



WISCONSIN DEPARTMENT
of HEALTH SERVICES

Tuberculosis Disease: Laboratory Results for Nurses

Andrea Liptack, MSN, RN
TB Nurse Consultant
Wisconsin TB Program

Accreditation Statement



Accreditation Statement

In support of improving patient care, Mayo Clinic College of Medicine and Science is jointly accredited by the Accreditation Council for Continuing Medical Education (ACCME), the Accreditation Council for Pharmacy Education (ACPE), and the American Nurses Credentialing Center (ANCC) to provide continuing education for the healthcare team.

Credit Statement(s):

AMA

Mayo Clinic College of Medicine and Science designates this live activity for a maximum of 18.25 *AMA PRA Category 1 Credits™*. Physicians should claim only the credit commensurate with the extent of their participation in the activity.



ACPE

Mayo Clinic College of Medicine and Science designates this educational activity for a maximum of 18.25 ACPE Knowledge-based contact hours. Participants should claim only the credit commensurate with the extent of their participation in the activity.

UAN Number: UAN JA0000238-0000-26-089-L99-P.

ANCC

Mayo Clinic College of Medicine and Science designates this activity for a maximum of 18.25 ANCC contact hours. Nurses should claim only the credit commensurate with the extent of their participation in the activity.



IPCE CREDIT™

This activity was planned by and for the healthcare team, and learners will receive 18.25 Interprofessional Continuing Education (IPCE) credit for learning and change.

Other Healthcare Professionals:

A record of attendance will be provided to all registrants for requesting credits in accordance with state nursing boards, specialty societies or other professional associations.

For disclosure information regarding Mayo Clinic School of Continuous Professional Development accreditation review committee member(s) and staff, please go here to the course accreditation page.

Available Credit

- 18.25 ACPE,
- 18.25 *AMA PRA Category 1 Credit™*
- 18.25 ANCC
- 18.25 Attendance
- 18.25 IPCE



Agenda

- Definitions, resources, background
- Most common active TB disease labs:
 - AFB smear microscopy
 - TB PCR/ NAAT
 - Culture
 - Susceptibility
- Other related labs you may see:
 - NTM cultures
 - IGRAs



Definitions of Abbreviations Used in This Presentation

AFB

Acid Fast Bacillus

MTBC

Mycobacterium tuberculosis complex

DST

Drug Susceptibility Testing

NTM

Non-tuberculous Mycobacteria

MAC

Mycobacterium avium complex

WSLH

Wisconsin State Laboratory of Hygiene

PCR and NAAT

Polymerase Chain Reaction

Nucleic Acid Amplification Testing

IGRA

Interferon Gamma Release Assay



Resources

- [TB Laboratory, Webinar Series, video recording and PDF slide set](#)
- [TB Lab Results Reference for Nurses](#)
- [WSLH Test Reference Manual](#)
- [Sputum collection instructions](#) (video by PHMDC)
- [Sputum collection instructions](#) (PDF publication)
- [Understanding TB Laboratory Testing for Public Health Nurses](#), APHL





