Wisconsin State
Laboratory of Hygiene
UNIVERSITY OF WISCONSIN-MADISON
Laboratory 101

Nate Simon
TB Laboratory Program Coordinator
Wisconsin State Laboratory of Hygiene
Objectives

- Specimen collection
- Specimen storage and transport
- Testing offered at WSLH
- Result reporting and interpretation
- Expected turn around times for results
Specimen Types

Almost any source is acceptable for AFB culture and smear

- **Sputum**
  - Induced
  - Expectorated
- Bronchial washing/BAL
- Gastric aspirate
- Fresh tissue
- Bone
- Blood

- Bone Marrow
- CSF
- Body fluids
- Abscess
- Stool
- Urine
- Skin
Sputum

- Recently discharged material from the bronchial tree, with minimal amounts of oral or nasal material
  - Expectorated: from deep productive cough
  - Induced: use of nebulization to increase fluid in the airway and ease clearance of sputum
Purposes of sputum collection:

- To establish an initial diagnosis of TB
- To monitor the infectiousness of the patient
- To determine the effectiveness of treatment

Image Credit: WHO
Specimen collection

- Supervise patient for at least the first specimen, until ability to properly collect the specimen has been demonstrated
- Patient should be in a negative pressure room
- Anyone in the room should wear a fit tested N-95 respirator
- All specimens are collected into sealed leak proof containers
- Label specimen with two patient identifiers, collection date/time and specimen type.
Specimen collection

- Optimal: collect a diagnostic specimen before the initiation of drug therapy
  - Collect a series of three sputum specimens, 8-24 hours apart, at least one of which is an early morning specimen

- Monitoring of therapy: Obtain sputum specimens for culture at least monthly until cultures convert to negative for TB
Specimen collection kits

WSLH Kit #8: Sputum collection
Order: 1-800-862-1088
KITS ARE FREE!

- Sterile tube with label
- Absorbent pad
- Specimen transport bag
- Cold pack
- Instruction sheet
- Insulated mailer with labels
Storage and Transport

- Sputum samples should be refrigerated if they cannot be transported immediately

- Deliver specimens to the laboratory as soon as possible—try not to batch!

- Recommended: Include a cold pack during specimen transport.
Storage and Transport

Why is this important?

- Minimize overgrowth of normal flora
- Viability of AFB
- Rapid turn-around times
  - Isolation precautions
  - Start/Stop treatment
Submission of Specimens to WSLH

- Requisition form A
- Order: 1-800-862-1088
- Preprinted with account information
- One form per specimen
# Submission of Specimens to WSLH

<table>
<thead>
<tr>
<th>Code</th>
<th>Test Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM00250</td>
<td>Mycobacteria (AFB) Smear and Culture</td>
</tr>
<tr>
<td>MM00253</td>
<td>Mycobacteria Isolate Identification</td>
</tr>
<tr>
<td>MM02881</td>
<td>Mycobacterium tuberculosis Isolate Genotyping</td>
</tr>
<tr>
<td>MM00204</td>
<td>Mycobacterium tuberculosis Susceptibility-1st Line Drugs</td>
</tr>
<tr>
<td>MM00202</td>
<td>Mycobacterium avium Complex (MAC) Susceptibility</td>
</tr>
<tr>
<td>MM00260</td>
<td>Mycobacterium avium Complex PCR Decontaminated? Yes No Smear Result</td>
</tr>
<tr>
<td>MM00256</td>
<td>Mycobacterium tuberculosis PCR Decontaminated? Yes No Smear Result</td>
</tr>
</tbody>
</table>
Courier information

Service is offered at no charge to submitters

Call to set up an account and schedule a pickup:
763-233-0099
Assessing Sputum Quality

Test results are used as an aid in patient diagnosis and treatment.

Test results are directly related to the quality of the specimen.
Assessing Specimen Quality

Collection Date/Time:

- CDC MMWR 2005: 54, RR-17: “Persons requiring sputum collection for smear and culture should have at least three consecutive sputum specimens obtained, each collected in 8-24 hour intervals, with at least one being an early morning specimen”
- Specimens >7 days old will be rejected
Assessing Specimen Quality

Sputum Quality

- Specimens are thick and contain mucopurulent material
- 3-5 ml in volume, but smaller quantities accepted if the quality is satisfactory
  - <1 ml sputum will be rejected
- Poor quality specimens are thin and watery—Saliva and nasal secretions are unacceptable
- Induced sputum should be indicated on requisition form to avoid rejection
Assessing Specimen Quality

Thick Mucopurulent

Watery (induced?)

