



Wisconsin State
Laboratory of Hygiene

UNIVERSITY OF WISCONSIN-MADISON

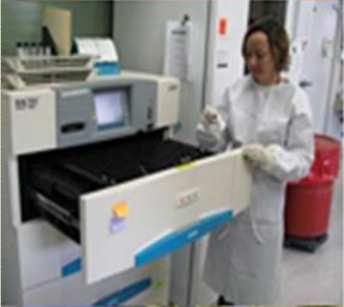
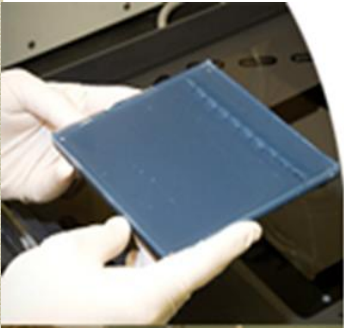


Laboratory 101

Nate Simon

TB Laboratory Program Coordinator

Wisconsin State Laboratory of Hygiene





Objectives

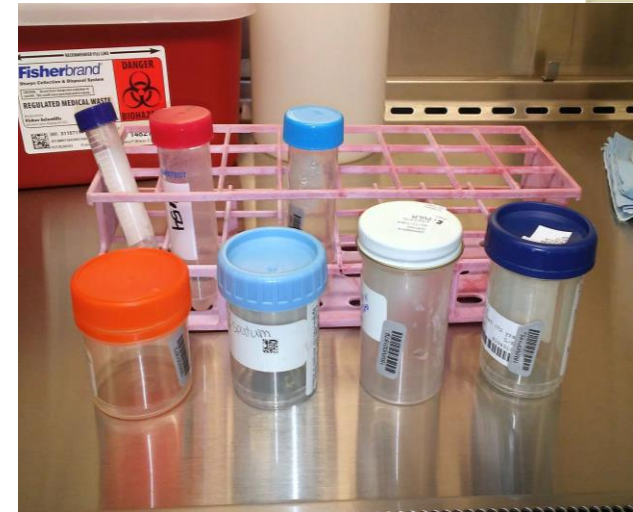
- Specimen collection
- Specimen storage and transport
- Testing offered at WSLH
- Result reporting and interpretation
- Expected turn around times for results



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
 - Expecterated: 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





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

Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN-MADISON
2601 Agriculture Drive
Madison, WI 53718

D.F.L. Kortyca, M.D., Medical Director
http://www.slh.wisc.edu
CDD Customer Service
Phone: 800-862-1013
Fax: 844-390-6233
Kits and Supplies: 800-862-1088

CDD Requisition Form (A) 10-1-17 FORM 4185

(Please type or print using black pen)

(1) Patient's Last Name Patient's First Name Mid. Init.
(2) Name Change? Former Last Name
(3) Patient's Address
(4) City State Zip
(5) Date of Birth (6) Age (7) Female Male
(9) Ethnicity: Hispanic/Latino NonHispanic/Latino (10) Race: Amer Indian Black/African Amer White Asian Pacific Islander Other
(11) Chart #/Patient ID Number (12) Submitter Specimen ID Number (16) Clinician: (17) UDIN # _____ NPI # _____

(13) Medicare generally does not cover routine screening tests. ABN attached? Yes No Please provide Third-Party information on the back side of this page
(18) Date and Time of Collection (Required) Date of Onset Outbreak Yes No Name of Outbreak: _____

Specimen Type (Required):
 _____ Acute Serum _____ Whole Blood (anticoagulant) _____ Sputum _____ Body Fluid (Site: _____) _____ Swab (Site: _____)
 _____ Convalescent Serum _____ Plasma (anticoagulant) _____ Stool _____ Slide/Smear (Site: _____) _____ Tissue (Site: _____)
 _____ Serum _____ CSF _____ Urine _____ Isolate (Site: _____) _____ Wash/Aspirate (Site: _____)

Clinical Data:
 _____ Asymptomatic _____ Fever _____ Abdominal Cramps _____ Acute Respiratory Disease
 _____ Postmeritum _____ Headache _____ Diarrhea _____ Conjunctivitis
 Date of Exposure _____ Lesion (Type _____) _____ Erythema _____ Cough
 Prenatal EDD _____ Lymphadenopathy _____ Meningitis _____ Croup
 Vaccination Date _____ Myalgia _____ Stiff Neck _____ Nasal Discharge
 Vaccination Type _____ Rash (Type _____) _____ Vomiting _____ Sore Throat

(21) For Third-Party payment ICD-10 codes are required:
To order a test please write the letter corresponding to the appropriate ICD-10 Code to the left of the test name. Note: ICD-10 Codes must support the medical necessity of the test for Medicare reimbursement.
ICD-10 Code (A) _____ ICD-10 Code (B) _____ ICD-10 Code (C) _____

Bacteriology:	BT Agent (Potential), please call 800-862-1013	Parasitology:
MP00476 Bacterial ID, Non-Enteric Public Health Panel (WHP); Suspect Organism	WSLH assigned	MP00801 Anthropod Identification
MP00623 Bordetella Culture	• <i>Bacteroides mallei/psuedomallei</i> • <i>Bacillus anthracis</i> • <i>Francisella</i> • <i>Bruceella</i> • <i>Yersinia pestis</i>	MP00802 Cryptosporidium/Giardia DFA
MP00311 Bordetella PCR (pertussis/pneumoniae/houseii)	Invasive Bacteriology Surveillance (IBLS):	MP00840 Ova & Parasites, Intestinal
Botulism Testing (Call 800-862-1013)	MP00651 Haemophilus influenzae	MP00880 Parasites Blood Smear (Travel history form required)
SC00111 Chlamydia GC/NAAT	MP00628 Listeria monocytogenes	Suspect Parasite
SC00118 Chlamydia trachomatis NAAT	MP00561 Neisseria meningitidis	MP00881 Parasites Tissue Smear (Travel history form required)
SC00112 Neisseria gonorrhoeae NAAT	MP00465 Streptococcus pneumoniae	Suspect Parasite
SC00200 Trichomonas vaginalis NAAT		MP00860 Parasitic Worm Identification
MP00580 Bacterial Characterization for Resistance Susceptibility results required. Organism _____	Mycobacteriology:	VR01703 Enterovirus PCR (CSF)
MP00380 Diphtheria Culture	MM00201 Mycobacteria (A/B) Smear and Culture	VR01704 Herpes Simplex Virus PCR (Genital/Dermal)
MP00460 Enteric Pathogen Isolate Identification (WEPIS)	MM00253 Mycobacteria Isolate Identification	VR01713 Measles Virus PCR-Requires WDPH Approval
Suspect Organism	MM02881 Mycobacterium tuberculosis Isolate Genotyping	VR01714 Mumps Virus PCR
MP00610 Haemophilus ducreyi Culture	MM00204 Mycobacterium tuberculosis Susceptibility-1st Line Drugs	VR01717 Norovirus PCR
MP00420 Legionella Clinical Culture	MM00302 Mycobacterium avium Complex (MAC) Susceptibility	VR01725 Rubella Virus PCR-Requires WDPH Approval
MP00421 Legionella PCR	MM00207 Mycobacteria Rapid Grower Susceptibility Organism ID	VR01727 Varicella Zoster Virus PCR
MP00549 Shiga Toxin EIA		SS02303 Chikungunya Virus PCR
MP00543 Shiga Toxin PCR Screen		SS02302 Dengue Fever PCR and Serotyping
MP00684 S. aureus for VISA/VRSA Confirmation		
MP00660 Stool Culture, Routine Suspect Organism _____ CDT? Yes _____ No _____ Method _____		Other Tests (Specify):
MP00593 Toxic Shock Syndrome Toxins		

