13th National Conference on Laboratory Aspects of Tuberculosis

June 12–13, 2023
Loews Atlanta Hotel

PROGRAM

Floor Plan	2
Schedule at a Glance	3
Welcome	5
Conference Agenda	7
Exhibitors	13
Posters	14
Attendee List	27

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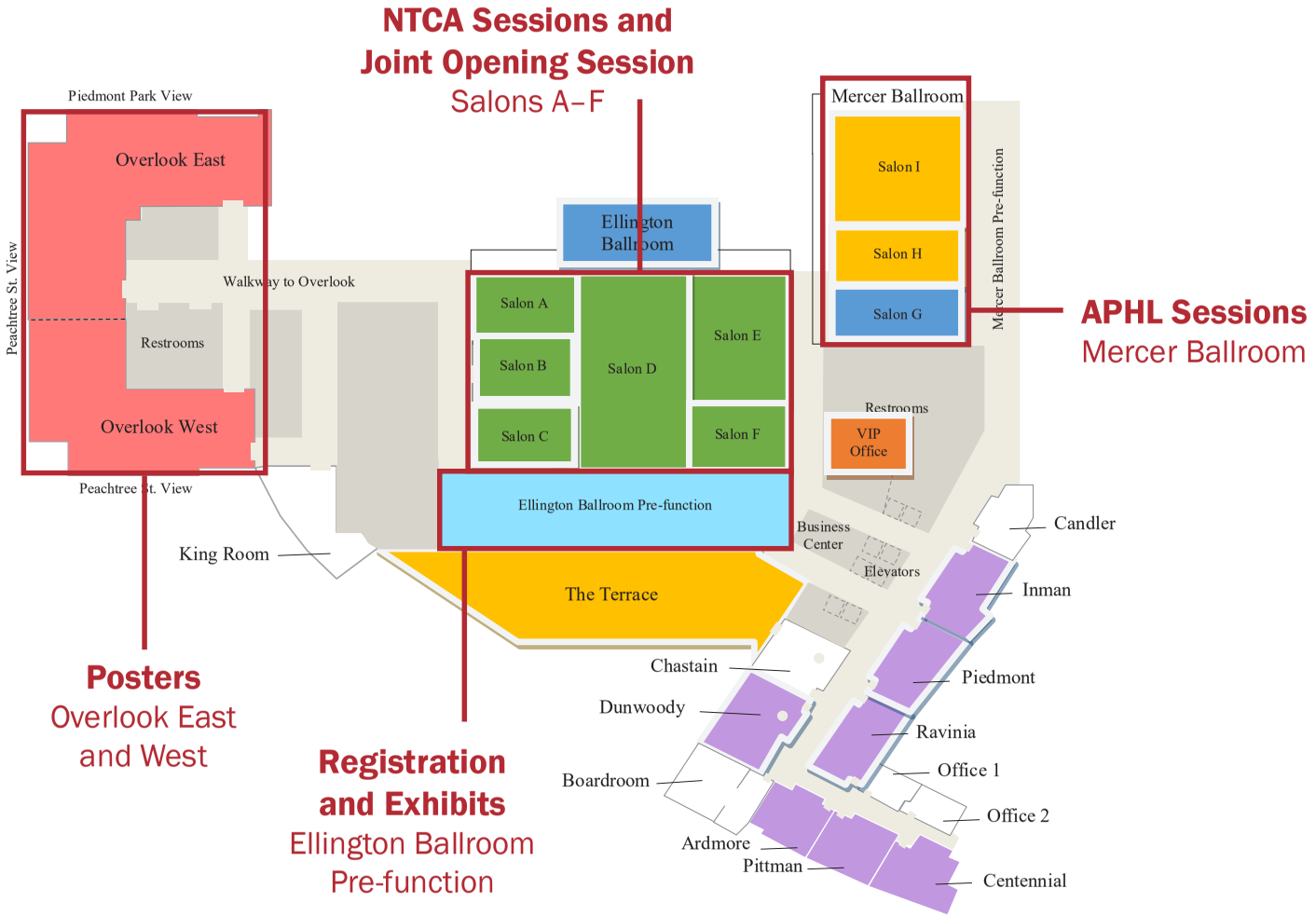
NTCA Sessions and Joint Opening Session
Salons A–F
Piedmont Park View
Overlook East
Overlook West
Restrooms
Peachtree St. View
Walkway to Overlook
Ellington Ballroom
Salon A
Salon B
Salon C
Salon D
Salon E
Salon F
Ellington Ballroom Pre-function
Mercer Ballroom
Salon I
Salon H
Salon G
Mercer Ballroom Pre-function
APHL Sessions
Mercer Ballroom
Restrooms
VIP Office
Business Center
Elevators
King Room
The Terrace
Candler
Inman
Piedmont
Ravinia
Office 1
Office 2
Centennial
Chastain
Dunwoody
Boardroom
Ardmore
Pittman
Posters
Overlook East and West
Registration and Exhibits
Ellington Ballroom Pre-function

AGENDA AT A GLANCE

Day 1 | Monday, June 12

7:00 am – 8:30 am	Poster Setup
7:00 am – 8:30 am	Breakfast (provided)
7:00 am – 5:30 pm	Registration
7:00 am – 7:00 pm	Exhibit Hall Open
8:30 am – 7:00 pm	Poster Viewing
8:00 am – 9:30 am	**Welcome and Keynote: Waiting on the World to Change**
9:30 am – 10:00 am	**Icebreaker: Getting to Know You, Getting to Know All About You**
10:00 am – 10:30 am	**Morning Break** (coffee and juice provided)
10:30 am – 12:00 pm	**Don't Stop IDin': Mycobacterial Identification Strategies**
12:00 pm – 1:00 pm	**Lunch in the Exhibit Hall** (box lunch provided by Cepheid)
1:00 pm – 1:30 pm	**Icebreaker: Shiny Happy People: Supporting Your Workforce**
1:30 pm – 3:00 pm	**Riffing on the Rifamycins**
3:00 pm – 3:30 pm	**Afternoon Break** (drinks and snacks provided)
3:30 pm – 4:30 pm	**Brand New: Novel Approaches Toward Identification and Characterization of an Old Pathogen**
4:30 pm– 5:00 pm	**Poster Flash Talks**
5:00 pm – 5:30 pm	**Break**
5:30 pm – 7:00 pm	**Opening Reception and Poster Session**

Day 2 | Tuesday, June 13

7:00 am – 8:00 am	Breakfast (provided)
7:00 am – 3:00 pm	Poster Viewing
7:00 am – 5:00 pm	Exhibit Hall Open
7:00 am – 5:30 pm	Registration
8:00 am – 10:00 am	**NTCA/APHL Joint Opening Session***
10:00 am – 10:30 am	**Morning Break** (coffee and juice provided)
10:30 am – 12:30 pm	**Should I Stay or Should I Go: Navigating the Landscape of tNGS for TB**
12:30 pm – 1:30 pm	**Lunch** (on your own)
1:30 pm – 2:15 pm	**Every Step You Take, I'll Be Watching You**
2:15 pm – 3:30 pm	**Stop, Collaborate and Listen**
3:30 pm – 4:00 pm	**I Am a Survivor: Surviving MDR TB**
4:00 pm – 5:00 pm	**Afternoon Break and Sponsor FlashTalks***
5:00 pm – 5:30 pm	**Awards and Closing Remarks**

* combined sessions with NTCA

Association of Public Health Laboratories

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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Welcome to the 13th National Conference on Laboratory Aspects of Tuberculosis!

