Clinician and Laboratory Collaboration to Solve Diagnostic challenges

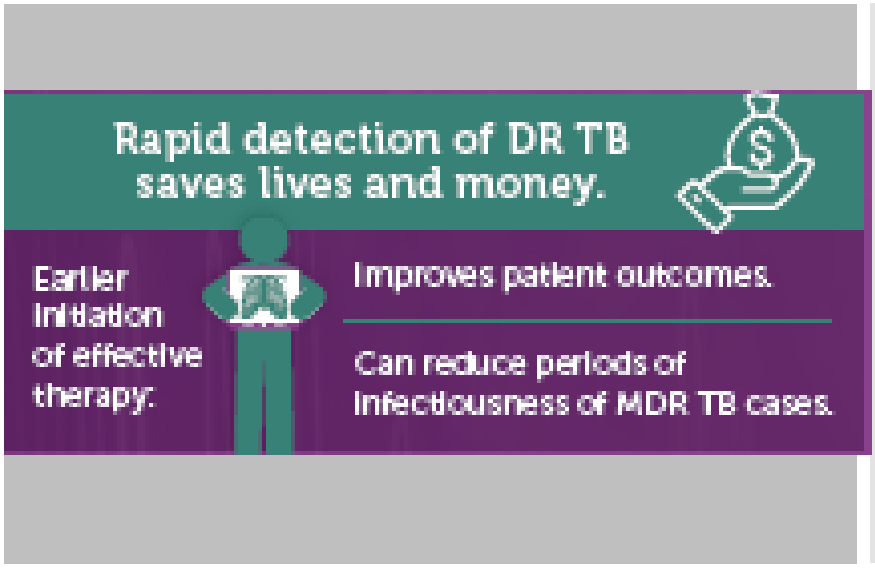
Amee Patrawalla, MD MPH
Global TB Institute
patrawam@njms.rutgers.edu

Connie A. Haley, MD MPH
Southeast National TB Center
connie.haley@medicine.ufl.edu

Learning Objectives

- Assess the role of the laboratory as a critical element of patient-centered care for TB patients and describe how early and continual engagement with the laboratory is beneficial for guiding appropriate test ordering and results interpretation for improved patient outcomes
- Utilize the appropriate molecular test to rapidly diagnose patients with pulmonary and extrapulmonary TB disease
- Discuss the importance of molecular detection of drug resistance in mixed cultures to guide accurate diagnosis and optimal treatment selection

Case 1.



Case 1: Presentation

- 46 y.o. female born in the Gambia, in U.S. for 12 years
- Sister died of TB
- Possible MS treated with interferon beta-1b
- Migraine, anxiety, depression and possible fibromyalgia
- Presented with cough, weight loss, fatigue and malaise
- CXR: right upper lobe cavity

How can the lab help with isolation decisions in a healthcare facility?

- Use AFB smear and Xpert MTB/RIF results (preferably 2 sputum specimens) to support clinician's decision for airborne infection isolation (AII)
- What if…
  - AFB smear +, Xpert MTB/RIF + for M.TB? **(TB is highly likely, continue AII)**
  - AFB smear +, Xpert MTB/RIF – for M.TB? **(Infectious TB is not likely, stop AIIR)**
  - AFB smear – and Xpert MTB/RIF – for M.TB ? **(Cannot rule out TB, not likely infectious, clinical decision to stop AII)**
    - If results of 2 specimens are discordant, use clinical judgement for AII decisions
- Can <u>any</u> TB NAA test be used for isolation decision-making?

NTCA/APHL Consensus Statement http://www.tbcontrollers.org/resources/airborne-infection-isolation/#.V7ddZ2VLWkg
Chaisson et al. JAMA Intern Med, 2018; Salfinger, JAMA Internal Medicine, 2018, Marks et al. CID, 2013; Cowan et al. CID, 2017

How can the lab help with initial treatment decisions?

Should you treat if….