***Mycobacterium tuberculosis* Complex**

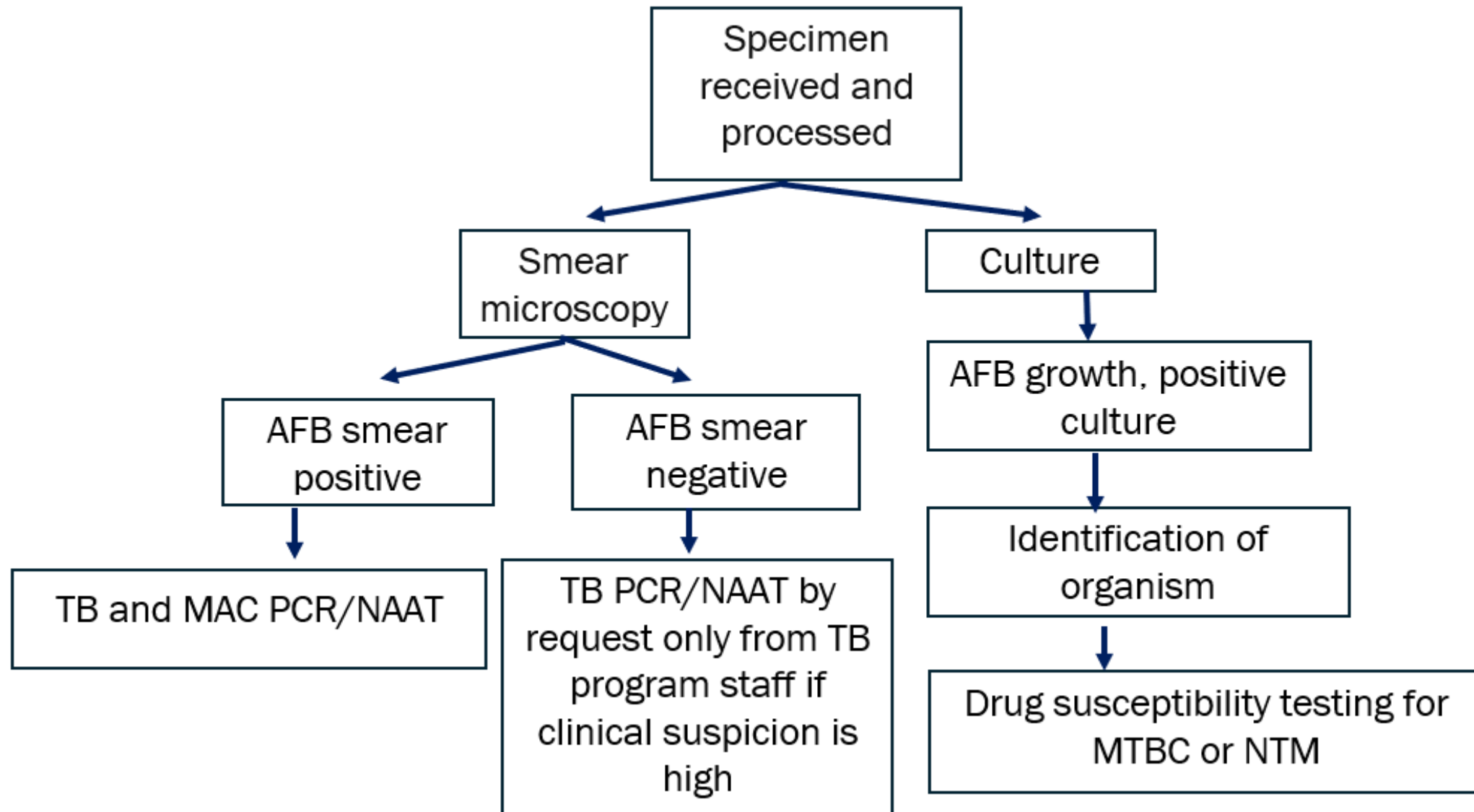
- Rod shaped, acid-fast, aerobic, slow-growing, intracellular pathogenic bacteria

- MTBC includes:

M. tuberculosis, M. bovis, M. africanum, M. microti, M. caprae, M. pinnipedii, M. canettii.



Testing Algorithm for WSLH





Smear Microscopy



AFB Smear

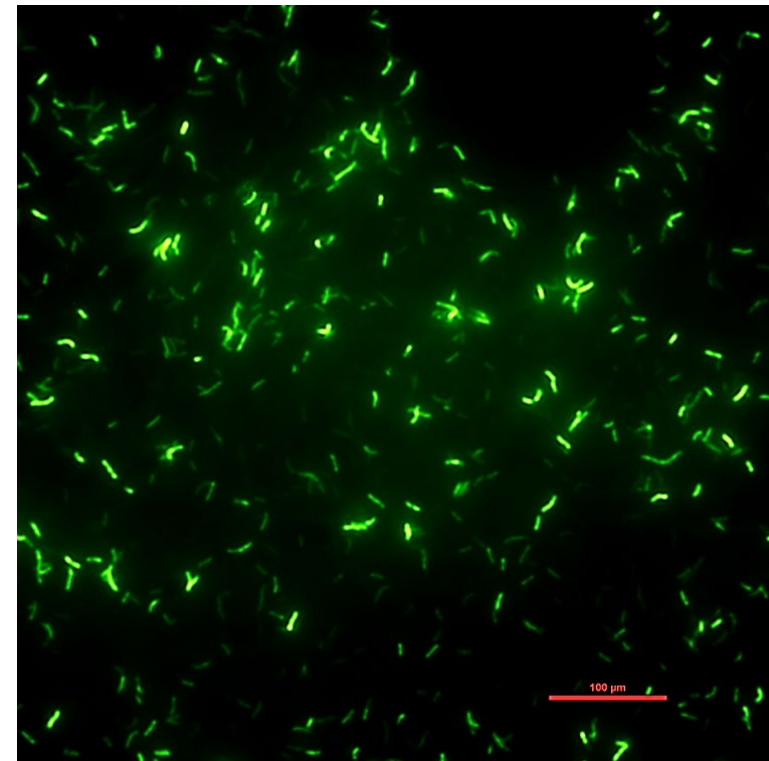


Smear Microscopy

Positive AFB Smear

This tells us the laboratorian saw AFB through the microscope. The waxy coating prevents the destaining agent from washing the stain off the TB.

What you might see through the microscope



Laboratory photos used with permission from Nate Simon, WSLH



Smear Microscopy

- You can remember this by thinking that the lab worker is “smearing” the specimen onto a glass slide to look at in their microscope.
- Because of this type of bacteria’s thick waxy coating, other organisms besides *Mycobacterium tuberculosis* may cause an AFB smear positive specimen.
- All this test type tells us is that there are bacteria in the specimen on this slide on the microscope that look like they may be TB.



Smear Microscopy

- This test is rapid and inexpensive for fast *indication* of TB disease but **is not confirmatory**.
- Most AFB smear positive test results in Wisconsin are for people with an NTM infection or with non-pathogenic (aren't causing illness) NTM in their respiratory tract.



Smear Microscopy, Result Grading

WSLH Result	Graded Scale	Qualitative Scale	Interpretation, <i>if infectious MTBC</i>
Negative	Negative	Negative	Potentially infectious
Rare (1-9 AFB per 100 fields)	1+	Positive	Likely low-level infectiousness
Few (1-9 AFB per 10 fields)	2+	Positive	Likely moderately infectious
Few (1-9 AFB per field)	3+	Positive	Likely moderately infectious
Many (>9 AFB per field)	4+	Positive	Likely highly infectious



Smear Microscopy Example



WEDSS
Electronic Disease Surveillance

SENDING LAB
Wisconsin State Laboratory of Hygiene (52D0661989)
2601 Agriculture Dr
Madison, WI 53718
(800) 862-1013

ACCESSION # 25MM0	PLACER ORDER # 25MM0
SPECIMEN COLLECTED DATE 05/05/2025 09:23	SPECIMEN RECEIVED DATE 05/06/2025 08:53
SPECIMEN SOURCE Sputum	RESULT
SPECIMEN NOTES	
RELEVANT CLINICAL INFORMATION	REASON FOR STUDY
REPORTED 05/06/2025 14:55	RESULTED 05/06/2025 14:54

Order Status: Final

PATIENT NAME			
PATIENT ID #	D.O.B	AGE	GENDER
ETHNICITY	RACE		
PATIENT ADDRESS			
PATIENT PHONE #		WORK PHONE #	
ORDERING FACILITY MILWAUKEE HEALTH DEPT/TB		REFERRING PHYSICIAN	

TEST ORDERED: MYCOBACTERIOLOGY STAIN

Culture report to follow

RESULT	VALUE	UNITS	REFERENCE RANGES	ABNORMAL	RESULT STATUS
MYCOBACTERIA SMEAR	Many (>9 acid fast bacilli per oil immersion field)			Abnormal	Final
Acid fast Stn XXX	Many (>9 acid fast bacilli per oil immersion field)			Abnormal	Final

Performing Organization: Wisconsin State Laboratory of Hygiene
Performing Organization Address: MADISON, WI



Smear Microscopy, Limitations



Is this TB?

Maybe. Could be another fluorescing bacilli such as NTM (most common cause of an AFB smear positive test) and some legionella species (limited specificity).



Smear Microscopy, Limitations



Is this TB?

Maybe. Could be another fluorescing bacilli such as NTM (most common cause of an AFB smear positive test) and some legionella species (limited specificity).

Is the TB alive?

Maybe. Could be nonviable TB or NTM. This is increasingly likely if the person has been on TB therapy for some time.



Smear Microscopy, Limitations



Is it TB?

Maybe. Could be another fluorescing bacilli such as NTM (most common cause of an AFB smear positive test) and some legionella species (limited specificity).

Is the TB alive?

