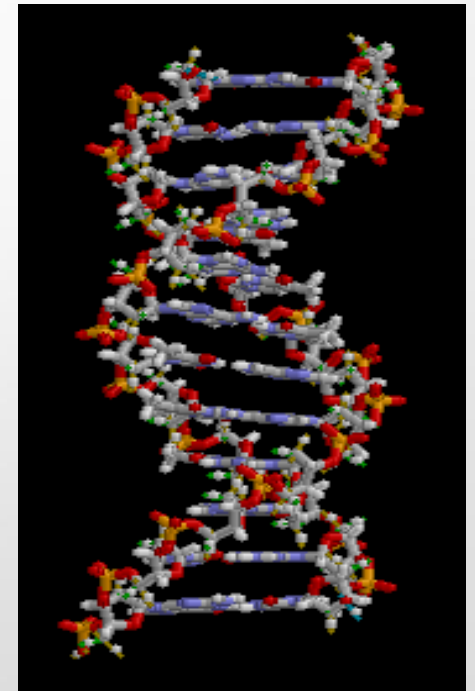


Tuberculosis Laboratory Overview

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What do all the words mean?



NAA

culture

Amplification

MGIT

WGS

MTD

16 S Sequencing

Susceptibility

Smear

Molecular

mutation

HPLC

MALDI-Tof

Gene Xpert

MDDR

NAAT

Genotyping

Learning Goals

- What do all the words mean?
- Timelines for testing and results
- Why is my specimen rejected?
- Identify new laboratory techniques and methods for detection of M. tuberculosis

Mycobacterial Examination

1. Proper specimen collection / specimen decontamination
2. Examination of acid-fast bacilli (AFB) smears
3. NAAT-nucleic acid amplification test
4. Specimen culturing and final identification
5. Drug susceptibility testing
6. TB genotyping

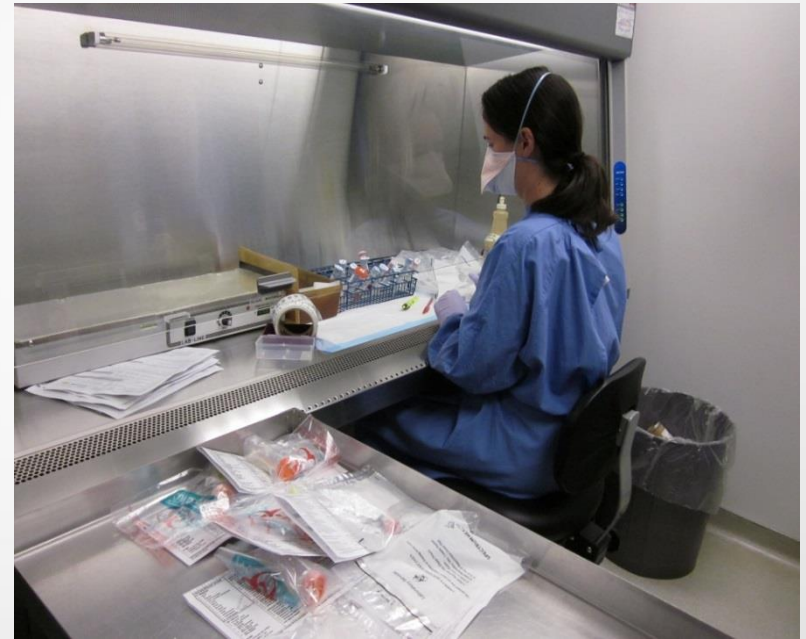
TB SPECIMEN SOURCES

- Sputum (primary)
- Pulmonary aspiration
- Gastric aspiration (less preferred, must reach the lab within 72 hours, must be neutralized to pH 6.0-8.0)
- Body fluids (CSF, pleural, peritoneal, etc)
- Tissues
- Blood
- Stool (special request)
- Other

Specimen Collection

- Collect in sterile, leak proof containers
- Refrigeration of specimen is recommended to reduce overgrowth of contaminating bacteria
- Deliver specimen to TB lab within 24 hrs.
- Always include patient name on both test request form and the specimen container

