Tuberculosis Laboratory

Overview

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

- Prevent Disease
- Promote Wellness
- Improve Quality of Life
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
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.
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

- Prevent Disease
- Promote Wellness
- Improve Quality of Life
A lot of tests with very little specimen

- Prevent Disease
- Promote Wellness
- Improve Quality of Life
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
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!

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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 primes 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
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*
• 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
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
Accuprobe and Biochemical Tests

**Accuprobe**

M. *tuberculosis* complex
M. *avium* complex
M. *kansasii*
M. *gordonae*
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 1\textsuperscript{st} and 2\textsuperscript{nd} 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-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

Prevent Disease • Promote Wellness • Improve Quality of Life
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