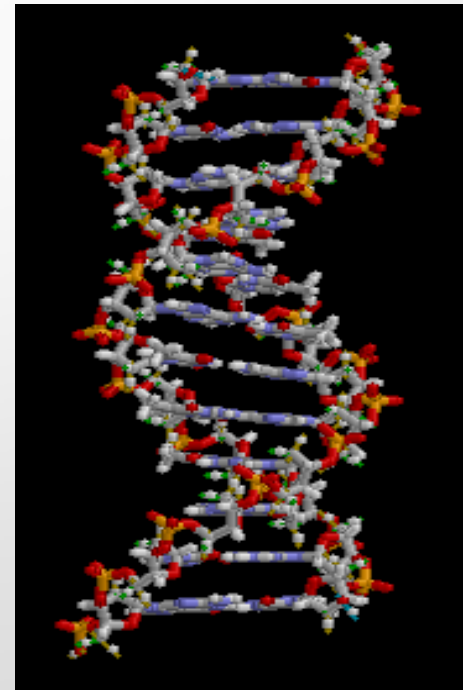


New Technologies in Laboratory Testing

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Learning objectives

- What do all the words mean?
- Identify new laboratory techniques and methods for detection of *M. tuberculosis*
- The advantages and disadvantages of new techniques as compared to more commonly used tests
- Timelines for testing and results

What do all the words mean?



NAA

Amplification MGIT

MTD

PCR

Pyrosequencing

Molecular

mutation

HPLC

MALDI-Tof

Gene Xpert

MDDR

NAAT

Genotyping

WGS

16 S Sequencing

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

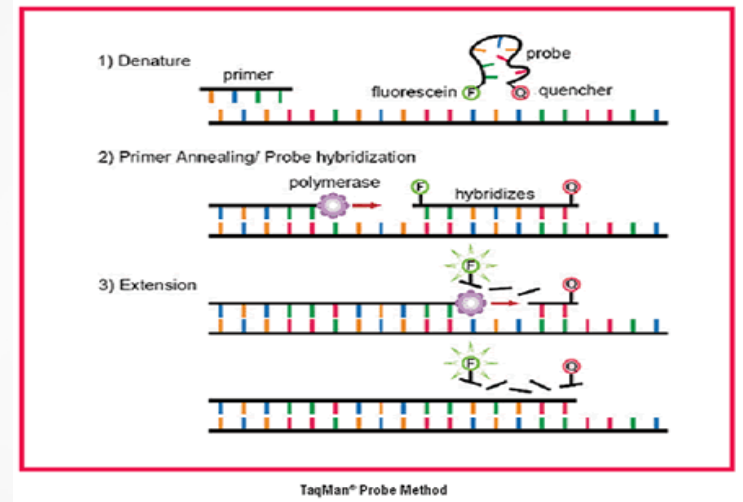


- PCR based assay in a cartridge
- Amplifies DNA from sputum (FDA) samples
- Detection method is fluorescent probe bound to DNA
- Also detects Rifampicin resistance
- Less than one minute hands on time, results in 90 min
- cost could be up to \$50/cartridge

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

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)

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

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:

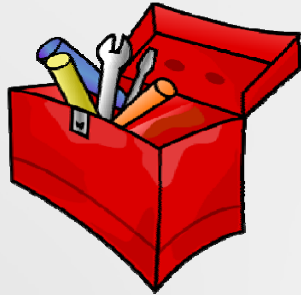
rpoB-Mutation Rifampin resistant (100% of our in house evaluation of 550 clinical isolates with this mutation are rifampin resistant)