- Xpert MTB/RIF + for *M.tb* and - for *rpoB* mutations? **(Treat as RIF-susceptible TB)**
- Xpert MTB/RIF + for *M.tb* and + *rpoB* mutations? **(Treat as RIF-Resistant TB)**
- Xpert MTB/RIF is negative for *M.tb*? **(Does not rule out TB, use clinical judgement)**

https://www.currytbcenter.ucsf.edu/products/view/drug-resistant-tuberculosis-survival-guide-clinicians-3rd-edition
Lewinsohn et al., CID 2017; Acharya et al. Molec Biol Rev, 2020

How can the lab help when…

- Can molecular testing for *M.tb* be used when…..
  - The sputum sample is AFB smear negative?
  - The specimen is a BAL or pleural effusion-can NAAT still be done?
  - I have a non-respiratory specimen?
- Where can I get molecular testing if my lab does not have Xpert MTB/RIF?
- Do I have to wait for the culture to grow before asking for MDDR?

TB is rarely an emergency to treat!!!

- Take time to get a molecular test for TB and drug resistance before starting treatment
  - Order Xpert MTB/RIF for all patients where TB suspected
    - Alternative--HAIN (detects *rpoB*, AND *katG*, *inhA* which confer INH resistance)
  - Always assess patient for clinical risk factors for drug resistance
  - Obtain CDC MDDR if rifamycin resistance
- Don't wait for the culture to grow – seek molecular test to r/o RIF-R

Case 1: Initial TB Treatment Challenges

- This patient's sputum smear is **AFB+, NAA + for *M.tb***
- 7/14/20: Started RIF, INH, PZA, EMB
- 7/31/20: Rash/itching developed, RIF→ rifabutin (RFB)
- 8/12/20: Vision changes→ EMB held
- 8/26/20: AST 205, ALT 231 → RFB, INH, PZA held
- 10/15/20: RFB, INH, Levaquin restarted
  Patient felt LQ caused LE weakness→ held LQ
  Continued RFB, INH, EMB
- 10/23: ALT 157, new rash/itching/muscle aches
- 11/8/20: **Sputum collected 10/22 reported culture +**
  Previously converted to neg 8/5/20

How can the lab help determine if this is TB treatment failure?

- Patient's AFB culture is positive, but DST is pending…..
- Is there a role for molecular after TB treatment started?
- What caveats does clinician need to consider?
- Does lack of FDA approval mean test shouldn't be used?

Case 1: Lab Collaboration for Diagnosis

- 11/24: Clinician was told the Xpert MTB/RIF was "positive for *M.tb* and for *rpoB* mutation" (Friday night, Thanksgiving weekend…..)
- Given the implications, the clinician called the lab supervisor the next day to confirm → Yes M.TB detected, but NO mutation at *rpoB*
- Clinician planned to use moxifloxacin in regimen, asked lab to perform rapid molecular test for quinolone resistance on AFB+ specimen
  - HAIN (can identify mutations for INH, RIF + FQ)

HAIN Results from Florida BPHL

3145	HAIN Test GenoType MTBDRplus	No rpoB point mutation detected No katG and No inhA point mutation detected	[redacted]

Note: --The HAIN test is investigational. Not intended for diagnostic purposes.
-- The clinical application of the HAIN results should be determined by the responsible treating care provider; for assistance with the interpretation of results of this test, please contact the TB Physicians' Consultation Network at 800-4TB-INFO (800-482-4636).
--All control bands present (conjugate, amplification, TB complex, *rpoB*, *katG*, and *inhA*)
--As with any DNA-based assay, this test only screens the nucleic acid sequence and not the amino acid sequence. Therefore, it is possible that mutations that do not cause an amino acid exchange (silent mutations) will still produce the absence of one of the wild type probes.
The **GenoType MTBDRplus** test only indicates those resistances of the *M. tuberculosis* complex that have their origins in the *rpoB*, *katG* and *inhA* regions examined here. Resistances originating from mutations of other genes or gene regions as well as other rifampin and isoniazid resistance mechanisms will not be detected by this test.
The presence of multiple bacterial species in the sample to be analyzed might hamper the interpretation of the test. Theoretically, a resistance can exist in spite of a wild type pattern. If, at investigation, the sample contains a strain that has developed only a partial resistance that is not covered by the mutation probes, the wild type pattern will appear. If the sample contains more than one *M. tuberculosis* strain (due to mixed culture or contamination) and one of these harbors a mutation that is not covered by the mutation probes, the wild type pattern will appear. As with other diagnostic assays, the results of this test may only be interpreted in combination with additional laboratory and clinical data available to the responsible treating care provider.

