TB LABORATORY UPDATE

LEARNING OBJECTIVES

Upon completion of this session, participants will be able to:

1. Utilize rapid identification methods for drug susceptibility for tuberculosis to improve patient outcomes

INDEX OF MATERIALS

<table>
<thead>
<tr>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. TB laboratory update – slide outline</td>
</tr>
</tbody>
</table>

Presented by: Max Salfinger, MD, FAAM, FIDSA

SUPPLEMENTAL MATERIAL


ADDITIONAL REFERENCES


TB Laboratory Update
Max Salfinger, MD, FIDSA, FAAM
Co-Lead, DrPH Laboratory Concentration
University of South Florida, College of Public Health
max@health.usf.edu
http://health.usf.edu/publichealth/

The journey sets the destination

1978-1981 University Hospital, Basel-Switzerland
1981-1992 University of Zurich, Dept. Medical Microbiology
1986-1988 Sabbatical – Denver, Colorado
1992-2006 National Jewish, University Hospital, Webb-Waring Lung Institute
2012-2018 Florida Department of Health, Tallahassee, Florida
2018 – present National Jewish Health, Denver, Colorado

University of South Florida, College of Public Health
Tampa, Florida
Topics

- Introduction
- Direct Detection (NAAT)
- Decision to Discontinue Airborne Infection Isolation in Healthcare Settings
- Acid-fast Bacilli (AFB) Smear Microscopy
- Growth Detection
- Identification (incl. NTM)
- Antimicrobial Susceptibility Testing (AST)
- Genotyping
- Systems / Algorithms

Reported Tuberculosis (TB) Cases
United States, 1982–2016 [CDC]

2017
9,093 new TB cases
2.8/100,000
TB Laboratory Update
Max Salfinger, MD, FIDSA, FAAM
University of South Florida, College of Public Health

TB Elimination

**Goal: One TB case per 1 Million Pop.**

<table>
<thead>
<tr>
<th>Year</th>
<th>Actual Cases</th>
<th>Goal Cases</th>
<th>Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017 US</td>
<td>9,093</td>
<td>323</td>
<td>28</td>
</tr>
<tr>
<td>Arizona</td>
<td>188</td>
<td>8</td>
<td>24</td>
</tr>
<tr>
<td>Colorado</td>
<td>84</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>New Mexico</td>
<td>37</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Utah</td>
<td>29</td>
<td>4</td>
<td>7</td>
</tr>
</tbody>
</table>

*Mycobacterium spp.*

Source: [http://www.bacterio.net/mycobacterium.html](http://www.bacterio.net/mycobacterium.html)

Number of species cited in this file: **198**
Number of subspecies cited in this file: **14**

TB Case Management and Contact Investigation Intensive
March 19-22, 2019
Pulmonary NTM – Medicare Beneficiaries

AJRCCM 2012 Adjemian et al

Burden of Pulmonary NTM Disease - US


Measurements and Main Results: In 2010, we estimated 86,244 national cases, totaling to $815 million, of which 87% were inpatient related ($709 million) and 13% were outpatient related ($106 million). Annual state estimates varied from 48 to 12,544 cases ($503,000–$111 million), with a median of 1,208 cases ($11.5 million). Oceanic coastline states and Gulf States comprised 70% of nontuberculous mycobacterial disease cases but 60% of the U.S. population. Medical encounters among individuals aged 65 years and older ($562 million) were twofold higher than those younger than 65 years of age ($253 million). Of all costs incurred, medications comprised 76% of nontuberculous mycobacterial disease expenditures. Projected 2014 estimates resulted in 181,037 national annual cases ($1.7 billion).
Laboratory’s charge

To provide the clinician with accurate results in a timely fashion

Toolbox 1

✓ Specimen – sputum, CSF, formalin-fixed tissue
  – NALC-NaOH versus Oxalic acid (CF w/history of Pseudomonas aeruginosa)
  – AFB microscopy
  – Solid (NTM plate) & broth-based media
  – NAAT-D (TB complex, NTM - mostly MAC)
  – NAAT-R (RIF, INH and more)
  – Direct AST