Specimen Receipt

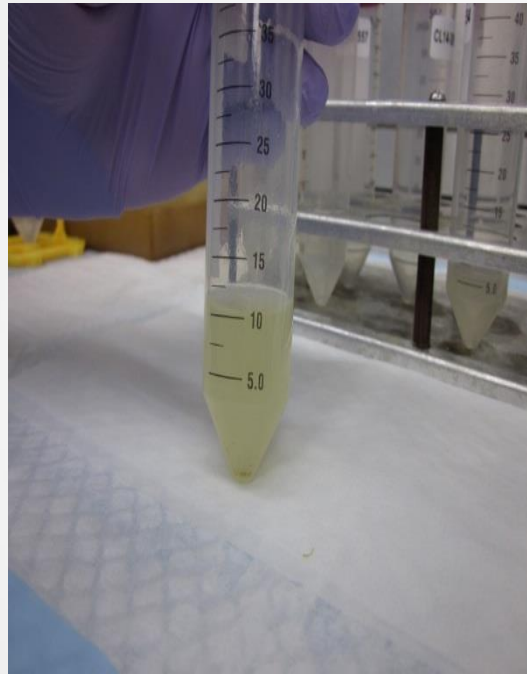


We receive 20-40 specimens a day, each specimen requisition is checked against the specimen tube, we are looking at names or unique identifiers, and if DOB and DOC are marked. Test requisitions are put in the autoclave before they are sent to our data coding unit

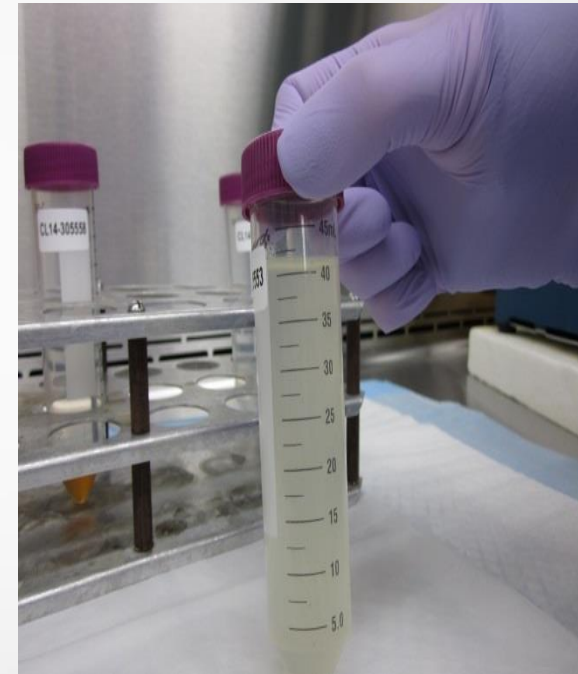
Specimen Processing



We will process all that we receive

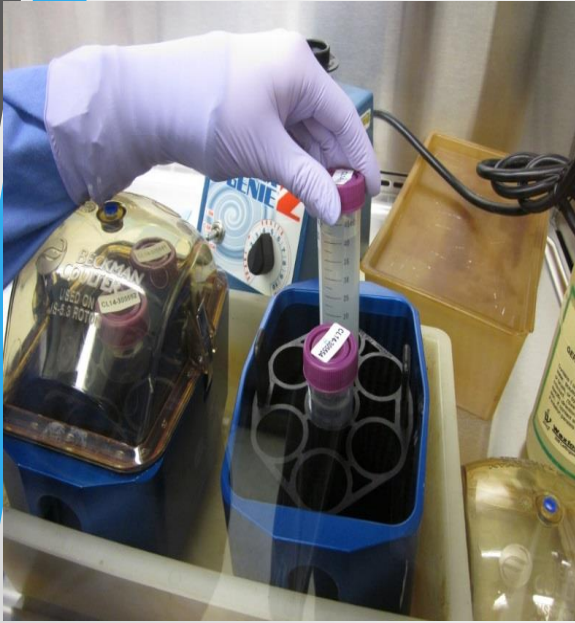


Equal volume of digestant (NaOH / N-Acetyl L Cysteine) added (3-15 min)



