Diagnosis of TB: Mycobacteria Laboratory
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PHLET-Before

PHLET-After
Mycobacteria Laboratory

Direct Tests ← Specimens
→ Detect & Isolate

ID → Drug Susceptibility Testing → DNA Fingerprinting

3200/yr.

Pulmonary Mycobacterial Disease

- Diagnosis
  - Clinical symptoms
  - X-rays
  - ppd skin test/Quantiferon/Tspot
  - AFB analysis of respiratory specimens by microbiology (confirms a dx. of TB)
Terminology/Acronyms

- Smear/stain (bugs)
- AFB-acid fast bacillus
- MOTT-mycobacteria other than TB; or NTM-non-tuberculous mycobacteria; or atypical mycobacteria
- MAC-M. avium & M. intracellulare complex
- Culture/Plates/Colonies (MGIT)
- Probes (DNA ID test done on organism)
- DAT-Direct amplified test (done on specimen)

Specimens

- Respiratory
  - sputum-natural or induced
  - tracheal aspirates
  - bronchial washings
  - bx.
  - gastric aspirates
Respiratory Specimens

Sputums:
* 3 good quality specimens (early morning)
* volume (no standards; 5 mL)
* minimize transit time to the laboratory to reduce contamination

Extrapulmonary Specimens

* blood
* bone marrow
* sterile fluids
* CSF
* stool
* urine
* tissue
* wound drainage
* how to send?
Specimen Processing

• Specimens from non-sterile sites are decontaminated (removes unwanted bacteria & yeast), then concentrated.
  ♦ e.g., respiratory, stool, gastrics, wounds
    ♦ Wounds-“direct” and processed

• Specimens from sterile sites are not decontaminated, only concentrated.
  ♦ CSF, aseptically collected biopsy tissues, blood

Specimen Processing

• Decontaminate (several methods)
  ♦ NALC-NaOH method
    ♦ alkaline, destroys routine organisms
    ♦ mucolytic - releases the AFB
    ♦ neutralize with phosphate buffer, pH 6.8
    ♦ 2nd processing; oxalic acid treatment

• Concentrate
  ♦ Centrifugation
Specimen Processing

Decontaminated Concentrated Respiratory Specimens
Microscopy

- Fluorochrome stain (auramine-rhodamine)
  - 250-400x
  - bacilli fluoresce green-yellow

- Acid-fast stains
  - 1000X
    - Ziehl-Neelsen
    - Kinyoun
  - bacilli appear red ("acid-fast bacilli")
AFB on Fluorochrome Stain

Ziehl-Neelsen AFB Stain 1000X

http://courses.nus.edu.sg/course/mictank/mbbs/mycobacteria/sld005
Microscopy

- Smear positive
  - 5,000-10,000 acid fast bacilli (AFB)/mL sputum

- Smear positive TB patients are considered more infectious than smear negative.

- Smears are graded as negative, or 1, 2, 3 or 4+ positive

Microscopy-fluorochrome x 250

- 1+ 1-9 AFB/10 fields
- 2+ 1-9 AFB/field
- 3+ 10-90 AFB/field
- 4+ >90 AFB/field

Microscopy

- Smear result should be available within 24 hours of specimen receipt

- Smears do not distinguish between different species of mycobacteria (not specific); e.g. can’t tell a MAC from a TB

- Scenario of smear +, and culture –
  - Drug therapy
  - Drug resistant TB
  - Due to processing
  - Artifact

AFB Culture
**AFB Culture**

“Gold Standard”

- **Solid Medium**
  - TB grows slowly (2-3 weeks)
  - semi-quantitative (plate count)
  - more sensitive than smear (10 AFB/mL)

**Middlebrook**

**Lowenstein Jensen**

**AFB Broth Culture**

- Broth culture: “rapid system”
- Newer instrumentation=better recovery of NTM, “2 medias better than 1”
- Contamination rates increase
  - Anti-microbial supplements
    - Not perfect for every contaminant
    - May slow or inhibit mycobacterial growth
- Multiple AFB species
AFB Broth Culture-Rapid

- Instrumentation
  - Bactec 460 (Becton-Dickinson)
  - MGIT 960 (Becton-Dickinson)
  - ESP Culture System II /VersaTrek (Trek diagnostic)
  - MB/BacT (Biomerieux)

Bactec 460 Instrument
radiometric CO₂ production
**Mycobacteria Growth Indicator Tube (MGIT)**