✓ Patient management (culture negativity after 2 months on treatment)

Ideally, molecular TB testing 7 Days a week
Toolbox 2

✓ **AFB positive culture (broth-, solid-based media)**
  - TB Yes/No (final identification within TB complex)
  - NAAT-R
  - Broth-based AST
  - Agar-based AST
  - Minimal Inhibitory Concentration (MIC)

✓ **Population management/genotyping**
  - RFLP-IS6110, Spoligo and MIRU
  - whole genome analysis
  - standardization through contracted PHL-MI

Quality Specimen

**Quality testing requires quality specimen**

[5 to 10 ml sputum]
Quality Specimen

**Sputum, expectorated or induced:**
Collection: Instruct patients on the proper method of sputum collection
- the material brought up from the lungs after a productive cough what is desired, and not nasopharyngeal discharge and saliva
- **5 - 10 mL sputum** collected in a sterile container.
- Difficulty in producing sputum
  - sputum induction by inhalation of an aerosol of sterile hypertonic saline (3%) or sterile water produced by a nebulizer that causes coughing. **Label as INDUCED**
- Perform in areas with adequate environmental controls under supervision.
- 3 consecutive specimens in 8- to 24-hour intervals, with at least one being an early morning specimen.
- Sputum specimens should not be pooled.

**CSF:**
- Collection: At least 5 mL of CSF should be aseptically collected.
- Minimum volume required: 2 to 3 mL; optimal volume is 10 mL.
- A separate sample should be collected for chemistry and hematology.

**Gastric Lavage:**
- Collection: Specimens should be collected in early morning before patients eat and while they are still in bed. The lavage should be performed with 25 to 50 mL of chilled, sterile, distilled water. Recovered sample should be placed in a leak-proof, sterile container (e.g., 50-mL conical tube).
- Transport: Gastric wash or lavage material should be submitted in a sterile leak-proof container, such as a sterile 50-mL conical tube or sterile urine collection container.
- Transport time and temperature: Specimens should be transported at room temperature as soon as possible.
  - If transport is delayed for more than one hour, specimens should be neutralized with 100 mg sodium carbonate within one hour of collection, and transported as soon as possible at room temperature.
**Quality Specimen**

**Abscess:**
- Tissue (at least 1 g, if possible) or fluid is preferred. Tissue should not be frozen or preserved.
- A swab is strongly discouraged unless it is the only specimen available. Swabs should be submitted in 2 to 3 mL sterile saline. Swabs submitted in transport medium or a commercial swab transport device are unacceptable.

**Blood:**
- **Collection:** Manufacturer's instructions for automated blood culture systems should be followed.
- **Alternatively,** 10 mL whole blood should be collected aseptically in a yellow-top collector tube containing SPS, or green-top collector tube containing heparin.
- Blood must not be collected in a red-top tube, EDTA (purple top), or ACD (yellow top).
- Minimum volume is 5 mL for adults; 1 mL for children.

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**Topics**

- Introduction
- **Direct Detection (NAAT)**
- Decision to Discontinue Airborne Infection Isolation in Healthcare Settings
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- Systems / Algorithms
TB NAAT

- FDA approved for respiratory specimens
  - Smear-positive (Dec. ‘95)
  - Smear-negative (Sept. ‘99)
- MMWR, January 16, 2009 [Universal]

In July 2013, the FDA granted Market Authorization to a cartridge-based assay. This NAA test can simultaneously identify *Mycobacterium tuberculosis* complex (TBC) and genetic mutations associated with resistance to rifampin from raw sputum and concentrated sputum sediments.

TB NAAT Recommendations

“NAA testing should be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management or TB control activities.”

