Studies: Lessons from the Field	
3:30pm								
4:00pm	Registration							
4:30pm								
5:00pm								
5:30pm				Special Session				
6:00pm								
6:30pm								
7:00pm								

Welcome Letter

Welcome to the 9th National Conference on Laboratory Aspects of Tuberculosis, co-located with the 2015 National TB Conference in Atlanta — the home of the Braves!

Where are we today? Since 1992, the case rate for tuberculosis has continued to decline in the United States and in 2012 there were 9,951 cases of TB reported — the first time this number was below 10,000 since records began in 1953. The trend has continued, and in 2014 there were 9,412 new cases of TB in the US. Despite this progress, our work is far from done:

- The 2014 incidence rate for TB is 3.0 cases per 100,000 population, the same as in 2013.
- Although the total number of TB cases continues to decline, 2014 showed the smallest decline in incidence in over a decade.
- The TB rate is 13.4 times higher in foreign-born persons than US-born persons and TB continues to disproportionately affect racial/ethnic minorities.
- TB drug resistance rates remain relatively stable, slightly above 1%, with the majority of the multi-drug resistant cases (90%) occurring in foreign-born persons.
- We have yet 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.

What are we doing in the laboratory? The TB laboratory plays an essential role in the fight against TB, striving to provide the highest quality testing as quickly as possible. There have been several noteworthy advances in TB testing since the 8th National TB Conference in 2013:

- Nucleic acid amplification testing continues to be adopted by laboratories performing diagnostic testing for TB.
- Availability of molecular methods for detection of drug resistance has increased.
- The Xpert MTB/RIF assay received FDA market authorization for expanded use to determine if patients with suspected TB can be removed from airborne infection isolation.
- CDC's Division of TB Elimination Laboratory Branch continues to offer reference services with the addition of the California Department of Health Microbial Disease Laboratory as a reference center for antimicrobial susceptibility testing for states with low TB incidence.
- Next generation sequencing methods are being increasingly utilized, particularly in the identification of outbreaks, enabling TB control programs to more rapidly determine clusters and prevent transmission of TB.

At the **9th National Conference** we will hear more about these advances. I hope you enjoy the presentations and take some time to interact with the poster presenters and visit the vendor exhibits. This year we are co-located with the National TB Conference organized by the National TB Controllers Association. We will share meeting breaks with our fellow TB clinicians and TB control program officials, and on Wednesday, June 10 we will host a joint session. Take advantage of the expertise at the meeting and the opportunity to meet face-to-face with colleagues. As in baseball, TB elimination is a team effort — let's have all the bases covered!

I would like to thank our planning committee for bringing us such an exciting agenda!



Marie-Claire Rowlinson, PhD, D(ABMM)

Chair, program planning committee, 9th National Conference on the Laboratory Aspects of Tuberculosis

About APHL

VISION: A healthier world through quality laboratory systems.

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.

The Association Of Public Health Laboratories (APHL) is a non-profit 501(c)(3) organization representing governmental laboratories that monitor and detect public health threats, including emerging infectious disease surveillance, detection of metabolic and genetic conditions in newborns, water contamination identification and foodborne outbreak detection. APHL's members are state, local, county and city public health laboratories, state and local environmental health laboratories, state agricultural laboratories, corporations, individual and student members with an interest in public health laboratory issues, and organizations that share common goals with APHL.

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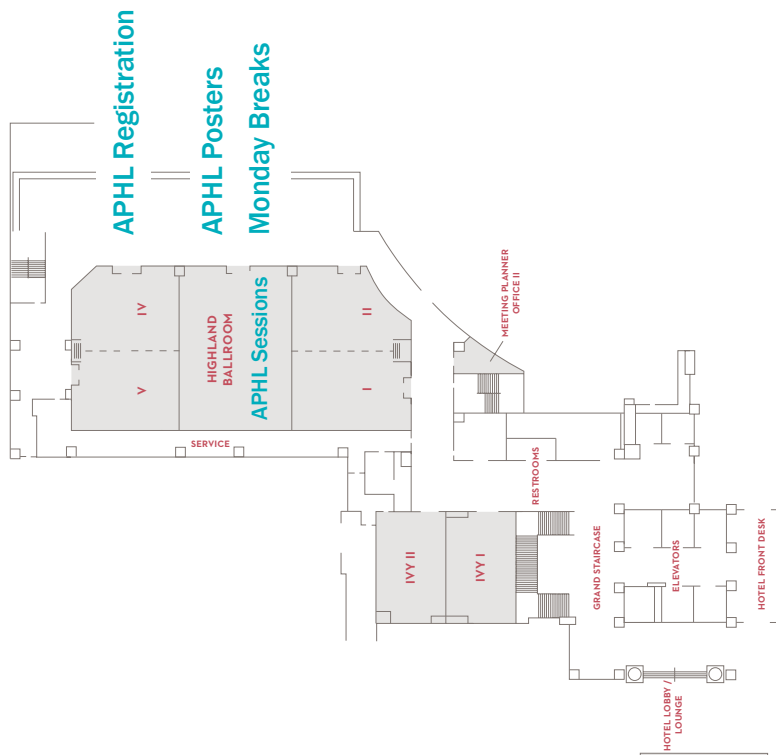
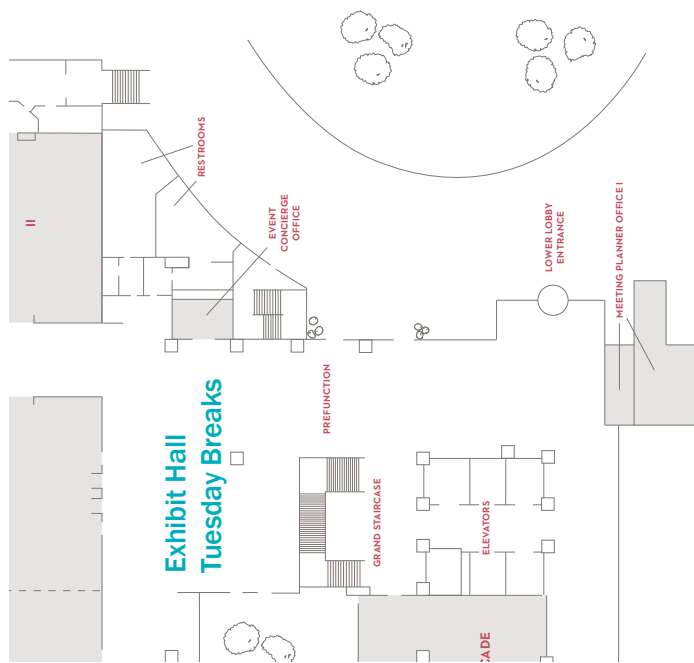
Paula M. Vagnone, MT(ASCP), Minnesota Department of Public Health