We would like to personally welcome each of you to the 13th National Conference on the Laboratory Aspects of Tuberculosis, co-located with the 2023 National TB Conference. Over the past year, public health laboratories have encountered new challenges in what is now accepted as the endemic era of COVID-19. Profound changes have occurred in the TB arena, particularly regarding treatment with new drugs and new regimens. These advances generated new needs for the health professionals therefore new requirements for diagnostics. Laboratories also faced the discontinuation of some of the cornerstone assays for mycobacterial detection and identification and had to start to adapt by integrating novel technologies into their workflows. In the face of these changes, our dependence on strong partnerships is more important than ever. We developed an agenda that will offer opportunities to collaborate and network with our fellow TB clinicians and TB care and prevention program officials. This includes a combined poster session on Monday June 12, a joint opening session on Tuesday June 13, as well as coordinated break and lunch schedules. We strongly encourage you to take full advantage of these opportunities and embrace the concept of partnership at this meeting.

It is an exciting time for public health laboratories and we intend to offer important topics inspired by the 2023 National TB Conference's theme of Partnership, Innovation and Equity. Our conference will navigate the numerous ways in which public health laboratories have responded to the evolving TB landscape including mycobacterial identification and utilization of targeted, next-generation sequencing for prediction of *Mycobacterium tuberculosis* resistance to antimicrobials. We will also reflect on how understanding and usage of the rifamycins in TB treatment has evolved and learn about compelling data that will help us to improve testing of MTB susceptibility to these drugs for the future.

Throughout the conference, we will showcase the work of many of our public health laboratories through poster flash talks and case studies that offer critical insight into how laboratories play a central role in the clinical and public health response to TB. Recent developments in TB diagnostics and treatment will be presented and we will highlight how services offered by TB laboratories remain at the cutting edge. To facilitate interaction and focus on laboratories' greatest resource (the people!), we will hold a few short sessions for discussion of various topics, ranging from how to strengthen the laboratory workforce and uplift morale, to how to handle a regulatory inspection.

On behalf of the planning committee for the 13th National Conference, it is my great pleasure to present you with this exciting and interactive agenda. I truly appreciate the thoughts and countless hours of hard work that all the members of the program planning committee have given to lead us to this day. It was a pleasure and privilege to work with all of them.

Vincent Escuyer, PhD, PharmD
Chair, Conference Planning Committee

GENERAL INFORMATION

Registration Desk

Sunday, June 11	4:00 pm – 7:00 pm
Monday, June 12	7:00 am – 5:30 pm
Tuesday, June 13	7:00 am – 5:30 pm

Exhibit Hall and Poster Viewing

Poster/Exhibitor Setup:

Sunday, June 11	3:00 pm – 6:00 pm
Monday, June 12	7:00 am – 8:30 am

Exhibit Hall Hours:

Monday, June 12	7:00 am – 7:00 pm
Tuesday, June 13	7:00 am – 5:00 pm

Poster Viewing Hours:

Monday, June 12	8:30 am – 7:00 pm
Tuesday, June 13	7:00 am – 3:00 pm

Health and Safety Protocols

All attendees are strongly encouraged to be fully vaccinated and boosted against COVID-19 and to wear well-fitting, high-quality masks (e.g., KF94, KN95 or better) if desired.

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 11.25 contact hours by attending the entire conference. To receive P.A.C.E. credit, proof of attendance must be provided for 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.

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AGENDA

Sunday, June 11

APHL Registration:	4:00 pm –7:00 pm
Exhibitor and Poster Setup:	3:00 pm – 6:00 pm

Day 1 | Monday, June 12

Registration:	7:00 am – 5:30 pm
Poster Setup:	7:00 am – 8:30 am
Breakfast:	7:00 am – 8:30 am
Exhibit Hall Open:	7:00 am – 7:00 pm
Poster Viewing:	8:30 am – 7:00 pm

Welcome and Keynote: Waiting on the World to Change

8:00 am – 9:30 am • 588-859-23 • 1.5 contact hours

In the wake of the COVID-19 pandemic, a decidedly mixed picture of tuberculosis diagnosis and care has emerged. On the one hand, a new generation of diagnostic assays and regimens are becoming available. On the other, the consequences of decades of underinvestment and of the dependence on a small number of diagnostic providers are becoming ever clearer. This session will examine how to navigate the landscape of tuberculosis laboratory science and how to best use emerging technologies to inform TB care and prevention activities.

Moderator: Vincent Escuyer, PharmD, PhD, Director, Mycobacteriology Laboratory, David Axelrod Institute New York State Department of Health, Wadsworth Center

Speaker: Claudio Köser, BA MA PhD, Consultant, TB Alliance, Becton Dickinson, WHO Global TB Programme, WHO Regional Office for Europe and Foundation for Innovative New Diagnostics, Geneva

Icebreaker: Getting to Know You, Getting to Know All About You

9:30 am – 10:00 am

This conference has brought us all together to learn more about laboratory aspects of TB. Conferences also provide the opportunity to meet colleagues you may otherwise not and visit with familiar faces. This facilitated session will help you get to know your colleagues and broaden your network!

Moderators: Association of Public Health Laboratories and **DTBE's Laboratory Capacity Team**

Morning Break (coffee and juice provided)

10:00 am – 10:30 am

Don't Stop IDin': Mycobacterial Identification Strategies

10:30 am – 12:00 pm • 588-860-23 • 1.5 contact hours

Although the Hologic® AccuProbe® *Mycobacterium* culture identification products were discontinued in late 2022, technological advances have left laboratories with a variety of options to choose from when it comes to mycobacterial identification. In this session we will hear from four laboratories that have evaluated and/or validated alternative identification methods. Panelists will give an overview of the workflow and method(s) their laboratory utilizes, discuss their implementation strategies and address advantages and disadvantages of the methods used.

Moderator: Stephanie Johnston, MS, Lead, Laboratory Capacity Team, Centers for Disease Control and Prevention

Speakers:

- **Derek Armstrong**, MHS, Microbiologist/International Laboratory Coordinator, TB Research Center, Johns Hopkins University School of Medicine
- **Dorothy Baynham, MT(ASCP)**, Manager Special Microbiology, Division of Laboratory Services, Tennessee Department of Health
- **Kathleen Milloy, MS, MLS (ASCP)^{CM}**, Principal Scientist — Microbial Reference Group, Virginia Division of Consolidated Laboratory Services
- **Latricia Lewis, BS**, Mycobacteriology Supervisor, City of Houston Public Health Laboratory

Lunch in the Exhibit Hall (box lunch provided by Cepheid)

12:00 pm – 1:00 pm

Icebreaker: Shiny Happy People: Supporting Your Workforce

1:00 pm – 1:30 pm

In this guided session, participants will discuss in groups strategies for boosting morale and ideas for retention and recruitment of the public health workforce. Participants will be provided with resources and information developed by APHL members and staff and other participants and partners.