Maybe. Could be nonviable TB or NTM. This is increasingly likely if the person has been on TB therapy for some time.

Is TB in there?

Maybe. Sputum specimen collection is one small sample of one small part of the lung during one moment in time. The person may still have TB, but no TB bacteria were seen on the AFB smear (limited sensitivity).





TB PCR/ NAAT

Rapid Diagnosis



WSLH PCR

Detects MTBC and Mycobacterium Avium Complex (MAC)

- Rapid identification of TB and MAC DNA
- Is **confirmatory** that TB DNA is present in the tested sample
- Not performed serially (once only for rapid diagnosis)



WSLH PCR

Detects MTBC and Mycobacterium Avium Complex (MAC)

Sensitivity for TB:

- Greater than 95% for AFB smear positive, culture confirmed
- 55–75% for AFB smear negative, culture confirmed



WSLH PCR

Detects MTBC and Mycobacterium Avium Complex (MAC)

- WSLH performs both tests (TB and MAC PCR) on all **newly smear positive** respiratory specimens.
Newly smear positive: no positive AFB smears or cultures in the last 12 months.
- Wisconsin TB Program needs to "approve" TB PCR on smear negative specimens for high-risk individuals.



WSLH PCR Result Interpretations

WSLH Lab Result	Interpretation
“ <i>Mycobacterium tuberculosis</i> complex DNA detected”	Positive, TB identified
“ <i>Mycobacterium avium</i> complex DNA detected”	Positive, MAC identified
“No <i>Mycobacterium tuberculosis</i> complex DNA detected”	Negative, TB not identified
“No <i>Mycobacterium avium</i> complex DNA detected”	Negative, MAC not identified
“Inhibitory substances that prevent nucleic acid amplification were detected”	Testing of no diagnostic help



TB PCR, Limitations

Is this TB?

Yes! We have identified the DNA of the organism that is causing the positive smear as being present in the tested sample. TB is **confirmed**.



TB PCR, Limitations

Is this TB?

Yes! We have identified the DNA of the organism that is causing the positive smear as being present in the tested sample. TB is **confirmed**.

Is the TB alive?

Maybe. Could be nonviable TB. This is increasingly likely if the person has been on TB therapy for some time. This still confirms a TB diagnosis, and treatment is initiated while the culture grows.



TB PCR, Limitations

Is this TB?

Yes! We have identified the DNA of the organism that is causing the positive smear as being present in the tested sample. TB is **confirmed**.

Is the TB alive?

Maybe. Could be nonviable TB. This is increasingly likely if the person has been on TB therapy for some time. This still confirms a TB diagnosis, and treatment is initiated while the culture grows.

Is TB in there?

If **positive**, yes; if **negative**, maybe. We are reliant on capturing a good specimen with lots of TB bacteria, and for this test, TB DNA must also be in the specimen at the time it was collected.





Culture



The Gold Standard of Diagnosis

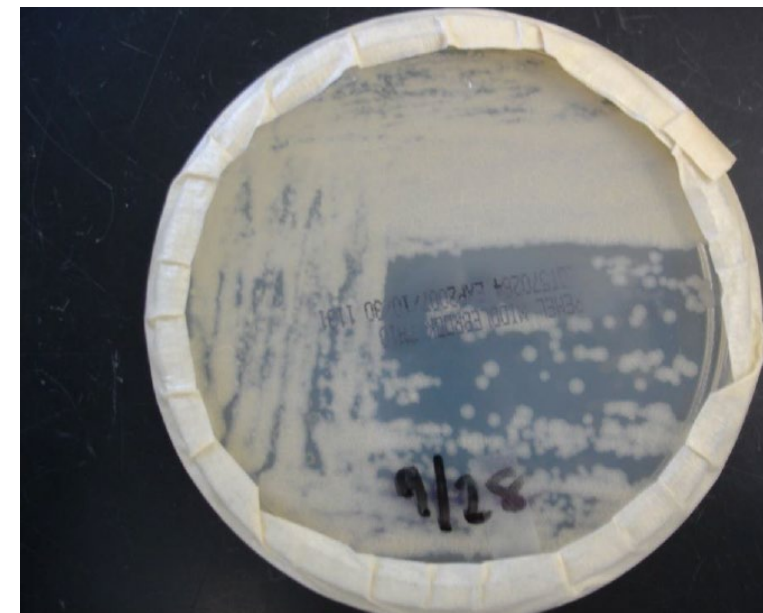


Culture

Positive MTBC Culture

This tells us that TB is alive and dividing in the growth media. Both solid and liquid growth media are used at WSLH. This is the most sensitive method of detection.