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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 in each attended session that credit is requested for 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.

APHL is an approved provider of Certified in Public Health (CPH) Recertification Credits through the National Board of Public Health Examiners (NBPHE). Attendees have the opportunity to earn up to 12 hours of credit by attending the entire conference. APHL will not issue certificates of attendance.

Hotel floor plans



Conference Schedule

SUNDAY, JUNE 7

Registration: 4:00 pm – 7:00 pm

MONDAY, JUNE 8

Registration: 7:00 am – 5:30 pm • Highland Prefunction

Poster viewing times: 8:00 am – 6:15 pm • Highland Prefunction

(Poster presenters should have their posters up by 8:00 am.)

DAY 1

588-831-15, 6.75 contact hours for the entire day

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

- Describe the applications of Next Generation Sequencing for a public health mycobacteriology laboratory.
- Discuss the issues surrounding discrepant Drug Susceptibility Test results and ways to address them.
- Explain approaches to improving biosafety when performing molecular testing for MTBC.
- Summarize the considerations for implementing MALDI-TOF MS for identification of mycobacteria.

8:00 am – 9:30 am • Highland I–III

Opening Session and Keynote

The opening session features a keynote address by Dr. David Dowdy on the role of diagnostic testing in both low- and high-burden settings, including the potential impact of diagnostic strategies on the epidemiology and economics of TB control in 2015.

Welcome to the 9th National Conference on Laboratory Aspects of TB

Marie-Claire Rowlinson, PhD, D(ABMM), Florida Bureau of Public Health Laboratories

Labs, Ledgers and Lives Saved: The Impact of Diagnostic Strategies on the Epidemiology and Economics of Tuberculosis

David Dowdy, MD, PhD, Johns Hopkins University Bloomberg School of Public Health

9:30 am – 10:00 am • Highland Prefunction

Morning Break

10:00 am – 12:00 pm • Highland I–III

Next Generation Sequencing and Applications for TB

The potential of next generation sequencing (NGS) in public health laboratories is beginning to be realized and now includes applications for TB. This session will provide an overview of NGS technology and considerations for successful implementation in a TB laboratory, as well as possible future directions.

Moderator: Anne Gaynor, PhD, Association of Public Health Laboratories

Overview of Next Generation Sequencing

David Engelthaler, PhD, The Translational Genomics Research Institute (TGen)

CDC's Use of Whole Genome Sequencing for Genotyping

James E. Posey, PhD, Center for Disease Control and Prevention

Perspectives From a Public Health Laboratory

Kimberlee A. Musser, PhD, Wadsworth Center, New York State Department of Health

12:00 pm – 1:00 pm

Lunch (on your own)

1:00 pm – 2:45 pm • Highland I–III

Navigating the World of Drug Susceptibility Testing

Drug resistance continues to be a major public health concern threatening progress made in TB care and control globally. Issues with culture-based methods and the introduction of advanced molecular methods for identifying drug resistance have added more complexity to our understanding of drug resistance. This session will address the ongoing questions with test methodology and results of drug susceptibility tests.

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

Evaluation of Phenotypic Drug Susceptibility Test Methods Project (Expanded MPEP Study)

Beverly Metchock, DrPH, D(ABMM), Centers for Disease Control and Prevention

Expected Discrepancies Between Molecular and Growth-based DST: Which Technology Is Giving the Right Answer?