3146	HAIN Test GenoType MTBDRsl	No gyrA/gyrB point mutation detected **rrs Indeterminate** No eis point mutation detected	[redacted]

Reasons for TB Treatment Failure

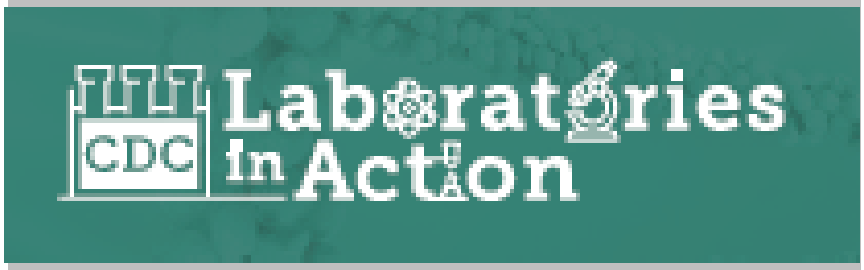
1. Non-adherence
2. Non-adherence
3. Non-adherence
4. Non-adherence
5. Non-adherence
6. Non-adherence
7. Non-adherence
8. Inadequate drug levels/malabsorption
9. **Acquired or previously undetected drug resistance
10. Foci of disease where TB drugs can't penetrate

**This is where molecular DST is helpful. New mutations suggest new drug resistance Negative NAA in setting of positive AFB suggests NTM, positive for M.TB *could* indicate relapse with same infection

Case 1: Follow-up

- Started liver-gentle regimen 12/2/20: **Rifabutin, moxifloxacin, linezolid**
- Follow up cultures grew *M.tb*, fully susceptible confirming MDDR
- No hepatotoxicity on RFB, Linezolid, Moxi
- The patient remains a challenge…….
  - Meds held again for neutropenia and thrombocytopenia
  - Adrenal insufficiency
  - Currently taking RFB, moxi, steroids –9 months treatment
  - Converted cultures to negative again

Case 2.



Case 2. Presentation

- 59 y.o. man from Brazil with no known medical history
- He complains of a cough for 2 weeks and weight loss. No fevers, nights sweats or other symptoms
- He has a known contact with pulmonary TB known to be pan-susceptible. However, he also mentions his brother had pulmonary TB 20 years ago and they were in close contact at that time
- Chest radiography demonstrates bi-apical pleural scar and patchy upper lobe consolidation with possible cavitation

Case 2. Initial Diagnosis and Treatment

- **AFB smear positive; Xpert MTB/Rif was *M.tb* detected, Rif resistance not detected**
- Patient was started on a standard 4 drug regimen with Rifampin, Isoniazid, Pyrazinamide and Ethambutol
- He had improvement in cough and weight over the next 3-4 weeks

Case 2. Additional Testing

- AFB culture reported as **positive for *M.tb***
- Growth based susceptibility testing from liquid culture reported **INH, SM, PZA resistance**
- Treatment was changed based on drug susceptibility results and MDDR was sent to CDC

How can the lab help?

- How would you approach these drug resistance findings from the lab point of view?
- How are molecular diagnostics helpful in this case?

Case 2. Follow Up

- MDDR returned without mutations
- Growth based susceptibility testing at CDC could not be performed as the culture was contaminated
- Subsequent AFB cultures at the local laboratory grew nontuberculous mycobacteria and a repeat DST on a pure MTB culture was pan susceptible
- Treatment regimen was adjusted back to 1st line treatment

Laboratory Discussion

- What are the limitations/ issues with liquid cultures in this scenario?
- What should raise suspicion for a clinician about a possible mixed-culture, and what should trigger a call to the lab for help?