WHITE-RETURN TO WSLH
YELLOW-KEEP FOR YOUR RECORDS

- Requisition form A
- Order: 1-800-862-1088
- Preprinted with account information
- One form per specimen



Submission of Specimens to WSLH

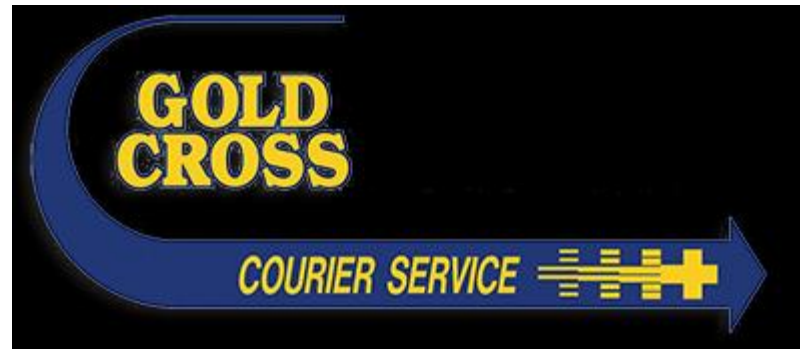
Code		Test Description
MM00250	✘	Mycobacteria (AFB) Smear and Culture
MM00253		Mycobacteria Isolate Identification
MM02881		Mycobacterium tuberculosis Isolate Genotyping
MM00204		Mycobacterium tuberculosis Susceptibility-1st Line Drugs
MM00202		Mycobacterium avium Complex (MAC) Susceptibility
MM00260		Mycobacterium avium Complex PCR Decontaminated? Yes No Smear Result _____
MM00256		Mycobacterium tuberculosis PCR Decontaminated? Yes No Smear Result _____



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

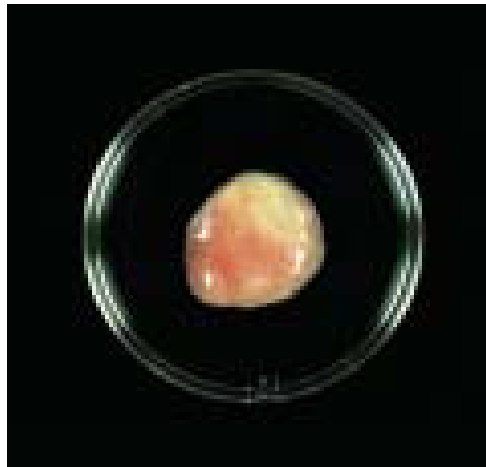
Thick
Mucopurulent



Watery
(induced?)