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

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

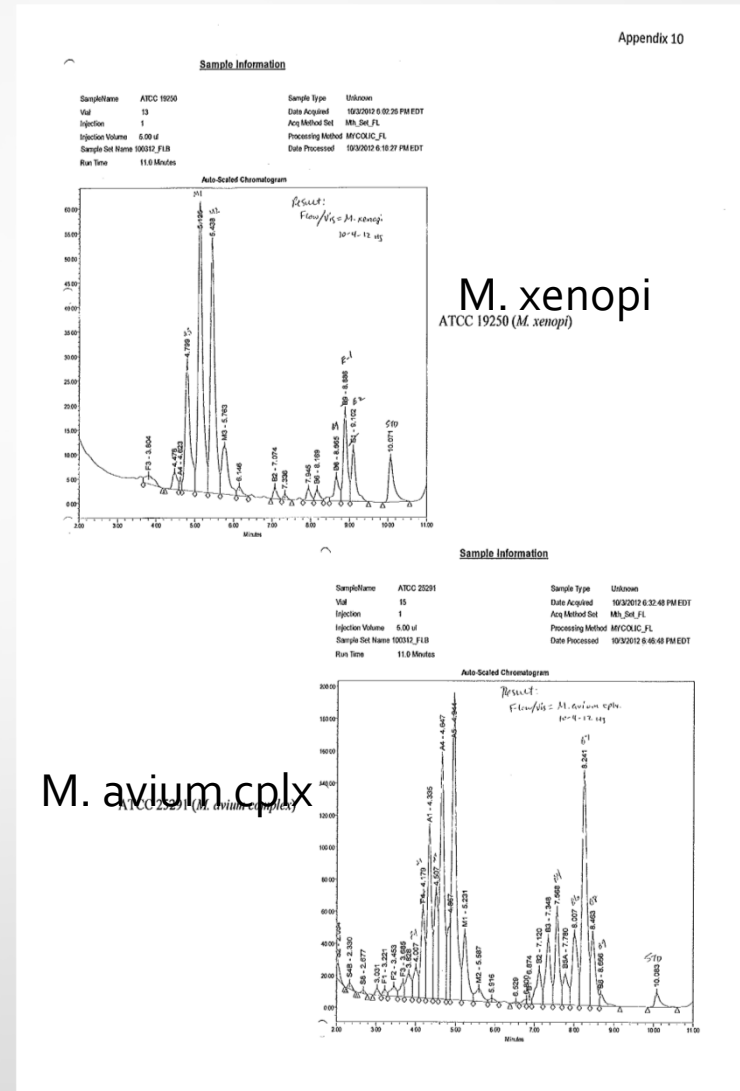


- HPLC-high performance liquid chromatography
- Maldi-Tof-Matrix-assisted laser desorption/ionisation Time of Flight
- Accuprobe-M. tb cplx., M.avium cplx., M. kansasii, M. gordonae
- Conventional biochemical testing

HPLC



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
 Run time per specimen is about 15 minutes



Maldi-Tof



Matrix-Assisted Laser Desorption/Ionization
Time of Flight
Extract and Analyze intrinsic proteins by mass
spec.
Spectral pattern of protein expression is
compared with reference patterns in a database
Run time on the instrument is a minute

Analyte20



Analyte Name: Ext Ctrl B
 Analyte Description: D:\data\MaldiBiotypeRealTimeClassification\160331-1120-100006248\Ext Ctrl B\0_A4\1\SLin
 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

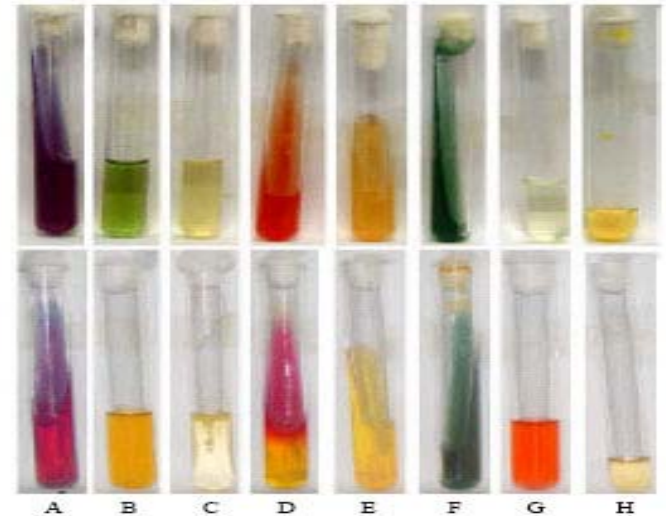
Accuprobe and Biochemical Tests

Accuprobe



M. tuberculosis complex
M. avium complex
M. kansasii
M. goodii
Solid or broth cultures, results in about 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

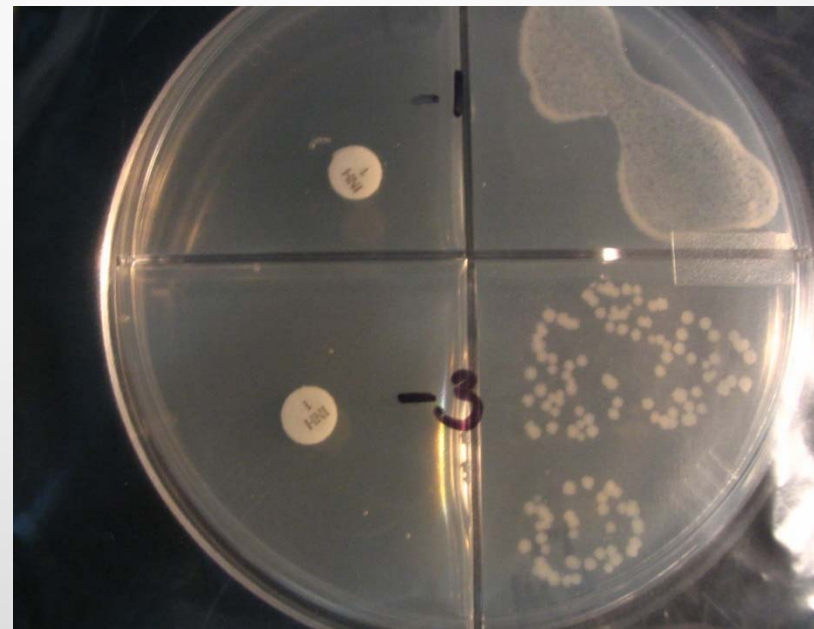
- Isoniazid
- Rifampin
- Ethambutol
- Pyrazinamide



Secondary Antibiotics

Results available about 3 weeks after resistance is detected

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



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-000000000003771 / 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 alone, Molecular testing and culture growth based testing are imperfect, used together, the accuracy and speed of detection of *Mycobacterium tuberculosis* and drug resistance is greatly improved