Hemoptysis

Salivary

http://www.theunion.org
Mycobacteriology at WSLH

- Full-service mycobacteriology laboratory
- BSL-3 facility
- Roles:
  - Primary Diagnostic Facility
  - Reference laboratory
  - Public Health Laboratory
Mycobacteriology at WSLH

- 22 laboratories around the state perform smear and culture
  - 5 labs perform some level of identification
  - Most others send to WSLH for identification
- WSLH receives clinical specimens from:
  - 2 large local hospitals
  - Health Departments (state-wide)
    - Milwaukee City TB Clinic
    - Madison Dane County Public Health
Mycobacteriology at WSLH

- Smear Microscopy
- PCR for direct detection
- Culture
- Identification
- Drug Susceptibility Testing
  - Molecular
  - Conventional
Mycobacteriology Testing at WSLH

Patient Specimen

Decontamination and Concentration

Smear Microscopy

AFB Smear Positive
- Same Day Nucleic Acid Amplification Testing (TB/MAC PCR)

AFB Smear Negative
- PCR Testing for patients with TB risk factors*

Culture

AFB positive growth
- ID by PCR, MALDI-TOF, DNA sequencing
  - Drug susceptibility Testing

Referred positive cultures
Smear Microscopy

- Small amount of concentrated patient specimen is placed on slide and stained with Auramine O fluorescent stain

- Rapid and inexpensive screening tool
  - First indication of mycobacterial infection and possible TB disease
  - Must be accompanied by additional testing including culture for confirmatory diagnosis
Auramine O Smear

Photo Credit: laboratoryinfo.com
## Smear Microscopy: Result Interpretation

<table>
<thead>
<tr>
<th>WSLH Report</th>
<th>Graded Scale</th>
<th>Qualitative Scale</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Potentially infectious</td>
</tr>
<tr>
<td>Rare (1-9 AFB per 100 fields)</td>
<td>1+</td>
<td>Positive</td>
<td>Low-level infectious</td>
</tr>
<tr>
<td>Few (1-9 AFB per 10 fields)</td>
<td>2+</td>
<td>Positive</td>
<td>Moderately infectious</td>
</tr>
<tr>
<td>Moderate (1-9 AFB per field)</td>
<td>3+</td>
<td>Positive</td>
<td>Highly infectious</td>
</tr>
<tr>
<td>Many (&gt;9 AFB per field)</td>
<td>4+</td>
<td>Positive</td>
<td>Highly infectious</td>
</tr>
</tbody>
</table>
Microscopy Results Guide Decisions

- Clinical Management
  - Patient therapy may be initiated for TB
  - Changes in smear status important to monitor response to therapy

- Public health interventions
  - Smear status and grade useful for identifying the most infectious cases
  - Contact investigation priority based on smear result
  - Decisions regarding respiratory isolation
Smear Microscopy: Limitations

- Does not distinguish between viable and dead organisms
- Limited sensitivity
  - High bacterial load: 5,000-10,000 AFB/mL is required for detection
  - Misses >45% of U.S. TB cases
- Limited specificity
  - All mycobacteria are acid fast
  - Does not provide species identification
- Cannot be done without a culture
Direct Detection using PCR

- AKA: Nucleic Acid Amplification Testing (NAAT)
WSLH PCR testing

- Detect *M. tuberculosis* complex (MTBC) and *M. avium* complex (MAC) directly from sputum (or other specimen) sediment
- PCR test developed at WSLH
  - 1 other laboratory in the state is performing NAAT (Cepheid GeneXpert)
- Testing takes about 2 hours
- Unable to distinguish live and dead bacilli
PCR testing (cont.)