Edward P. Desmond, PhD, D(ABMM), California Department of Public Health, Microbial Diseases Laboratory

Issues in Tuberculosis Drug Susceptibility Testing: TB Subcommittee White Papers

David Warshauer, PhD, D(ABMM), Wisconsin State Laboratory of Hygiene

2:45 pm – 3:15 pm • Highland Prefunction

Afternoon Break and Poster Viewing

Poster presenter should stand by their posters from 2:45 pm to 3:15 pm

3:15 pm – 5:15 pm • Highland I–III

Practical Approaches to Success in the TB Laboratory

With advances in methodologies come opportunities to improve the way classical TB diagnostic methods are delivered. This session will examine various approaches to improving the quality, efficiency and safety of your TB Laboratory.

Moderator: Roy Tu'ua, M(ASCP), Missouri State Public Health Laboratory

Specimen Collection, Packaging and Shipping

William Slanta, M(ASCP), Arizona Department of Health Services

Biosafety and Risk Assessment for New Molecular Methods

Michael Pentella, PhD, D(ABMM), William S. Hinton State Laboratory Institute (MA)

Quality Assurance Issues in the TB Lab

Mike Loeffelholz, PhD, D(ABMM), University of Texas Medical Branch, Galveston

Implementing MALDI-TOF MS

Julie Tans-Kersten, MS, MT (ASCP), Wisconsin State Laboratory of Hygiene

5:30 pm – 6:15 pm • Highland I–III

Special Session

APHL in collaboration with CDC established a quality-assured Drug Susceptibility Testing Reference Center at the California Department of Public Health, Microbial Diseases Laboratory. This session will be an opportunity to learn about enrollment, the role of the reference center, the services provided and its relationship with the Molecular Detection of Drug Resistance (MDDR) testing service provided at CDC.

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

National Public Health Laboratory Drug Susceptibility Testing Reference Center for *Mycobacterium tuberculosis*

California Department of Public Health, Microbial Diseases Laboratory

TUESDAY, JUNE 9

Registration: 7:00 am – 5:15 pm • Highland Prefunction

Exhibit Hall Open: 9:45 am – 4:00 pm • Grand Ballroom Prefunction,
Lower Level Lobby

Poster viewing times: 8:00 am – 5:00 pm • Highland Prefunction

DAY 2

588-832-15, 6.75 contact hours for the entire day

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

- Discuss how the predictive value and impact of NAAT varies with different patient populations.
- Explain the methods CDC uses to monitor performance of the TB Laboratory System, and describe the changes that have occurred in the past 5 years.
- Describe ways NTM can be identified through sequencing methods.
- List ongoing TB research activities in the field of drug resistance.

8:00 am – 9:45 am • Highland I–III

Perspectives on TB Molecular Diagnostics

Molecular diagnostics such as nucleic acid amplification testing (NAAT) have become standard for rapid detection of M. tuberculosis complex (MTBC) and more frequently for detection of drug resistance. This session will examine how clinical and public health laboratories have integrated rapid molecular diagnostics into their TB testing algorithms and assessed their impact in various settings.

Moderator: Marie-Claire Rowlinson, PhD, D(ABMM), Florida Bureau
of Public Health Laboratories

NAAT in the Clinical Laboratory and Impact on Infection Control

Susan Novak, PhD, D(ABMM), Southern California Permanente Medical Group

Xpert MTB/RIF Performance Characteristics in a State Public Health Laboratory Context

Ken Jost, MT(ASCP), Texas Department of State Health Services

Experience With Xpert MTB/RIF in a Low Incidence State

Nancy Robinson, MPH, M(ASCP), Alabama Bureau of Clinical Laboratories

9:45 am – 10:15 am • Grand Ballroom Prefunction

Morning Break in the Exhibit Hall

10:15 am – 11:00am • Highland I–III

Setting the Standard: The TB Laboratory System in the US

This session will explore data from two evaluations from the CDC TB Cooperative Agreement grantees. Presenters will discuss trends in testing volumes, turnaround times and methodologies over the last 5 years, as well as discuss the use of appropriate, realistic and evidence-based recommendations to monitor performance.

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

It's All About That Data: Five-Year Laboratory Trends From TB Elimination Cooperative Agreements

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

Evaluation of TB Laboratory Performance Indicators

Tracy Dalton, PhD, Centers for Disease Control and Prevention

11:00 am – 12:00 pm • Highland I–III

Highlighting the Work of Our Laboratories

There is much to be learned from work done in the field. This session will highlight the findings of three outstanding abstracts that have broad applications to TB laboratories.

Moderator: William Murtaugh, MPH, Association of Public Health Laboratories

Development of an Individualized Quality Control Plan (IQCP) for MGIT Pyrazinamide (PZA) Drug Susceptibility Testing (DST)

Denise Hartline, MT(ASCP), Centers for Disease Control and Prevention

Nontuberculous Mycobacteria (NTM) Species Identified Using *rpoB*/*hsp65* Gene Sequencing and *erm*(41) Gene Analysis

Max Salfinger, MD, FIDSA, FAAM, National Jewish Health

Improvements in Efficiency and Safety: Phenol/Alcohol Fixing of AFB Smears

Jessica Gentry, Indiana State Department of Health

12:00 pm – 1:00 pm

Lunch (on your own)

1:00 pm – 2:30 pm • Highland I–III

Developments in TB Research

The session will provide three cutting-edge projects in TB research, including innovative diagnostics, treatment regimens and the new science of understanding drug resistance.