Moderators: Association of Public Health Laboratories and **DTBE's Laboratory Capacity Team**

Riffing on the Rifamycins

1:30 pm – 3:00 pm • 588-861-23 • 1.5 contact hours

Since rifampin was first used to treat pulmonary *Mycobacterium tuberculosis* (MTB) infection in the late 1960s, rifamycins have remained essential components of MTB treatment regimens. Our understanding of MTB resistance to rifamycins has continued to evolve with respect to both phenotypic and genotypic resistance, but much remains poorly defined. In this session participants will learn about two timely and relevant studies that aim to improve our understanding and usage of rifamycins for MTB treatment. First we will consider the revised rifamycin critical concentrations (CC) recommended by the WHO and examine the impact of reducing the CC further than the most recently recommended 0.5 mg/mL. Then we will learn about how the same MTB *rpoB* mutations impact resistance to rifamicin, rifabutin and rifapentine. This has important implications for laboratories that utilize rifamicin as a surrogate for other rifamyins in phenotypic studies.

Moderator: Calvin Lung, MSc, PHM, M(ASCP)^{CM}, Senior Public Health Microbiologist, Contra Costa Public Health Laboratory

Speakers:

- **Donna Kohlerschmidt, MT (ASCP)**, Research Scientist, David Axelrod Institute New York State Department of Health, Wadsworth Center
- **Wenming Zhu, PhD**, Microbiologist, Applied Research Team, Centers for Disease Control and Prevention
- **Glenn Morlock, MS**, Microbiologist, Applied Research Team, Centers for Disease Control and Prevention

Afternoon Break (drinks and snacks provided)

3:00 pm – 3:30 pm

Brand New: Novel Approaches Toward Identification and Characterization of an Old Pathogen

3:30 pm – 4:30 pm • 588-862-23 • 1.0 contact hour

Mycobacterium tuberculosis (MTB) has been known as a prominent pathogen for well over a century. Our understanding of the organism and the tools used to detect, characterize and treat MTB have continually evolved with time. In this session we will hear about new research aimed improving detection and prediction of MTB drug resistance. First, we will learn about an ultra-sensitive clustered regularly-interspaced short palindromic repeats (CRISPR) approach to detect MTB cell-free DNA in blood samples and a portable device to read point of care test results. Then we will learn about a study evaluating the potential role of *ahpC* in isoniazid resistance.

Moderator: Caitlin Miranda, M(ASCP), Microbiologist III, Oregon State Public Health Laboratory

Speakers:

- **John Christie, MBA**, Chief Operating Officer, Intelligenome, Inc.
- **Scott Burns, MS**, Biologist, Division of Tuberculosis Elimination — Applied Research Team, Centers for Disease Control and Prevention

Poster Flash Talks

4:30 pm – 5:00 pm

This session will feature "flash" presentations from each of our accepted poster presenters.

Moderator: Sarah Buss, PhD, D(ABMM), Association of Public Health Laboratories

Speakers: Poster Presenters (Posters 1–20)

Break

5:00 pm – 5:30 pm

Opening Reception and Poster Session

5:30 pm – 7:00 pm

This session will be held in the exhibit hall with all the posters to allow ample time for viewing of all posters that are accepted at the meeting.

Day 2 | Tuesday, June 13

Registration: 7:00 am – 5:30 pm
Exhibit Hall Open: 7:00 am – 5:00 pm
Poster Viewing: 7:00 am – 3:00 pm
Breakfast: 7:00 am – 8:00 am

Joint Opening Session

8:00 am – 10:00 am • 588-863-23 • 2.0 contact hours

The recent COVID-19 epidemic has exposed many challenges in diagnosing disease and delivering care in the communities where people who are sick live and work. From the work of Renee Dubois to Paul Farmer, we know that the social determinants of health are intricately tied to the risk of TB and disease outcomes. This talk will demonstrate how understanding and addressing the social determinants of TB are critical in the struggle to eliminate TB and are the foundation of equitable health care in communities.

Moderators:

- **NTCA: Joseph Burzynski, MD, MPH**, Assistant Commissioner and Director Bureau of TB Control, NYC Department of Health and Mental Hygiene
- **APHL: Vincent Escuyer, PhD, PharmaD**, Director, Mycobacteriology Laboratory, David Axelrod Institute New York State Department of Health, Wadsworth Center

Speakers:

- **NTCA: Salmann Keshavjee MD, PhD, ScM**, Director, Center for Global Health Delivery, Harvard Medical School
- **APHL: Cassandra Kelly Cirino PhD**, Vice President Disease Programs, FIND

Morning Break (coffee and juice provided)

10:00 am – 10:30 am

Should I Stay or Should I Go: Navigating the Landscape of tNGS for TB

10:30 am – 12:30 pm • 588-864-23 • 2.0 contact hours

Targeted next generation sequencing (tNGS) is a valuable tool in TB diagnostics, however it is not the right technology for every laboratory. In this session, panelists will give an overview of their TB diagnostics workflow, highlighting the ways in which they use tNGS technologies. We will hear from laboratories that utilize a variety of different tNGS protocols, including tNGS via referral. The panelists will discuss how their laboratories work to meet the unique needs of their jurisdiction using tNGS, how they evaluated the available methodologies, and the strengths and weaknesses of their approach.

Moderator: Varvara Kozyreva, PhD, PHM-CA(AAB), HCLD(ABB), Section Chief of Mycobacterial, Mycotic, and Parasitic Diseases, MDL, California Department of Public Health

Panelists:

- **Shannon Murphy PhD**, APHL/CDC Antimicrobial Resistance Post-doctoral Fellow, Wadsworth Center, New York State Department of Health
- **Atanaska Marinova-Petkova, DVM, MS, PhD**, Team Lead, Reference Laboratory, Laboratory Branch, Centers for Disease Control and Prevention
- **Toby Bennett, MS, MLS(ASCP)**, Supervising Clinical Laboratory Scientist, Special Pathogens & Biothreat Laboratory, Rhode Island Department of Health
- **Matthew D. Schimenti, MPH, M(ASCP)**, Medical Laboratory Scientist IV, Molecular Mycobacteriology Florida Department of Health

Lunch (on your own)
12:30 pm – 1:30 pm

Every Step You Take, I'll Be Watching You
1:30 pm – 2:15 pm

In this guided session, participants will discuss in groups their recent experiences with CLIA inspections. Specifically, we will focus the questions (and discussion) on the pre-analytical processes in your laboratory.

Moderators: Association of Public Health Laboratories and **DTBE's Laboratory Capacity Team**

Stop, Collaborate and Listen
2:15 pm – 3:30 pm • 588-865-23 • 1.25 contact hours

This session will feature three interesting case studies that highlight the importance of collaboration among Clinical and Public Health Laboratory partners and TB Care and Prevention Programs to successfully diagnose and treat TB disease.

Moderators:

- **Benjamin Alpers**, Application Scientist, Texas Department of State Health Services
- **Sarah Buss, PhD, D(ABMM)**, Association of Public Health Laboratories

Speakers:

- **Nikki Parrish PhD, MHS, D(ABMM)**, Director, Johns Hopkins Hospital Medical Mycobacteriology
- **Cherie Stafford, MSN/MPH**, Nurse Coordinator, Tuberculosis Control Program, Arizona Department of Health Services
- **Drew Francis, M(ASCP)^{CM}**, Arizona State Laboratory Services
- **Atanaska Marinova-Petkova, DVM, MS, PhD**, Team Lead, Reference Laboratory, Laboratory Branch, Centers for Disease Control and Prevention
- **Sarah Labuda, MD, MPH**, Medical Officer, Centers for Disease Control and Prevention

I Am a Survivor: Surviving MDR TB
3:30 pm – 4:00 pm • 588-866-23 • 0.5 contact hours

Preenie Gill will discuss her experience and journey with TB — from diagnosis to post treatment. Being diagnosed during the COVID-19 pandemic with MDR-TB, Preenie experienced unique challenges.