Case 3

Case 3. Presentation

- 56yo F born in Alabama, no known TB exposure/risk
- Pelvic mass and lymphadenopathy
- Total hysterectomy, bilateral salpingo-oophorectomy
- Histopathology: Fallopian tube with fibrotic granulomas of varying sizes, some with central caseating necrosis expanding the interstitial connective tissue and obliterating the mucosal folds and lamina propria
  - Formalin-fixed, paraffin-embedded
  - AFB stain: rare structures suggestive of mycobacteria
- T-spot positive

How can the lab help confirm the diagnosis?

Common scenario….TB not considered until after a tissue sample collected and placed in fixative

- Culture and molecular testing cannot be done after specimen is placed in formalin/paraffin
- What can be done to enable diagnosis and DST at this point??

Centers for Disease Control and Prevention
National Center for Emerging and Zoonotic Infectious Diseases (NCEZID)
Division of High-Consequence Pathogens & Pathology (DHCPP)
Infectious Diseases Pathology Branch (IDPB)
Pathology Report

Diagnosis:

Fallopian tube, bilateral salpingo-oopherectomy:
-tuberculous salpingitis
--immunohistochemical and molecular evidence of a Mycobacterium tuberculosis complex species (see comments)

See comments and footnotes, as applicable.

Comments:

DNA extracted from formalin-fixed, paraffin-embedded tissue sections will be submitted to the Laboratory Branch in the Division of Tuberculosis Elimination at CDC to test for molecular detection of drug resistance. Results from those evaluations will be provided in a separate report from that laboratory.

Correlation of any reported results with clinical, epidemiological, and other laboratory information is highly recommended.

Infectious Diseases Pathology Branch	Pathology Report Continued IDPB No: 2020-0808	Centers for Disease Control and Prevention

Results:

Specimen	Test	Result
IHC		
Rt & lt proximal fallopian tube stumps (5/28/20)	Mycobacterium species (1687)	Immunoreactive
PCR		
Rt & lt proximal fallopian tube stumps (5/28/20)	Mycobacterium genus 16S rRNA	Positive for Mycobacterium tuberculosis complex species
Rt & lt proximal fallopian tube stumps (5/28/20)	Mycobacterium tuberculosis complex (IS6110)	Positive for Mycobacterium tuberculosis complex species

TB Laboratory Resources

- Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention Clinical Practice Guidelines: Diagnosis of TB in Adults and Children https://academic.oup.com/cid/article/doi/10.1093/cid/ciw694/2629583/Official-American-Thoracic-Society-Infectious
- Curry TB Center's A Clinician's Guide to the TB Laboratory http://www.heartlandntbc.org/assets/products/case_studies_tb_ncm_training_tools.pdf
- Drug Resistant TB: A Survival Guide for Clinicians, https://www.currytbcenter.ucsf.edu/products/view/drug-resistant-tuberculosis-survival-guide-clinicians-3rd-edition
- APHL Training Modules: https://www.aphl.org/programs/infectious_disease/tuberculosis/Pages/Training-Modules.aspx
- Guide to Application of Genotyping to TB Prevention and Control, http://www.cdc.gov/tb/programs/genotyping/manual.htm
- Webinars about using GeneXPERT for Airborne infection isolation decisions https://sntc.medicine.ufl.edu/Webinars.aspx#.V8BNfPkrJhE
- https://www.vdh.virginia.gov/content/uploads/sites/112/2016/11/VDH-Guidance-for-Using-Xpert-for-AII-Decisions_corrected.pdf

Case 3: Timeline of Testing

- 5/28/2020 Sample collected in surgery
- 7/9/2020 TB COE consulted
- 7/15/2020 Tissue block submitted to CDC IDPB
- 9/4/2020 Report issued from IDPB

➢ The SOONER clinicians get samples to lab, the sooner diagnosis can be made

➢ The effort to track down samples, send to lab is worth it!!!