$O_2$ consumption = fluorescence

**ESP II/Versatrek**

gas pressure & temp measurements

**Myco Bottle**
BacT ALERT
colorimetric detection of CO₂ production

IDENTIFICATION
**Identification**

- **Phenotypic**
  - High performance liquid chromatography (HPLC)
- **AccuProbe™ (DNA Probe)**
- **PCR-based technologies**
  - Gene sequencing (16S rRNA)
  - Line Probe Assay (LiPA)-Innogenetics
  - GenoType- Hain LifeScience
  - Home Brew
    - *(PRA)*

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**High Performance Liquid Chromatography (HPLC)**

- Used predominantly by public health laboratories
- Specialized instrumentation
- Extract cell wall mycolics & separate by column chromatography
- Pattern of peaks usually unique to the species
**HPLC**

- Identifies MTB complex
- Can be performed on smear-positive sputum concentrates. Texas DSHS: Identifies TB or MAC 50-70% of the time
- Less accurate with infrequently isolated NTM
- Can’t separate rapidly growing Mycobacteria sp.
  - “M. fortuitum group” or
  - “M. abscessus/chelonae group”
    - Outbreaks - pedicure salons, lasik surgery, liposuction
DNA Probe – AccuProbe™

Gen-Probe

● 2-4 hour test
● Test growth from solid or liquid culture (not direct specimens)
● DNA probe binds to rRNA generates a chemiluminescent signal
● Only identifies 5 species of Mycobacteria
  - M. tuberculosis complex
  - M. avium
  - M. intracellulare
  - M. kansasi
  - M. gordonae
MicroSeq 500 system
Applied Biosystems

- Extract the Mycobacterial DNA & perform PCR
- Sequence 500-bp of a gene fragment (16S rRNA)
- Different species have differences in the gene sequences
- Compare the un-identified Mycobacteria to a computer database of known sequences of Mycobacteria (in development)
Direct Tests

Direct Amplified Nucleic Acid Test (DAT)

Nucleic Acid Amplification Test (NAA)

**DAT**

- *M. tuberculosis* direct test (MTD) (Gen-Probe)
  - FDA approved for **smear + & - respiratory specimens**
  - 4 hrs. transcriptase mediated amplification (amplifies 16 S rRNA), followed by hybridization step specific for TB

- **Amplicor TB test (Roche)**
  - FDA approved for **smear + respiratory specimens**
  - 6.5 hours-PCR -amplifies 584 bp segment of T6S rDNA, followed by hybridization step specific for TB
DAT Advantages

- Direct specimen test: Rapid detection & highly specific
- Affects treatment decisions
  - TB or NTM such as MAC??
- Reduces health care costs
  - isolation rooms
  - contact investigation

DAT Limitations

- Tests for only MTBC
- Expensive, high complexity test
- Validated with respiratory specimens
- The lower the target material the lower the sensitivity (smear neg/TB culture += 48-52% sensitivity)
- Cannot be used to monitor therapy
DAT Interpretation
MMWR July 7, 2000; 49(26): 593-594

Susceptibility Testing
Susceptibility Testing
First-Line Drugs

Testing is done on the initial TB isolate

- Isoniazid
- Rifampin
- Ethambutol
- Pyrazinamide (problematic; pH)

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Susceptibility Testing

- Susceptibility testing is repeated if a culture becomes positive for TB after a time period of successful conversion to negative, or if conversion does not occur.

- Additional drugs are tested if a TB isolate is resistant to rifampin or two or more first line drugs.
Susceptibility Testing

- **Agar-based**
  - Proportion & agar disk elution (3-weeks)

- **Broth-based system**
  - Bactec 460 (4-12 days)
  - ESP Culture System II (4-12 days)
  - MGIT (4-14 days)

Proportion Susceptibility Test
First-line TB Drugs
# Public versus Private Sector Issues

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*Note: This table is illustrative and does not reflect the actual content of the document.*
Issues

- **Communication.** Fewer labs are doing full service testing. Results are difficult to obtain since testing is done at community hospitals/labs, private labs and state labs (HIPPA).

- Delays in lab testing can lead to a delay in diagnosis, initiation of therapy & reporting.

- Laboratories are experiencing a workforce shortage, this affects testing proficiency and TAT.

Initiatives

- **California**
  - MGIT by mail system, local labs process, do smear & culture and ship MGIT to the state lab

- **New York**
  - Fast Track-165 labs send spu from smear + or TB suspects to the state lab

- **Michigan**
  - Statewide wide courier system to deliver specimens & pos broths to the health department
Future Goals

- Partnering of labs to expedite isolate submission for rapid ID, susceptibility, and molecular typing (fingerprinting).

- Development of internet based statewide communication systems to network health care providers, public health and laboratories.

- Addressing laboratory personnel shortages & funding issues.

- Incorporate rapid molecular tests (under research development), into the clinical lab setting. Slow and expensive process.