Moderator: Erin Estes, MBA, MLS(ASCP)^{CM}, Association of Public Health Laboratories

Speaker: Preenie Gill

Afternoon Break and Sponsor FlashTalks
4:00 pm – 5:00 pm

Awards and Closing Remarks

5:00 pm – 5:30 pm

We will recognize outstanding members of our community with the following three awards:

On the Front Lines of TB Testing Award

Honors a bench-level scientist from a state or local public health laboratory who has made significant contributions to the advancement of TB testing as part of public health laboratory science and/or practice.

NTCA Ed Desmond TB Laboratorian Award

The National Tuberculosis Controllers Association (NTCA) has annual awards, including the Ed Desmond TB Laboratorian Award, which honors exemplary service, dedication or leadership and is awarded to a TB laboratory professional. This year the award will be given at our meeting so that the recipient may be recognized amongst their peers.

David Warshauer TB Lifetime Achievement Award

Honors public health laboratory scientists that have dedicated their career to TB through the TB Lifetime Achievement Award.

Moderators:

- **Vincent Escuyer, PharmD, PhD**, Director, Mycobacteriology Laboratory, David Axelrod Institute New York State Department of Health, Wadsworth Center
- **Kimberlee Musser, PhD**, Clinical Laboratory Director, David Axelrod Institute, New York State Department of health, Wadsworth Center
- **Kelly Wroblewski, MPH, MT(ASCP)**, Director, Infectious Diseases, APHL

About the David Warshauer TB Lifetime Achievement Award

Historically, APHL has awarded the Tuberculosis Lifetime Achievement award during the National Conference on Laboratory Aspects of Tuberculosis to laboratorians who have made significant contributions to TB laboratory science and public health. This year, we have the distinct honor of announcing the winner of the first ever David Warshauer Tuberculosis Lifetime Achievement Award. The award will continue to be offered to an individual for their lifetime of distinguished service, leadership and excellence in the field of TB diagnostics and training. We feel that establishing this award in memory of Dr. David Warshauer is an appropriate way to honor Dave's legacy.

Dr. Dave Warshauer (left) receives the APHL Silver award from Dr. A. Christopher Whelen.

Dr. Warshauer served as the Chief Bacteriologist and Technical Director of the Communicable Disease Division for the Wisconsin State Laboratory of Hygiene (WSLH) from 2000 through his retirement in 2019. During his tenure at WSLH Dave received the APHL Silver Award (2017), the APHL TB Lifetime Achievement Award (2019) and the Ed Desmond Laboratorian Award (2014), given by the National TB Controllers Association for "exemplary service, dedication and leadership of a tuberculosis laboratory professional." His laboratory was named TB Elimination Champions by the Centers for Disease Control and Prevention's Division of TB Elimination (2016) "to recognize accomplishments and learn best practices from people who are making a significant contribution to preventing and controlling TB."

Dave was credited for building "the Wisconsin public health mycobacteriology laboratory into a superb laboratory, serving as a reference lab for many others, and acting as a hub for Wisconsin laboratory services, data, and education provided to private, clinical and other public health laboratories throughout the state." It was stated that Dave "encourages growth and expertise among his staff, and many of them also are nationally known in their areas of expertise". For many of us in the public health community, Dave was a dear colleague, teacher, mentor, friend or some combination of all four. We are grateful to have known and worked with Dave and are proud to rename this award in his honor.

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Rutgers Global Tuberculosis Institute
Mayo Clinic Center for Tuberculosis
Southeastern National TB Center
Stop TB USA

POSTER ABSTRACTS

Poster 1

Rifampin Mono-resistant Tuberculosis in NYC, 2010–2021

JA Lindsey, AV Easton, H Modestil, J Burzynski, FF Dworkin, DN Nilsen | New York City Department of Health and Mental Hygiene, New York City, USA

Background: Identification and appropriate treatment of rifampin (RIF) mono-resistant tuberculosis (RR-TB) is necessary to avoid the development of multidrug-resistant TB.

Design/Methods: Patients with RR-TB in New York City (NYC) between 2010 and 2021 were identified using NYC's TB registry. RR-TB was defined based on molecular or phenotypic drug susceptibility test (mDST/pDST) results. Most susceptibility testing was conducted at the New York State Wadsworth Laboratory, which began conducting whole genome sequencing on all TB isolates in 2016.

Results: Of 7,097 TB cases reported during 2010-2021, 31 (<1%) were RR-TB. Among 27 cases with available susceptibility data, 15 (56%) were RIF-resistant by both mDST and pDST; 7 (26%) were RIF-sensitive by pDST but RIF-resistant by mDST; 4 (15%) were RIF resistant by mDST and pDST was not done; and 1 (4%) was RIF resistant by pDST and mDST was not done. Thus, 11 (7+4) RR-TB cases were identified based on mDST alone. Eight of the 27 (30%) RR-TB cases with available susceptibility data were diagnosed during 2010-2015; the other 19 (including 9 of the 11 diagnosed by mDST alone) were diagnosed during 2016-2021. Of the 31 patients with RR-TB, 18 (58%) have completed treatment, with an average treatment duration of 17 months. There was no significant change in treatment duration between 2010 and 2021.

Conclusions: mDST identified cases of RR-TB that would not have been recognized based on pDST results alone. Unfortunately, patients with RR-TB experienced long treatment duration throughout the study period. New short-course regimens will hopefully reduce treatment times for these patients.

Poster 2

Incremental Yield of Sputum Samples for Diagnosis of Infectious Pulmonary Tuberculosis Among Immigrants and Refugees Admitted to the United States from the Electronic Disease Notification System, January 2017 – October 2022

GS Lamb, K Hailu, SR Toney, C Phares, and K Skrobarcek | Immigrant, Refugee, and Migrant Health Branch, Division of Global Migration and Quarantine, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Background: The World Health Organization recommends two sputum samples for the diagnosis of pulmonary tuberculosis (TB); however, the mandatory overseas TB screening for United States (U.S.)-bound immigrants and refugees requires three sputum samples for applicants with clinical or radiologic evidence of TB disease or known HIV infection. We evaluated the incremental yield of a third sputum sample for diagnosis of infectious pulmonary TB during the overseas medical examination.

Methods: We identified a subset of refugees and immigrants that arrived in the U.S. during January 1, 2017–October 17, 2022 who had at least one positive culture for Mycobacterium tuberculosis from a set of three cultures during the overseas examination. We calculated the incremental yield of cultures.

Results: In total, 1,984 persons met our inclusion criteria, including 261 (13.2%) refugees and 1723(86.8%) immigrants. Median age was 46 years (IQR 31-60 years), and 8 (0.4%) had known HIV infection. Among individuals with positive cultures, 276 (13.9%) had growth on only the first culture, 1,739 (87.7%) had growth on the first and/or second culture, and 245 (12.3%) were positive on only the third culture.

Conclusions: Among U.S.-bound refugees and immigrants with positive cultures, most positive cultures were identified on the first or second sputum sample. Approximately 250 cases would have been missed if the third culture was not performed. Among those with positive cultures, performing a third culture increased the diagnostic yield by 12.3%. Additional evaluation is needed before recommendations for updating the overseas TB screening for immigrants and refugees can be made.

