

TB ToolKit

Michigan Department of Community Health Laboratory Testing for Tuberculosis

- 1. Submission Guidelines**
- 2. Shipping Unit Request Form**
- 3. Test Request Form**
- 4. Description of AFB Slide and Isolation Test**
- 5. Description of Direct Specimen PCR Test for TB**
- 6. Description of AFB Identification Test**
- 7. Description of AFB Susceptibility Test**
- 8. AFB Test Fact List**
- 9. AFB Test Flow Chart**
- 10. AFB Test Report Timeline**
- 11. AFB Test Reports**
- 12. Glossary**
- 13. References**

Guidelines for Submission of Specimens for Mycobacterial Examination to Michigan Department of Community Health Bureau of Laboratories

Specimen Submission Guidelines

The accuracy and clinical usefulness of laboratory analysis is limited by the quality and appropriateness of the specimen. The techniques used for the collection and submission of the specimens can influence the outcome of testing results.

Appropriate specimens are submitted by using collection units supplied by the Laboratory Support Unit of the Michigan Department of Community Health, Bureau of Laboratories. The Laboratory Support Unit is responsible for assembling the various units that are distributed to health departments, licensed physician's offices and licensed health care facilities. Order units by completing the Requisition for Clinical Specimen Containers (DCH-0568). The correct collection unit is listed as unit number 12. Orders may be submitted by mail, fax (517-335-9039) or by phone (517-335-9867).

Please ship specimens to the following address:

Michigan Department of Community Health
Bureau of Laboratories
3350 North Martin Luther King Jr. Blvd.
Building 44 Room 155
P.O. Box 30035
Lansing, MI 48909

NOTE: All packages containing clinical specimens and/or etiologic agents must conform to current regulations for the transporting of specimens of this type. These regulations are subject to change. Current regulations may be found at the following sites:

Federal Postal Regulations	www.cdc.gov/od/ohs/biosfty/shipregs.htm
United Postal Service	www.ups.com
Federal Express	www.fedex.com/us
DHL	www.dhl.com

The shipper is responsible for being sure that their package is in compliance with current regulations!

Guidelines for Submission of Specimens for Mycobacterial Examination to Michigan Department of Community Health Bureau of Laboratories

Specimen Rejection Policy

All specimens are subject to rejection if they:

- Are received with either specimen container unlabeled or incomplete test requisition form, or the specimen label not matching the test requisition form.
- Are leaking and can easily be replaced by re-collection. In the event that a specimen is received leaking, every attempt will be made to salvage leaking or improperly submitted samples of cerebrospinal fluid or samples attained through surgical means providing that the safety of the laboratory worker is not compromised.
- Are submitted in an inappropriate manner, i.e., are not shipped according to International Aviation Transportation Authority (IATA) regulations or other applicable standards.

Important Information to be Aware of:

- If the submitter of any specimen has not provided all required information on the test requisition, the form will be returned. The testing will not be reported until the completed test requisition is received. If the delay in testing will compromise the test results, the submitter will be contacted by telephone or fax to clarify the test requisition.

Reporting Results:

MDCH Bureau of Laboratories makes every effort to process, test, and report out specimens as quickly as possible. New positive results are communicated to the submitter by phone the same day as the results are reported.

For additional information and to download the Requisition for Clinical Specimen Containers (DCH-0568) form and test requisitions (Microbiology/Virology Test Requisitions Forms DCH-0583), visit the MDCH Laboratory web site at www.Michigan.gov/mdchlab.

Guidelines for Submission of Specimens for Mycobacterial Examination to Michigan Department of Community Health Bureau of Laboratories

Instructions for Submission of Specimens

NOTE: Specimens will not be tested if not properly labeled, test requisition is not completed or the specimen label does not match the test requisition.

MATERIALS NEEDED:

- Tuberculosis and Fungal Diagnosis Specimen container
- Microbiology/Virology Test Requisition form (DCH-0583)

SPUTUM

1. An early morning sample is desired. The specimen should be coughed up from deep within the chest—it should **not** be saliva from the mouth.
2. After coughing, patient should hold the sterile tube up to the mouth and expel the sputum into the tube. Fill the tube about one-half full, if possible. **Do not get sputum on the outside of the tube.**

OTHER TYPES OF SPECIMENS

Consult the Michigan Department of Community Health, Division of Infectious Diseases at 517-335-8067.

INSTRUCTIONS FOR SPECIMEN SUBMISSION:

1. Screw cap on the tube as tightly and evenly as possible to prevent from leaking. Using parafilm or vinyl tape (**not** scotch or masking tape), tape the cap on the tube to secure the cap and prevent from leaking.
2. Label the tube with the same name/unique identifier used on the test requisition.
3. Record the name/unique identifier on tube and test requisition for your records. You will use it to link the specimen to the patient.
4. Place the properly labeled tube, **wrapped in absorbent material such as paper towel or tissue and enclosed in the plastic bag provided**, into aluminum screw-capped can and tighten the cap securely.
5. Wrap **completed** test requisition around the **outside** of aluminum screw-capped can.
6. Place aluminum can with completed test requisition into screw-capped cardboard mailing container and secure cap with tape.