- Automatically performed on new smear positive patients
- Fee-exempt testing for smear positive specimens and patients suspected of having active TB (approved by WI TB Program)
- Sensitivity
  - >95% for AFB smear-positive, culture-confirmed TB patients
  - 55-75% of AFB smear-negative, culture-confirmed TB patients
## TB/MAC PCR Specimen Types

<table>
<thead>
<tr>
<th>Test</th>
<th>Specimen Type</th>
<th>Smear Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB PCR</td>
<td>Respiratory</td>
<td>Smear positive and smear negative*</td>
</tr>
<tr>
<td>TB PCR</td>
<td>Non-respiratory</td>
<td>Smear positive only</td>
</tr>
<tr>
<td>MAC PCR</td>
<td>Respiratory and non-respiratory</td>
<td>Smear positive only</td>
</tr>
</tbody>
</table>

*Smear-negative TB PCR requires approval from the TB program for fee-exempt testing. Submitters may choose to pay for TB PCR testing on smear-negative respiratory specimens.*
Who should be tested?

- CDC recommendation: First sputum of all patients suspected to have TB for whom the test result would alter case management or TB control activities
  - Should not be routinely ordered when clinical suspicion of TB is low.
- PCR testing is diagnostic only! Not to be used in place of smear to remove patients from isolation!

*Updated Guidelines for the Use of Nucleic Acid Amplification Tests in the Diagnosis of Tuberculosis*; MMWR 2009; 58 (01); 7-10
Wisconsin TB PCR Criteria (Fee-exempt testing)

- Patient must have signs and symptoms of pulmonary TB
- Patient must be reported to the local or state public health department as a suspect TB case as required by Wisconsin Statute Chapter 252.05 and Wisconsin Administrative Code Chapter HFS 145.04 (3)(a).
- Patient must be in respiratory isolation
Wisconsin TB PCR Criteria (Cont.)

- Patient must not have been diagnosed with TB or a non-tuberculous mycobacterial infection within the last 12 months

- Patient must have received $\leq 7$ days of anti-mycobacterial therapy or no such treatment within the last 12 months
## Interpretation of PCR Results

<table>
<thead>
<tr>
<th>WSLH Lab Report</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>“<em>Mycobacterium tuberculosis</em> complex DNA detected”</td>
<td>Positive for TB</td>
</tr>
<tr>
<td>“<em>Mycobacterium avium</em> complex DNA detected”</td>
<td>Positive for MAC</td>
</tr>
<tr>
<td>“No <em>Mycobacterium tuberculosis</em> complex DNA detected”</td>
<td>Negative for TB</td>
</tr>
<tr>
<td>“No <em>Mycobacterium avium</em> complex DNA detected”</td>
<td>Negative for MAC</td>
</tr>
<tr>
<td>“Inhibitory substances that prevent nucleic acid amplification were detected”</td>
<td>Test is of no diagnostic help</td>
</tr>
</tbody>
</table>
Advantages of PCR Testing

- More rapid diagnosis
- Diagnosis in smear negative patients
- Initiation of earlier treatment
- Cost savings for patient isolation
- Faster reporting to TB programs
- Fewer transmissions
TB/MAC PCR Goals

Identify smear-positive TB patients within 48 hours (Target: 77%)
  - Respiratory isolation
  - Start therapy

Identify smear positive MAC patients
  - Release from isolation
  - Alter therapy decisions

Presumptive rapid results for about 60% of smear-positive patients
Percentage of Culture-Confirmed Pulmonary TB Cases Detected within 48 hours by NAAT in Wisconsin
Mycobacterial Culture

- Used to detect viable mycobacteria from patient specimens
- Most sensitive method for mycobacterial detection ("Gold Standard")
  - ~10 viable bacilli/ml for culture compared to >5000 bacilli/ml for microscopy
- Slowest method
  - Average time to detection for MTBC = 15 days
  - Range for detection of MTBC = 8-30+ days
## Mycobacterial Culture

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Broth</strong></td>
<td>• In automated instrument, read hourly for 42 days</td>
</tr>
<tr>
<td>“MGIT” tube—Mycobacteria Growth Indicator Tube</td>
<td>• O₂ consumption detected through fluorescent pad</td>
</tr>
<tr>
<td><strong>Solid</strong></td>
<td>• Visually inspected once per week for 6 weeks</td>
</tr>
<tr>
<td>Middlebrook 7H11 plate (only on non-respiratory and known TB patients)</td>
<td></td>
</tr>
</tbody>
</table>
Bactec MGIT 960
# Mycobacteria ID Methods at WSLH

