Laboratory Update Characterization, NAAT, and DST

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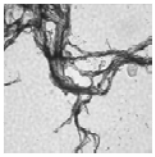
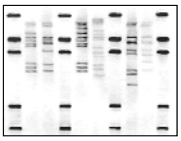
Microbial Identification and Characterization





Phenotypic
- Biological & biochemical
- DNA Culture Probes
- Antibiotic & drug susceptibility testing

Genotypic
- DNA Fingerprinting
  - Spoligotyping
  - RFLP
  - VNTR analysis
- Probe hybridization
  - Accu Probe
- Nucleic Acid Amplification (NAA)
  - GenProbe MTD, Cepheid GenXpert
  - 16 S sequencing

2011 Mycobacteriology Testing
Annual Workload

Primary Isolation
- 5007 patient specimens
- 1805 individual patients
- 88 individual patients for whom at least one culture was positive for *M. tuberculosis* complex.

Reference Culture Identification
- 638 cultures submitted
- 125 individual patients that had at least one reference isolate identified as *M. tuberculosis* complex.

NAAT Testing (MTD)
- 109 patients that had the MTD test performed
- 35 patients that MTD test detected M.tuberculosis DNA.

2011 Mycobacteriology Testing
Drug Susceptibility

First Line
- 190 first line drug tests on all initial *M. tuberculosis* isolates

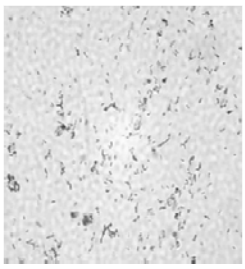
Second Line
- 32 Second line drug tests on all resistant *M. tuberculosis* isolates.

Drugs for most other *Mycobacteria* spp. available through National Jewish Hospital upon request.

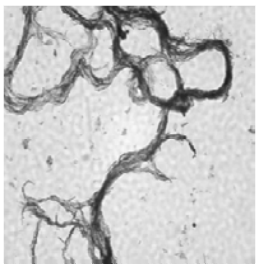
MGIT 960
Used for Primary isolation, 1st and 2nd line DST

- Continuous incubation at 37°C
- Continuous monitoring for fluorescence
- Growth of any organism is detected
  - Mycobacteria, yeast, other bacteria
- Smears prepared from broth
  - Growth determined to be acid-fast

Acid Fast Bacilli in Culture
Stained with Kinyoun (Carbol-fuchsin)

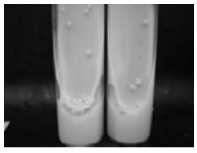


Pleomorphism and branching often seen in *M. avium* complex



"Cording" typical of *M. tuberculosis*

Growth on LJ Slant



M. tuberculosis

Non photochromagen
M. avium

Positive DNA Culture Probe for *M. tuberculosis* complex

- *M. tuberculosis* complex results phoned for all new patients
  - Diagnostic of the clinical syndrome, tuberculosis
- Preliminary Culture Report mailed
- Conventional biochemicals for speciation of *M. tb* complex by request or when testing indicates possible *M. bovis*.
  - Average of 3 weeks
- Final Culture Report mailed
  - *M. tuberculosis* complex

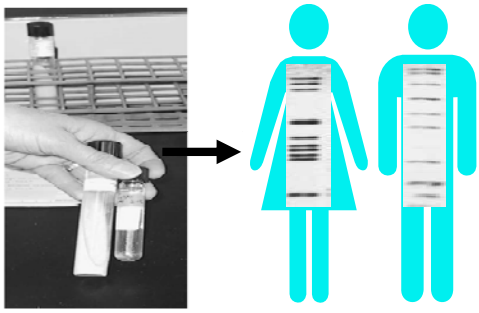
Drug Susceptibility Testing

Mycobacterium tuberculosis

- First line drug testing
  - Isoniazid (INH), Rifampin, Ethambutol Streptomycin, Pyrazinamide (PZA)
- Results available within 7-12 days after speciation
- Resistant strains - results phoned to submitter
- Second line drug testing
  - Ethionamide, Capreomycin, Ofloxacin, INH at a higher concentration
  - Sent to CDC for additional drug susceptibility testing