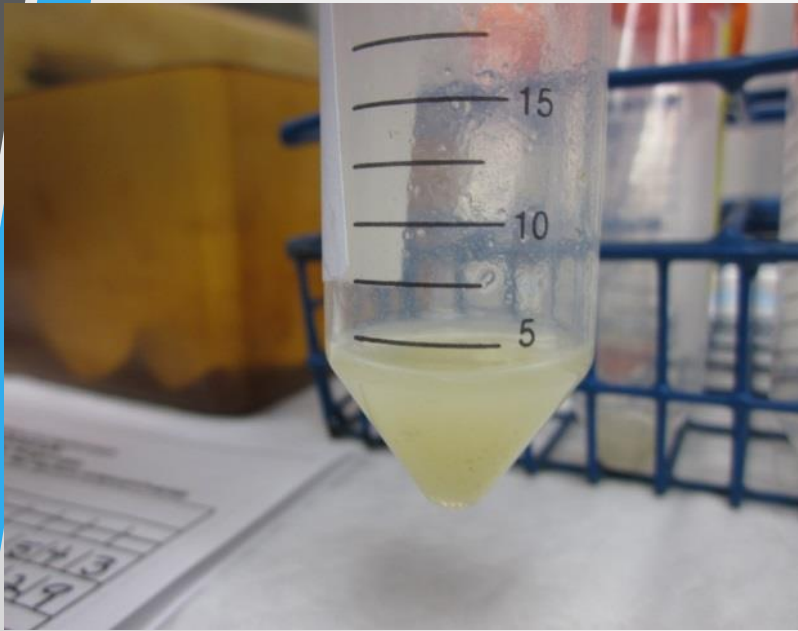
Add buffer to stop the reaction

Specimen Processing

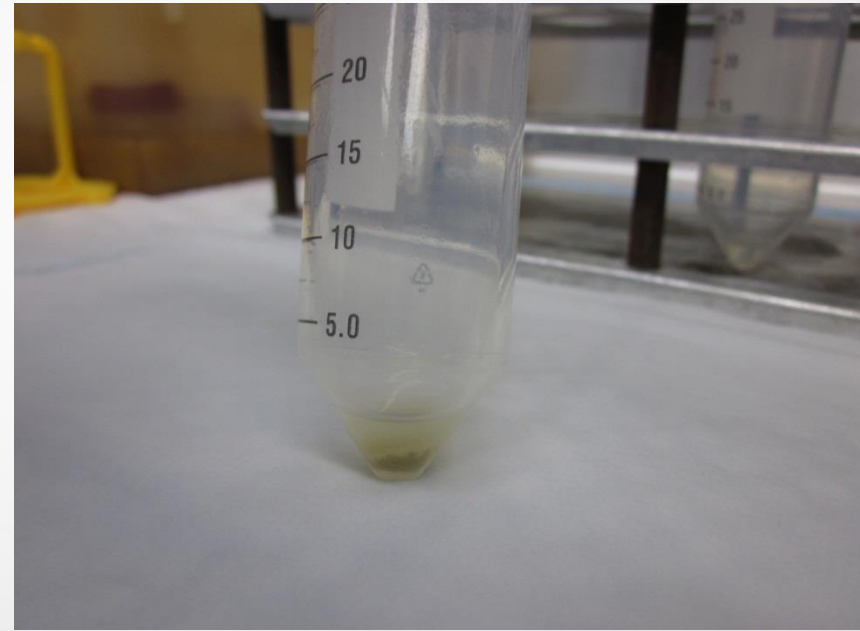


Centrifugation concentrates all of the organisms into a pellet – supernatant is discarded

Specimen Processing

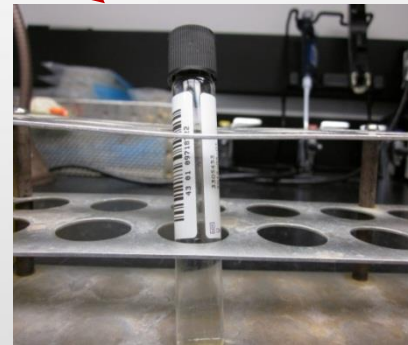
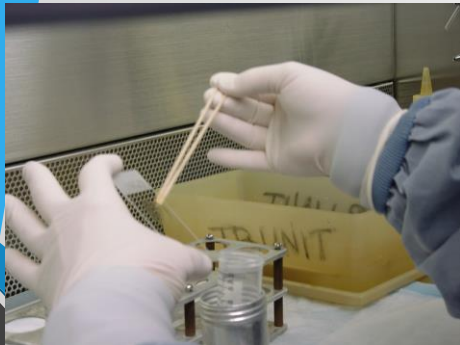
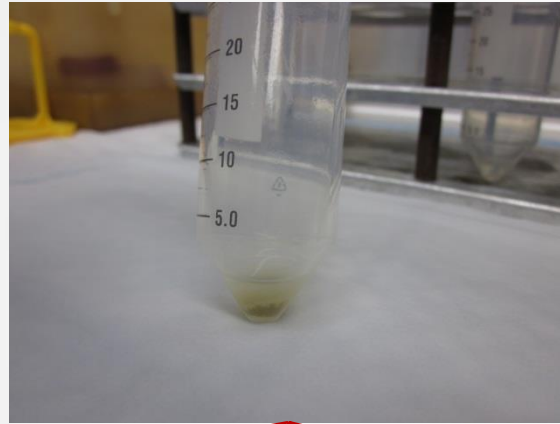