Hemoptysis



Salivary





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

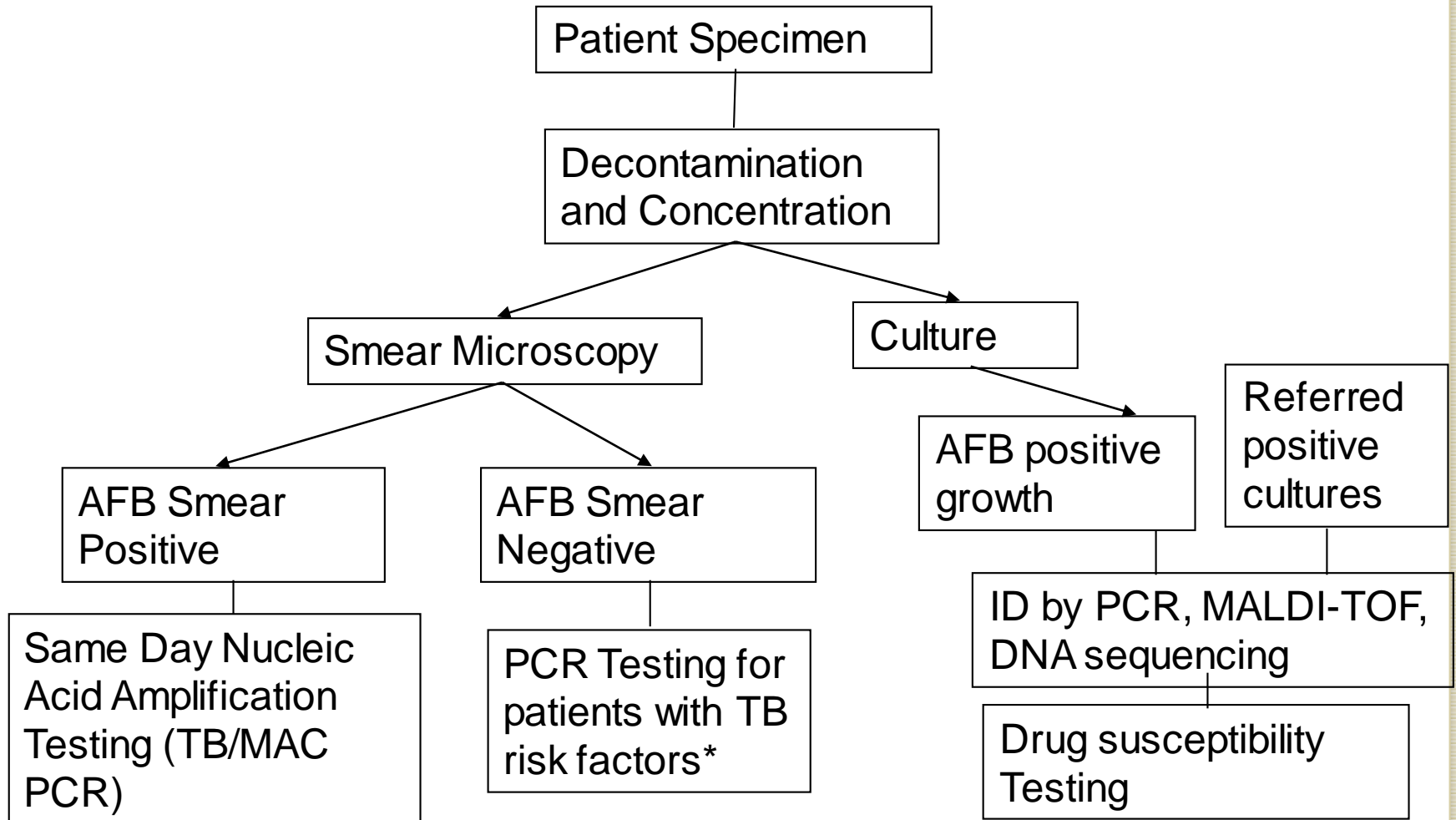


Mycobacteriology at WSLH

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



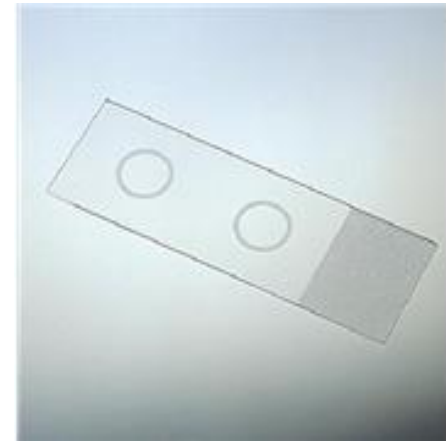
Mycobacteriology Testing at WSLH





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

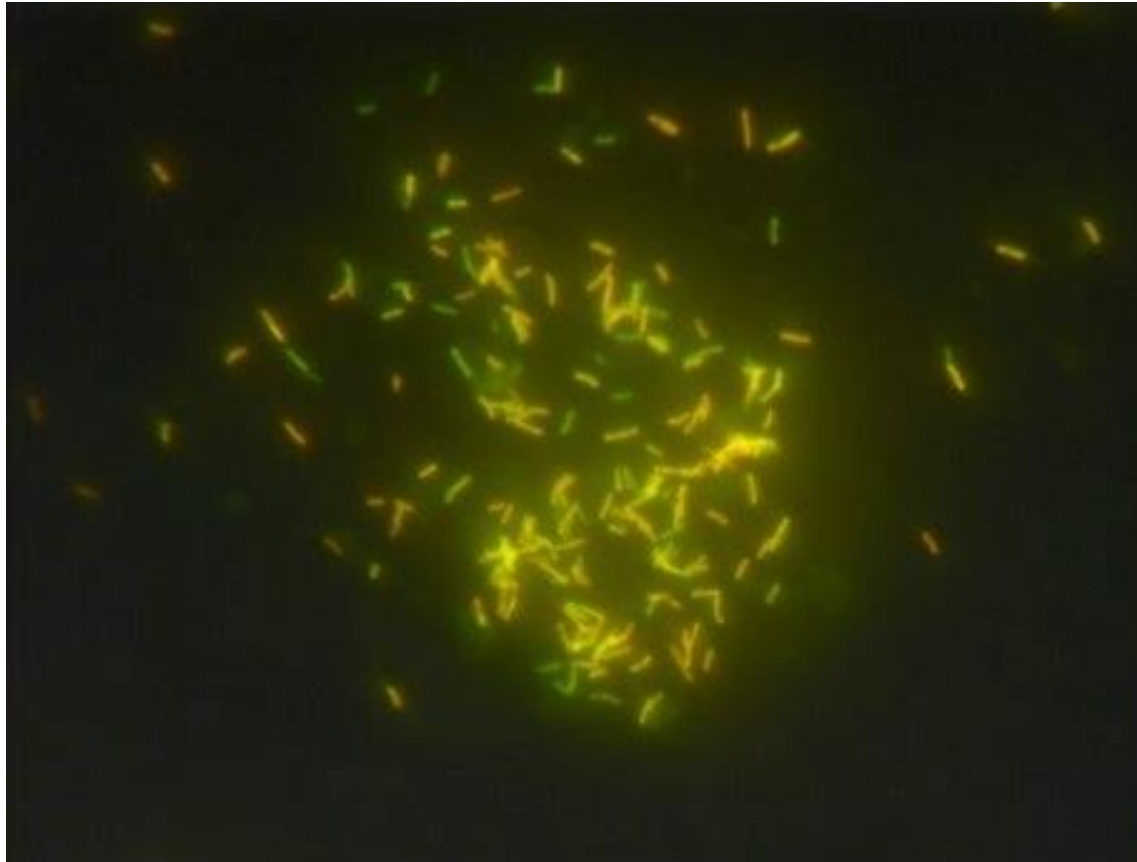


Photo Credit: laboratoryinfo.com



Smear Microscopy: Result Interpretation

WSLH Report	Graded Scale	Qualitative Scale	Interpretation
Negative	Negative	Negative	Potentially infectious
Rare (1-9 AFB per 100 fields)	1+	Positive	Low-level infectious
Few (1-9 AFB per 10 fields)	2+	Positive	Moderately infectious
Moderate (1-9 AFB per field)	3+	Positive	
Many (>9 AFB per field)	4+	Positive	Highly infectious



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

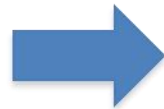


Direct Detection using PCR

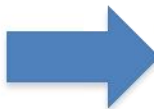
- AKA: Nucleic Acid Amplification Testing (NAAT)



Sputum



DNA extraction



Amplification



MTBC or MAC
detected

MTBC or MAC
not detected



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

Test	Specimen Type	Smear Result
TB PCR	Respiratory	Smear positive and smear negative*
TB PCR	Non-respiratory	Smear positive only
MAC PCR	Respiratory and non-respiratory	Smear positive only

*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!