What you would see



Laboratory photos from Nate Simon, WSLH



Culture

Either liquid (broth) or solid growth media are used for the incubation period.

- **Liquid broth:** Mycobacteria Growth Indicator Tube (MGIT) is used, it is an automated instrument, it detects oxygen consumption through a florescent pad (glows and alerts lab tech if positive).
- **Solid media:** Middlebrook 7H11 plates are used for non-respiratory and specimens from people with known TB. These are visually inspected at least once per week for 6 weeks.



Culture, Example

Specimen Details

	Collected	Type	Source	Submitter ID
25MM00[REDACTED]	4/20/2025 0620	Sputum		

Mycobacteria Culture (Final result)

ID:	25MM00[REDACTED]	Type/Src:	Sputum
-----	------------------	-----------	--------

Mycobacteria Culture Result

Mycobacterium tuberculosis complex (A)

Comments:

The M. tuberculosis complex includes the species M. tuberculosis, M. bovis, M. bovis BCG, M. africanum and M. microti. 98% of the isolates in the M. tuberculosis complex identified at the State Laboratory of Hygiene are M. tuberculosis.

WSLH automatically performs culture-based drug susceptibility testing on an initial isolate from each TB patient.

A laboratory developed real-time PCR assay was used for identification from culture growth. This test was developed and its performance characteristics determined by the Wisconsin State Laboratory of Hygiene, a Clinical Laboratory Improvement Amendments (CLIA) certified, high complexity clinical laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration.

Cultures are incubated for a total of six weeks. Further reports will be issued if additional Mycobacteria species are detected.



Culture, Limitations

Is this TB?

Yes! We have identified the organism growing and dividing in the growth media is MTBC. TB is **confirmed**. Isolation of MTBC *almost always* signifies disease.



Culture, Limitations

Is this TB?

Yes! We have identified the organism growing and dividing in the growth media is MTBC. TB is **confirmed**. Isolation of MTBC *almost always* signifies disease.

Is the TB alive?

Yes! Since TB grew in culture, it is alive and viable. If the TB has been exposed to TB drugs for more than a few days, it is likely already experiencing changes that make it harder to grow and less infectious.



Culture, Limitations

Is this TB?

Yes! We have identified the organism growing and dividing in the growth media is MTBC. TB is **confirmed**. Isolation of MTBC *almost always* signifies disease.

Is the TB alive?

Yes! Since TB grew in culture, it is alive and viable. If the TB has been exposed to TB drugs for more than a few days, it is likely already experiencing changes that make it harder to grow and less infectious.

Is TB in there?

Yes! Culture is the *gold standard* for TB diagnosis. It is the **slowest** method. It can take up to 42 days to wait to see if it is growing and dividing in the liquid and solid media (not good for rapid diagnosis).





Susceptibility



Phenotypic and Molecular DST



DST, Phenotypic and Molecular

- Performed for all newly culture-confirmed specimens
- Guides treatment plan and regimen such as medication changes and duration of therapy
- Allows for appropriate treatment of contacts with appropriate regimens (susceptible to TB drugs)



Phenotypic vs. Molecular DST

Phenotypic

Incubates a known concentration of MTBC in a known concentration of drug to observe if it grows or is inhibited from growing.

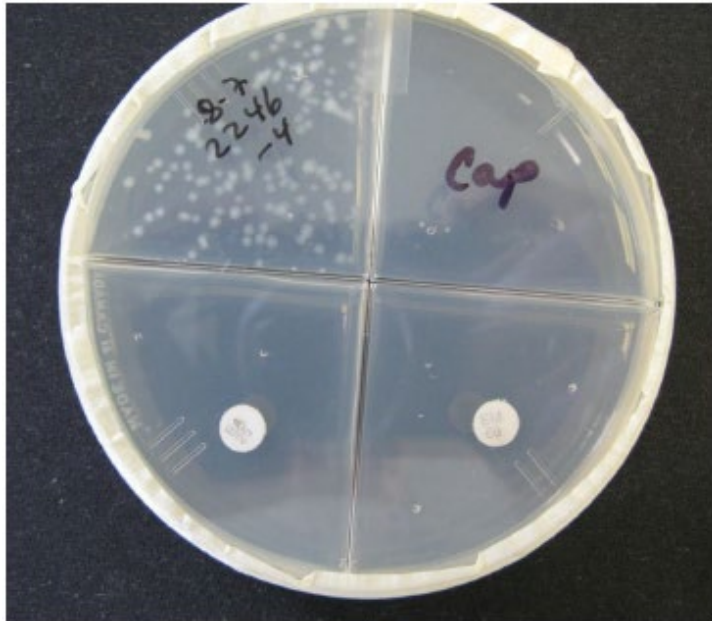
Molecular (or genotypic)