Moderator: Jafar Razeq, PhD, Dr. Katherine A. Kelley Public Health Laboratory,
Connecticut Department of Public Health

Development and Evaluation of New Diagnostic Tests for TB

Susan Dorman, MD, Johns Hopkins University, Department of Medicine

Pharmacokinetics in Pulmonary Lesions/MALDI-MS Imaging Studies of Drug Distributions

Brendan Prideaux, PhD, Rutgers University, Public Health Research Institute

Shortening Drug Regimens: New Agents for Combination Therapy

Eric Nuermberger, MD, Johns Hopkins University, Department of Medicine

2:30 pm – 3:00 pm • Grand Ballroom Prefunction

Afternoon Break in the Exhibit Hall

3:00 pm – 4:45 pm • Highland I–III

TB Case Studies: Lessons From the Field

In practice, there are situations in which the laboratory test results are not clear cut or are difficult to interpret, requiring persistence, good communication and investigative decision making. This session will present four examples of these challenging cases in an interactive format.

Moderators: Marie-Claire Rowlinson, PhD, D(ABMM),
Florida Bureau of Public Health Laboratories
Beverly Metchock, DrPH, D(ABMM),
Centers for Disease Control and Prevention

Discordance and Low-Level Resistance

Megan Ninneman, MMS, PA-C, Jackson Memorial Hospital
Beverly Metchock, DrPH, D(ABMM), Centers for Disease Control and Prevention

Mixed Infections

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

Use of Whole Genome Sequencing for Outbreak Investigation and Control

Edward P. Desmond, PhD, D(ABMM), California Department of Public Health,
Microbial Diseases Laboratory

Martin Cilnis, MPH, MS, Outbreak Prevention and Control Section, Tuberculosis
Control Branch, California Department of Public Health

Diagnosis of TB from Pathology Tissue Specimens

Julu Bhatnagar, PhD, Centers for Disease Control and Prevention, Infectious Disease
Pathology Branch

4:45 pm – 5:15 pm • Highland I–III

Conference Wrap Up and Closing Remarks

Marie-Claire Rowlinson, PhD, D(ABMM), Florida Bureau of Public Health
Laboratories

5:15 pm

Conference Conclusion

Visit the Exhibitors

Thank you to the exhibitors for their support of our conference!
Please visit them on Tuesday.

9:45 – 4:00 pm

Grand Ballroom Prefunction, Lower Lobby Level

Aeras

AiCure

Alpha-Tec System

Atlas Medical

Bruker Corporation

Cepheid

Consilience Software, A Xerox Company

Covaris

IMMY

LW Scientific

MIDI, Inc.

Oxford Immunotec, Inc.

QIAGEN

Thermo Fisher Scientific

WestPrime Healthcare

Poster Abstracts

Poster 1:

Improvement to a multiplex real-time PCR assay to detect IS6110-negative strains of *Mycobacterium tuberculosis* complex DNA in New York State

T. Halse, T. Quinlan, V. Escuyer, K. Musser, New York State Wadsworth Center, Albany, New York

Objective: To improve the existing multiplex real-time PCR assay that detects *Mycobacterium tuberculosis* complex (MTBC) and *Mycobacterium avium* complex (MAC), targeting IS6110 and 16S-23S ITS respectively, so that rare IS6110-negative strains are detected.

Study Design: The IS6110 is an insertion element that is found exclusively within the MTBC and has become an important diagnostic tool in the identification of MTBC. However, it is known that rare IS6110-negative strains of MTBC exist. In 2012 and 2013, two isolates that lack IS6110 were received at the Wadsworth Center. The testing of these isolates resulted in false negative real-time PCR results. One isolate was later identified as MTBC on day 11 by DNA sequence analysis and one isolate was later identified as MTBC on day 6 by MALDI-TOF MS analysis. To improve our detection of MTBC, a conserved target, ext-RD9, was added to the existing multiplex real-time PCR. A sensitivity comparison of the original MTBC-MAC assay and the new assay containing the ext-RD9 primers/probes was performed as well as a retrospective blinded comparison study on 80 primary specimens.

Results: We found no loss in sensitivity with the new assay containing the ext-RD9 primers and probe. As expected, 78/80 (98%) specimens were concordant, as the new assay detected both IS6110-negative isolates. Two additional isolates missing IS6110, received in 2014, were identified as MTBC on day 1 using the new assay.

Conclusions: We found the addition of the ext-RD9 detection improves the diagnostic capability of the multiplex real-time PCR assay by detecting IS6110-negative strains and will prevent false negative reporting.

Poster 2:

Evaluation of 10 Week Protocol

D. Sickels, B. Burns, T. Choudhry, T. King, P. Seitz, D. Galkowski M.D., NJ PHEAL, Ewing, New Jersey

Objective: Routine monitoring of cultures in the Mycobacteriology laboratory is six weeks. In 2013, a new protocol was introduced, derived from pg.489 of the 10th Edition of the “Manual of Clinical Microbiology” to screen smear positive and culture negative specimens for an additional 4 weeks. NJ PHAEL evaluated the “10 Week Protocol” to determine value and the reliability. Data evaluation included positive AFB cultures and 10 week negative cultures.