Genotyping

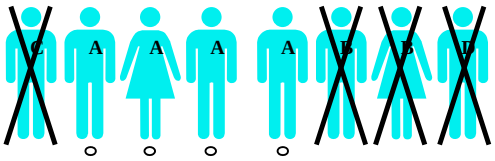
Mycobacterium tuberculosis



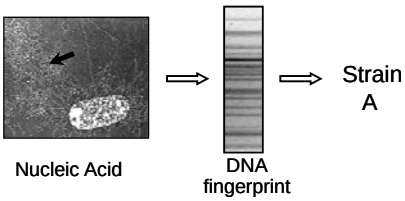
Different Strains

M. tuberculosis Strain Typing

- Commonly Used Methods
  - IS6110 Restriction Fragment Length Polymorphism (RFLP)
  - Spoligotyping
  - Mycobacterial Interspersed Repetitive Units – Variable Number Tandem Repeat (MIRU – VNTR) typing
- Testing performed by Regional Lab (MI) or the CDC
  - In 2011 DCLS sent 208 samples for typing



Genotyping (DNA fingerprinting) allows for the tracking of TB strains circulating in a population

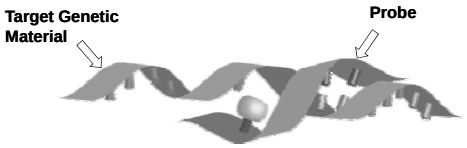


Advantages of TB Genotyping

- Increases the understanding of TB transmission within VA and nationally
- Can help to uncover information that may not be obtained with traditional epidemiologic methodologies and investigations
  - Contact investigations can often be limited to individuals directly exposed.
  - Often more precise as compared to phenotypic methods
- Can often discriminate between strains and determine whether infections are due to:
  - Recent transmission
  - Reactivation of a previous infection
  - Infection with a new strain

Nucleic Acid Amplification Tests (NAATs)

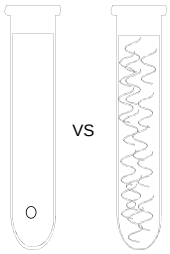
- Identify a region of genetic material unique to a particular organism (ie. *M. tuberculosis*)
- FDA approved the GenProbe Amplified *M. tuberculosis* Direct Test for AFB smear (+) respiratory specimens in 1995 and for smear (-) respiratory specimens in 1999



Why is an amplification test for TB needed?

- Direct detection with probes is not possible
- Microscopic AFB smears are rapid, but insensitive and non-specific
- Culture is sensitive and specific, but too slow (2-8 weeks).
- Clinical Significance
  - Isolate patients to prevent spread of disease
  - Treatment decisions: Is it *M.tb* or MOTT?
  - Reduce morbidity and mortality
  - Reduce health care costs for unnecessary isolation/treatment

AMPLIFIED *Mycobacterium Tuberculosis* (MTD) Assay



- Amplified molecular assay detects *M. tuberculosis* directly from sputum samples in less than 3.5 hours
- Utilizes a Transcription-Mediated Amplification system (TMA) to detect rRNA
- One *M. tuberculosis* organism can contain up to 10,000 copies of rRNA (biological amplification)

AMPLIFIED MTD Test Detects All Members of *M. tuberculosis* Complex

Mycobacterium africanum
Mycobacterium bovis
Mycobacterium microti
Mycobacterium tuberculosis
Mycobacterium canetti

What is the MTD test FDA approved and not approved for?

Approved for:

- Testing smear (+) and (-) specimens (NOTE: Smear (-) specimens NOT tested at DCLS)
- Testing patients who have taken TB medications for LESS than 7 days
- Patients with high clinical suspicion of TB

NOT Approved for:

- Specimens from patients receiving TB medications in the past 12 months
  - NOT a test of cure; MTD can detect nucleic acids from dead and live organisms, so may remain positive long after treatment is completed and the culture is negative
- Testing children or patients unable to produce sputum

How good is this test*?