MMWR Jan 16, 2009
Cartridge-based NAAT


US Study - California

217 clinical specimens/suspicion of TB

- **98%**  AFB smear pos.; culture pos.
- **72%**  AFB smear neg.; culture pos.
- **41 NTM**  negative
- **1 M. bovis**  positive

J Clin Microbiol 49:1621-23 (2011)
Xpert only performed

…the following actions must be taken:

- It is strongly recommended that specimen be sent to a reference laboratory for AFB smear and culture as soon as possible regardless of the NAA result. If there is a sufficient volume of raw sputum, split the specimen and send to a reference laboratory for both concentrated AFB smear and culture. The sample must be split prior to the laboratory mixing a sputum sample with the Sample Reagent (or SR). If volume is insufficient, request an additional sputum specimen for AFB smear and culture.
- Report results from a cartridge-based assay as soon as available while awaiting culture confirmation.
- If RIF resistance is detected, a specimen should be sent to a reference laboratory to confirm the resistance by DNA sequencing as soon as possible.

APHL Factsheet Sept 2013

TB NAAT Comparison

<table>
<thead>
<tr>
<th></th>
<th>AFB Smear +</th>
<th>Smear -</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTD*</td>
<td>97</td>
<td>76</td>
</tr>
<tr>
<td>Laboratory Developed Test**</td>
<td>99.6</td>
<td>75.4</td>
</tr>
<tr>
<td>Xpert***</td>
<td>100</td>
<td>71.7</td>
</tr>
<tr>
<td>Xpert – Ultra****</td>
<td>-&amp;+/+</td>
<td>90</td>
</tr>
<tr>
<td>Xpert****</td>
<td>-&amp;+/+</td>
<td>77</td>
</tr>
</tbody>
</table>


Ultra: CE-marked – Not FDA approved
TB NAAT - CSF

- HIV-infected adults with suspected meningitis at Mbarara Regional Hospital (Uganda). Centrifuged CSF, resuspended the pellet in 2 mL of CSF, and tested 0·5 mL with mycobacteria growth indicator tube culture, 1 mL with Xpert, and cryopreserved 0·5 mL, later tested with Xpert Ultra.


TB NAAT - CSF

- Xpert Ultra sensitivity was 70% (95% CI 47–87; 16 of 23 cases) for probable or definite tuberculous meningitis compared with 43% (23–66; 10/23) for Xpert and 43% (23–66; 10/23) for culture.
- **Testing 6 mL or more of CSF** was associated with more frequent detection of tuberculosis than with less than 6 mL (26% vs 7%; p=0·014).

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Decision to Discontinue Airborne Infection Isolation in Healthcare Settings

NTCA/APHL Consensus Statement on the Use of Cepheid Xpert MTB/RIF Assay in Making Decisions to Discontinue Airborne Infection Isolation in Healthcare Settings

- It is important to note that the process described herein is not to be used alone to rule out TB; Xpert negative or acid-fast bacilli (AFB) smear-negative sputum may contain viable organisms and represent infectious tuberculosis.
- Furthermore, NAA testing should not be used to monitor response to treatment or to release a newly confirmed TB patient from A.I.I.

April 2016
Decision to Discontinue Airborne Infection Isolation in Healthcare Settings

Interpretation of an Xpert result must be made in the context of the clinical and radiographic presentation and the clinician’s suspicion for infectious TB. A decision to remove a patient with a negative Xpert result from AII must consider the clinical presentation and the risk of possible transmission of TB from an infectious patient to others. Such a decision should not be based on sputum test results alone. The sensitivity of sputum testing for TB is subject to variability from a variety of factors, including sampling (e.g., poor specimen quality), inappropriate transport and processing of the specimen, errors in performance of the assay itself, and errors in labelling or reporting.

NTCA/APHL GeneXpert Consensus Statement – April 2016
San Francisco study

• In a prospective cohort study with a pragmatic, before-and-after implementation design, the authors analyzed 621 consecutive hospitalized patients undergoing sputum examination for evaluation of active pulmonary TB from January 2014 to January 2016 at the Zuckerberg San Francisco General Hospital and Trauma Center. JAMA Intern Med. 2018; 178(10):1380-1388

San Francisco study

• The mean hospital costs per molecular TB test-negative patient decreased from $46,921 to $33,574 after implementation of the algorithm, providing an average savings of $13,347 per patient.