Poster 3

Characterization of Pathogen-host Interaction in Mtb-derived Extracellular Vesicles

Li Yang, Qingbo Shu, Wenshu Zheng, Lin Li, Julian G. Saliba, Christopher J Lyon, Tony Hu | Center for Cellular and Molecular Diagnostics, Tulane University School of Medicine, New Orleans, LA / Department of Biochemistry and Molecular Biology, Tulane University School of Medicine, New Orleans, LA

Background: Extracellular vesicles (EVs) can transfer factors that play important roles in immune function and disease processes, and can thus serve as diagnostic biomarkers, candidate therapeutic targets, and indicators of effective treatment responses. We therefore hypothesized that circulating EVs of patients with tuberculosis (TB), a leading cause of death from infectious disease, could identify host and pathogen-derived factors associated with *Mycobacterium tuberculosis* (Mtb) infection, latency, reactivation, and escape from the host immune system.

Methods: We analyzed EVs isolated from THP-1 macrophages before and after infection with Mtb H37Rv to model the infection process leading to TB, and evaluate whether these EVs carried host and pathogen derived factors associated in Mtb infection status that could be linked to specific host and Mtb processes. Peptides derived from these EVs were processed using a modified single-pot, solid-phase-enhanced sample preparation method and analyzed by mass spectrometry (MS) for bottom-up proteomics using a data-independent acquisition (DIA) and data-dependent acquisition (DDA) method, and Spectronaut to identify specific peptides from the MS spectra.

Results: MS data from the infected and uninfected macrophage EVs identified similar numbers of proteins (4000-4500) in both these groups. Bioinformatics identified eight Mtb-derived EV membrane proteins associated with response to hypoxia (hspX, groEL2, tuf, and Rv2623), energy metabolism (atpD and atpG), virulence (cfp29), and intracellular growth (pspA). Bioinformatics also identified 173 proteins that were differentially expressed (68.2% were up-regulated) in infected versus uninfected macrophage EV samples, including ferritin light chain, IL-27B, RBM12, and other proteins related to host antiviral and antibacterial responses.

This analysis also detected membrane proteins that were upregulated (TNFAIP2, LY75, EHD1, and TFG) or downregulated after (SORL1, CD36, SCRAB1, and CD68) Mtb infection. Notably, the former proteins are associated with Mtb recognition, phagosome maturation, and innate immunity, while the latter are associated with phagosome delivery and granuloma formation to limit Mtb growth or act as a receptor for an Mtb virulence factor.

Conclusions: This data suggests that analysis of membrane proteins on EVs derived from individuals with suspected TB could rapidly provide important diagnostic and prognostic information, including the potential to permit early TB diagnosis (incipient and/or subclinical TB), Mtb-HIV co-infection, and treatment monitoring.

Poster 4

Determination of MICs for Rifampin (RIF), Rifabutin (RFB) and Rifapentine (RFP) in *Mycobacterium tuberculosis* Strains Possessing Various Missense Mutations in rpoB

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Background: Resistance to the rifamycins RIF, RFB and RFP results from mutations in rpoB. Cross-resistance among these drugs in *M. tuberculosis* strains remains poorly defined. The objective of this study was to correlate rifamycin MICs with specific rpoB mutations.

Method: MIC testing was performed according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) method. Isolates were selected based on the presence of missense mutations in the rpoB rifampicin resistance determining region (RRDR). Seventy-seven isolates representing 22 distinct rpoB missense mutations, 18 wild-type isolates and the susceptible reference strain H37Rv were tested.

Results: For H37Rv, MICs for RIF, RFB and RFP ranged from 0.125 – 0.25 µg/ml, 0.0039 – 0.0156 µg/ml, and 0.0156 – 0.0313 µg/ml, respectively. MICs for 18 isolates with wild-type rpoB ranged from 0.0313 – 0.5 µg/ml for RIF, 0.0078 – 0.125 µg/ml for RFB and 0.0078 – 0.125 µg/ml for RFP. Twenty-seven isolates, representing eight rpoB missense mutations, including those coding for Asp435Ala and Asp435Gly, had a RFB MIC of 0.5 µg/ml, similar to wild-type strains, but had high MICs for RIF (MIC ≥32 µg/ml) and RFP (MIC ≥8 µg/ml). Twenty-four isolates representing 11 different rpoB point mutations displayed similar MICs demonstrating complete cross-resistance. The remaining 8 isolates inclusive of three unique missense mutations had inconclusive RIF MIC results.

Conclusion: These results indicate certain rpoB mutations do not confer the same level of resistance to RIF, RFB and RFP. Eight rpoB mutations (27 isolates) evaluated had RFB MICs similar to wild-type isolates. These results may inform the treatment of patients with rifamycins.

Poster 5

Comparison of Molecular and Culture-based Drug-susceptibility Testing for Tuberculosis Cases in Minnesota 2016–2021

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Background: This study aims to assess the accuracy and timeliness of molecular drug-susceptibility testing compared to the gold-standard culture-based method for detecting drug-resistant tuberculosis (TB) for the four first-line TB drugs and its impact on patient treatment. Previous analyses of comparability have been published by laboratories and do not reflect the TB program experience of receiving information from multiple laboratories using a variety of instruments and implementing them in clinical decision-making. This analysis quantifies the importance of access to reliable and rapid drug susceptibility testing using TB program data.

Methods: This cross-sectional study includes 221 verified tuberculosis patients in Minnesota that had molecular drug susceptibility testing performed between 2016-2021. Sensitivity, specificity, positive predictive values, and negative predictive values were calculated for molecular drug susceptibility tests, using culture-based drug susceptibility results as the gold standard. Paired T-tests were used to compare differences in result turnaround in culture-based and molecular-based drug susceptibility tests and differences in inadequate treatment durations or treatment delays.

Results: The results of molecular drug-susceptibility testing were comparable to culture-based drug susceptibility testing with high sensitivity, specificity, positive predictive values, and negative predictive values for rifampin and isoniazid resistance. Ethambutol and pyrazinamide resistance have more variable sensitivity, specificity, positive predictive values, and negative predictive values. Molecular drug-susceptibility testing resulted in a mean 7-10-day shorter time from specimen collection to results compared to other methods. The use of molecular tests was associated with a mean 7-day shorter duration of inappropriate drug treatments when cases exhibited resistance to rifampin or isoniazid and was also associated with a mean 20-day reduction in waiting time for treatment initiation in cases where treatment start dates followed the availability of molecular drug-susceptibility results.

Conclusions: Molecular drug susceptibility testing is comparable to culture-based tests at detecting rifampin and isoniazid resistance. Rapid molecular tests' association with shorter time to initiation of adequate TB treatment supports the need for widespread programmatic access to rapid molecular testing. Based on this analysis, state public health labs should consider investing in tools for rapid detection of rifampin resistance, particularly considering CDC TB Lab's February 2023 discontinuation of pyrosequencing on NAAT+ sediments.

Poster 6

Public Health and *M. tuberculosis* Complex Nucleic Acid Amplification (Naa) Testing: Finding A Balance

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Background: In June 2021 the State of Arizona Tuberculosis Program (AZ-TB) launched a 12-month NAA testing collaborative with the State Public Health Lab (AZ-Lab). AFB smear(-) clinical sputa, not normally triggering NAA reflux, would be assessed for possible NAA testing. A goal was to determine the right balance of testing based on resources and patient impact.