- Respiratory smear (+) specimens from untreated patients with high suspicion for TB.
  - Sensitivity = 95%
  - Specificity = 98%
- Respiratory smear (-) specimens from untreated patients with high suspicion for TB.
  - Sensitivity = 66%
  - Specificity = 98%

*GenProbe Package Insert

Interpretation of Results

- Not a perfect test – false positive and false negatives can occur
  - Poor specimen quality
  - Contamination
  - Low numbers of Mycobacterium
  - Inhibited due to a naturally occurring inhibitor in the specimen or processing reagent (ex. blood)
  - Cross-reactivity (rare!!)
- Does not replace culture results which are the "gold standard".
- Interpret within the context of the patient's symptoms, chest x-ray, smear and culture

DCLS Data

October 2011 to September 2012

Number of MTD Performed – 59

- 20 smear Negative
  - 19 MTD negative
    - Culture: 2 MOTT; 17 No AFB Isolated
  - 1 MTD positive
    - Culture: *M. tb* complex

DCLS Data
Continued

- 39 smear positive
  - 13 MTD negative
    - Culture: 12 MOTT, 1 *M. tb* complex (mixed with M.avium)
  - 26 MTD positive
    - Culture: 26 *M. tb* complex

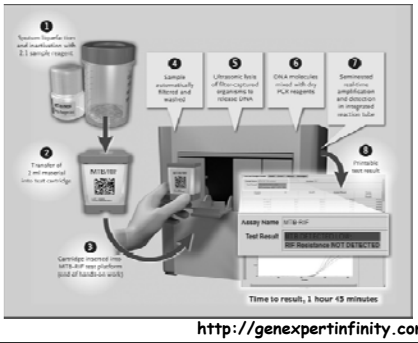
DCLS Data
Continued

- Sensitivity and Specificity of MTD test with smear and culture results.
  - Sensitivity is 96%
  - Specificity is 95%
- Sensitivity and Specificity of MTD test with smear results only
  - Sensitivity is 66%
  - Specificity is 95%

Cepheid GeneXpert: MTB/RIF

- Integrated, hands free specimen processing
- Molecular detection within a closed cartridge containing reagents for lysis, nucleic acid extraction, amplification, and amplicon detection
- Detection of *M.tuberculosis* complex and resistance to rifampin all in one test.
- Test is not currently FDA approved
- DCLS plans to complete validation studies in 2013.

Cepheid GeneXpert: MTB/RIF



http://genexpertinfinity.com/

CDC MDDR Assay *

- Service to rapidly and accurately detect drug resistance.
- Can test NAAT + sputum sediments and isolates
- Looks for genetic mutations associated with Rifampin(RMP), isoniazid(INH) resistance.
- If resistance to RMP is detected
  - Resistance to Ethambutol(EMB), Pyrazinamide(PZA), 2nd line drugs fluoroquinolones(FQ) and injectables: Amikacin(AMK), Kanamycin(KAN), and Capreomycin(CAP) are tested.

* Information provided in the CDC users guide

CDC MDDR Assay

- Two to three day TAT
- Includes a screening PCR/Sequencing assay.
- If RMP mutation is observed a conventional DNA sequencing assay is performed to look for mutations in the other 1st line drugs, FQ, 2nd line, and injectables.

CDC MDDR Assay

- Limitations of molecular DST testing
  - Gaps in knowledge
  - Limits of detection in assays used
  - Not all mechanisms of resistance are known
- Absence of a mutation is not confirmation of drug susceptibility.
- Requires conventional DST's to confirm the molecular testing results.

Conclusion

- NAAT provide additional laboratory results to base decisions upon.
- CDC MDDR service can help with early detection of drug resistance. Requests for testing must go through TB Control in conjunction with DCLS.
- Culture and Drug Susceptibility Testing are still the gold standard.
- DCLS and TB Control are available to assist with patient consultations and interpretation of results!

DCLS Acknowledgements

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