Uses DNA amplification and detection to identify gene mutations that are known to confer resistance to TB drugs.



Phenotypic vs. Molecular DST

Phenotypic DST, Agar



Laboratory photos used with permission
from Nate Simon, WSLH

Molecular DST, GeneXpert ®

Type/Src:	Aspirate/Neck	
Result		Units
MTB complex DNA detected.	(A)	
Rifampin gene mutation detected.	(A)	
Possible rifampin resistance.		



DST, Phenotypic

- Usually takes 2–4 weeks after a positive culture
- Phenotypic DST provides results for first line TB drugs: isoniazid (INH), rifampin (RIF), ethambutol (EMB,) and pyrazinamide (PZA)



DST, Phenotypic Result Interpretation

Result	Interpretation
Susceptible	TB strain is likely to show responsiveness to drug (TB will not grow and divide well or at all).
Resistant	TB strain is unlikely to show responsiveness to drug (TB will probably continue to grow and divide, evading the effect of the drug).



DST, Phenotypic Example

Susceptibility	
Mycobacterium tuberculosis complex Iso1	
MYCOBACTERIAL SUSCEPTIBILITY	
Isoniazid (0.1 mcg/mL)	Susceptible
Isoniazid (100 mcg/mL)	Susceptible

Results apply only to sample tested.
A=Abnormal; AA=Panic; H=High, L=Low

Report ID: [REDACTED]
Page: 1 of 2

Report may only be reproduced in its entirety

05-12-2025 11:30 AM UW Madison → WISCONSIN DIVISION OF PUBLIC HEALTH pg 3 of 5



Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN-MADISON

Wisconsin State Laboratory of Hygiene
Communicable Disease Division
2601 Agriculture Dr, PO Box 7904
Madison, WI 53718
(800) 862-1013

Laboratory Report

James J. Schauer, Ph.D., PE, MBA, Director
Errin C. Rider, Ph.D., D(ABMM), M(ASCP)CM,
Director of Clinical Laboratory Services

Rifampin (1 mcg/mL)	Susceptible
Ethambutol (5 mcg/mL)	Susceptible
Pyrazinamide (100 mcg/mL)	Susceptible

Iso1 - Mycobacterium tuberculosis complex:
This is a final report.

Reported results were determined using the MGIT 960 Broth System.
Antibiotic values (mcg/ml) shown are the Equivalent Reference Method concentrations.

PAGE 3/5 REC'D 5/12/2025 11:30:33 AM TC



DST, Molecular

If TB PCR positive, rapid molecular testing can be performed

- Cepheid GeneXpert® MTB/RIF testing is performed.
- Predicts if the TB bacteria will be susceptible to **rifampin** (fastest predictor of MDR-TB).
- Detects mutations in the *rpoB* genetic loci in 1–2 business days. A mutation in the *rpoB* gene loci predicts rifampin resistance.



DST, Molecular

Other considerations

- May detect "silent" mutations or mutations of unknown significance— ones that do not confer resistance. Phenotypic DST confirms resistance.
- GeneXpert® only predicts resistance to rifampin.
- Performed on primary **respiratory specimens**— you need a culture to grow to perform this testing on non-respiratory specimens.



DST, Molecular Result Interpretation

Result	Interpretation
MTB DETECTED; Rif Resistance DETECTED	Likely TB is resistant to rifampin (rifampin likely can not be used as an effective drug in the regimen).
MTB DETECTED; Rif Resistance NOT DETECTED	Likely TB is susceptible to rifampin (rifampin can be used as an effective drug in the regimen).



