TB/MAC PCR Specimen Types

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

*Smear negative requires approval from the TB program for fee-exempt testing

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.
- 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 NAAT Criteria (fee exempt)

- 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 (for pulmonary disease)

Wisconsin NAAT 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
"*Mycobacterium tuberculosis* complex DNA detected"	Positive for TB
"*Mycobacterium avium* complex DNA detected"	Positive for MAC
"No *Mycobacterium tuberculosis* complex DNA detected"	Negative for TB
"No *Mycobacterium avium* complex DNA detected"	Negative for MAC
"Inhibitory substances that prevent nucleic acid amplification were detected"	Test is of no diagnostic help

Advantages of NAAT

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

WSLH TB/MAC PCR Goal

Identify smear positive TB patients within 48 hours (HP 2020 Goal—Target: 77%))

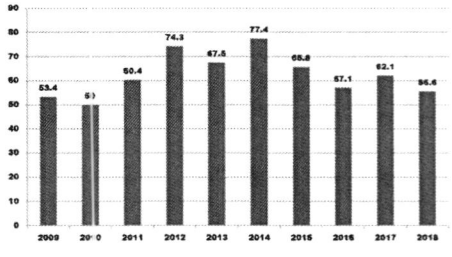
- Respiratory isolation
- Start therapy

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 (HP2020 Goal) by NAAT in Wisconsin



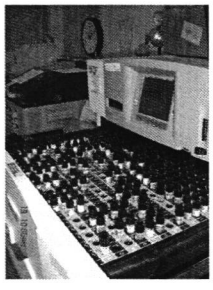
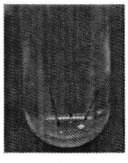
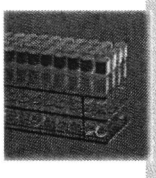
Mycobacterial Culture

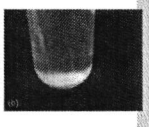
- Used to detect viable mycobacteria from patient specimens
- Most sensitive method for mycobacterial detection ("Gold Standard")
  - ~ 10 viable bacilli/ml for culture compared to >5000 bacilli/ml for microscopy
- Slowest method
  - Average time to detection for MTBC = 15 days
  - Range for detection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

Mycobacterial Culture

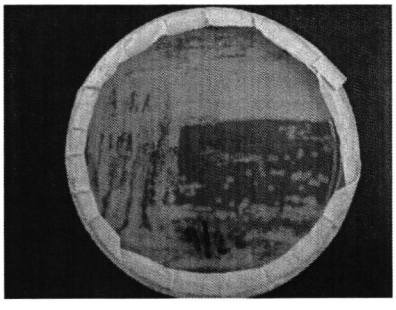
Media	Incubation
Broth "MGIT" tube—Mycobacteria Growth Indicator Tube	• In automated instrument, read hourly for 42 days • O2 consumption detected through fluorescent pad
Solid Middlebrook 7H11 plate (only on non-respiratory and known TB patients)	• Visually inspected once per week for 6 weeks

Bactec MGIT 960



7H11 Plate



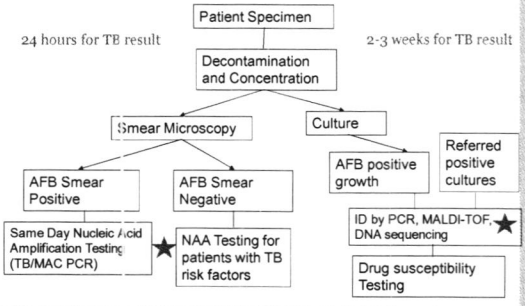
Mycobacteria ID Methods at WSLH

Method	Benefits	Limitations
TB or MAC PCR	• ID from scant growth • Rapid ID for >60% of new positive MGIT tubes	• Can only ID MTBC and *M. avium* complex
MALDI-TOF	• Good identification from solid media • Good ID of rapid growers	• Need good, pure growth • Extraction method • Poor ID from positive MGIT broth
DNA Sequencing	• "Gold standard", good ID to species level	• 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 (do not need to order)
- 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 DST

- WSLH is the only laboratory in the state that performs culture-based TB drug susceptibility testing (DST)
- 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.