Before



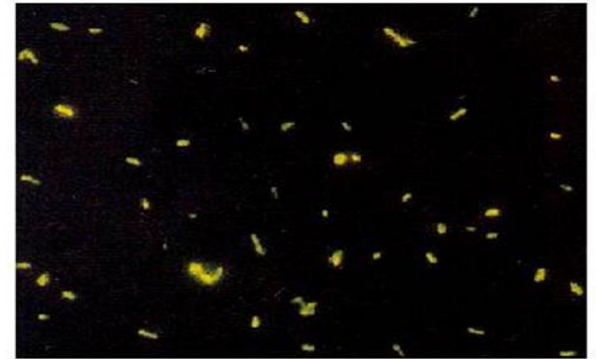
After

A lot of tests with very little specimen



Fluorescent Acid Fast Microscopy

- Least sensitive of all AFB Tests / first test result available
- Requires 10,000 AFB/ml for a slide to be positive
- If positive, the patient can infect others
- Positive slide cannot determine AFB viability
- Positive slide does not determine TB or MOTT (Mycobacteria other than TB)
- Reported within 24 hours of receiving the specimen in the laboratory



Auramine-O staining of AFB under Fluorescence Microscopy



MTB Identification by molecular methods

- Next step after Smear Results
- Nucleic Acid Amplification Test (NAAT) - Test that amplifies the Genetic Material of an organism for identification.
- Amplify = make copies of a certain area of the nucleic acid using an enzyme.
- Polymerase Chain Reaction (PCR) – Used to detect target DNA in a sample or to amplify DNA for Sequencing.
- NAAT yields RAPID results!



MTD-Hologic and Gene Xpert-Cepheid are the only FDA approved methods

MTD



- Mycobacterium tuberculosis* Direct (MTD) Test
- Transcription Mediated Amplification
 - Amplifies rRNA from decontaminated sediment (sputum, bronchial wash, tracheal aspirates)
 - Detection method is a complimentary strand DNA probe specific to MTBC
 - Bound probe gives off signal that is read in a luminometer
 - 3 hour hands on time, approximately \$23/sample

Gene XPERT

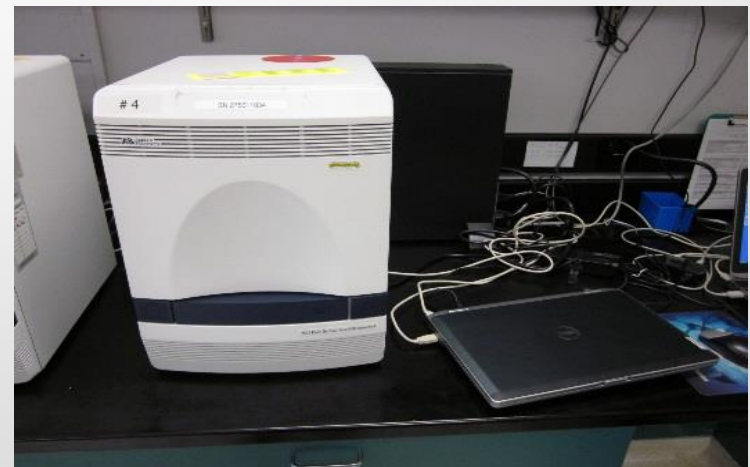
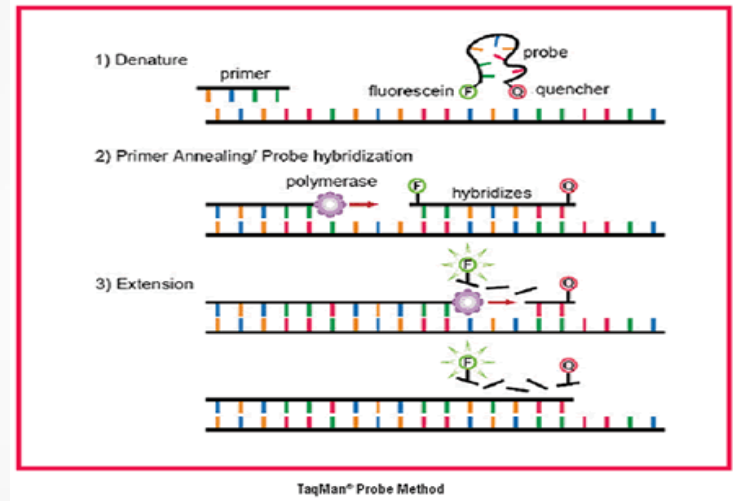