Study Design: From January 1, 2014 to December 31, 2014 six week smear positive and culture negative specimens were screened weekly for an additional 4 weeks. A transilluminator was used to manually screen MGIT tubes and a stereoscope was used for the 7H11plates. Kinyoun AFB staining was done to detect the presence of AFB, NON-AFB and/or mixed cultures. AFB positive MGIT tubes were worked up as were suspect colonies on 7H11plates. A preliminary report to notify clinics of the additional four week protocol and a 10 week final report were created.

Results: Of the 2180 specimens processed, 71 cultures (3.2%) were held for the 10 week protocol. Total percent of additional mycobacteria isolated was 0.6% with 0.5% identified as MTBC (two new patients). Of the 71 cultures held, 58 (81.7%) were reported as final negative. Of the 71 cultures held, 15 (21.1%) mycobacteria were isolated, 10 (14.0%) were identified as MTBC and 5 (7.0%) were NTM. Of the 15 mycobacteria isolated, 10 (66.6%) were MTBC and 5 (33.3%) were NTM.

Conclusions: Based on the number of mycobacteria isolated including two new MTBC patients detected, it was decided to retain the protocol. Holding smear positive and culture negative specimens for an additional 4 weeks, allows for the detection of slower growing mycobacteria.

Poster 3:

Combining Mycobacterium Testing Methods to Deliver Enhanced Laboratory Diagnostics and Clinical Patient Outcomes: A Proposed Algorithm Using MycoDDR and Xpert MTB-RIF

W. Candelaria, S. Namdarian, C. Magee, K. Maneclang, Clinical Laboratory, Maricopa Integrated Health System, Phoenix, Arizona

Objective: To decrease the time to definitive diagnosis utilizing the specimen processing system, IMMY MycoDDR and the Cepheid Xpert MTB-RIF nucleic acid amplification test (NAAT).

Study Design: Three sputum specimens were collected 8–24 hours apart and followed through the traditional algorithm, as well as, the newly proposed algorithm for clinical diagnosis from sputum sample processing until confirmed diagnosis. Comparison of the time to diagnosis, patient management and cost savings were used to determine which algorithm was more beneficial overall.

Results: Use of the MycoDDR for sample processing produced improved AFB smears and yielded positive cultures, on average, one day sooner than other specimen processing methods. The MycoDDR system along with the Xpert MTB-RIF resulted in confirmed identification of TB in 1–3 days. This is a significant improvement compared to the 16–18 days to confirmed identification of TB using culture confirmation and off-site NAAT. The decreased time to diagnosis can result in a significant estimated cost savings to the hospital (~\$33,600) and a substantial health savings to the patient.

Conclusions: The results of this study suggest that the new proposed algorithm utilizing the MycoDDR sample processing system and the Xpert MTB-RIF for TB diagnosis results in an increased positive patient outcome by providing positive diagnosis faster, which could result in a significant cost savings to the patient and treatment facility.

Poster 4:

Integrated Microfluidic Card with TaqMan Probes and High Resolution Melt Analysis to Detect Tuberculosis Drug Resistance Mutations across 10 Genes

S. Pholwat¹, J. Liu¹, S. Stroup¹, J. Gratz¹, S. Banu², S.M. M. Rahman², S. S. Ferdous², S. Foongladda³, D. Boonlert³, O. Ogarkov⁴, S. Zhdanova⁴, G. Kibiki⁵, S. Heysell¹, and E. Houpt¹

¹University of Virginia, ²International Center for Diarrheal Diseases and Research, Bangladesh, ³Mahidol University, Thailand, ⁴Russian Academy of Medical Sciences, Russia, ⁵Kilimanjaro Clinical Research Institute, Tanzania

Objective: To develop a microfluidic TaqMan array card that utilizes both sequence-specific probes and high resolution melt analysis (TAC-HRM) to detect TB drug resistance mutations.

Study Design: Forty seven targets were designed to interrogate critical regions of the *inhA*, *katG*, *rpoB*, *embB*, *rpsL*, *rrs*, *eis*, *gyrA*, *gyrB*, and *pncA* genes. The assay optimization of each target was performed on 384 well PCR format. The optimized assays were spotted into microfluidic card; DNA sample was mix with PCR reagent then load in to card and performed PCR.

Results: The method was evaluated on 230 clinical *M. tuberculosis* isolates from Bangladesh, Thailand, Russia, Tanzania and the US; yielded 96.1% (range 81%–99%) accuracy versus Sanger sequencing and 87% (range 72%–94%) accuracy versus the culture-based susceptibility results.

Conclusions: This TAC-HRM method yields a fast, comprehensive, and accurate drug susceptibility result for the 9 major antibiotics, and could be performed at public health laboratories without sequencing capabilities while awaiting MDDR and phenotypic DST.