<table>
<thead>
<tr>
<th>Method</th>
<th>Benefits</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB or MAC PCR</td>
<td>• ID from scant growth</td>
<td>• Can only ID MTBC and <em>M. avium</em> complex</td>
</tr>
<tr>
<td></td>
<td>• Rapid ID for &gt;60% of new positive MGIT tubes</td>
<td></td>
</tr>
<tr>
<td>MALDI-TOF</td>
<td>• Good identification from solid media</td>
<td>• Need good, pure growth</td>
</tr>
<tr>
<td></td>
<td>• Good ID of rapid growers</td>
<td>• Extraction method</td>
</tr>
<tr>
<td>DNA Sequencing</td>
<td>• “Gold standard”, good ID to species level</td>
<td>• Poor ID from positive MGIT broth</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Labor intensive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Slow</td>
</tr>
</tbody>
</table>
Significance of MTBC culture results

- MTBC identification is the most important finding in the clinical mycobacteriology laboratory
  - MTBC is not found in the environment
  - Isolation of MTBC almost always signifies disease
- Necessary for species identification, drug susceptibility testing, genotyping
- Monitor patient response to treatment
Mycobacteriology Testing at WSLH

Patient Specimen

Decontamination and Concentration

Smear Microscopy

AFB Smear Positive

Same Day Nucleic Acid Amplification Testing (TB/MAC PCR)

AFB Smear Negative

NAA Testing for patients with TB risk factors

Culture

AFB positive growth

ID by PCR, MALDI-TOF, DNA sequencing

Drug susceptibility Testing

Referred positive cultures

24 hours for TB result

2-3 weeks for TB result
Drug Susceptibility Testing (DST) for MTBC

- Automatically performed on all new culture-confirmed TB-patients in WI
- Used as a guide in choosing treatment plan—provide the best chance of a cure
- Stop transmission of TB by ending infectious period as quickly as possible
- Initiate appropriate treatment for contacts
Culture-based Drug Susceptibility Testing

- WSLH is the only laboratory in the state that performs culture-based TB drug susceptibility testing
- Rarely, DST for a WI TB patient is performed at Mayo
- WI Statutes require that an isolate from each culture-positive TB patient be submitted to WSLH for DST, genotyping, and repository.
Culture-based Drug Susceptibility Testing

A.K.A. phenotypic or conventional DST

Principle: Incubate a known concentration of MTBC isolate with a known concentration of a drug and observe for growth, or inhibition of growth
First Line Drugs

MGIT 960 broth system

- INH (0.2 ug/ml)
- INH (1.0 ug/ml)
- rifampin (1.0 ug/ml)
- ethambutol (5.0 ug/ml)
- PZA (100 ug/ml)

- Confirm resistance by repeat testing
**MGIT Method**

Organism suspension added to drug-free tube: **diluted**

Organism suspension added to drug-containing tubes: **undiluted**

- **IIRE**
  - Growth Control

- **INH low**
- **INH high**
- **RMP**
- **EMB**

- **PZA**
  - Growth Control
**Critical Concentration**

Critical concentration is the lowest concentration of a drug that:

- Inhibits growth of all susceptible strains
- AND
- Allows growth of all resistant strains

Growth of MTBC at critical concentration = **RESISTANT**

No Growth of MTBC at critical concentration = **SUSCEPTIBLE**
## Interpretation of Drug Susceptibility Results

<table>
<thead>
<tr>
<th>Result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>Strain is likely to show responsiveness to the drug</td>
</tr>
<tr>
<td>Resistant</td>
<td>Strain is unlikely to show responsiveness to the drug</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Test is of no help in prediction of responsiveness to the drug</td>
</tr>
</tbody>
</table>

2-4 weeks after positive culture—How do we get quicker results?
Molecular Detection of Drug Resistance (MDDR) Testing