DST, Molecular Example

TEST ORDERED: CEPH ID GENEXPERT MTB/RIF

This real-time PCR assay simultaneously detects the presence of both *M. tuberculosis* complex DNA and genetic mutations associated with rifampin resistance from raw (induced or expectorated) or concentrated sputum sediments.

The assay is FDA cleared only for sputum specimens from patients for whom there is clinical suspicion of tuberculosis and who have received no antituberculosis therapy, or less than three days of therapy. It has not been approved for specimens from pediatric patients or to be used as a test of cure.

RESULT	VALUE	UNITS	REFERENCE RANGES	ABNORMAL	RESULT STATUS
MYCOBACTERIUM TUBERCULOSIS COMPLEX TARGET GENE	MTB complex DNA detected.			Abnormal	Final
M TB rpoB XXX QI PCR	MTB complex DNA detected.			Abnormal	Final
RESULT	VALUE	UNITS	REFERENCE RANGES	ABNORMAL	RESULT STATUS
RPOB GENE MUTATION	No rifampin gene mutation detected.				Final
M TB rpoB XXX QI PCR	No rifampin gene mutation detected.				Final
RESULT	VALUE	UNITS	REFERENCE RANGES	ABNORMAL	RESULT STATUS
RIFAMPIN INTERPRETATION	Likely rifampin susceptible.				Final
M TB rpoB XXX QI PCR	Likely rifampin susceptible.				Final

Performing Organization: Wisconsin State Laboratory of Hygiene
Performing Organization Address: MADISON, WI



Public Health / International Submitter IDs

Patient ID: [redacted]
Specimen ID: [redacted]

Alt. Patient ID:
Alt. Specimen ID:

CDC Specimen ID: [redacted] CDC Unique ID: [redacted] CDC Local Aliquot ID: [redacted]

Rifampin (RIF)	<u>Result</u>	<u>Interpretation</u>
RIF interpretation		RIF susceptible
rpoB	No mutation	
Isoniazid (INH)	<u>Result</u>	<u>Interpretation</u>
INH interpretation		Cannot rule out INH resistance.
inhA	No mutation	
fabG1	No mutation	
katG	No mutation	
Ethambutol (EMB)	<u>Result</u>	<u>Interpretation</u>
EMB interpretation		Cannot rule out EMB resistance.
embB	No mutation	
Pyrazinamide (PZA)	<u>Result</u>	<u>Interpretation</u>
PZA interpretation		Cannot rule out PZA resistance.
pncA	No mutation	
Fluoroquinolones (FQ)	<u>Result</u>	<u>Interpretation</u>
FQ interpretation		Cannot rule out FQ resistance.
gyrA	No mutation	
gyrB	No mutation	

DST, Molecular, CDC Expanded Panel

Molecular detection of drug resistance (MDDR) can be done at CDC. This provides an expanded result panel for first- and second-line TB drugs. This testing will be requested by the TB program or automatically sent out based on first line resistance results.





Other TB Labs



NTM and IGRA



NTM PCR and Cultures

NTM are commonly found in soil and water

Mycobacterium peregrinum (A)

Mycobacterium avium complex (A)

Comments:

SUPPLEMENTAL REPORT: 4/23/2025

Please note that *M. avium* complex has been isolated in addition to *M. peregrinum* reported on 4/22/2025.

RESULT	VALUE
MYCOBACTERIA CULTURE RESULT	MYCOBACTERIUM AVIUM COMPLEX
Mycobacterium XXX Cult	Mycobacterium, avium-intracellulare group
Mycobacterium avium complex	

Can cause similar symptoms to TB disease in those with underlying lung disease or immune compromise. Are not contagious from person to person. May require treatment with antibiotics. MAC is the most common NTM.



IGRAs

QuantiFERON®-TB Gold (QFT)

Detects CD4 and CD8 T cell interferon-gamma response to MTB antigens

Four numeric results. Positive, negative, or indeterminate

T-SPOT ®.TB

Detects memory T cells that produce interferon-gamma in response to MTB antigens

Results as number of “spots”. Positive, negative, or borderline



QFT, Numeric Vales

Mitogen	Positive Control: Ensures the sample can respond to interferon gamma (baseline immune response)
Nil	Negative control: Adjusts for background interferon gamma
TB1	Primarily detects CD4 T cell response
TB2	Optimized for CD4 and CD8 T cell detection



IGRA, Result Interpretation

IGRA Test Result	QuantiFERON	T-Spot	Notes
Positive	TB-Nil is higher than or equal to 0.35 IU/mL	8 spots or more	Infection is likely in individuals with risk factors. Consider retesting in low- or no-risk individuals.
Negative	TB-Nil is lower than 0.35 IU/mL	4 spots or less	Infection is unlikely.
Indeterminate or invalid	High nil value or low mitogen value	High nil value or low mitogen value	Collect another specimen for retesting since these results cannot be interpreted. This occurs if controls do not perform as expected.
Borderline (not clear)	Not applicable	5, 6 or 7 spots	Uncertain likelihood of TB infection. Collect another specimen for retesting.



