An Introduction to Laboratory for TB Nurses

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Has the following disclosures to make:

- No conflict of interests
- No relevant financial relationships with any commercial companies pertaining to this educational activity

An Introduction to Laboratory for TB Nurses

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TB Diagnostic Methods

- **IGRA (Interferon Gamma Release Assay)**
- **AFB Smear**
- **Nucleic Acid Amplification**
- **AFB Culture**

- Clinical Presentation
- TST (Tuberculin Skin Test)
- X-ray

Modified from Denise Dunbar

IGRAs

- Which tube do I use?
  - T-spot (heparin – green top tube)
  - QFT – tubes that come with the kit or a heparin – green top (if your lab will let you…..)
- Which lab do I send it to? How do I get it there?
- How quickly do I need to have it to the lab?
- How do I store the tube until transport?

QuantiFERON®-TB Gold Plus

Mitogen – Positive Control Low response may indicate inability to generate IFN-γ	
Nil – Negative Control Adjusts for background IFN-γ	
TB1 – Primarily detects CD4 T cell response	
TB2 – Optimized for detection of CD4 and CD8 T cell responses	

- Essentially 2 tests in one blood draw
- TB1 and TB2 should be close in value

Sputum specimens

- Who should I collect sputum from
  - Patients with respiratory symptoms
  - Patients with an abnormal CXR

Bacteriologic and histologic Examinations

Especially when lung or larynx is site of disease:

- 3 sputum specimens for AFB smear and culture
- Collected 8-24 hours apart with at least 1 early morning specimen

Specimens should be obtained in an isolated, well-ventilated area or sputum collection booth

Bacteriologic and histologic Examinations

- Sputum collection should be directly supervised
- For patients unable to cough up sputum, deep coughing may be induced

Bacteriologic and histologic Examinations

Extrapulmonary Specimens

- Urine
- Cerebrospinal fluid ✱
- Pleural fluid ✱
- Pus
- Biopsy specimens

✱recovery poor

Laboratory Examination

AFB Smear

- First clue
- Presumptive diagnosis only

- Fluorochrome staining preferred method
- Results available in 24 hours
- Many patients have negative AFB smears