Poster 5:

Validation of MALDI-TOF for identification of Mycobacteria

Donald Busalacchi, Julie Tans-Kersten and Dave Warshauer, Wisconsin State Laboratory of Hygiene, Madison, Wisconsin

Objective: Recent advancements in Matrix-assisted Laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) have permitted its application to the field of clinical microbiology. We validated the Bruker Biotyper system for identification of mycobacteria and integrated this method as a key component of our novel testing algorithm.

Study Design: The Bruker MycoEX protein extraction protocol was optimized through the addition of a secondary liquid media wash step, and the post-extraction viability of MTBC was determined. Variables known to affect the quality of MALDI identification scores including culture age and media type were evaluated. Most importantly, the ability of MALDI to identify acid-fast organisms directly from newly-positive MGIT broth culture was evaluated.

Results: We analyzed over 450 independently identified strains representing 40 species of mycobacteria and fully validated our testing algorithm for identification of *M. tuberculosis* complex, *M. avium* complex and 14 species of non-tuberculous mycobacteria. MALDI could identify pure growth of mycobacteria from 7H10 plate, 7H9 broth and LJ slant with little difference in score and no difference in identification. Approximately 6×10^7 organisms were needed for adequate identification, and MALDI scores were shown to decrease as cultures age. Direct MALDI identification from positive MGIT cultures inoculated with primary patient specimens proved difficult due to low numbers of cells and high background from patient inoculum proteins. Only 25% of mycobacteria-containing, instrument-positive MGIT tubes could be identified within one day of positivity.

Conclusions: Although Identification could not routinely be obtained from MGIT cultures, MALDI has the potential to yield quicker, less expensive, more automated, and more accurate results than previous identification systems if carefully integrated into an identification work flow.

Poster 6:

Correlation of *erm41* sequevar with the time to detection of inducible macrolide resistance in *M. abscessus* group organisms.

S. Christianson, W. Grierson, J. Wolfe, M. Sharma. Public Health Agency of Canada, Winnipeg, Manitoba

Objective: A T28C mutation in the *erm41* gene is associated with inducible macrolide resistance in *Mycobacterium abscessus* group organisms. A previous study by these authors showed that the majority of *M.abscessus* strains show inducible resistance by day 7, but some take 10–14 days. This study aimed to determine if there was a correlation between the length of time it takes to detect inducible macrolide resistance in *M.abscessus* group organisms and their *erm41* sequevar.

Study Design: We amplified and sequenced the *erm41* genes of a total of 81 *M.abscessus* group isolates. Sequevars were determined as previously described. The isolates were tested for phenotypic clarithromycin resistance using Trek Diagnostics Sensititre RAPMYCO microbroth dilution plates. The clarithromycin MIC was recorded at days 7, 10 and 14. The *erm41* gene sequences were correlated with time to detection of resistance.

Results/Conclusions: Of the 81 isolates tested, 15 had truncated *erm41* genes and 66 had the full length gene. Nine isolates with full length genes had a T28C mutation. All isolates with a truncated gene and 8/9 of isolates with T28C mutations were sensitive after a 14 day incubation. Of the remaining isolates, 53 had inducible resistance that was identified at 7 days and 4 had inducible resistance that was identified after 10 days. These 57 isolates fell into 12 sequevars. Three sequevars contained the 4 strains with 10 day detection times. Based on this data, there does not appear to be a correlation between *erm41* sequevar and time to the detection of inducible macrolide resistance.

Poster 7:

Analysis of *rpoB* Mutations with Variable Rifampin Results in the Model Performance Evaluation Program (MPEP)

C. Stafford, B. Metchock. Centers for Disease Control and Prevention, Atlanta, Georgia

Objective: Certain mutations in the *rpoB* gene of *Mycobacterium tuberculosis* complex (MTBC) result in borderline or low-level rifampin (RMP) resistance that increases the RMP minimum inhibitory concentration (MIC) above that of RMP-susceptible isolates lacking a detectable *rpoB* mutation. Growth-based drug susceptibility testing (DST) methods may not detect this low-level resistance. CDC's MPEP, which assesses laboratories' DST practices, evaluated the ability of participating laboratories to detect RMP resistance in isolates with these types of *rpoB* mutations.

Study Design: MPEP shipped three MTBC isolates with Asp516Tyr, His526Leu, and His526Asn *rpoB* mutations in the 2013 and 2014 biannual surveys. Self-reported DST results were analyzed in aggregate, by mutation, and by method (i.e., MGIT, agar proportion [AP], Sensititre, and VersaTrek).

Results: In aggregate, 18% (57/313) of growth-based DST results indicated resistance to RMP. Isolates with Asp516Tyr and His526Asn mutations had 2% and 0% of results reported as resistant, respectively. For the isolate with a His526Leu mutation, 56% of all results were reported as resistant; stratification by method showed 50% resistance for MGIT and 83% for AP.

Conclusions: Growth-based DST methods continue to produce variable RMP results for isolates with certain *rpoB* mutations. Further investigation is needed in terms of how DST results for isolates with these types of *rpoB* mutations shape treatment regimens and affect clinical outcomes.