- Confirming TB diagnosis helps engage/convince the patient, have legal basis for health orders, start effective treatment and reduce infectivity/transmission
- Using appropriate TB therapy avoids unnecessary toxicity

Infectious Diseases Pathology Branch (IDPB)

UNEXPLAINED INFECTIOUS DEATH?

IDPB conducts investigations and studies of infectious diseases of unknown cause or origin to help identify previously unrecognized or new infectious agents.

WHAT WE DO

Diagnostics

IDPB has diagnostic assays for more than 150 etiologic agents, including viral, bacterial, parasitic, and fungal organisms, and provides tissue-based diagnoses to medical examiners, community-based pathologists, and public health departments utilizing:

Immunohistochemistry (IHC) **Molecular evaluation**
Ultrastructural study **Microbiological methods**

Pathogenesis

IDPB employs pathology, the study of the cause of progression of infectious diseases in the human host. IDPB employs modern molecular technologies and electron microscopy that can collect information at a cellular level. IDPB used this approach in the recognition of Lyme carditis as a cause of sudden cardiac deaths (See story below).

In an ongoing study of Nodding Disease, IDPB examines the nature of unusual deposits in the brains of children who died of Nodding Disease. IDPB is also involved in the study of the pathology of the current outbreak of cases of fatal chikungunya.

Expert Consultation

Each year, laboratory specimens from all over the world are sent to IDPB, often in cases where the cause of illness is unknown. IDPB's expert pathologists and scientists routinely provide consultation and perform a range of laboratory tests. Testing by IDPB has resulted in the identification of novel disease agents of public health importance.

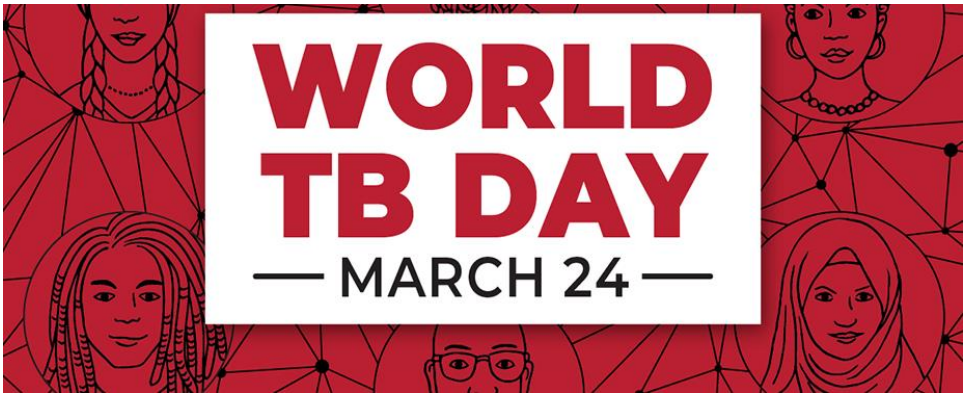
Notable Contributions to Public Health

IDPB scientists have played a critical role in the identification, diagnosis, and description of many diseases, including:

Fungal meningitis (U.S.)
Transplant-associated infections (U.S.)
Pandemic H1N1 influenza
Severe acute respiratory syndrome (worldwide)
Monkeypox (U.S.)
Bioterrorism-related anthrax (U.S.)
Hantavirus (U.S.)
Enterovirus 71 (Taiwan)
West Nile virus (U.S.)
Leptospirosis (U.S.)
Nipah virus (Malaysia & Singapore)

Visit IDPB Online at http://www.cdc.gov/ncezid/dhcpp/idpb/

National Center for Emerging and Zoonotic Infectious Diseases
Division of High-Consequence Pathogens and Pathology



CDC TB Centers of Excellence for Training, Education, and Medical Consultation

https://www.cdc.gov/tb/education/tb_coe/default.htm