• The authors estimated utilization and costs for approximately 250 patients completing TB evaluation each year and projected a total annual savings to the hospital of $3.3 million.
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*Mycobacterium tuberculosis* - Ziehl-Neelsen Staining
Reading/Interpretation: ZN & F Stain

<table>
<thead>
<tr>
<th>AFB Number per view fields (1000 X oil immersion)</th>
<th>AFB Number per view fields (250 X)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None per 300 fields</td>
<td>None per 30 fields</td>
</tr>
<tr>
<td>1-2 per 300 fields</td>
<td>1-2 per 30 fields</td>
</tr>
<tr>
<td>1-9 per 100 fields</td>
<td>1-9 per 10 fields</td>
</tr>
<tr>
<td>1-9 per 10 fields</td>
<td>1-9 per field</td>
</tr>
<tr>
<td>1-9 per field</td>
<td>10-90 per field</td>
</tr>
<tr>
<td>&gt;9 per field</td>
<td>&gt;90 per field</td>
</tr>
</tbody>
</table>

A quantification of the numbers of acid-fast organisms per field should be rated 1+ to 4+. The number of tubercle bacilli in pulmonary secretions is directly related to the risk of transmission.
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Demanding Instant Results!

20 Min    20 Hours
Processing Sputum Samples

- Procedures kill all but 10-20% of the mycobacteria
- Contamination
  2-5% of sputum specimens on Loewenstein-Jensen medium (LJ)

Growth Detection

- **M. leprae** – in armadillo for research; NOT in clinical laboratories
- **Suspicion for M. ulcerans, M. marinum, M. haemophilum**: additional media incubated at 30 to 32°C Celsius
- **Suspicion for fastidious organisms**
  - (M. haemophilum, M. genavense, M. paratuberculosis) require supplements – hemin, mycobactin, etc.
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Mycobacterium spp.

198 Species and 14 Subspecies in genus Mycobacterium as of March 18, 2019

M. tuberculosis complex

M. tuberculosis; M. bovis; M. bovis BCG; M. africanum;

M. caprae; M. microti; M. canettii;
M. pinnipedii; M. mungi; M. orygis
TB complex (2001-2004)

<table>
<thead>
<tr>
<th></th>
<th>NUMBER</th>
<th>PERCENT</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. tuberculosis</em></td>
<td>1,594</td>
<td>94.6%</td>
</tr>
<tr>
<td><em>M. africanum</em></td>
<td>31</td>
<td>1.8%</td>
</tr>
<tr>
<td><em>M. bovis</em></td>
<td>36</td>
<td>2.1%</td>
</tr>
<tr>
<td><em>M. caprae</em></td>
<td>1</td>
<td>0.1%</td>
</tr>
<tr>
<td><em>M. bovis BCG</em></td>
<td>23</td>
<td>1.4%</td>
</tr>
</tbody>
</table>

Wadsworth Center – NYS-DOH

Human tuberculosis (N=35) caused by *Mycobacterium bovis*
New York City
2001 – 2004

Winters et al 2005 MMWR 54:605-608
**M. bovis NYC 2001 - 2004**

![Bar chart showing the number of cases of M. bovis from different countries.](chart)

- **US-born (origin of parents)**
- **Non-US**

<table>
<thead>
<tr>
<th>Country</th>
<th>Number of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominican Republic</td>
<td>5</td>
</tr>
<tr>
<td>Guatemala</td>
<td>0</td>
</tr>
<tr>
<td>Guyana</td>
<td>0</td>
</tr>
<tr>
<td>Mexico</td>
<td>30</td>
</tr>
</tbody>
</table>

**Tuberculosis? 78 year-old Male**

![Thoracic X-ray and MRI scan showing lung and bladder abnormalities.](images)

Bladder Cancer with BCG Treatment

**Identification**

- Nucleic acid probe kits
- High Performance Liquid Chromatography (HPLC) – cell wall
- PCR Restriction Analysis (PRA)
- Line Probe Assays
- DNA sequencing
- Whole Genome Sequencing (WGS)
- Biochemicals are second