Guidelines for Submission of Specimens for Mycobacterial Examination to Michigan Department of Community Health Bureau of Laboratories

7. Complete and apply the return address, Biological Substance label to cardboard container and ship immediately by the most convenient means available (i.e., courier, U.S. First Class, Priority or Express mail etc.) to:
Michigan Department of Community Health
Bureau of Laboratories
3350 North Martin Luther King Jr. Blvd.
Building 44 Room 155
P.O. Box 30035
Lansing, MI 48909

1	IF REQUESTING EXAMINATION FOR: HEPATITIS B TEST CODE 2740 COMPLETE ALL THAT APPLY															
<input type="checkbox"/> Pregnancy (HBsAg)			<input type="checkbox"/> Exposure to someone with Hepatitis B			INFECTED PERSON'S DATE OF BIRTH			M	M	D	D	Y	Y	Y	Y
INFECTED PERSON'S NAME																
IF AN INFANT, MOTHER'S NAME																
<input type="checkbox"/> Other (Specify):												<input type="checkbox"/> Court Order		<input type="checkbox"/> At Risk		
2	IF REQUESTING EXAMINATION FOR: SYPHILIS - DFA TEST CODE 2105 COMPLETE THIS SECTION															
Duration of Lesion			<input type="checkbox"/> Days <input type="checkbox"/> Months <input type="checkbox"/> Years			Specify Site:										
3	IF REQUESTING EXAMINATION FOR: RABIES ANTIBODY SEROLOGY TEST CODE 2810 COMPLETE THIS SECTION															
Date of Last Rabies Vaccination			M	M	D	D	Y	Y	Y	Y						
4	IF REQUESTING EXAMINATION FOR: LYME BORRELIOSIS TEST CODE 2111 COMPLETE THIS SECTION															
ONSET DATE			M	M	D	D	Y	Y	Y	Y	EARLY DISEASE		<input type="checkbox"/> Valid Early Disease		<input type="checkbox"/> Erythema migrans (5 cm at least in diameter)	
LATE DISEASE		<input type="checkbox"/> Neurologic <input type="checkbox"/> Cardiologic <input type="checkbox"/> Rheumatologic			State/County of Exposure											
5	IF REQUESTING EXAMINATION FOR: AEROBIC/ANAEROBIC CULTURE TEST CODES 0200/0300 COMPLETE ALL THAT APPLY															
<input type="checkbox"/> Aerobe <input type="checkbox"/> Anaerobe <input type="checkbox"/> Microaerophile			Gram <input type="checkbox"/> Positive <input type="checkbox"/> Negative <input type="checkbox"/> Variable			<input type="checkbox"/> Rod <input type="checkbox"/> Coccus <input type="checkbox"/> Diplococcus										
Bacterial Growth Char.: MacConkey <input type="checkbox"/> Pos <input type="checkbox"/> Neg			Oxidase <input type="checkbox"/> Pos <input type="checkbox"/> Neg			Catalase <input type="checkbox"/> Pos <input type="checkbox"/> Neg			Dextrose <input type="checkbox"/> Oxidation <input type="checkbox"/> Fermentation							
<input type="checkbox"/> Other: _____																

2270	ADENOVIRUS BY CULTURE	2110	LEGIONELLA - DFA
0004	AFB SUSCEPTIBILITY - Cultural Isolate	0402	LEGIONELLA - HA
0300	ANAEROBIC CULTURE – ID Complete # 5	0708	LYME DISEASE CULTURE (Human)
2771	ARBOVIRUS ENCEP. PANEL (IgM) §	0718	LYME DISEASE CULT. (Non-Human)
0709	AUTOCLAVE TEST STRIPS	2111	LYME DISEASE - EIA Complete # 4 Above
2145	BRUCELLA SEROLOGY	2113	LYME DISEASE-IFA (Tick or Culture)
2200	CHLAMYDIA TRACHOMATIS – Culture	0801	NEISSERIA GONORRHOEAE - Isolation
2230	CYTOMEGALOVIRUS CULTURE	0851	NEISSERIA - REFERRED CULTURE
2580	CYTOMEGALOVIRUS IgG	0502	PARASITOLOGY - BLOOD
2400	ENTEROVIRUS BY CULTURE	0503	PARASITOLOGY - WORM
0603	E. COLI (SLT) TOXIN & SEROLOGY	0750	PERTUSSIS PCR
0701	FOODBORNE ILLNESS - Stool or Food	2105	SYPHILIS DFA Complete # 2 Above
2516	FUNGAL IMMUNODIFFUSION	2103	SYPHILIS VDRL - CSF Only
0103	FUNGAL SLIDE & CULTURE	2121	TETANUS TOXIN EIA
	Clinical Specimens	2130	TOXOPLASMA GONDII - IgG
2155	FRANCISELLA SEROLOGY	2140	TOXOPLASMA GONDII – IgM
2860	HANTAVIRUS	2220	VARICELLA ZOSTER – CULTURE
2800	HEPATITIS A VIRUS (IgM)	2350	VIRAL RESPIRATORY PANEL – CULT.
2590	HERPES SIMPLEX VIRUS IgG		
2952	HCV - PCR		
0400	LEGIONELLA CULTURE		

²The Following Tests Must Have

Prior MDCH Approval

2961	BACTERIAL TYPING – PFGE
0702	BOTULISM TOXIN
2973	ENTEROVIRUS - PCR
2954	HEPATITIS A VIRUS – PCR
2950	HIV – PCR
2250	MUMPS - CULTURE
2983	MUMPS - PCR
2820	MEASLES IgM
4309	NOVAL INFLUENZA A - PCR
2951	NOROVIRUS – PCR
0450	PERTUSSIS CULTURE
2830	RUBELLA IgM
0602	SALMONELLA SEROTYPING (Non-Human)
2102	SYPHILIS FTA - ABS DS
2109	SYPHILIS IgM WESTERN BLOT
0705	TOXIC SHOCK TESTING

***Sexually Transmitted Diseases – Definitions**

Symptoms:	Patient requesting examination due to symptoms, or, symptoms discovered on examination.
Infected Partner:	Patient has known exposure to STD (self-reported or documented).
Partner Risk:	Patient has multiple sex partners.
History of STD:	Patient has been diagnosed with a sexually transmitted disease within last 3 years.
Prenatal Visit:	Patient examination is part of prenatal visit.
Age recommended:	Recommended age criteria for screening female patients is ≤ 24 for family planning clinics, adolescent and juvenile detention sites, and all ages for STD clinics.
“Plan First!” Clients:	A “Plan First!” client seeking family planning services will receive screening and teaching. As a Title X Standards & Guideline requirement, <i>Chlamydia trachomatis</i> and <i>Neisseria gonorrhoeae</i> screening must be offered to “Plan First!” clients < 24 years of age, prior to provision of a contraceptive method, if risk factors are reported.
IUD Insertion:	Title X mandates that clients who are provided with Intrauterine Device (IUD) insertion must be tested for <i>N. gonorrhoea</i> and <i>Chlamydia trachomatis</i> for diagnostic purposes and/or for maintenance of health status.