Intervention/Response: AZ-TB evaluated whether broader NAA testing, alongside the existing reflux after AFB sputum smear(+) result, would promote earlier treatment initiation. Inclusion criteria were: 1-public health submitters without in-house NAA capabilities; 2-clinical sputum sample; 3-AFB smear(-); and 4-no prior NAA or culture(+) within the last 365-days. AZ-TB reviewed samples daily and notified AZ-Lab which sample(s) to include.

Results: During the 12-month process, 214 samples met inclusion criteria, resulting in an increase of 12.5 NAAs per month. Four samples were AFB smear(-) and NAA(+), accounting for <2% of all NAAs. Of those four, treatment started 1-day prior to NAA result for one patient and 3, 7, and 23-days after NAA result for the remaining three patients.

Conclusions: Highlights of the testing collaborative included exploring a deeper connection with laboratory data, automating morning emails for eligible samples to review, an expansion to selected correctional partners, and reenforcing the lab/program partnership. It's unclear if universal NAA testing on sputum smear(-) samples impacts treatment initiation and public health interventions among patients already followed by public health. The NAA testing collaborative continues and will be adapted based on ongoing analysis and building upon lessons learned.

Poster 7

Determination of Minimal Inhibitory Concentrations (MICs) of Drug-resistant and Susceptible *Mycobacterium tuberculosis* isolates to the novel antibiotic Teixobactin

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Background: The effective treatment of drug-resistant tuberculosis not only benefits the patient but also helps control the emergence and dissemination of these strains. The recently described Teixobactin (TXB) represents a new class of antibiotics with antimicrobial activity against gram positive bacteria. We report MICs for TXB against susceptible and drug-resistant *M. tuberculosis* strains.

Design/Methods: A microdilution broth assay consisting of a two-fold dilution series of TXB concentrations ranging from 0.0156 – 8.0 mg/L was used to determine MICs to TXB in the presence or absence of 0.025% Tween 80. Four susceptible, 6 multidrug-resistant (MDR), 3 pre-extensively drug-resistant (XDR) and 3 XDR clinical *M. tuberculosis* isolates, as well as the fully susceptible laboratory strain H37Rv, were tested.

Results: MICs were impacted by the presence of Tween 80 in the culture medium. In the absence of Tween 80, MICs were > 8 mg/L for all strains tested. In medium containing Tween 80, the MIC range for strain H37Rv was 1.0 – 2.0 mg/L and among the clinical isolates the MIC ranges were ≤ 0.0156 – 0.5 mg/L, ≤ 0.0156 – 1 mg/L, and ≤ 0.0156 – 0.0625 mg/L for susceptible strains, MDR strains and pre-XDR and XDR strains respectively.

Conclusion: The biochemical properties of TXB requires medium containing Tween 80 for MIC testing. TXB MICs for drug-resistant strains were similar to those of susceptible strains. Our results indicate that TXB is a promising investigational drug for the treatment of drug-resistant tuberculosis.

Poster 8

Establishment of Whole Genome Sequencing to Predict Resistant Strains of *Mycobacterium tuberculosis*

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Background: Diagnostic delay is defined as the time interval between the onset of symptoms and confirmed diagnosis of a disease. Current diagnostic tools for *Mycobacterium tuberculosis* (Mtb) limit the scope of detecting patterns due to this delay. The goal of this project is to develop the detection of Mtb outbreaks including patterns of Rifampin-resistant Mtb and other local strains present in the population utilizing whole genome sequencing (WGS).

Methods: This project has been simplified to six steps: identify, culture, extract, sequence, analyze, and educate. New Mtb patients will be identified through standard mycobacteria isolation protocols for further characterization. These specimens are cultured through BACTEC MGIT 960® to provide ample template for extraction and downstream applications. Positive cultures are heat-killed and tested for viability. Relevant MTb complex (MtbC) nucleic acid is extracted from a primary BACTEC MGIT 960+ culture for the purposes of whole genome sequencing. MTbC DNA is prepped from standardized protocols for WGS organisms using the Illumina DNA Flex library preparation kit then subsequently sequenced on an Illumina platform, thus ensuring inter-laboratory comparability of sequencing results. Isolates can then be clustered which may elucidate epidemiologically relevant data.

Results: Any epidemiologic relationships identified with this process will be interpreted and informative of cases and potential outbreaks. This interpretation will then be compiled into a format that is understood for health officials and other medical professionals to utilize to educate the general public that may be most affected by these trends.

Poster 9

Development and Validation of a Multiplex Real-time PCR Assay to Identify New York State's Most Commonly Encountered Non-Tuberculous *Mycobacteria* (NTM) in Clinical Specimens

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Background: Non-tuberculous *Mycobacteria* (NTM) are *mycobacteria* other than *Mycobacterium tuberculosis* complex (MTBC) and *Mycobacterium leprae*. They are found in the environment and can cause infections in humans. NTM infections are emerging globally and can outnumber MTBC infections in some developed countries. The Mycobacteriology Laboratory at the New York State (NYS) Wadsworth Center (WC) already has in place molecular assays that detect MTBC and the two most common NTMs in NYS: *Mycobacterium avium* complex (MAC) and *Mycobacterium abscessus* (MAB). Recently, Hologic announced the discontinuation of the Accuprobe *Mycobacterium kansasii* test used by many clinical microbiology laboratories, creating the need for alternative(s) to detect this organism. Additionally, retrospective data analysis showed that *Mycobacterium chelonae*, *Mycobacterium fortuitum, Mycobacterium kansasii*, and *Mycobacterium xenopi* are the next most identified NTMs after MAC and MAB in NYS. Therefore, we decided to develop a multiplex real-time PCR

assay to detect these NTMs in cultured material by targeting the genes ITS in *M. chelonae*, erm39 in *M. fortuitum*, 32kDa protein in *M. kansasii*, and gyrA in *M. xenopi*.

Results: A panel of 186 isolates including reference strains and clinical isolates was used to assess specificity. This panel included 84 distinct *Mycobacterium* species, bacteria from the normal lung/skin flora, and organisms other than *mycobacteria* causing respiratory, skin and soft tissue infections. All samples containing *M. chelonae, M. fortuitum, M. kansasii*, and *M. xenopi* were identified by the assay. For 48 of 50 (98%) of positive samples, cycle threshold (Ct) value was <30. Cross-reactivity was observed with *Mycobacterium franklinii, Mycobacterium mageritense, Mycobacterium heckeshornense* and *Mycobacterium stephanolepidis*. However, Ct value was >30 when this occurred. Inter- and intra-assay studies showed that the assay is reproducible. A prospective study tested 157 clinical samples with the NTM real-time PCR assay in parallel with the assays currently used in our testing algorithm. Results agreed for 153 of 157 samples (97.5%).

Conclusion: This assay is accurate, reproducible and will rapidly identify four of the most common pathogenic NTMs encountered in NYS. Its implementation will significantly reduce the workload of MALDI-TOF MS and Sanger sequencing, while dramatically decreasing turnaround time.