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 ≤ 7 days of anti-mycobacterial therapy or no such treatment within the last 12 months



Interpretation of PCR Results

WSLH Lab Report	Interpretation
" <i>Mycobacterium tuberculosis</i> complex DNA detected"	Positive for TB
" <i>Mycobacterium avium</i> complex DNA detected"	Positive for MAC
"No <i>Mycobacterium tuberculosis</i> complex DNA detected"	Negative for TB
"No <i>Mycobacterium avium</i> complex DNA detected"	Negative for MAC
"Inhibitory substances that prevent nucleic acid amplification were detected"	Test is of no diagnostic help



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

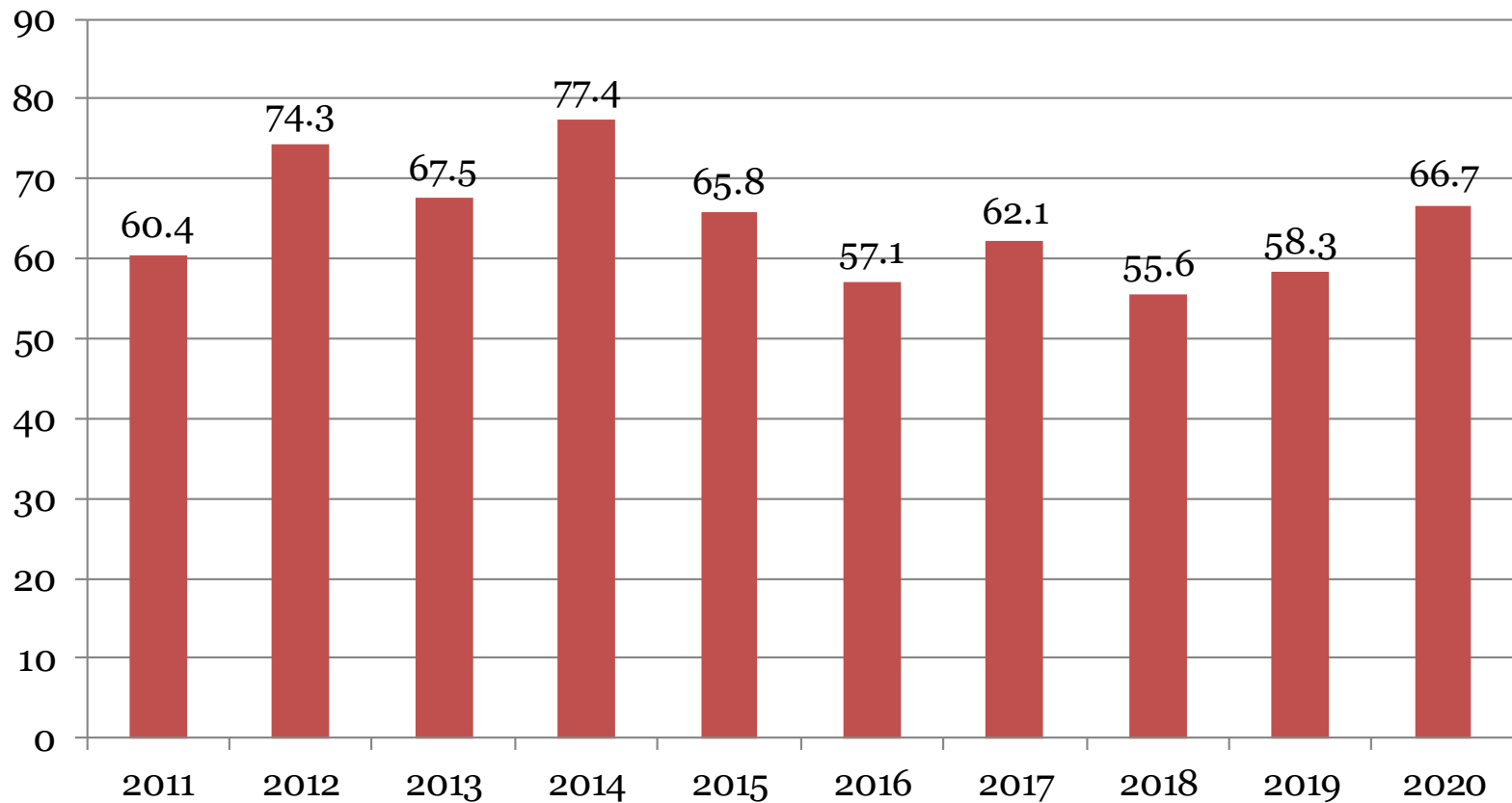
Identify smear positive MAC patients

- 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

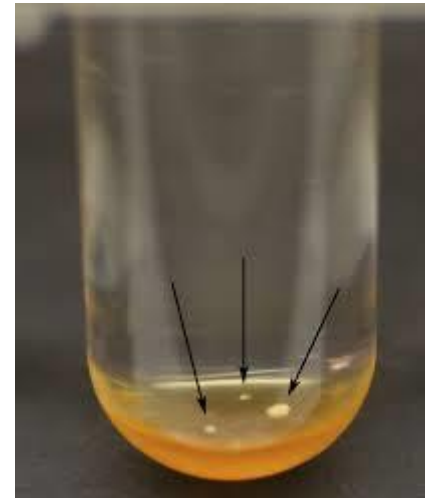
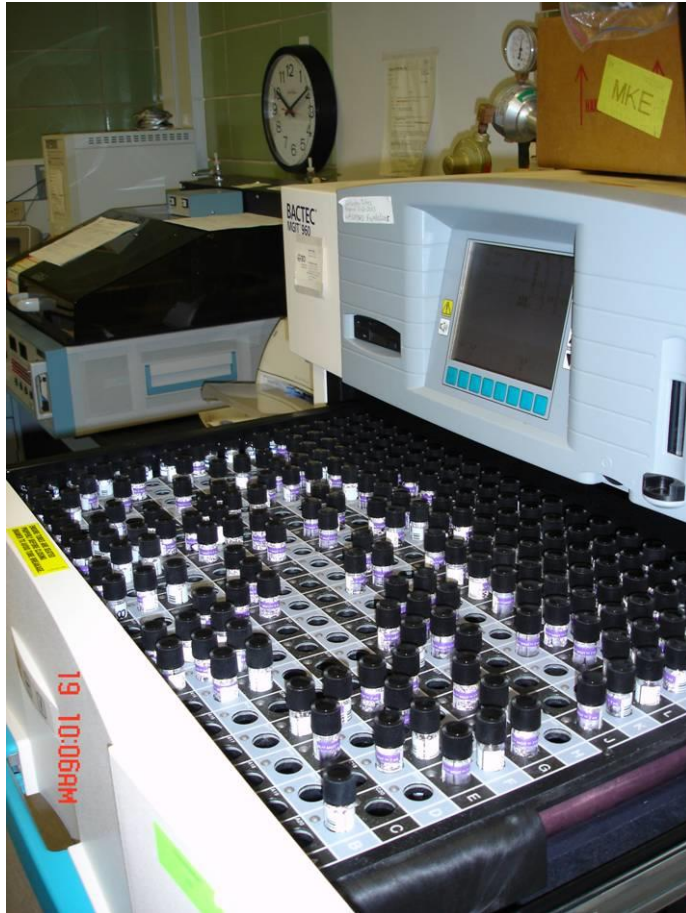