AKA: genotypic testing, DNA-based

Principle: Use DNA amplification and detection methods to identify specific gene mutations that are known to confer resistance to antituberculosis drugs.
Molecular Drug Susceptibility Testing

Advantages:
- Rapid turnaround time—result in 1-2 days vs. 2-3 weeks
- Test can be performed on mixed or non-viable cultures
- Characterization of new mutations

Disadvantages:
- Interpretation of uncommon or unknown mutations
## Examples of Molecular DST

<table>
<thead>
<tr>
<th>Genetic loci</th>
<th>Cepheid GeneXpert® MTB/RIF</th>
<th>Sanger Sequencing</th>
<th>Pyrosequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>rpoB (for RMP)</td>
<td>Varies but can include rpoB, inhA, katG, aphC, embB (EMB), pncA (PZA), gyrA (FQ), and rrs (injectables)</td>
<td>Varies but can include rpoB, inhA, katG, aphC, gyrA, and rrs</td>
<td></td>
</tr>
<tr>
<td>Format</td>
<td>Semi-automated real-time PCR</td>
<td>DNA sequencing</td>
<td>DNA sequencing</td>
</tr>
<tr>
<td>FDA approved</td>
<td>Yes</td>
<td>N/A (laboratory developed test)</td>
<td>N/A (laboratory developed test)</td>
</tr>
<tr>
<td>Expected turnaround time from receipt in laboratory</td>
<td>1-2 working days</td>
<td>1-2 working days (depends on how often performed in lab)</td>
<td>1-2 working days (depends on how often performed in lab)</td>
</tr>
</tbody>
</table>
MDDR Testing at WSLH

- WSLH performs GeneXpert MTB/RIF assay on all new TB patients identified in WI (sputum sediment, BAL sediment, or broth culture—MGIT)
  - Any other specimen type is sent to CDC or Milwaukee City Public Health Department for testing

- Used as a rapid method to detect Rif-resistance/potential MDR TB
GeneXpert MTB/RIF

Workflow: Self contained cartridge – just add sample

1. Pour Sample Reagent into sample tube. Incubate for 15 minutes at room temperature. (Acceptable sample types: unprocessed sputum or sediment from concentrated specimen.)

2. Pipette diluted sample into cartridge.

3. Insert cartridge and start assay.

TOTAL HANDS-ON TIME = 2 MINUTES
## GeneXpert Result Interpretation

<table>
<thead>
<tr>
<th>Result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTB DETECTED; Rif Resistance DETECTED</td>
<td>Likely Resistant to Rifampin</td>
</tr>
<tr>
<td>MTB DETECTED; Rif Resistance NOT DETECTED</td>
<td>Likely Susceptible to Rifampin</td>
</tr>
</tbody>
</table>

If RIF resistance mutation is detected, specimen is sent to CDC for full MDDR panel and 2\textsuperscript{nd} line drug agar proportion testing
Results for Molecular Detection of Drug Resistance (Sanger Sequencing, complete panel); Conventional Drug Susceptibility Test in progress.

<table>
<thead>
<tr>
<th>Locus (region) examined*</th>
<th>Result</th>
<th>Interpretation (based on in-house evaluation of 550 clinical isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rpoB (RRDR)</td>
<td>Mutation: TCG&gt;TGG; Ser531Trp</td>
<td>Rifampin resistant. (100% of isolates in our in-house evaluation of 550 clinical isolates with this mutation are RMP-R.)</td>
</tr>
<tr>
<td>inhA (promoter)</td>
<td>No mutation</td>
<td>Cannot rule out INH resistance. (86% of INH-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at one or both of these loci.)</td>
</tr>
<tr>
<td>katG (Ser315 codon)</td>
<td>No mutation</td>
<td>Cannot rule out ethambutol resistance. (78% of EMB-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at this locus.)</td>
</tr>
<tr>
<td>embB (Met306,Gly406)</td>
<td>No mutation</td>
<td>Cannot rule out PZA resistance. (86% of PZA-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at this locus.)</td>
</tr>
<tr>
<td>pncA (promoter, coding region)</td>
<td>No mutation</td>
<td>Cannot rule out fluoroquinolone resistance. (80% of FQ-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at this locus.)</td>
</tr>
<tr>
<td>gyrA (QRDR)</td>
<td>No mutation</td>
<td>Cannot rule out resistance to injectable drugs (kanamycin, capreomycin, amikacin). (In our in-house evaluation of 550 clinical isolates: 91% of AMK-R isolates have a mutation in the rrs locus; 87% of KAN-R isolates have a mutation in either the rrs locus or the els locus; 58% of CAP-R isolates have a mutation in either the rrs locus or the tlyA locus.)</td>
</tr>
<tr>
<td>rrs (1400 region)</td>
<td>No mutation</td>
<td></td>
</tr>
<tr>
<td>els (promoter)</td>
<td>No mutation</td>
<td></td>
</tr>
<tr>
<td>tlyA (entire ORF)</td>
<td>No mutation</td>
<td></td>
</tr>
</tbody>
</table>