- PCR based assay in a cartridge
- Amplifies DNA from sputum (FDA) samples
- Detection method is fluorescent probe bound to DNA
- Also detects Rifampicin resistance
- Less than one minute hands on time, results in 90 min
- cost could be up to \$50/cartridge

NAA tests are available that are not FDA approved, such as real time PCR assays

- Amplifies target DNA in real time
- Uses probes with fluorescent labels
- Each probe fluoresces at a different wavelength
- Multiplex Real-Time PCR - multiple probes and primers in one reaction tube
- Half an hour to 1 hour of hands on time, 1 hour on instrument.
- Approximately \$7 /sample

MDHHS performs a real time lab developed PCR test to detect MTBC and MAC using the ABI 7500 Fast DX

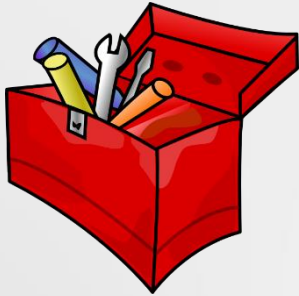


ABI 7500 Fast DX

Limitations of NAAT

- NAA tests that are used to for identification of MTBC are usually only validated for respiratory specimens
- Cannot differentiate among members of MTBC
- NAAT results may be affected by specimen processing conditions/storage or shipping conditions
- Inhibitors may be present that affect amplification.
- A positive result does not indicate active disease.
- A negative test does not exclude the possibility of culturing MTBC

Mycobacterium identification by culture based methods (Which tools to use)



- HPLC: High Performance Liquid Chromatography
- MALDI-TOF: Matrix-Assisted Laser Desorption Ionization - Time of Flight
- Accuprobe: M. tb cplx., M.avium cplx., M. kansasii, M. gordonae
- Conventional biochemical testing

AFB Culture

More sensitive than AFB smear

10 AFB/ml can produce a positive result

Culture may be AFB positive even if smear was reported negative for AFB

Rapid broth testing – normally positive within 1-2 weeks. Requires 6 weeks to report culture as negative

Positive culture result may be either *Mycobacterium tuberculosis* complex or atypical *Mycobacterium*



HPLC

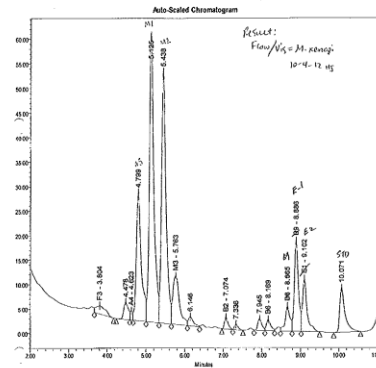


- High Performance Liquid Chromatography
- Mycobacteria contain mycolic acids
- These mycolic acids are extracted and produce a profile by (HPLC)The profiles are identified by an HPLC library
- Extraction time ~2 hours
- Run time per specimen is about 15 minutes

Appendix 10

Sample Information

SampleName	ATCC 19250	Sample Type	Unknown
Vial	15	Date Acquired	10/30/2012 6:02:29 PM EDT
Injection	1	Acq Method Set	Mh_Set_FL
Injection Volume	5.00 ul	Processing Method	MYCOLIC_FL
Sample Set Name	100312_FLB	Date Processed	10/30/2012 6:10:27 PM EDT
Run Time	11.0 Minutes		

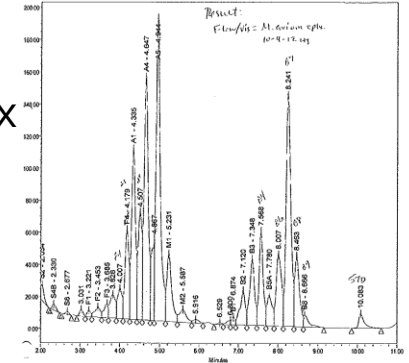


M. xenopi
ATCC 19250 (*M. xenopi*)

Sample Information

SampleName	ATCC 25291	Sample Type	Unknown
Vial	15	Date Acquired	10/30/2012 6:32:49 PM EDT
Injection	1	Acq Method Set	Mh_Set_FL
Injection Volume	5.00 ul	Processing Method	MYCOLIC_FL
Sample Set Name	100312_FLB	Date Processed	10/30/2012 6:46:48 PM EDT
Run Time	11.9 Minutes		