¹All tests positive for *Chlamydia* will automatically be tested for *N. gonorrhoeae*.

Mycobacterial Slide and Isolation Examination

TEST CODE: 0001

USE OF TEST: To determine the presence of *Mycobacterium* spp. from clinical specimens obtained from human patients: with clinical illness suggestive of tuberculosis; who have recently been exposed to a person with an active case of tuberculosis; or who although clinically well, recently developed a positive tuberculin skin test and abnormal chest x-ray.

TEST PERFORMED: Lansing/Monday through Friday.

ANALYTIC TIME: Slide - same day received.

Isolation - up to 42 days.

For genetic amplification testing refer to Mycobacterial Exam (Genetic Amplification Probe) *M. tuberculosis* complex.

INTERPRETATION:

Slide:

1. The presence of acid fast bacilli (AFB) in a clinical specimen may indicate the presence of mycobacterial disease. A negative microscopic examination for AFB does not exclude this diagnosis as AFB are detected in less than 60% of the culture positive specimens.

Culture:

1. A report of "Mycobacteria not found" indicates that *Mycobacterium* spp. were not detected.
2. Refer to "Identification of Mycobacteria" for additional information.

SPECIMEN TYPE: Sputum, urine, cerebrospinal fluid, tissues, bone marrow, blood, gastric contents (must be neutralized to pH 7.0) and other body fluids. For isolates refer to *Mycobacterium* spp. Identification of Culture.

VOLUME: Minimum quantity of 5 ml of liquid specimen (1.0 ml of cerebrospinal fluid) or a piece of tissue 0.5 cm³ to 2.0 cm³ in size.

UNIT NUMBER: Unit 12.

MICHIGAN DEPARTMENT OF COMMUNITY HEALTH
BUREAU OF LABORATORIES

Mycobacterial Slide and Isolation Examination, Page 2 of 2

Rev. 2/04

CONTAINER: Sterile, 50 ml, screw-capped, polypropylene tube which fulfills all federal shipping requirements.

FORM NUMBER: DCH 0583.

NOTE

1. Prior consultation with the laboratory is required before fecal specimens will be examined for *Mycobacteria* spp.
2. Although all cerebrospinal fluids will be examined upon request, optimal results will be obtained from larger volumes (3 ml or greater).
3. Occasionally, other microorganisms such as fungi and *Nocardia* spp. survive the decontamination process. These microorganisms can be isolated and may be identified.
4. Neutralized gastric contents (pH 7) must be received at MDCH within 72 hours of obtaining the specimen to be tested. Specimens which are not neutralized or which are greater than 72 hours old upon receipt will not be tested.

Mycobacterial Exam (Genetic Amplification Probe) *M. tuberculosis* complex

TEST CODE: 0005

USE OF TEST: To determine the presence of *Mycobacterium tuberculosis* complex by amplification of acid fast, smear positive respiratory clinical specimens. This is performed directly on the processed clinical specimen. Genetic amplification testing will not be repeated on a previously positive specimen.

TEST PERFORMED: Lansing/Monday through Friday.

ANALYTIC TIME: Specimens received Monday through Thursday - 24 hrs.

Specimens received Fridays - 72 hrs.

INTERPRETATION: 1. Positive for *Mycobacterium tuberculosis* complex indicates that *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum*, or *M. microti* have been detected in the clinical specimen.

2. Negative for *Mycobacterium tuberculosis* complex indicates that members of the complex have not been detected.

3. Positive test results are not quantitative, cannot determine bacteriologic cure and cannot determine the organism's viability.

SPECIMEN TYPE: Acceptable specimen types include: sputum (induced or expectorated), bronchial specimens (bronchoalveolar lavage or bronchial aspirate), or tracheal aspirates from patients not receiving antimicrobial therapy for tuberculosis.

VOLUME: Minimum quantity of 0.5 ml of processed spun sediment.

UNIT NUMBER: Unit 12.

***Mycobacterium* spp. Identification of Culture**

USE OF TEST: To speciate mycobacteria obtained from human clinical specimens.

TEST PERFORMED: Lansing/Monday through Friday.

ANALYTIC TIME: TB: 7 - 10 days.

MOTT (*Mycobacterium* other than TB): 21 days.

INTERPRETATION: Speciation can confirm a previously identified mycobacterium or provide a specific identification if not already known. However, unless the isolate is *Mycobacterium tuberculosis*, its significance and relationship to the patient's pathologic process cannot be determined in the laboratory.

SPECIMEN TYPE: Acid fast bacterial growth on egg based or synthetic agar slants in screw-cap tubes or from positive bottles from rapid broth AFB detection systems. For clinical specimens refer to Mycobacterial Slide and Isolation Examination.

VOLUME: N/A

UNIT NUMBER: Unit 42

FORM NUMBER: DCH 0583

NOTE

1. Please contact the Microbiology Laboratory with any questions concerning the submission of specimens or cultures for Mycobacteriology testing.
2. Susceptibility testing is only performed when requested or when appropriate.