Mycobacterial Culture

Media	Incubation
Broth “MGIT” tube— <u>M</u> ycobacteria <u>G</u> rowth <u>I</u> ndicator <u>T</u> ube	<ul style="list-style-type: none">• In automated instrument, read hourly for 42 days• O₂ consumption detected through fluorescent pad
Solid Middlebrook 7H11 plate (only on non-respiratory and known TB patients)	<ul style="list-style-type: none">• Visually inspected once per week for 6 weeks

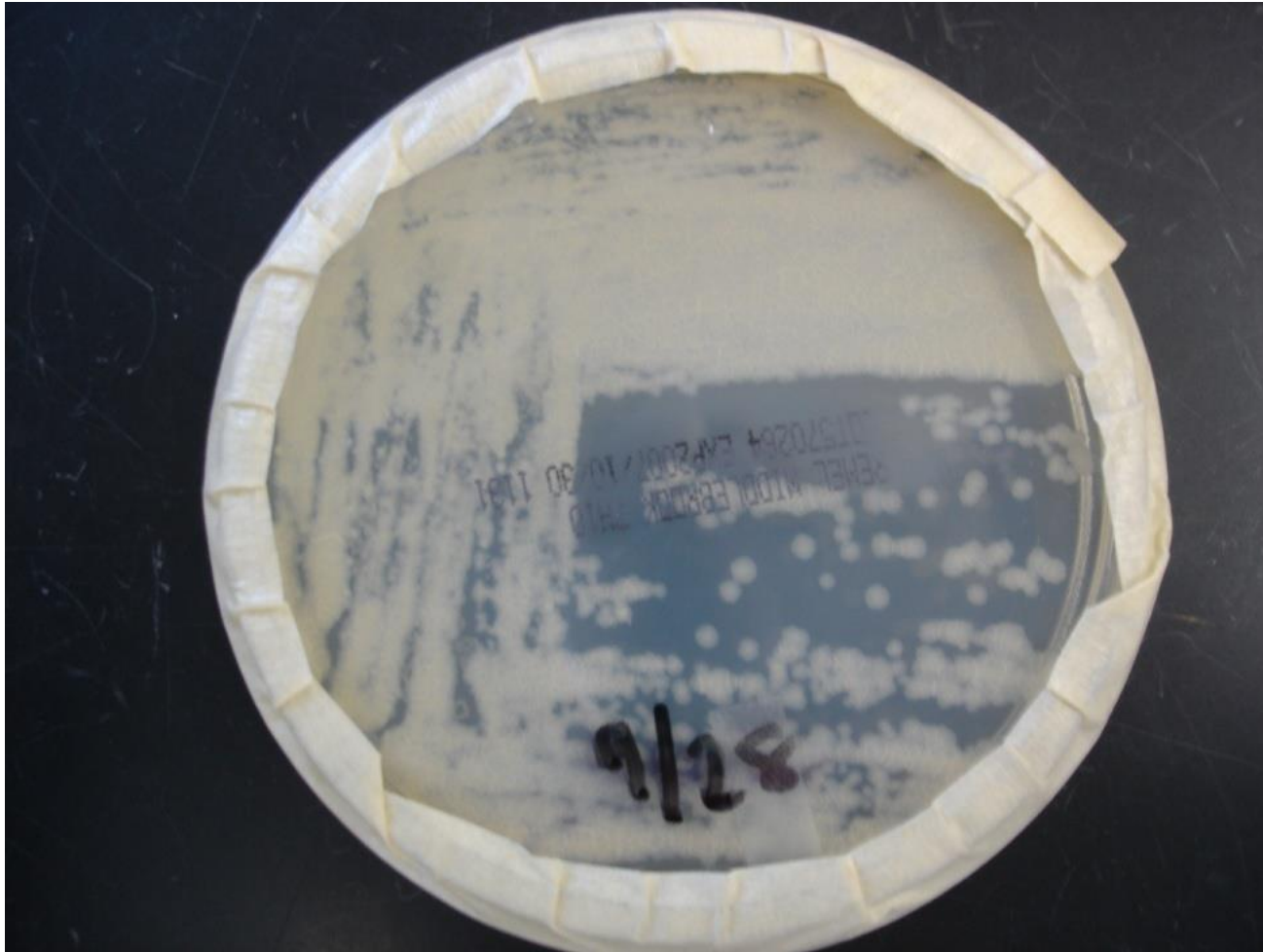


Bactec MGIT 960





7H11 Plate





Mycobacteria ID Methods at WSLH

Method	Benefits	Limitations
TB or MAC PCR	<ul style="list-style-type: none">• ID from scant growth• Rapid ID for >60% of new positive MGIT tubes	<ul style="list-style-type: none">• Can only ID MTBC and <i>M. avium</i> complex
MALDI-TOF	<ul style="list-style-type: none">• Good identification from solid media• Good ID of rapid growers	<ul style="list-style-type: none">• Need good, pure growth• Extraction method• Poor ID from positive MGIT broth
DNA Sequencing	<ul style="list-style-type: none">• “Gold standard”, good ID to species level	<ul style="list-style-type: none">• Labor intensive• Slow