Auto-Scaled Chromatogram



M. avium cplx
ATCC 25291 (*M. avium* complex)

MALDI-TOF



- Matrix-Assisted Laser Desorption Ionization - Time of Flight
- Extract and Analyze intrinsic proteins by mass spectrometry
- Spectral pattern of protein expression is compared with reference patterns in a database
- Extraction time ~2 hour
- Run time on the instrument approx. 1 minute

Analyte20



Analyte Name: Ext Ctrl B
 Analyte Description: D:\data\MaldiBiotypeRealTimeClassification\160331-1120-100006248\Ext Ctrl B\0_A4\1\1SLin
 Analyte ID: 2c1e06c7-a987-4e81-92f6-cdee44737e61
 Analyte Creation Date/Time: 3/31/2016 4:03:03 PM
 Applied MSP Library(ies):
 Applied Taxonomy Tree: Bruker Taxonomy

Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (++)	Mycobacterium avium 08 TWF	2.281	1764
2 (++)	Mycobacterium avium [ssp hominissuis] 142_10 HLG b	2.203	127778581
3 (++)	Mycobacterium avium [ssp hominissuis] 276_04 FZB b M	2.174	127778581
4 (++)	Mycobacterium avium 12029441 MVD b	2.094	127778581
5 (++)	Mycobacterium avium [ssp hominissuis] 7881_10 FZB b	2.02	127778581
6 (++)	Mycobacterium avium ssp avium CCUG 28067 CCUG b	2.02	127778581
7 (++)	Mycobacterium avium ssp avium 212_11 FZB b	2.012	127778581
8 (++)	Mycobacterium avium [ssp hominissuis] 1840_09 FZB b	2.001	127778581
9 (+)	Mycobacterium avium 22_027242 MML b	1.976	127778581
10 (+)	Mycobacterium avium NO1578 LIG b	1.975	127778581

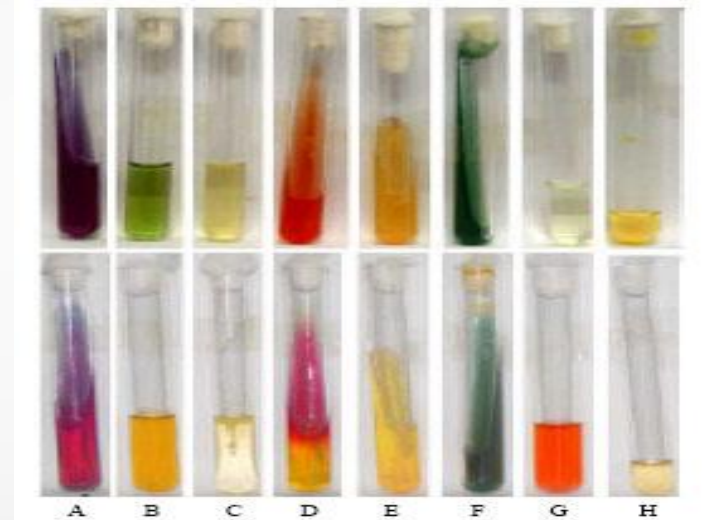
Accuprobe and Biochemical Tests

Accuprobe



M. tuberculosis complex
M. avium complex
M. kansasii
M. goodii
Solid or broth cultures, results in ~2 hours

Biochemicals

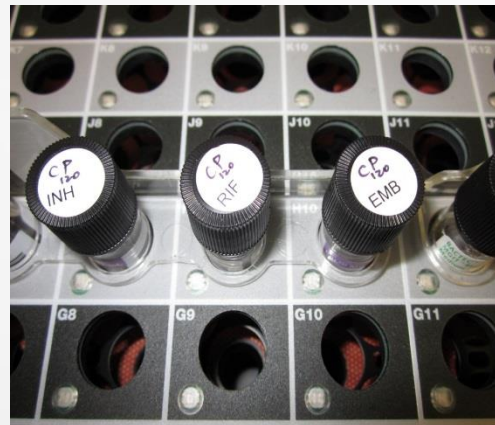


Biochemicals are used for identification confirmation when the identification by other methods failed to produce a clear result

Primary TB Antibiotics

Most results are available within 7-14 days of *M. tuberculosis* complex Identification