Mycobacterial Susceptibility Testing

TEST CODE: 0004

USE OF TEST: For determining the susceptibility of *Mycobacterium tuberculosis* to anti-tuberculosis antibiotics commonly used to treat tuberculosis.

TEST PERFORMED: Lansing/Monday through Friday.

ANALYTIC TIME: 14-21 days.

INTERPRETATION: Results of susceptibility testing of *Mycobacterium tuberculosis* anti-mycobacterial antibiotics is expressed as Susceptible or Resistant to:

Isoniazid
Streptomycin
Ethambutol
Rifampin
Pyrazinamide
Kanamycin
Ethionamide
Capreomycin
Cycloserine
Ciprofloxacin
Ofloxacin
Amikacin
PAS (p-aminosalicylic acid)

SPECIMEN TYPE: Pure and viable cultures of *Mycobacterium tuberculosis* submitted on solid culture medium.

VOLUME: N/A

UNIT NUMBER: Unit 42

FORM NUMBER: DCH 0583

NOTE

1. Please contact the Microbiology Laboratory with questions concerning submission of cultures for susceptibility testing.

MICHIGAN DEPARTMENT OF COMMUNITY HEALTH
BUREAU OF LABORATORIES
Mycobacterial Susceptibility Testing, Page 2 of 2

Rev. 3/07

2. Susceptibility testing of *Mycobacterium tuberculosis* is only performed and reported when a written request is received at MDCH or when testing is deemed essential by MDCH.

AFB LAB TESTING

SPECIMEN

1. REQUIRES THREE SPECIMENS ON THREE SUCCESSIVE DAYS.
2. BEST RESULTS COME FROM MATERIAL EXPECTORATED BY PATIENT SOON AFTER AWAKENING IN THE MORNING.
3. SPECIMEN SHOULD BE DELIVERED TO THE LAB ASAP.

AFB SLIDE EXAMINATION (1 DAY)

1. LEAST SENSITIVE OF ALL AFB TESTS
2. REQUIRES 100,000 AFB/ML FOR SLIDE TO BE POSITIVE
3. IF POSITIVE THE PATIENT CAN INFECT OTHERS.
4. POSITIVE SLIDE – CANNOT DETERMINE WHETHER TB OR MOTT
5. REPORT – WITHIN 24 HOURS OF RECEIVING SPECIMEN AT LAB.

DIRECT PCR TB PROBE TEST (1-2 DAYS)

1. VERY SENSITIVE AND VERY SPECIFIC
2. REQUIRES ONLY ONE AFB TO BE POSITIVE
3. PERFORMED ONLY ON NON-BLOODY PULMONARY SPECIMENS.
4. PERFORMED ON ONE OF THE NEW PATIENT'S FIRST SLIDE POSITIVE SPECIMENS (NOT AFTER TREATMENT IS STARTED)
5. POSITIVE (RELIABLE) - *Mycobacterium tuberculosis* complex (NOT MOTT)
6. REPORT – WITHIN 24 HOURS OF THE SLIDE POSITIVE REPORT
7. TEST NOT OFFERED BY MOST LABS.

AFB CULTURE (7-10 DAYS)

1. MORE SENSITIVE THAN SLIDE
2. ONLY REQUIRES 10 AFB/ML OF SPECIMEN
3. CULTURE MAY BE AFB POSITIVE EVEN THOUGH THE SLIDE WAS REPORTED AFB NEGATIVE.
4. RAPID BROTH TESTING – POSITIVE WITHIN IN 1-2 WEEKS
5. AFB POSITIVE CULTURES ARE EXPECTED TO BE REPORTED IN LESS THAN 21 DAYS OF RECEIPT OF SPECIMEN IN THE LAB.
6. NEGATIVE CULTURE REQUIRES 6 WEEKS TO REPORT.

AFB IDENTIFICATION (10-12 DAYS)

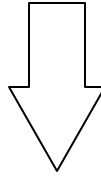
1. PERFORMED AS SOON AS CULTURE BECOMES POSITIVE
2. RAPID PRELIMINARY IDENTIFICATION –MAY BE EXECTED TO BE REPORTED WITHIN 1-3 DAYS OF POSITIVE CULTURE REPORT.
3. PRELIMINARY IDENTIFICATION IS BASED UPON HPLC OR GENETIC PROBE.
4. GENETIC PROBE (NON-PCR) – REPORTS *M.tuberculosis* complex or MOTT ID
5. HPLC – REPORTS MOST *Mycobacterium* species

SUSCEPTIBILITY (21 DAYS)

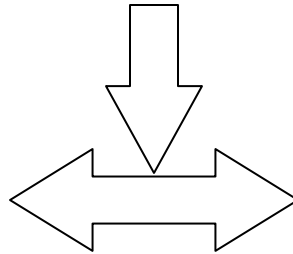
1. PERFORMED ON GROWTH FROM CULTURE
2. PRIMARY ANTIBIOTIC SUSCEPTIBILITY REPORT MAY BE EXPECTED ONE WEEK FROM *M.tuberculosis* complex ID REPORT.
3. PYRAZINAMIDE SUSCEPTIBILITY REPORT MAY BE EXPECTED ONE WEEK AFTER PRIMARY DRUG REPORT.
4. SECONDARY AND TERTIARY ANTIBIOTICS MAY BE REQUESTED AND ARE REPORTED APPROXIMATELY THREE WEEKS AFTER INITIATED.