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**Mycobacterial Species in Pulmonary NTM**

Four integrated health care delivery systems*, 1991-2007

- *M. avium* complex 1,495 (80.1%)
- *M. chelonae/abscessus* 225 (12.1%)
- *M. fortuitum* 106 (5.6%)
- *M. kansasii* 102 (5.5%)
- *M. simiae* 53 (2.8%)
- *M. xenopi* 33 (1.7%)

*KP Southern California, KP Southern Colorado, Group Health, Geisinger Am J Respir Crit Care Med 2010 Prevots et al.*
MALDI-TOF MS

**MALDI-TOF MS can reliably and rapidly identify**

- Approximately 88% of *Mycobacterium* species, 90% of *Nocardia* species, and 51% of other aerobic actinomycetes encountered in routine clinical practice at a tertiary medical center/reference laboratory.
  - Using a custom, enhanced library and a streamlined extraction procedure
- Described the ability of the manufacturer’s library to identify these groups of organisms and described the effects of lowering the accepted cutoff score from 2.0 to 1.7
  - As the manufacturer continues to expand its database, many laboratories will have the ability to identify many of the isolates they routinely encounter using MALDI-TOF MS.
  - An expanded custom library may ultimately be the most useful tool for identification of the uncommon species encountered most often in a reference laboratory setting.


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**Notifiable NTM by State - 2016**
NTM Reporting Requirements

<table>
<thead>
<tr>
<th>State</th>
<th>Reporting Time for NTM</th>
<th>What is Required to be Reported (websites accessed on 12-11-2016)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maryland</td>
<td>within one working day</td>
<td>Mycobacterium spp., other than Mycobacterium tuberculosis complex or Mycobacterium leprae</td>
</tr>
<tr>
<td>Mississippi</td>
<td>one week</td>
<td>Nontuberculous mycobacterial disease</td>
</tr>
<tr>
<td>Missouri</td>
<td>within 3 days</td>
<td>Nontuberculosis mycobacteria (NTM)</td>
</tr>
<tr>
<td>Nebraska</td>
<td>within 7 days</td>
<td>Mycobacterium spp. (including M. tuberculosis complex organisms [for genotyping] and all “atypical” species, to include culture, nucleic acid tests, or positive histological evidence indicative of tuberculosis infection or disease)</td>
</tr>
<tr>
<td>Nevada</td>
<td>not specified</td>
<td>Submission of isolates of Mycobacterium spp.</td>
</tr>
<tr>
<td>New Jersey</td>
<td>within 72 hours</td>
<td>Mycobacterium, atypical</td>
</tr>
<tr>
<td>New Mexico</td>
<td>within 24 hours</td>
<td>Tuberculosis or other nontuberculous mycobacterial infections (including Mycobacterium avium complex or leprosy)</td>
</tr>
<tr>
<td>Ohio</td>
<td>close of the next biz. day</td>
<td>Mycobacterial disease other than tuberculosis (MOTT)</td>
</tr>
<tr>
<td>Oregon</td>
<td>one working day</td>
<td>Nontuberculous mycobacterial infection (nonrespiratory)</td>
</tr>
<tr>
<td>Virginia</td>
<td>immediate</td>
<td>Results of cultures positive for any member of the Mycobacterium tuberculosis complex (i.e., M. tuberculosis, M. bovis, M. africanum) or any other mycobacteria. Results of rapid methodologies, including acid hybridization or nucleic acid amplification, which are indicative of M. tuberculosis complex or any other mycobacteria.</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>within 72 hours</td>
<td>Mycobacterial disease (nontuberculous)</td>
</tr>
</tbody>
</table>

WGS - NYS

Comprehensive Whole-Genome Sequencing and Reporting of Drug Resistance Profiles on Clinical Cases of Mycobacterium tuberculosis in New York State