*A negative result (e.g., no mutation) does not rule out contributory mutations present elsewhere in the genome.*
Agar Proportion Method

*3 weeks incubation at 35-37°C
**AP Result Interpretation**

Susceptibility Testing Method: Indirect agar proportion, 7H10 medium; Susceptibility is defined as < 1% resistance compared to colonies that develop on drug-free media

### RESULTS:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Percent Resistance</th>
<th>Interpretation</th>
<th>Drug</th>
<th>Percent Resistance</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid 0.2 ug/ml</td>
<td>0%</td>
<td>S</td>
<td>Kanamycin 5.0 ug/ml</td>
<td>0%</td>
<td>S</td>
</tr>
<tr>
<td>Isoniazid 1.0 ug/ml</td>
<td>0%</td>
<td>S</td>
<td>Ethionamide 10.0 ug/ml</td>
<td>0%</td>
<td>S</td>
</tr>
<tr>
<td>Isoniazid 5.0 ug/ml</td>
<td>0%</td>
<td>S</td>
<td>Capreomycin 10.0 ug/ml</td>
<td>0%</td>
<td>S</td>
</tr>
<tr>
<td>Rifampin 1.0 ug/ml</td>
<td>100%</td>
<td>R</td>
<td>PAS 2.0 ug/ml</td>
<td>0%</td>
<td>S</td>
</tr>
<tr>
<td>Ethambutol 5.0 ug/ml</td>
<td>0%</td>
<td>S</td>
<td>Ofloxacin 2.0 ug/ml</td>
<td>0%</td>
<td>S</td>
</tr>
<tr>
<td>Streptomycin 2.0 ug/ml</td>
<td>0%</td>
<td>S</td>
<td>Amikacin 4.0 ug/ml</td>
<td>0%</td>
<td>S</td>
</tr>
<tr>
<td>Streptomycin 10.0 ug/ml</td>
<td>0%</td>
<td>S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rifabutin 2.0 ug/ml</td>
<td>50%</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin 2.0 ug/ml</td>
<td>0%</td>
<td>S</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Susceptibility Testing Method: MGIT 960

**Pyrazinamide 100 ug/ml** : Susceptible

**Comments:** Molecular Detection of Drug Resistance (MDDR) report was issued 6/27/2017.

These conventional agar proportion results agree with the MDDR results.
Agar Proportion Limitations

- Slow---3 week incubation
  - Compared to 4-12 days with broth method
- Media preparation—cannot purchase commercially
- CDC goal
  - Report RIF DST result within 17 days of organism ID (impossible to meet!)
**Turn around Times**

- **Primary specimen**
- **Smear/PCR** 24-48 hours
- **Molecular Detection of Resistance** 1-3 days
- **Smear positive respiratory**
- **Culture** 7-21 days
- **Identification** 0-2 days
- **TB first-line drugs** 4-20 days
- **TB second-line drugs** 3-4 weeks

**Conventional (Culture Based):**
- 4-10 weeks
Acknowledgements

Dave Warshauer, PhD
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Laura Louison, MLS(ASCP)
The WSLH TB Laboratory Staff
Questions?

Nate Simon
WSLH - TB Laboratory Program Coordinator
608-224-4265
Nathan.simon@slh.wisc.edu

WSLH Customer Service: 800-862-1013

Wisconsin TB Program: 608-261-6319