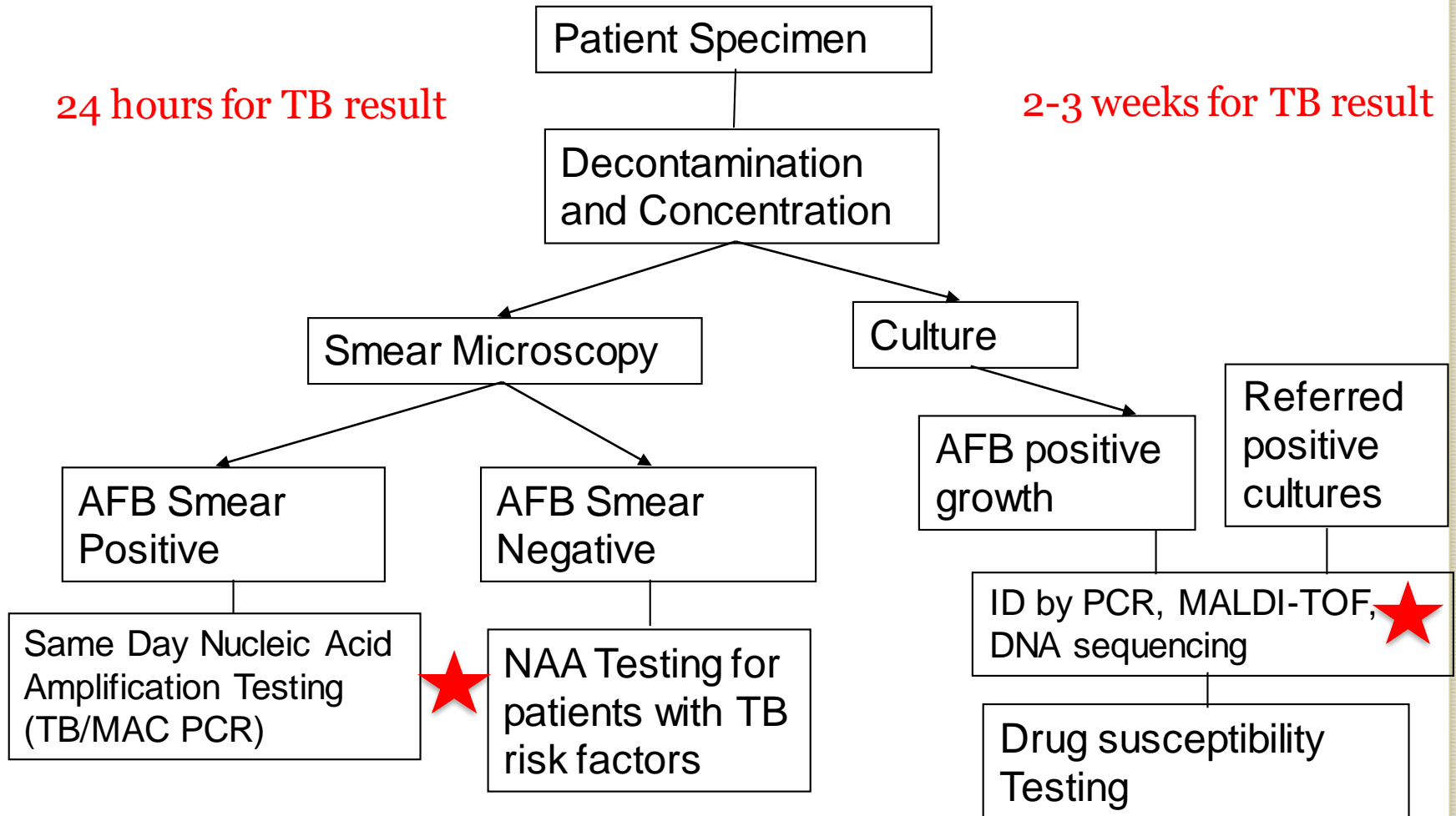


Significance of MTBC culture results

- 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





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

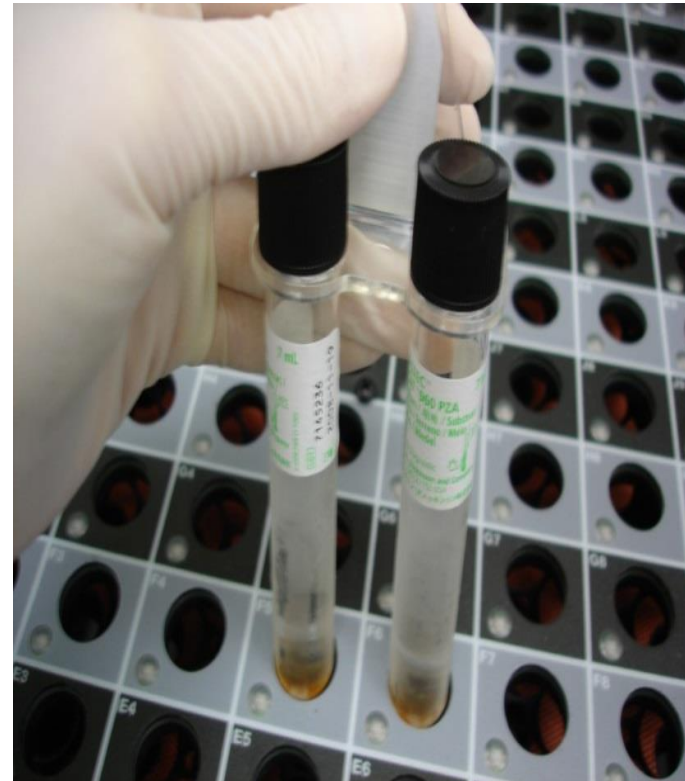


First Line Drugs

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



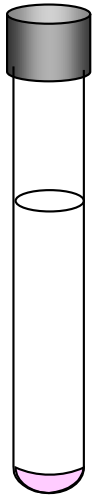


MGIT Method

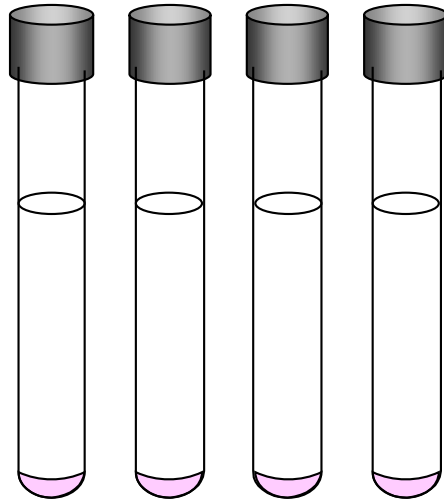
Organism suspension
added to drug-free
tube: **diluted**