ABSTRACT Whole-genome sequencing (WGS) is a newer alternative for tuberculosis (TB) diagnostics and is capable of providing rapid drug resistance profiles while performing species identification and capturing the data necessary for genotyping. Our laboratory developed and validated a comprehensive and sensitive WGS assay to characterize Mycobacterium tuberculosis and other M. tuberculosis complex (MTBC) strains, composed of a novel DNA extraction, optimized library preparation, paired-end WGS, and an in-house-developed bioinformatics pipeline. This new assay was assessed using 608 MTBC isolates, with 146 isolates during the validation portion of this study and 462 samples received prospectively. In February 2016, this assay was implemented to test all clinical cases of MTBC in New York State, including isolates and early positive Bectec mycobacterial growth indicator tube (MGIT) 960 cultures from primary specimens.

WGS – added value

✓ Faster turn-around time
✓ More comprehensive results
  ❖ Detect mixed infections
  ❖ Many predictors of drug resistance
  ❖ Emerging resistance
✓ Cost effective
  ❖ Replace existing assays (real-time PCR, pyrosequencing, spoligotyping)
  ❖ Staff time savings

WGS to diagnose tuberculosis

Posted on March 29, 2017 at 12:46 pm

• Public Health England has announced that Whole Genome Sequencing (WGS) is now being used to identify different strains of tuberculosis (TB).

• This is the first time that WGS has been used as a diagnostic solution for managing a disease on this scale anywhere in the world. The technique, developed in conjunction with the University of Oxford, allows faster and more accurate diagnoses, meaning patients can be treated with precisely the right medication more quickly. Where previously it could take up to a month to confirm a diagnosis of TB, confirm the treatment choices and to detect spread between cases, this can now be done in just over a week by Public Health England’s Birmingham laboratory. This slows the spread of the disease and boosts the fight against anti-microbial resistance.

• This world first service has been developed in partnership with Genomics England, National Institute for Health Research (NIHR) and Wellcome Trust. The implementation of this technology will contribute to achieving the aims of the 100,000 Genomes Project.
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Primary MDR TB, United States, 1993–2017*

* Based on initial isolates from persons with no prior history of TB; multidrug-resistant TB (MDR TB) is defined as resistance to at least isoniazid and rifampin.
Reference AST

Agar Proportion Method [CLSI M24]:
- % resistant colonies
- Recognition of mixed cultures
- Up to 3 weeks’ incubation
- Direct AST (AFB+ smears)

• Broth-based methods [WHO]:
  - Susceptible vs. resistant
  - Shorter TAT
  - Walk-away system
  - Strains with elevated MICs under-recognized

Advantages of MIC

• Microtiter platform for first- and second-line anti-TB drugs available
• Multiple concentrations tested – internal control
• MIC will detect strains with altered susceptibilities
• Value of MIC result is enhanced by the result of the therapeutic drug monitoring
**CLSI M24**

M24 recognizes agar proportion as the reference methodology on which all other methodologies are based. In addition, this standard includes recommendations for using commercial broth susceptibility methods with shorter incubation times, which are now in widespread use for MTBC susceptibility testing, and information on molecular methods for detecting drug resistance and their integration with culture-based methods.

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**CLSI M24 MGIT & VersaTrek**

M24, 3rd ed.

**Appendix C. Drugs Available for *Mycobacterium tuberculosis* complex Susceptibility Testing Using Regulatory Organization–Cleared or –Approved Commercial Short-Incubation Liquid Media Systems’ and Their Equivalence in the Agar Proportion Method**

<table>
<thead>
<tr>
<th>Antituberculous Agent</th>
<th>System and Concentration, µg/mL</th>
<th>System and Concentration, µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fluorescence-based Detection System</td>
<td>Pressure-based Detection System</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Isomiazid</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Rifampin</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Ethambutol hydrochloride</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Ethambutol hydrochloride</td>
<td>7.5</td>
<td>8.0</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>4.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*Closed for use as of this standard’s completion.

1 Not available for sale in the United States.

2 Not available or not recommended.
CLSI M24 7H10, 7H11, LJ

M24, 3rd ed.