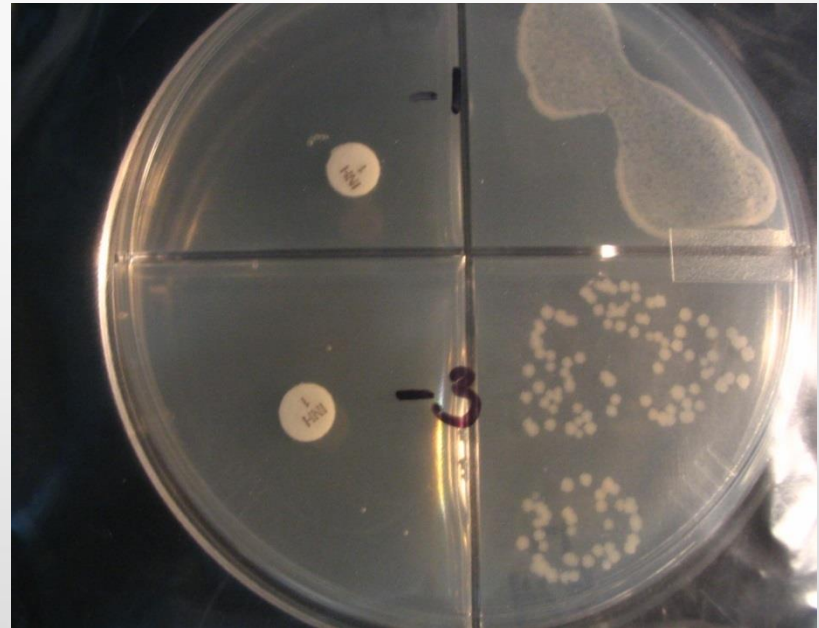
- Isoniazid
- Rifampin
- Ethambutol
- Pyrazinamide



Secondary Antibiotics

Results available about 3 weeks after resistance is detected

- Fluoroquinolone (ciprofloxacin, ofloxacin, levofloxacin or moxifloxacin)
- Ethionamide
- Cycloserine
- Capreomycin
- Amikacin
- Kanamycin
- Streptomycin
- PAS



Molecular Detection of TB Drug Resistance (MDDR)

- Testing performed by CDC
- Rapid testing for DNA sequences associated with 1st and 2nd line drug resistance
- NAAT (+) sputum sediment or growth based culture isolates
- 3-4 day turn-around-time
- Only requested by state health lab
- Submission criteria :
 - ✓ Known Rifampin resistance
 - ✓ Known MDR
 - ✓ High risk of Rifampin resistance or MDR-TB (e.g. previous TB, MDR-TB contact, foreign born)
 - ✓ High profile patient (e.g. daycare worker, nurse)
 - ✓ Mixed or non-viable culture
 - ✓ Adverse reaction (e.g. RIF allergy)

CDC MDDR

Mutations that CDC testing detects

- **First-line** MDDR to detect MDR-TB-Pyrosequencing
 - *rpoB* (Rifampin)
 - *inhA* and *katG* (Isoniazid)
- **Second-line** MDDR to detect XDR-TB-Sanger sequencing
 - *gyrA* (Fluoroquinolones)
 - *rrs* (Kanamycin, Amikacin, Capreomycin)
 - *eis* (Kanamycin)
 - *tlyA* (Capreomycin)
 - *pncA* (Pyrazinamide)
 - *embB*(Ethambutol)

What do the results mean



When a mutation is DETECTED, the report will read:

rpoB-Mutation Rifampin resistant (100% of our in house evaluation of 550 clinical isolates with this mutation are rifampin resistant)

When a mutation is NOT DETECTED, the report will read:

rpoB-No Mutation Probably rifampin susceptible (97% of our rifampin resistant isolates in our in-house evaluation of 550 clinical isolates have a mutation at this locus)

A negative result (e.g., No Mutation) **DOES NOT RULE OUT** out contributory mutations present elsewhere in the genome

TB DNA Genotyping Universally Offered by CDC

Genotyping provides a fingerprint of each isolate

Michigan performs MIRU-VNTR testing within 2 days , CDC performs the Spoligo testing:

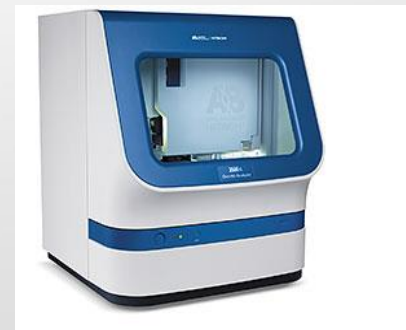
Spoligo-00000000003771 / MIRU-223325173533 / 445644423328