QFT, Example

TEST ORDERED: QUANTIFEROB TB PLUS

RESULT	VALUE	UNITS
QUANTIFEROB PLUS INTERPRETATION	Positive	

M TB IFN-g Bld-Imp POSITIVE

M. tuberculosis infection possible. This test should be used in conjunction with other tests (e.g., AFB smear and culture, chest x-ray).

See Directory of Services for Interpretive Information

RESULT	VALUE	UNITS
PNIL	0.15	[IU]/mL

Gamma interferon background Bld IA-aCnc	0.15	[IU]/mL
--	------	---------

RESULT	VALUE	UNITS
P TB AG-1 NIL	0.52	[IU]/mL

M TB IFN-g CD4+ bckgrnd cor Bld-aCnc	0.52	[IU]/mL
---	------	---------

RESULT	VALUE	UNITS
P TB AG2-NIL	0.54	[IU]/mL

M TB IFN-g CD4+CD8+ bckgrnd cor Bld-aCnc	0.54	[IU]/mL
---	------	---------

RESULT	VALUE	UNITS
P MITOGEN-NIL	0.28	[IU]/mL

Mitogen IFN-g bckgrnd cor Bld-aCnc	0.28	[IU]/mL
---------------------------------------	------	---------



Resources for IGRAs

- Follow-Up for IGRA Reports: Checklist for Public Health Staff ([P-02540](#))
- TB Blood Test: Interferon Gamma Release Assay ([P-01182](#))
- [SNTC Webinars: IGRA for Nurses](#), webinar recording
- [MCCT: All About IGRA for Civil Surgeons](#), webinar recording



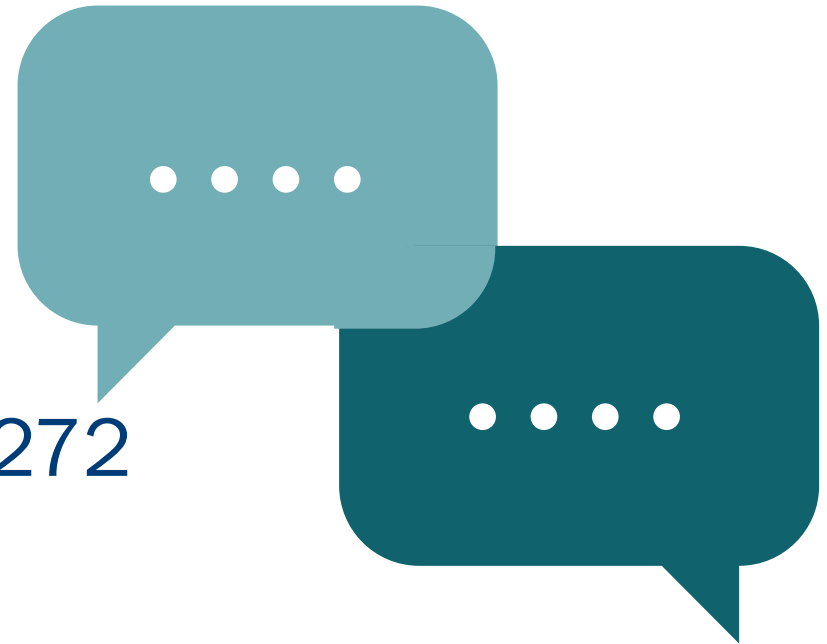
Contact Us

TB Program: 608-261-6319

WSLH Customer Service: 608-224-4272

dhswitbprogram@dhs.wisconsin.gov

<https://www.dhs.wisconsin.gov/tb/index.htm>





Thank you!

WSLH laboratory staff

Nate Simon, PhD,
TB Lab Coordinator at
WSLH for info, lab
photos, and review