Poster 10

Continuing Public Health Laboratory Operations Through Challenging Circumstances — A Continuity of Operations Plan Toolkit for Public Health Mycobacteriology Laboratories

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Background: Public health laboratories (PHL) can experience situations that disrupt testing including pandemics, natural disasters, and facility/maintenance issues. These interruption of service events (ISEs) may result in a temporary inability to use equipment or laboratory space, compromise staffing and infrastructure, and may require extensive disinfection and decontamination. A continuity of operations plan (COOP) allows for development of specific approaches and processes to minimize interruptions to laboratory testing. PHL COOPs may not include specific individual laboratory departments such as mycobacteriology, and staff may or may not be aware of or have access to the COOP.

Methods: To determine the types of ISEs experienced in mycobacteriology laboratories and availability of COOPs, we distributed a CDC OMB-approved RedCap questionnaire to the 58 CDC Tuberculosis (TB) Elimination and Laboratory Cooperative Agreement public health laboratory awardees, downloaded and analyzed survey responses, and created 13 focus groups based on responses. Focus group participants discussed how their mycobacteriology laboratory prepared for and responded to recent ISEs, described gaps and lessons learned, and discussed COOP availability. Survey and focus group information was summarized to create a Continuity of Operations Plan Toolkit for Public Health Mycobacteriology Laboratories.

Results: The toolkit focuses specifically on the importance of continuity of testing services for the diagnosis of TB and provides various templates and checklists that can be adapted to meet specific local needs. Also included are ISEs that have or may affect PHL testing services that should be considered in planning as well as guidance for referral laboratory identification. Other items addressed include necessary actions for advanced preparation of ISEs, when a mycobacteriology laboratory COOP is activated, and when it is inactivated.

Conclusion: With our findings and development of this toolkit, PHL are encouraged to develop or update their COOP and to identify a referral laboratory for mycobacteriology testing. The toolkit contains information for creating, modifying, or implementing a mycobacteriology COOP, including considerations for leveraging partnerships that will help public health mycobacteriology laboratories be better prepared for a future ISE.

Poster 12

Incorporating Molecular Drug Susceptibility Test Results into Drug Resistant Tuberculosis Definitions for U.S. Surveillance

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Cynthia L Adams | Peraton

Background: Before the advent of molecular drug susceptibility testing (mDST), tuberculosis (TB) drug susceptibility or resistance was defined in the US National TB Surveillance System (NTSS) exclusively based on growth-based DST results. Updates to the TB surveillance data collection tool, Report of Verified Case of TB (RVCT), were implemented during 2020–2023 to allow for mDST result reporting. This change necessitates new surveillance procedures and definitions to ensure drug resistant TB cases are clearly and accurately described based on current knowledge and laboratory testing methods.

Methods: In 2019, the Centers for Disease and Control Prevention's Division of TB Elimination formed a workgroup comprised of surveillance, data management, medical, and laboratory professionals to address questions about the interpretation of mDST data for TB surveillance. The main questions facing the group: how to combine results from multiple specimens and testing modalities to arrive at a complete resistance pattern for a patient; how to evaluate novel gene mutations; and how to handle data from manual data entry, data imports, and electronic lab reporting. The group systematically addressed these and other questions to make decisions about the interpretation of mDST data for surveillance purposes. The workgroup convened again in 2022 to finalize and implement decisions.

Results: Molecular DST, both sequencing and non-sequencing based, is now readily available in the United States. In at least one state laboratory, it has already replaced some growth-based DST. The workgroup decided to expand the drug resistant TB surveillance definition to include mDST results. Initial resistance of *M. tuberculosis* identified from either growth-based or mDST from any specimen site is now considered categorically "resistant". Algorithms to determine resistance using mDST for surveillance are initially based on the presence of high-confidence mutations in genetic loci known to confer resistance to INH and RIF. A new category, "presumed resistant" was created to describe cases in which only non-sequenced rpoB data, without confirmatory sequence data, is available.

Conclusions: Establishing a framework for mDST interpretation facilitates the incorporation of newly reported molecular data into national surveillance reporting of drug-resistant TB cases from 2023 forward.

Poster 13

Portable, Battery-operated, Lab-in-tube Detection of TB Disease and Drug-resistance

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Background: Tuberculosis (TB) remains a leading cause of global mortality, and despite the urgent need for accurate and reliable case identification, 4.2 million (39.6%) of active TB cases were missed in 2021 and only 57% of diagnosed cases are confirmed by gold-standard TB tests. Rapid and reliable assays are needed at the point-of-care (POC) to accomplish TB diagnosis at sites of patient care and sample collection in resource-limited settings without the need for sample transportation, specialized personnel, and expensive, bulky equipment.

Methods: Here, we develop a Lab-in-Tube TB (LIT-TB) diagnostic device: a low-cost, ultraportable, battery-operated device that mediates CRISPR-based TB diagnosis and drug-resistance detection in a single sputum collection tube. The lyophilized detection reagents do not require cold-chain storage or transportation and the smartphone readout allows for automated fluorescence intensity analysis and diagnostic result reporting. The complete protocol is performed in a single-tube, closed-system protecting users from TB contamination without the need for external power supply.

Results: The lab-in-tube and battery-powered device can diagnose TB infection and detect drug resistant mutations from patient sputum samples following a protocol than can be performed without highly skilled technicians. Utilizing DNA isolation-free approach, the LIT-TB system displayed 89% sensitivity and 100% specificity in patient sputum samples. With specific design of CRISPR gRNAs to detect mutations, rifampicin-resistant mutation S450L of the rpoB gene is accomplished to inform clinicians on treatment choice.

Conclusions: The LIT-TB device is a lightweight, field-deployable microincubator and smartphone imager, that can be transported to sites of sample collection and performed by individuals with minimal training at the point-of-care. We believe the enhanced portability and inexpensive equipment will improve accessibility to TB healthcare in developing countries.

Poster 14

Multicenter Evaluation and Validation of a Customized MIC Panel for Antimicrobial Susceptibility Testing for *Mycobacterium tuberculosis*

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Objective: A multi-center study to validate a customized minimum inhibitory concentration (MIC) method for phenotypic antimicrobial susceptibility testing (AST) in *Mycobacterium tuberculosis* complex (MTBC).

Study Design: Validation of a customized MIC panel was conducted at two sites, the State of Florida Bureau of Public Health Laboratories (BPHL) and the New York State Wadsworth Center (WC). The customized MIC plate was developed in collaboration with the Florida TB Physician's Network to include drugs that recently became important in the treatment of tuberculosis (TB), including bedaquiline, linezolid and clofazimine. Twelve drugs were included on the 96-well plate, manufactured by Thermo Scientific. A standardized protocol was developed, including specifications about plate inoculation, incubation, reading time (days 10, 14 and 21), and results interpretation. Each site was allocated 51 plates to test a panel of pre-characterized isolates from BPHL and WC cases, except two linezolid resistant strains that were purchased.

Results: Isolates tested included pan-susceptible strains of varying lineages, multidrug resistant (MDR), extensively drug-resistant (XDR) and strains with various resistance patterns including resistance to bedaquiline and clofazimine, heteroresistance to fluoroquinolones and low-level rifampin resistance. Since no comparator method was available for several drugs on the panel and clinical concentration or breakpoints had not yet been established in the US, World Health Organization guidelines were followed where applicable. Results were compared to prior phenotypic results obtained with other methods and genotypic results (whole genome sequencing). Concordance was high, with strains predicted resistant by WGS showing 98% agreement with MIC results. A notable increase in MIC was observed for both BDQ and RIF at day 21 for strains carrying mutations predicting low-level resistance that was not previously detected using other phenotypic methods.