State Cluster: MI_0016 State Cluster Name2: MI_0016_003

GENType: G00012 Genotyping Lineage: East Asian (L2)

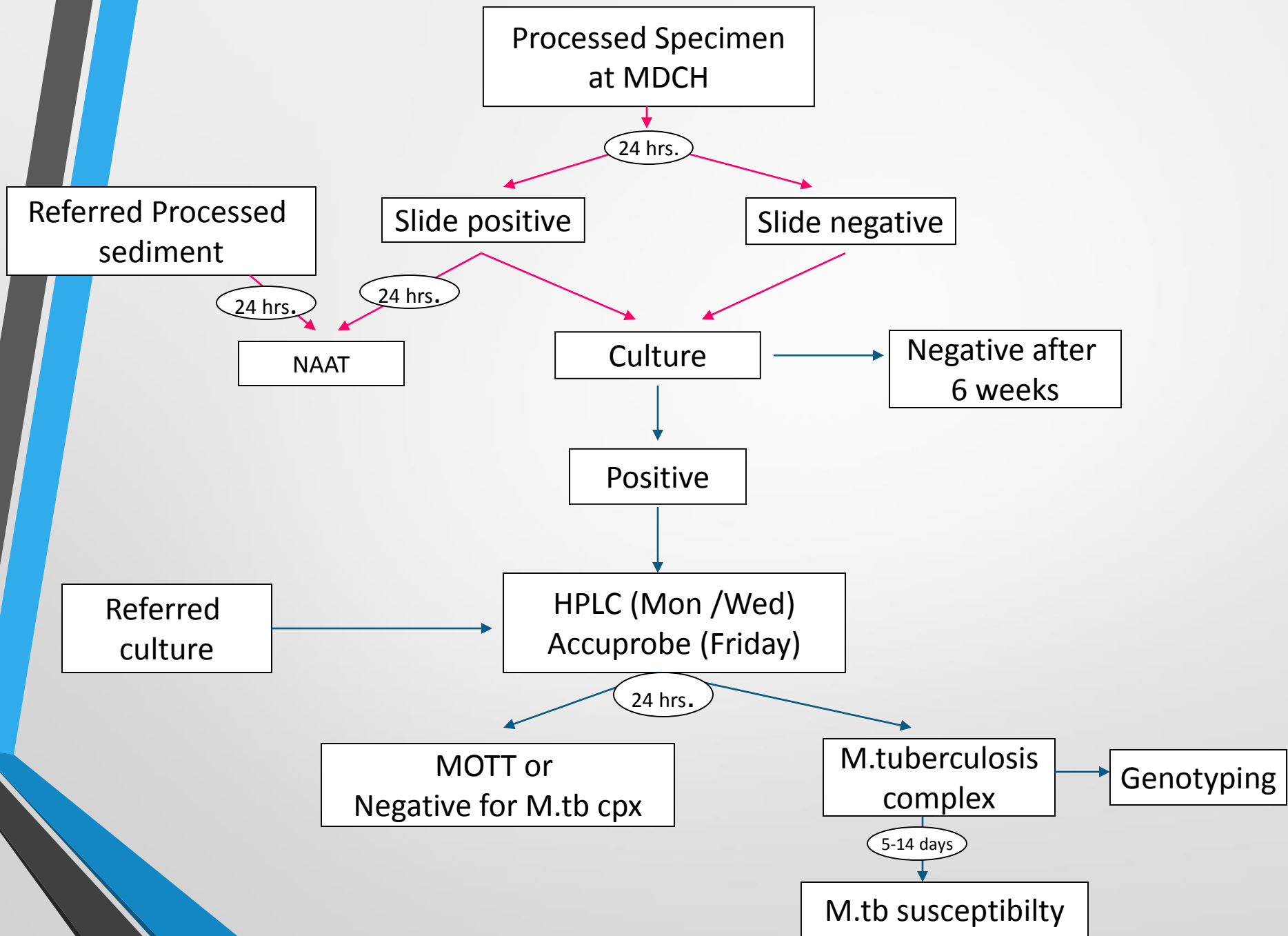
Used with traditional investigations, genotyping has

- Identified outbreaks not previously recognized
- Confirmed/detected transmission
- Identified risk factors for recent infection
- Demonstrated re-infection with different strains
- Documented lab cross-contamination



Used separately, Molecular and culture growth based testing are imperfect, used together, the accuracy and speed of detection of *Mycobacterium tuberculosis* and drug resistance is greatly improved







◦ Prevent Disease ◦ Promote Wellness ◦ Improve Quality of Life ◦