Flow Diagram for TB Specimens

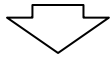
SPECIMEN



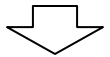
DIGESTION / DECONTAMINATION



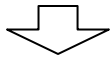
**Media Inoculation
(Medium Slants & Broth)**



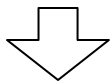
Culture Examination



AFB Positive Growth

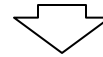


**Genetic Probe/HPLC
(AFB Identification)**

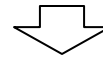


Indirect Susceptibility

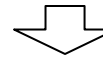
**Smear Preparation & Staining
(AO & ZN)**



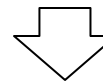
AFB Slide Examination



AFB Positive Slide (New)

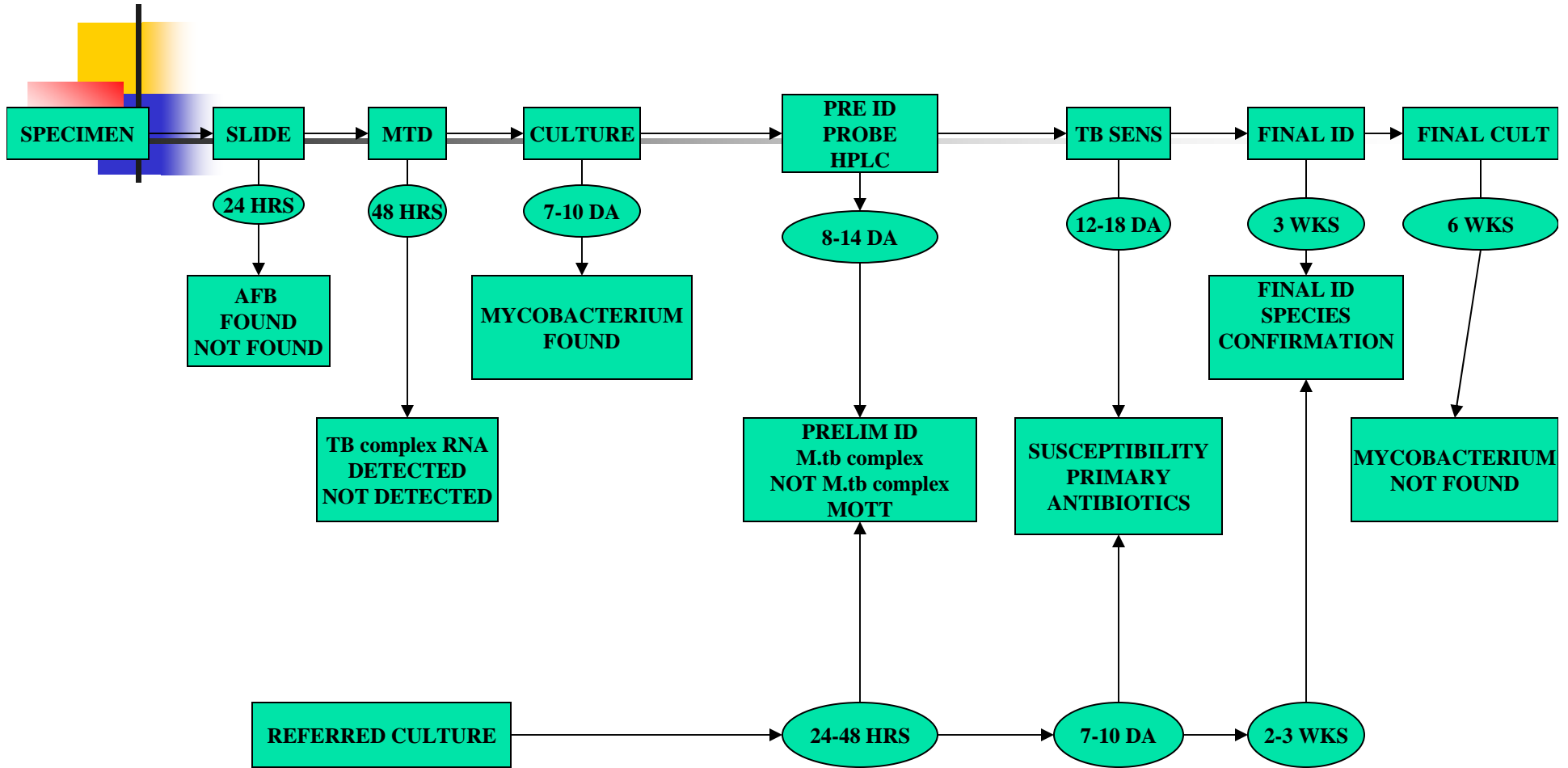


**Direct Amplified Probe (MTD)
(AFB Identification)**



Direct Susceptibility

AFB TEST TIME-LINE



MDCH LAB REPORTS

“AFB SLIDE EXAMINATION REPORT”

(24 hours after specimen is received at the laboratory)

1. “Acid fast bacilli not found”

Acid fast bacilli are not present in high enough numbers to be seen on the microscopic slide examination. Report does not eliminate the possibility that TB bacillus is present. Patient is not considered infectious and does not need to be isolated from others.

2. “Acid fast bacilli found”

Acid fast bacilli were found and in very high numbers, high enough to consider patient to be very infectious. Patient may be infected with TB bacillus. Isolate patient from others.

3. “Few Acid fast bacilli found”

Acid fast bacilli were found in high enough numbers to be considered infectious. Patient may be infected with TB bacillus. Isolate patient from others.

NOTE:

- a) **LEAST SENSITIVE OF ALL AFB TESTS**
- b) **REQUIRES 100,000 AFB/ML FOR SLIDE TO BE POSITIVE**
- c) **POSITIVE PATIENT CAN INFECT OTHERS.**
- d) **POSITIVE SLIDE – CANNOT DETERMINE WHETHER TB OR MOTT**
- e) **REPORTED WITHIN 24 HOURS OF RECEIVING SPECIMEN AT LAB.**

“AMPLIFIED TB PROBE (PCR) REPORT”

(24 hours after receiving a positive AFB slide report)

1. Mycobacterium tuberculosis complex rRNA “DETECTED”

Genetic material (RNA) has been found, which indicates that TB bacillus is present in the patient. TB RNA may be present as a result of active infection with *M.tuberculosis* or *M.bovis*. However, TB RNA may also be present following successful therapy which has rendered the TB bacillus to be non-viable or inactive. (Report on RVCT)

2. Mycobacterium tuberculosis complex rRNA “NOT DETECTED”

TB RNA is “NOT” present in the patient’s specimen. So, the AFB found on the positive AFB slide examination is due to another mycobacterial species and is not normally considered to be communicable or infectious to others.