Appendix B. Antituberculous Drugs and Their Recommended Concentrations in Middlebrook 7H10 and 7H11 Agar Mediaa and LJ Mediumb

<table>
<thead>
<tr>
<th>Antituberculous Drugs</th>
<th>Concentrations, μg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Middlebrook 7H10</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>0.2</td>
</tr>
<tr>
<td>Rifampin</td>
<td>1.0</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>5.0</td>
</tr>
<tr>
<td>Pyrazinamide1</td>
<td>NR</td>
</tr>
</tbody>
</table>

Second Linea

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sens.</th>
<th>Spec.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>4.0</td>
<td>30</td>
</tr>
<tr>
<td>Capreomycin</td>
<td>10.0</td>
<td>40</td>
</tr>
<tr>
<td>Ethionamide</td>
<td>5.0</td>
<td>40</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>5.0</td>
<td>30</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>1.0</td>
<td>30</td>
</tr>
<tr>
<td>Merthiolacet</td>
<td>0.5</td>
<td>30</td>
</tr>
<tr>
<td>µ-Aminosalicylic acid</td>
<td>2.0</td>
<td>30</td>
</tr>
<tr>
<td>Prothionamide</td>
<td>NR</td>
<td>40</td>
</tr>
<tr>
<td>Rifabutin</td>
<td>0.5</td>
<td>20</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>2.0</td>
<td>4.0</td>
</tr>
</tbody>
</table>

1The concentrations listed are breakpoints.

Molecular testing

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>RIF</td>
<td>rpoB</td>
<td>97.1</td>
<td>97.4</td>
</tr>
<tr>
<td>INH</td>
<td>katG, inhA</td>
<td>86.0</td>
<td>99.1</td>
</tr>
<tr>
<td>EMB</td>
<td>embB</td>
<td>78.8</td>
<td>94.3</td>
</tr>
<tr>
<td>PZA</td>
<td>pncA</td>
<td>86.0</td>
<td>95.9</td>
</tr>
<tr>
<td>F-quinolones</td>
<td>gyrA</td>
<td>79.0</td>
<td>99.6</td>
</tr>
</tbody>
</table>

Molecular Testing

Current methods for detection of drug resistance in *M. tuberculosis* complex:

<table>
<thead>
<tr>
<th>Method</th>
<th>Assay &amp; Time</th>
<th>RIF/INH,oth.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cepheid</td>
<td>Clin. &amp; Conc.</td>
<td>2.5 h</td>
</tr>
<tr>
<td>Hain</td>
<td>Conc. &amp; Cult.</td>
<td>6-7 h</td>
</tr>
<tr>
<td>Pyroseq.</td>
<td>Conc. &amp; Cult.</td>
<td>5-6 h</td>
</tr>
<tr>
<td>Sanger seq.</td>
<td>Conc. &amp; Cult.</td>
<td>1-2 days</td>
</tr>
</tbody>
</table>

_Curry Center: Drug-resistant tuberculosis – A survival guide for clinicians, 3rd ed. 2016_

Molecular Testing - Limitations

- Potential to identify mutations that do not confer phenotypic resistance
- Not all genetic loci associated with resistance are known; therefore,

  ‘no mutation detected’ does not rule out resistance
**Frequency of Silent rpoB Mutations**

<table>
<thead>
<tr>
<th>Location</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuwait</td>
<td>1/12</td>
<td>8%</td>
</tr>
<tr>
<td>(JCM April 2015)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haiti</td>
<td>2/153</td>
<td>1.3%</td>
</tr>
<tr>
<td>(PLOSOne Sept 2014)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>2/8</td>
<td>25%</td>
</tr>
<tr>
<td>(JCM Oct 2011)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>California PHL</td>
<td>26/154</td>
<td>17%</td>
</tr>
<tr>
<td>(pers. comm.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Texas PHL</td>
<td>3/19</td>
<td>16%</td>
</tr>
<tr>
<td>(pers. comm.)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Requesting molecular detection of drug resistance**