Poster 8:

Development of an Individualized Quality Control Plan (IQCP) for MGIT Pyrazinamide (PZA) Drug Susceptibility Testing (DST)

D. Hartline, L. Diem, J. Spencer, B. Metchock. Centers for Disease Control and Prevention, Atlanta, Georgia

Objective: IQCP is a 3-step process incorporating risk assessment, quality control (QC) planning, and quality assessment (QA) monitoring. The process identifies the specific QC requirements for a particular test. Our laboratory developed an IQCP for MGIT PZA DST based on manufacturer's instructions—weekly QC (vs. each run) and only one control (susceptible to PZA). IQCP is expected to allow our laboratory to follow these QC practices and meet CLIA requirements.

Study Design: Laboratory operating and QC procedures were reviewed and a risk assessment performed to identify the potential to cause harm in five test process components (specimen, test system, reagents, environment, testing personnel). Pre-analytical, analytical and post-analytical processes were assessed. Established or proposed controls were documented, risks characterized by probability and severity of harm, and acceptability of residual risk determined.

Results: We identified >25 risks. Controls were already in place for the majority. No unacceptable residual risk was identified. A QC plan has been developed, along with QA monitoring to periodically review the QC plan for effectiveness.

Conclusions: We have created a customized QC plan, which supports performing the weekly QC for MGIT PZA DST and provides equivalent quality testing to meet the CLIA QC regulations.

Poster 9:

Detection of resistance to first-line anti-tuberculosis drugs: Correlation between molecular and growth-based antimicrobial susceptibility testing methods

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Objective: BPHL performs antimicrobial susceptibility testing (AST) of *Mycobacterium tuberculosis* complex (MTBC) by microtiter plate method. A major challenge with this method is longer turnaround times (TAT). To provide faster AST for first-line drugs, BPHL performs molecular methods which have much shorter TAT and in some cases can be performed directly on the specimen.

Study Design: DNA sequencing of the *pncA* gene was performed to determine mutations associated with resistance to pyrazinamide (PZA). Growth-based AST for PZA was performed at NJH by MGIT960 method for MTBC with *pncA* mutations. **GenoType MTBDR_{plus}** (Hain) test was performed to detect mutations associated with resistance to isoniazid (INH) and rifampin (RIF). Growth-based AST was performed by the TREK Sensititre® method. 960 specimens from first time TB-patients were analyzed.

Results: Results from 960 MTBC positive cultures were reviewed and analyzed. Overall, we observed a concordance of 98.8 % (948/960) for INH and RIF between the **Hain** test and Sensititre results. 46 non-synonymous *pncA* mutations were detected by sequencing and of these mutations 74% (34/46) were concordant, and found to be resistant, by growth-based testing methods.

Conclusions: Molecular testing is an important part of the algorithm for detecting resistance to the first-line drugs: RIF, INH and PZA. In combination with growth-based susceptibility testing, these methods provide valuable information to the clinicians in a timely manner.

Poster 10:

Ontario Universal Typing — Tuberculosis (OUT-TB) — To The Web and Beyond

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Objective: Tuberculosis (TB) control requires coordination of health system activities and resources. Case investigation and contact tracing activities generate data that is essential to TB surveillance and prevention, requiring significant information management.

Study Design: In 2008, the Public Health Ontario Laboratories implemented the OUT-TB program to monitor the spread of TB strains within Ontario. Engaging health-unit stakeholders, OUT-TB Web was developed using a relational database platform with GIS technology, combining information about TB cases and associated TB isolates, in a visual display and line-list format. OUT-TB Web, using secure internet access, allows authorized users to view TB genotyping matches and laboratory results within the context of relevant clinical and epidemiological data.

Results: OUT-TB Web is currently available to 8 public health units, accounting for >85% of all TB cases in the province, and is an essential tool for TB case-management. User group sessions and ad-hoc feedback from end-users has identified key features implemented in application enhancements, including an email alert function, customizable heat maps for visualizing TB and drug-resistant cases, socioeconomic map layers, a dashboard providing health unit specific and provincial TB surveillance metrics, and a time slider feature “animating” the geographic spread of strains over time. The latest application version now provides reporting of closely related matches.

Conclusions: An award-winning application, OUT-TB Web has proven to be a useful tool, with development and enhancements determined through user feedback. Planned future versions will include addition of other data sources, and development of a mobile app.

Poster 11:

Method for Improved Fluorescent Acid Fast Staining Using an Acetone Step and Bulk Staining

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Objective: To compare a bulk staining method employing an acetone step (remove background debris) to the traditional auramine rhodamine rack staining method.

Study Design: 1) Slides are routinely stained individually using racks over a sink; in order to incorporate the acetone step and to minimize the release of acetone vapour into the environment, the use of bulk staining in containers was assessed to determine the potential for cross-contamination of negative smears from positive smears. 2) Bulk staining was then performed with no acetone and with acetone, and the results compared to the conventional individual rack staining protocol for quality of the smear, time required to read, and accuracy.