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

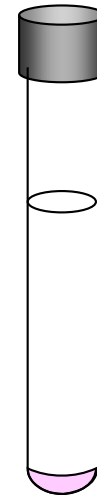
Organism suspension added to
drug-containing tubes:
undiluted



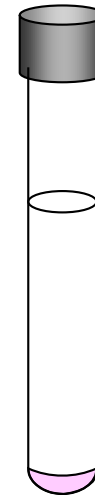
IIRE
Growth
Control



INH INH RMP EMB
low high



PZA



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

Result	Interpretation
Susceptible	Strain is likely to show responsiveness to the drug
Resistant	Strain is unlikely to show responsiveness to the drug
Indeterminate	Test is of no help in prediction of responsiveness to the drug

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

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



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

Pour Sample Reagent into sample tube.

Incubate for 15 minutes at room temperature.

(Acceptable sample types: unprocessed sputum or sediment from concentrated specimen.)



1

Pipette diluted sample into cartridge.



2

Insert cartridge and start assay.



3

TOTAL HANDS-ON TIME = 2 MINUTES



GeneXpert Result Interpretation

Result	Interpretation
MTB DETECTED; Rif Resistance DETECTED	Likely Resistant to Rifampin
MTB DETECTED; Rif Resistance NOT DETECTED	Likely Susceptible to Rifampin

If RIF resistance mutation is detected, specimen is sent to CDC for full MDDR panel and 2nd line drug agar proportion testing

CDC MDDR Result Interpretation



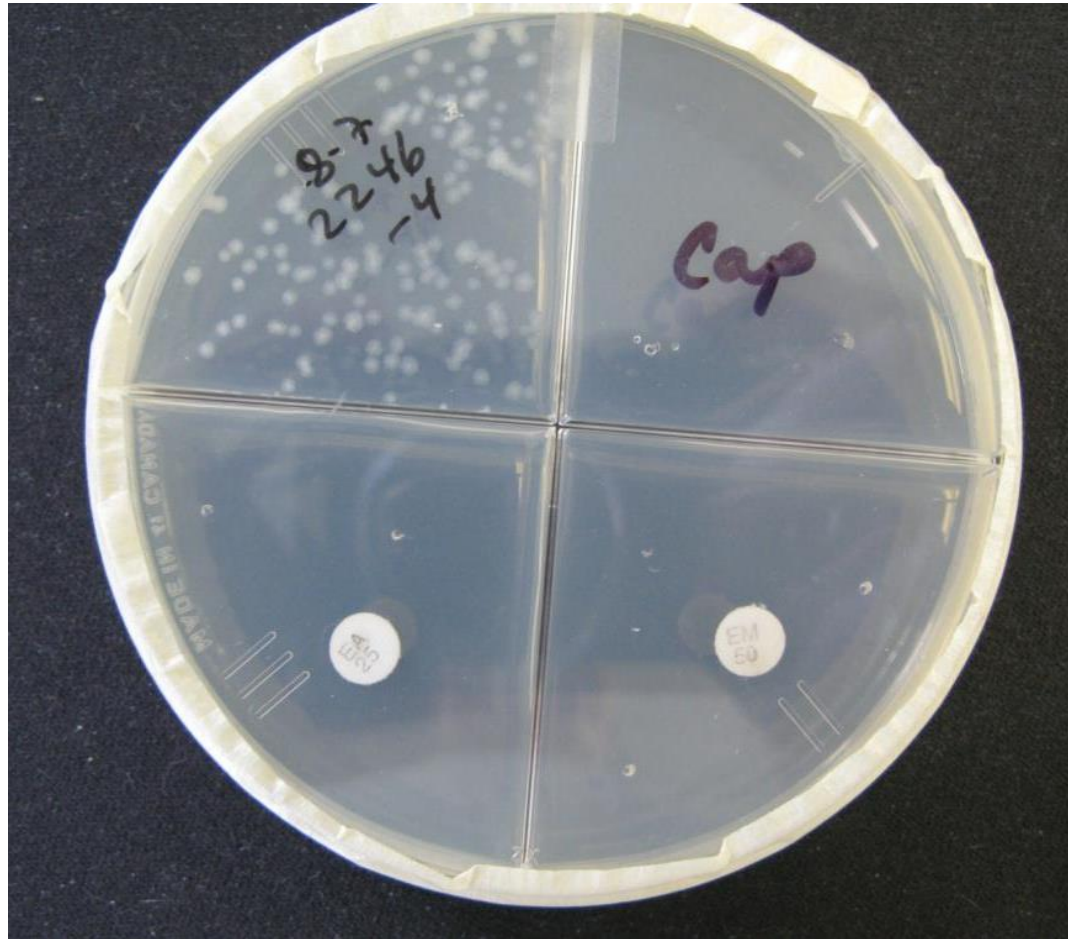
**Results for Molecular Detection of Drug Resistance (Sanger Sequencing, complete panel);
Conventional Drug Susceptibility Test in progress.**

Locus (region) examined*	Result	Interpretation (based on in-house evaluation of 550 clinical isolates)
rpoB (RRDR)	Mutation: TCG>TGG; Ser531Trp	Rifampin resistant. (100% of isolates in our in-house evaluation of 550 clinical isolates with this mutation are RMP-R.)
inhA (promoter)	No mutation	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.)
katG (Ser315 codon)	No mutation	
embB (Met306,Gly406)	No mutation	Cannot rule out ethambutol resistance. (79% of EMB-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at this locus.)
pncA (promoter, coding region)	No mutation	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.)
gyrA (QRDR)	No mutation	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.)
rrs (1400 region)	No mutation	Cannot rule out resistance to injectable drugs (kanamycin, capreomycin, amikacin). (In our in-house evaluation of 550 clinical isolates: <ul style="list-style-type: none"> • 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 eis locus; • 55% of CAP-R isolates have a mutation in either the rrs locus or the tlyA locus.)
eis (promoter)	No mutation	
tlyA (entire ORF)	No mutation	

*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 35-37C**



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:

	Percent Resistance	Interpretation		Percent Resistance	Interpretation
Isoniazid 0.2 ug/ml	0%	S	Kanamycin 5.0 ug/ml	0%	S
Isoniazid 1.0 ug/ml	0%	S	Ethionamide 10.0 ug/ml	0%	S
Isoniazid 5.0 ug/ml	0%	S	Capreomycin 10.0 ug/ml	0%	S
Rifampin 1.0 ug/ml	100%	R	PAS 2.0 ug/ml	0%	S
Ethambutol 5.0 ug/ml	0%	S	Ofloxacin 2.0 ug/ml	0%	S
Streptomycin 2.0 ug/ml	0%	S	Amikacin 4.0 ug/ml	0%	S
Streptomycin 10.0 ug/ml	0%	S			
Rifabutin 2.0 ug/ml	50%	R			
Ciprofloxacin 2.0 ug/ml	0%	S			

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.