NOTE:

- a) **VERY SENSITIVE AND VERY SPECIFIC**
- b) **REQUIRES ONLY ONE AFB TO BE POSITIVE**
- c) **PERFORMED ONLY ON NON-BLOODY PULMONARY SPECIMENS.**
- d) **PERFORMED ON ONE OF THE NEW PATIENT’S FIRST SLIDE POSITIVE SPECIMENS (NOT AFTER TREATMENT IS STARTED)**
- e) **POSITIVE (RELIABLE) - *Mycobacterium tuberculosis* complex (NOT MOTT)**
- f) **REPORT – WITHIN 24 HOURS OF THE SLIDE POSITIVE REPORT**
- g) **TEST NOT OFFERED BY MOST LABS.**

“CULTURE ISOLATION REPORT”

(AFB positive reports may require less than 6 weeks/AFB negative culture reports require at least 6 weeks)

1. “ACID FAST BACILLI FOUND ON CULTURE/IDENTIFICATION REPORT WILL FOLLOW”

Acid fast bacilli have been found on culture media which may be due to growth of a mycobacterial species including the possibility of the TB bacillus, *M.tuberculosis*. A rapid identification test will be performed within 24 hours to determine whether the bacterium found is *M.tuberculosis* or not. (A preliminary mycobacterial identification report may be expected within 24 -48 hours)

2. “MYCOBACTERIUM NOT FOUND”

No mycobacterial growth has been found, including *M.tuberculosis*, after 6 weeks of incubation of culture media.

NOTE:

- a) **MORE SENSITIVE THAN SLIDE**
- b) **ONLY REQUIRES 10 AFB/ML OF SPECIMEN**
- c) **CULTURE MAY BE AFB POSITIVE EVEN THOUGH THE SLIDE WAS REPORTED AFB NEGATIVE.**
- d) **RAPID BROTH TESTING – POSITIVE WITHIN 1-2 WEEKS**
- e) **AFB POSITIVE CULTURES ARE EXPECTED TO BE REPORTED IN LESS THAN 21 DAYS OF RECEIPT OF SPECIMEN IN THE LAB.**
- f) **NEGATIVE CULTURE REQUIRES 6 WEEKS TO REPORT.**

“AFB IDENTIFICATION REPORT”

Preliminary Identification:

(24-48 hours after culture medium has produced AFB growth, growth is referred for preliminary mycobacterial identification testing)

1. “PRELIMINARY REPORT (PROBE/HPLC) Mycobacterium tuberculosis complex ADDITIONAL REPORT WILL FOLLOW”

The culture isolate referred for identification has been identified as *M.tuberculosis* complex. (Report on RVCT). Expect to receive another report confirming and clarifying this report.

2. “PRELIMINARY REPORT (PROBE/HPLC) Mycobacterium sp. Other than M.tuberculosis ADDITIONAL REPORT WILL FOLLOW”

The culture isolate referred for identification has been determined be a *Mycobacterium species* other than *M.tuberculosis* complex (MOTT). (No RVCT report is necessary) Expect another report clarifying the specific mycobacterial species.

Final Identification:

(2-3 weeks after the preliminary ID)

1. “FINAL REPORT Mycobacterium tuberculosis”

M. tuberculosis has been confirmed by testing subsequent to genetic probe or HPLC testing. (RVCT should have already been submitted)

2. “FINAL REPORT Mycobacterium spp.”

A *Mycobacterium* species other than *M.tuberculosis*, such as *M.gordonae*, *M.avium* complex, or *M.fortuitum* complex, has been confirmed by testing subsequent to genetic probe or HPLC testing. (RVCT is not required)

NOTE:

- a) **PERFORMED AS SOON AS CULTURE BECOMES POSITIVE**
- b) **RAPID PRELIMINARY IDENTIFICATION –MAY BE EXPECTED TO BE REPORTED WITHIN 1-3 DAYS OF POSITIVE CULTURE REPORT.**
- c) **PRELIMINARY IDENTIFICATION IS BASED UPON HPLC OR GENETIC PROBE.**
- d) **GENETIC PROBE – REPORTS *M.tuberculosis* complex or MOTT ID**
- e) **HPLC – REPORTS MOST *Mycobacterium* species.**

“TB SUSCEPTIBILITY REPORT”

(Primary antibiotics reported 7 days after preliminary ID report / secondary antibiotics 3 weeks later)

1. “SUSCEPTIBILITY REPORT

Mycobacterium tuberculosis

Antibiotic		Interpretation
Isoniazid	0.2 mcg/ml	RESISTANT
Isoniazid	1.0 mcg/ml	SUSCEPTIBLE
Ciprofloxacin	2.0 mcg/ml	SUSCEPTIBLE
Streptomycin	2.0 mcg/ml	SUSCEPTIBLE
Streptomycin	10.0 mcg/ml	SUSCEPTIBLE
Ethambutol	5.0 mcg/ml	SUSCEPTIBLE
Rifampin	1.0 mcg/ml	RESISTANT
Kanamycin	6.0 mcg/ml	SUSCEPTIBLE
Ethionamide	5.0 mcg/ml	SUSCEPTIBLE
Cycloserine	30.0 mcg/ml	SUSCEPTIBLE
Capreomycin	10.0 mcg/ml	SUSCEPTIBLE
Pyrazinamide	100.0 mcg/ml	SUSCEPTIBLE
Ethambutol	10.0 mcg/ml	SUSCEPTIBLE
Amikacin	6.0 mcg/ml	SUSCEPTIBLE
Ofloxacin	2.0 mcg/ml	SUSCEPTIBLE
P-aminosalicylic acid	2.0	SUSCEPTIBLE
P-aminosalicylic acid	10.0	SUSCEPTIBLE