Results: 1) Ten sets of smears combining a total of 120 heavy positives with 120 negatives were passed through the same staining baths in various configurations and all negatives remained negative. No cross contamination occurred during the bulk staining tests. 2) Method comparison by staff ranked acetone method over rack staining to be improved for quality, as well as accuracy (11% avium / 2% MTBC). Enumeration of true positive smears was consistently higher using the acetone method (15% for avium / 20% for MTBC). Time taken to read the smears was reduced by 11% per smear. No improvement was seen from bulk staining without acetone.

Conclusions: Based on the results, we incorporated this staining method into our routine work flow. In the first year of use, we saw a significant increase in the proportion of MTBC isolates that were smear positive, combined with a decrease in number of smear positives that did not grow.

Poster 12:

Nontuberculous Mycobacteria (NTM) Species Identified using *rpoB*/*hsp65* Gene Sequencing and *erm*(41) gene analysis

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Objective: Accurate identification of NTM is an ever evolving challenge. Newer assays allow the distinction between *M. abscessus*, *M. massiliense* and *M. bolletii*. Patients with *M. massiliense* respond more favorably to appropriate antimicrobial therapy and for this reason clinicians should request a final identification of organisms within the *M. abscessus* group.

Study Design: NJH performs identification on over 3,600 isolates annually using *rpoB* gene sequencing. Our current identification algorithm starts with *rpoB* gene sequence analysis, which reflexes to *erm*(41)/*hsp65* analysis for species identification within the *M. abscessus* group.

Results: More than 8,700 isolates were analyzed over 26 months using *rpoB* gene sequencing. Seven Mycobacterium species accounted for ~80% of all isolates tested: 24.4% *M. abscessus* group, 19.9% *M. avium*, 16.4% *M. intracellulare*, 6.0% *M. chimaera*, 5.1% *M. fortuitum*, 3.8% *M. gordonae*, and 3.7% *M. chelonae*. Over 2,000 clinical isolates were reflexed to *erm*(41)/*hsp65* analysis for species differentiation within the *M. abscessus* group. 18.4% resulted in a species within the *M. abscessus* group that was different from the identification returned by *rpoB* gene sequencing. 71.7% were identified as *M. abscessus*, 20.5% *M. massiliense* and 5.4% *M. bolletii*.

Conclusion: Reflex sequencing of *hsp65* and gel analysis of the *erm*(41) gene product is required for accurate identification to the species level.

Poster 13:

Improvements in Efficiency and Safety: Phenol/Alcohol Fixing of AFB Smears

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Objective: To decrease the testing time, improve the sensitivity, and decrease the risks associated with heat fixing smears of *Mycobacterium tuberculosis* complex

Study Design: Our Tuberculosis Laboratory prepared duplicate smears with 40 previously tested processed sputum specimens and 10 AFB cultures (five MGITs and five 7H11 plates). One set was fixed with the traditional heat fixing method on a slide warmer set at 65–75 ° C for two hours and a second set was fixed using 5% phenol in 70% ethanol for five minutes in the BSC. Both sets of slides were then removed from the BSC and stained using Auramine-O (sputa and MGITs) or Kinyoun (7H11). Slides were viewed under low power (200X) and counted under high power (500X). The microscopic results of both methods were compared.

Results: The phenol/alcohol fixed smear results were in 100% agreement with the heat fixed smear results, with slightly higher AFB counts, indicating increased sensitivity of the new method. The smear fixing time is significantly decreased, resulting in AFB results being reported to the submitter nearly two hours earlier in the day.

Conclusion: The new method has resulted in more sensitive and rapid detection of AFB. Reporting the results out earlier in the day allows the submitters to take action on the positive results the same business day. With the previous method, the results were reported so late in the day that this was usually not possible. Additionally, PCR extractions can be performed on AFB positive smears the same day, resulting in more rapid confirmation of MTBC. The chemical fixing of the smears in the BSC effectively kills any MTBC that is present, decreasing the risks to analysts.

Poster 14:

Evaluation of a Testing Algorithm Utilizing a Multiplexed PCR and Melt Curve Analysis Method

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Objective: To evaluate the performance of a relatively inexpensive, simple, multiplexed PCR method in the rapid identification of various *Mycobacterium* species as an adjunct to MALDI-TOF MS at the Arizona State Public Health Laboratory (ASPHL).

Study Design: Various isolates grown on solid or liquid media identified by MALDI-TOF, HPLC or 16SrDNA partial sequencing will be tested by the multiplexed PCR/melt curve analysis for comparison.

Results: A total of 72 isolates grown either from solid or liquid media were tested. Of the 72 isolates tested, 51 isolates were members of *Mycobacterium* genus representing 13 species frequently identified at ASPHL. The multiplex PCR method performed as expected accurately identifying species that method is capable of targeting in both liquid and solid media. The method showed high specificity as only one sample demonstrated cross-reactivity.

Conclusions: Though a more comprehensive study needs to be conducted, the data suggests that the relatively inexpensive PCR/melt curve method can be used reliably for the rapid identification of various *Mycobacterium* species (including MTBC) from both liquid and solid media. Currently, ASPHL identifies all isolates from solid media by MALDI-TOF but is dependent on HPLC identification from liquid media. As this method is still undergoing extensive evaluation, ASPHL is currently examining the feasibility of implementing this method as a replacement for HPLC in the current testing algorithm.

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