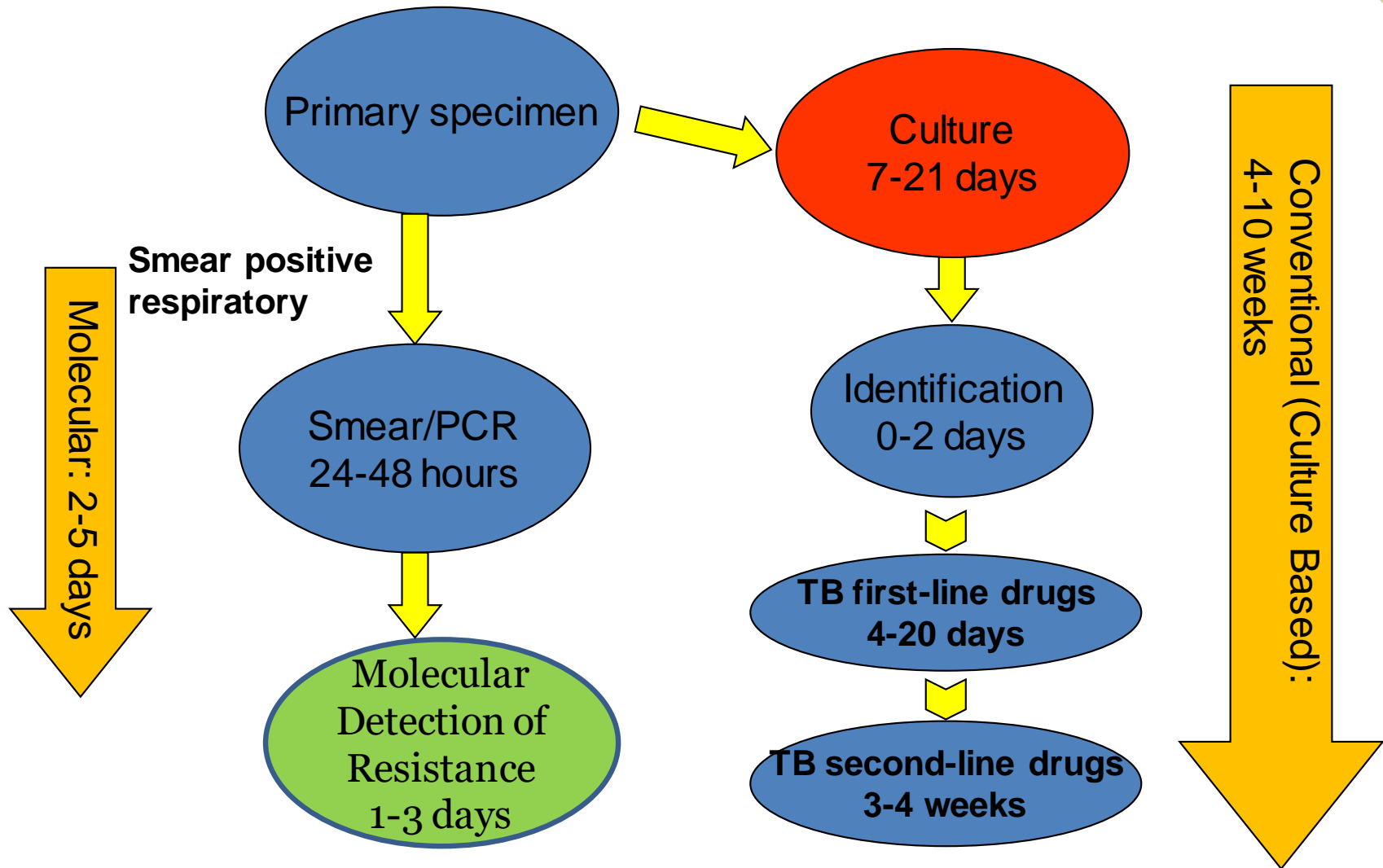


Agar Proportion Limitations

- 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





Acknowledgements

Dave Warshauer, PhD

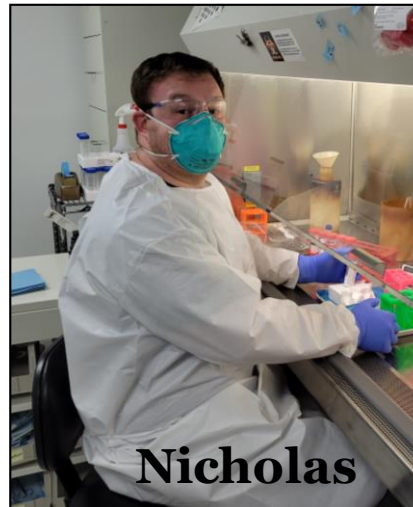
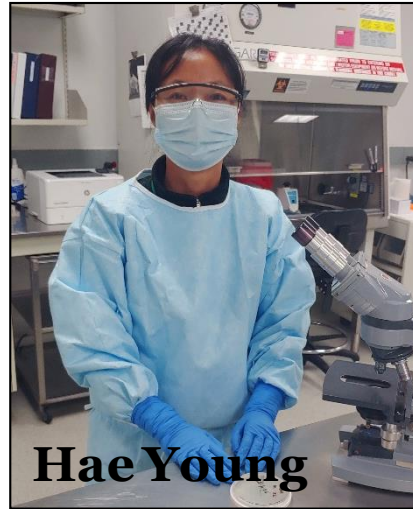
Julie Tans-Kersten

Laura Louison, MLS(ASCP)

The WSLH TB Laboratory Staff



WSLH TB Laboratory Team





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