NOTE:

- a) **PERFORMED ON GROWTH FROM CULTURE**
- b) **PRIMARY ANTIBIOTIC SUSCEPTIBILITY REPORT MAY BE EXPECTED ONE WEEK FROM *M.tuberculosis* complex ID REPORT**
- c) **PYRAZINAMIDE SUSCEPTIBILITY REPORT MAY BE EXPECTED ONE WEEK AFTER PRIMARY DRUG REPORT**
- d) **SECONDARY AND TERTIARY ANTIBIOTICS MAY BE REQUESTED AND ARE REPORTED APPROXIMATELY THREE WEEKS AFTER INITIATED.**

“TB GENOTYPING (DNA FINGERPRINTING)”

(Results are reported to MDCH TB epidemiology within 7 days after *M.tuberculosis* complex identification)

Genotyping results:

- 1. Are not needed if local TB control program staff has already identified an epi-link between patients as a result of contact investigations.**
- 2. Are only used when contact investigation has failed to determine epidemiological links.**
- 3. May associate a case with a cluster of cases of the same genotype.**
- 4. Are sent by Michigan’s TB genotyping laboratory to MDCH epidemiology staff for analysis.**
- 5. Are communicated to local health departments when relatedness to another case is suggested.**

NOTE:

- a) Contact investigation precedes genotyping.**
- b) Same genotype suggests relatedness but does not confirm relatedness.**
- c) Cases with the same genotype may be related.**
- d) Cases with the same genotype may not be related at all.**
- e) Genotyping may be needed by the laboratory to determine whether a new positive case is due to cross contamination or lab error.**

GLOSSARY OF TB LABORATORY TERMS

Acid-fast bacilli (AFB): Bacteria that retain certain dyes after being washed in an acid solution. Most acid-fast organisms are mycobacterium. *Nocardia* spp. and *Rhodococcus* spp. may also be weakly acid-fast. When AFB are seen on a stained smear of sputum or other clinical specimen, a diagnosis of TB should be suspected; however, the diagnosis of TB is not confirmed until an identification of *M. tuberculosis* complex has been reported, either by an amplified genetic TB probe testing, i.e. MTD/PCR performed directly on the specimen or by HPLC or non amplified genetic TB Probe testing performed on bacterial growth detected on culture medium.

Amplified Genetic Probe (Gen Probe's MTD test): A laboratory procedure used to test directly a patient's pulmonary specimen without waiting for growth from culture. This test procedure amplifies genetic material, DNA or RNA, which has been extracted from AFB which are found in the specimen. It uses a polymerase chain reaction (PCR) to provide enough genetic material for rapid and precise genetic probe identification of *M. tuberculosis* and *M. bovis*. The identification test can be performed directly on the patient's specimen and can be completed within 2 hours.

BACTEC®: Is a commonly used rapid broth culture method for early growth of acid fast bacilli. It provides rapid growth (in 7-14 days) and rapid drug-susceptibility testing (in 5-6 days). When BACTEC® is used in conjunction with rapid identification methods, *M. tuberculosis* complex can be identified within 10-14 days of specimen collection.

Culture: (noun) Bacterial growth on microbiological media. To "culture" (verb) refers to the process of growing bacteria in the laboratory so that organisms can be tested for identification, antibiotic susceptibility and genotyping. To culture can also mean to inoculate a patient's specimen to microbiological culture media in an attempt to grow bacteria, (i.e. a "culture" (noun) of bacteria).

DNA Probe: A laboratory procedure used for testing bacterial growth from culture which provides rapid and precise identification of AFB (e.g. *M. tuberculosis* and *M. bovis*). The identification test can be completed within 2 hours after AFB growth has been detected in culture media.

Droplet nuclei: Microscopic liquid particles produced when a person coughs, sneezes, shouts, or sings. The droplets produced by an infectious TB patient can carry tubercle bacilli and may remain suspended in the air for prolonged periods of time being suspended in normal air currents in the room.

Drug resistance, acquired: A resistance to one or more anti-TB drugs that develops while a patient is receiving therapy and which usually results from the

patient's nonadherence to therapy or the prescription of an inadequate regimen by a health care provider.

Drug resistance, primary: A resistance to one or more anti-TB drugs that exists before a patient is treated with the drug(s). Primary resistance occurs in persons exposed to an infected case which has been diagnosed with a drug-resistant strain of *M. tuberculosis*.

Drug susceptibility pattern: The anti-TB drugs to which the tubercle bacilli cultured from a TB patient are susceptible or resistant based on drug susceptibility tests.

Drug susceptibility tests: Laboratory tests that determine whether the tubercle bacilli cultured from a patient are susceptible or resistant to various anti-TB drugs.

EMB: Ethambutol – a primary anti-TB drug used to treat tuberculosis caused by *M.tuberculosis* or *M.bovis*.

Exposed (to TB): Having come in contact with a case of active tuberculosis, who is considered to be infectious, i.e. AFB slide positive and producing sputum or coughing.

Fluorochrome stain: A technique for staining a clinical specimen with fluorescent dyes to perform a microscopic examination (smear) for mycobacteria. This technique is preferable to other staining techniques because the mycobacteria can be seen easily and the slides can be read quickly.