Conclusions: MIC testing is a valuable method for AST of MTBC, however there are challenges in validation and implementation especially in a setting with low incidence of drug resistance. Nevertheless, in light of new drugs and new drug regimens for drug-resistant TB, e.g., BPaL, and a move toward all oral regimens for treatment, this testing will become more crucial to ensuring that MDR and XDR TB patients receive the appropriate treatment regimens.

Poster 15

Sensitivity of Interferon Gamma Release Assay in Culture or Biopsy Confirmed Tuberculosis Patients

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Introduction: Interferon Gamma Release Assays (IGRA) can be used to aid the diagnosis of tuberculosis (TB) infection when used in conjunction with clinical presentation, risk assessment and diagnostic evaluations such as radiography, culture and biopsy results. The sensitivity of IGRA is reported between 65% to 92% in various studies but many of the published studies had few patients only, also included patients with suspected TB or used interpretation criteria different from currently approved by FDA.

Montgomery County had the highest number of TB cases in Maryland from 2016 to 2020. The QuantiFERON-TB Gold In-Tube (QFT) was routinely performed for patients with suspected TB. We assessed the sensitivity of QFT in patients with culture or biopsy confirmed active TB treated at our public health department over 5 years.

Methods: Total 346 patients were treated for active TB from January 2016 to December 2020. 246 patients with positive culture, positive GeneXpert or granulomatous disease on pathology were included for analysis in this study. The 100 patients who were excluded either had IGRA not performed (47) or were treated empirically as clinical TB cases (53). Among 246 patients with confirmed active TB, 230 (93%) had positive culture or GeneXpert while 16 (7%) had negative cultures but had granulomatous disease on biopsy. 154 (63%) of 246 included patients had pulmonary TB and 92 (37%) had extra-pulmonary TB. The QFT was positive in 196 (80%), indeterminate in 10(4%) and negative in 40 (16 %) confirmed TB cases. Among 154 patients with pulmonary TB, 118 (77 %) had positive QFT, 10(6%) had indeterminate and 26 (17%) had negative QFT. 78 (85%) of 92 extra-pulmonary TB patients had positive QFT and 14 (15%) had negative QFT. 18 patients with TB had HIV coinfection, 14 (78 %) had positive QFT, 3 (17%) had indeterminate and 1 (5%) had negative QFT.

Conclusions: The sensitivity of QFT was 80% in culture, GeneXpert or biopsy confirmed patients with active tuberculosis in our population. The QFT sensitivity was nearly same in patients with pulmonary as well as extra-pulmonary tuberculosis and among patients with HIV coinfection.

Poster 16

Nanoplasmonic Detection of *M. tuberculosis*-specific Extracellular Vesicles in Peripheral Blood for Pediatric TB Diagnosis

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Background: Most pediatric tuberculosis (TB) deaths occur in children <5 years, primarily in untreated children with missed TB diagnoses. Current TB assays rely on the respiratory samples can miss >50% of pediatric TB, and sensitivity is lower in children living with HIV, who have higher TB mortality. Detection of *Mycobacterium tuberculosis* (Mtb)-derived biomarkers in serum provides direct evidence of TB. Extracellular vehicles (EVs), including exosomes, are abundantly secreted into the extracellular space by most cells, from where they can ultimately accumulate in circulation. EVs actively participate in infectious diseases and can thus carry information for analysis and diagnosis of active TB and TB cases that can be challenging to diagnose by current TB assays and clinical algorithms.

Methods: We developed a nano plasmonic-enhanced immunoassay (NEI) to detect two factors we discovered, whose co-expressed on extracellular vesicles (EVs), where EVs are captured directly from serum by an EV-specific antibody, then hybridized with gold nanorods conjugated with TB-specific antigen antibodies. after which nanorod light scattering is captured using a benchtop-based or portable smartphone-based dark-field microscope (DFM) and the specific signal from target EVs expressing Mtb-associated factors is quantified by image processing.

Results: NEI shows high sensitivity toward pediatric TB cases diagnosed by microbiologic (83%) and clinical findings (73%). NEI measurement showed different negative rates to unlikely pediatric TB cases who did (46%) and did not meet any criterion for clinical TB diagnosis (83%), indicating the potential for undiagnosed TB cases in the former group, and higher specificity was detected in other non-HIV-exposed pediatric cohorts (92%-100%). NEI signal decreased in pediatric TB cases after anti-TB treatment, to reflect Mtb containment or clearance during anti-TB treatment or immune reconstitution.

Conclusions: Our results imply that the analysis of serum EVs by detecting Mtb-derived biomarker signatures can identify active TB and TB cases that are challenging to diagnose by current TB assays and clinical algorithms. Notably, this approach should also be adaptable to other types of diseases, including non-tuberculous *mycobacteria* superfamily members, which can also be difficult to diagnose.

Acknowledgments: The work was primarily supported by research funding provided by NIH (R01HD090927, R01AI122932, R01AI113725, R21EB026347, and R21AI126361, U01CA214254,), Department of Defense (PR181319) and Arizona Biomedical Research Commission (ABRC) young investigator award.

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Poster 17

iValiD-TB: A Fully-characterized *Mycobacterium tuberculosis* Dataset for Antimicrobial Resistance Bioinformatics Workflow Validations

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Background: A crucial aspect of validating any clinical assay is the availability of well-characterized samples to assess the specificity and sensitivity of the methodology being tested. However, due to logistical constraints, the nature of the specimen studied, or geographic diversity, many laboratories struggle to access adequate clinical specimens or isolates. Consequently, a diverse and well-characterized dataset for standardized assay validation is needed. The Wadsworth Center, the New York State Department of Health's laboratory, has been performing whole genome sequencing (WGS) on every TB case in the state since 2016, along with comprehensive phenotypic drug susceptibility testing (DST) of all drug resistant isolates. This work has resulted in a large collection of fully characterized clinical M. tuberculosis complex isolates, complete with WGS data and paired phenotypic drug resistance profiles.

Methods: To support the TB community, we have compiled a comprehensive dataset from 50 of these well-characterized isolates, sequenced using the Illumina MiSeq platform. This dataset has been curated to be inclusive of a broad range of lineage diversity, drug resistance type, and mutation type.

Results: This dataset is now available to the community for method development and bioinformatics pipeline validation, serving as a valuable resource to advance research and enhance clinical WGS assays.

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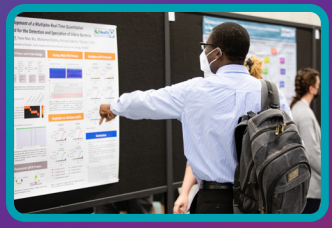

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Issues in *Mycobacterium tuberculosis* Complex Drug Susceptibility Testing

Current guidelines, recommendations and research for drug susceptibility testing with Rifampin, Ethambutol and Pyrazinamide.

Find these and more TB resources at www.aphl.org/TB

This publication was 100% funded by Cooperative Agreement # NU60OE000104 funded by the Centers for Disease Control and Prevention. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of CDC or the Department of Health and Human Services. Office of Surveillance, Epidemiology and Laboratory Services (OSELS), National Center for HIV, Viral Hepatitis, STDs and TB Prevention (PS), National Center for Zoonotic, Vector-borne, and Enteric Diseases (CK), National Center for Immunization and Respiratory Diseases (IP), National Center for Environmental Health (NCEH), National Center for Birth Defects and Developmental Disabilities (NCBDD).

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