- Acid-fast smear-positive specimen
- Some of the specimen sediment is available for sending to reference lab (State Public Health Lab)
- Drug resistance is suspected, or
- A susceptible population has been exposed, or
- The culture is non-viable, so regular antimicrobial susceptibility testing can’t be done
- CDC has the Molecular Detection of Drug Resistance (MDDR) program: tests for mutations associated with resistance to additional drugs—ethambutol, pyrazinamide
Topics

- Introduction
- Direct Detection (NAAT)
- Decision to Discontinue Airborne Infection Isolation in Healthcare Settings
- Acid-fast Bacilli (AFB) Smear Microscopy
- Growth Detection
- Identification (incl. NTM)
- Antimicrobial Susceptibility Testing (AST)
- Genotyping
- Systems / Algorithms

Genotyping Methods

- **Spoligotyping** (spacer oligonucleotide typing)
- **MIRU/VNTR** (mycobacterial interspersed repetitive units/ variable number of tandem repeats)
- **RFLP** fingerprinting (restriction fragment length polymorphism)
- **Whole genome sequencing** (WGS)
Spoligotyping

- Gives a result as a number, so to tell if 2 strains are different, just see if they have different numbers
- Not too powerful at discriminating different strains. Sometimes strains that are not part of the same outbreak will have the same spoligotype—e.g., Manila strain & Beijing strain
- Is now performed at CDC, using DNA sequencer

MIRU

- A PCR-based method, like spoligotyping
- Like spoligotyping, the result is a number (24 digits)
- Uses a DNA sequencer instrument to analyze the PCR products
- Like spoligotyping, MIRU sometimes doesn’t discriminate between unrelated strains
- Since April 2009, a new 24 locus MIRU protocol is in use, making it more powerfully discriminatory
Using MIRU and Spoligotyping together

- Both are PCR-based strain typing methods, so you can do them with just a small amount of DNA
- If 2 strains of *M. tuberculosis* are different from one another, it is unlikely that they will have the same spoligotype and the same MIRU type
  - Possible exceptions include Manila strains and Chinese “Beijing” strains

Universal Genotyping Approach (MI Lab)

- **All isolates**
  - MIRU-VNTR
  - ~ 2 weeks

- **WGS** with spoligotype inferred from sequence
  - additional 3 weeks
Contribution of Genotyping

1. Cross contamination studies
2. Outbreak investigation
3. TB Control needs, such as identifying settings where transmission occurs

**Note:** when genotyping links patients in a cluster, but no epi links are found, whole genome sequencing may be helpful in identifying which patients are truly linked in the same chain of transmission
### Topics

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- Direct Detection (NAAT)
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*Genotype cluster is defined as two or more cases with matching spoligotype and 24-locus MIRU-VNTR (GenType) within a county during the specified 3-year time period.*
Practice Guidelines for Clinical Microbiology Laboratories: Mycobacteria

In-house AFB service performed - APHL/CDC Survey 2011
Healthy People 2010, 2020, 2030?

- IID-32 Increase the proportion of culture-confirmed TB patients with a positive nucleic acid amplification test (NAAT) result reported within 2 days of specimen collection
- Baseline: 32.0 percent of culture-confirmed TB patients with a positive nucleic acid amplification test (NAAT) had their test results reported within 2 days of specimen collection in 2008
- Target: 77.0 percent
- Target-Setting Method: Maintain consistency with national programs, regulations, policies, and laws.
- Data Source: National TB Surveillance System (TB), CDC/NCHHSTP

Healthy People 2020 – status I

In 2015, the performance toward the objective was 46% in public health laboratories; no data were collected for non-public health laboratories.
Healthy People 2020 – status II

During the postimplementation period, clinicians at the Zuckerberg San Francisco General Hospital and Trauma Center ordered a NAAT on 75% of possible TB patients in airborne infection isolation. However, California data are lower, with 30% receiving a NAAT in 2017 (but a significant increase from 9% in 2010).

March 24 – World TB Day
TB Laboratory Update
Max Salfinger, MD, FIDSA, FAAM
University of South Florida, College of Public Health

Maroon Bells, Colorado

Thank you!
max@health.usf.edu

Roseate Spoonbills, Florida