Genetic Probe: A laboratory procedure used for testing bacterial growth for DNA or RNA unique to a cultured bacterial isolate, which can be used to provide rapid and precise identification of AFB (e.g. *M tuberculosis* and *M. bovis*). The identification test can be completed within 2 hours after AFB growth has been detected.

High Performance Liquid Chromatography (HPLC): Detects differences in the spectrum of mycolic acids in the cell wall harvested for culture growth, equally rapid as a nucleic acid probe, and can identify most pathogenic mycobacteria species.

INH: Isoniazid – a primary anti-TB drug used to treat tuberculosis caused by *M.tuberculosis* or *M.bovis*.

Infected with TB: to have inhaled droplet nuclei containing the tubercle bacillus.

Infectious: An active case of tuberculosis with AFB which can be seen by microscopic examination of the person's pulmonary secretions (sputum).

MOTT: Mycobacterium other than *Mycobacterium tuberculosis* (TB).

Mycobacterium Tuberculosis direct test (MTD): Amplified genetic probe test performed for the detection of *M. tuberculosis* in both smear positive and smear negative clinical specimens.

Multidrug-resistant tuberculosis (MDR-TB): Active TB caused by *M. tuberculosis* organisms that are resistant to more than one anti-TB drug.

***M tuberculosis* complex:** A group of closely related mycobacterial species that can cause active TB (e.g., *M. tuberculosis*, *M. bovis*, *M. africanum* and *M. microti*); most TB in the United States is caused by *M. tuberculosis*.

Nucleic acid probes: A rapid method of species identification. Once a culture is grown nucleic acid probes can identify species in 2-4 hours.

PZA: Pyrazinamide – a primary anti-TB drug used to treat tuberculosis caused by *M. tuberculosis* or *M. bovis*.

PAN Sensitive: TB case whose TB infection has been determined to be susceptible to “Primary anti-tuberculosis drugs”, i.e. INH, EMB, RA, Strep and PZA.

QuantiFERON® - TB Gold test (QFT-G): Whole blood test for use as an aid in diagnosing Mycobacterium tuberculosis infection, including latent tuberculosis infection and tuberculosis disease. This test was approved by the U.S. Food and Drug Administration in 2005.

Resistance: The ability of some strains of bacteria, including *M. tuberculosis*, to grow and multiply in the presence of certain drugs that ordinarily kill them; such strains are referred to as drug-resistant strains.

RA: Rifampin– a primary anti-TB drug used to treat tuberculosis caused by *M. tuberculosis* or *M. bovis*.

SIRE: the antimycobacterial drugs, Streptomycin, Isoniazid, Rifampin and Ethambutol, which are four primary antibiotics to test the susceptibility of *M. tuberculosis* and *Mycobacterium bovis*.

Smear (AFB smear): A laboratory technique for visualizing mycobacteria. The specimen is applied (smeared) onto a microscope slide, fixed and stained, then examined using a microscope. Smear results should be available within 24 hours. If mycobacteria (AFB) are observed on an AFB smear, it is usually an indication that the patient is infectious. However, a positive result does not

confirm the diagnosis of TB because organisms other than *M. tuberculosis* (MOTT) may also be seen on the smear as AFB.

Specimen: Any body fluid, secretion, or tissue sent to the laboratory for the purpose of laboratory testing, such as for AFB smear and to culture for *M.tuberculosis*.

Sputum: Phlegm coughed up from deep within the lungs. If a patient has pulmonary disease, an examination of the sputum by smear and culture can be helpful in evaluating the organism responsible for the infection.

Sputum smear, positive: when AFB are visible on the sputum smear viewed using a microscope. Persons with a sputum found to be smear positive for AFB are considered more infectious than those with smear-negative sputum.

TAT: Turn around time

Tubercle bacilli: *M.tuberculosis* bacilli.

Virulence: The degree of pathogenicity of a microorganism as indicated by the severity of the disease produced and its ability to invade the tissues of a host, *M. tuberculosis* is a virulent organism.

References

1. Grosset, J.H. 1993. Bacteriology of Tuberculosis, pp.60-61. *In* Reichman, L.B., E.S. Hershfield, and Claude Lenfant (ed.), *Tuberculosis: A Comprehensive International Approach*. Marcel Dekker, Inc., New York, New York.
2. Isenberg, Henry (ed). 2004 *Clinical Microbiology Procedures Handbook*., 2nd Edition, Vol. 2. pp. 7.1.2.1 – 7.1.2.9. American Society for Clinical Microbiology, Washington D.C.
3. Kent, P.T., and G.P. Kubica. 1985. *Public Health Mycobacteriology/A Guide for the Level III Laboratory*. U.S. Department of Health and Human Services. Public Health Service. (CDC) Atlanta, Georgia.
4. Pfyffer, G.E. 2007. Mycobacterium: General Characteristics, Laboratory Detection, and Staining Procedures pp. 543-572. *In* P.R. Murray, E.J. Baron, J.H. Jorgensen, M.L. Landry, M.A. Pfaller (ed). *Manual of Clinical Microbiology*, 9th ed. American Society for Microbiology, Washington, D.C.
5. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Institutes of Health, Fifth Edition, Feb. 2007. US Government Printing Office Washington: 2007. <http://www.cdc.gov/od/ohs/biosfty/bmb16/bmb15toc.htm>

Federal Shipping Regulations www.cdc.gov/od/ohs/biosfty/shipregs.htm

MDCH Bureau of Laboratorie's guidelines and forms www.michigan.gov/mdchlab

Centers for Disease Control and Prevention (CDC) www.cdc.gov

CDC Guidelines for use of Quantiferon test www.cdc.gov/mmwr/

American Lung Association of Michigan www.alam.org

MI-ACET www.michigantb.org