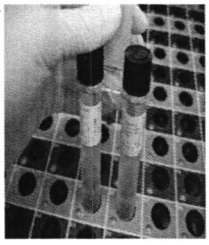
Culture-based DST

A.K.A. phenotypic or conventional DST

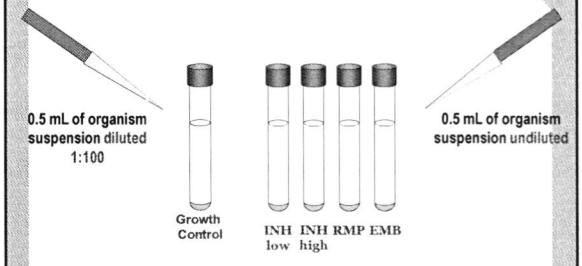
Principle: Incubate a standardized concentration of M. tuberculosis isolate with a known concentration ("critical concentration") of a drug and observe for growth or inhibition of growth

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



Critical Concentration

Lowest concentration that inhibits 95% of "wild strains" of MTBC that have never been exposed to the drugs.

- Does not inhibit strains isolated from patients failing to respond to therapy
- Ideally the critical concentration is the lowest concentration of a drug that discriminates between susceptible and resistant strains of MTBC
  - Inhibits growth of all susceptible strains

  AND
  - Allows growth of all resistant strains

Critical Concentration

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

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 DST

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 mutations

Examples of Molecular DST

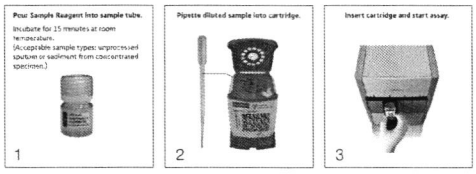
	Method		
	Cepheid GeneXpert® MTB/RIF	Sanger Sequencing	Pyrosequencing
Genetic loci	*rpoB* (for RMP)	Varies but can include *rpoB*, *inhA*, *katG*, *aphC*, *embB* (EMB), *pncA* (PZA), *gyrA* (FQ), and *rrs* (injectables)	Varies but can include *rpoB*, *inhA*, *katG*, *aphC*, *gyrA*, and *rrs*
Format	Semi-automated 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 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 potential MDR TB

GeneXpert MTB/RIF

Workflow: Self contained cartridge – just add sample



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. (88% 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. (80% 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: • 91% of AMK-R isolates have a mutation in the rrs locus; • 67% of KAN-R isolates have a mutation in either the rrs locus or the eis locus; • 59% 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.

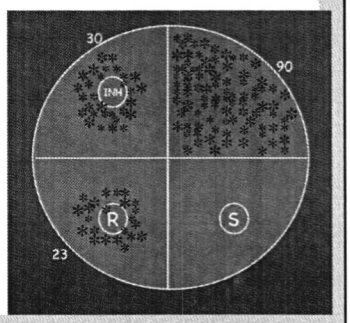
Agar Proportion (AP) Method

Growth control (no drug) quadrant
90 colonies

INH quadrant
30 colonies
INH 30 / 90 = 33% resistant

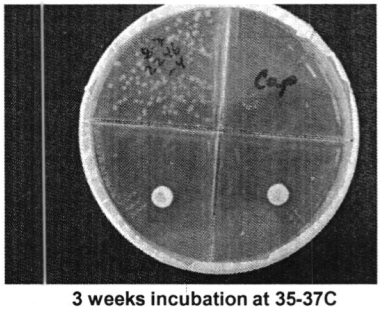
RMP (R) quadrant
23 colonies
RMP 23 / 90 = 25% resistant

Streptomycin (S) quadrant
No colonies = susceptible



Curry Center. 2008. Drug-Resistant Tuberculosis: A Survival Guide for Clinicians

Agar Proportion Method



3 weeks incubation at 35-37C

AP 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!)

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			

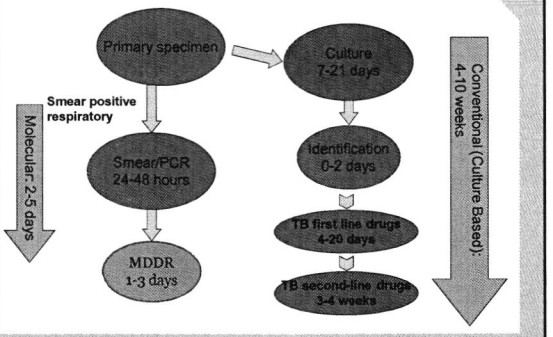
Susceptibility Testing Method: MGIT 960

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.

Turn around Times



Acknowledgements

Dave Warshauer, PhD
Julie Tans-Kersten
The WSLH TB Laboratory Staff

WSLH TB Laboratory Team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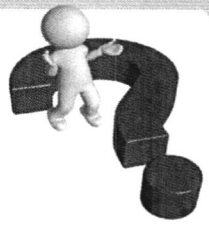
Questions?



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