Specimen Quality

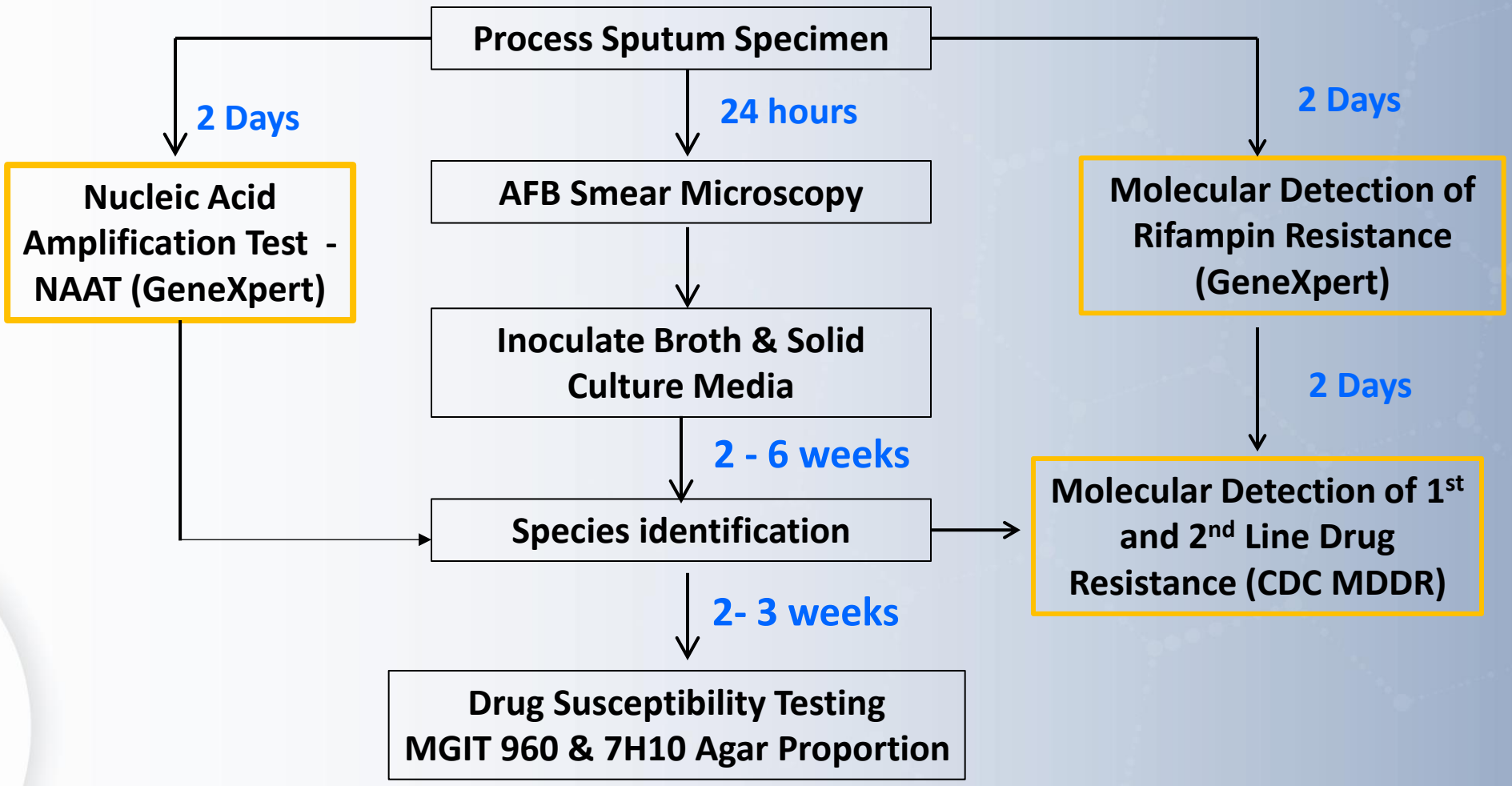
- Accurate laboratory results are directly proportional to the quality of the specimen

- Sputum
  - *Recently* discharged material from the bronchial tree, with minimal amounts of upper respiratory tract secretions
    - Well coached patient, collect at least 3 ml
    - Label tube, form, and indicate test:
      - Initial Dx: Smear, NAAT, & Culture
      - Follow-up: Smear and Culture
      - Release from respiratory isolation?
        - Order Smear only

- Transport to lab cool and quickly

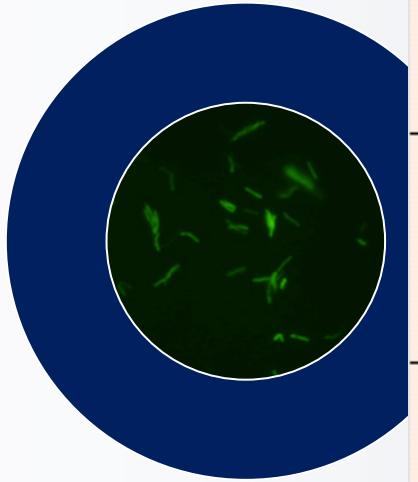
Courtesy of Denise Dunbar

TB Laboratory Testing Algorithm



Courtesy of Denise Dunbar

AFB Smear



one microscopic field

CAP	ATS	Interpretation	AFB/ml sputum	Infectiousness of patient
negative	negative	negative	<5,000	probably not infectious
1 or 2 per smear	1 or 2 per smear	weakly positive	~5,000	probably infectious
<1 per field	1+	moderately positive	~10,000	probably infectious
	2+	moderately positive	~100,000	probably infectious
1-10 per field	3+	strongly positive	~1,000,000	probably very infectious
>10 per field	4+	strongly positive	>1,000,000	probably very infectious

Courtesy of Denise Dunbar

Nucleic Acid Amplification Tests (NAAT)

- Tiny amounts of DNA/RNA are amplified (copied) until there is enough for easy detection

- DNA/RNA is examined
  - Identification
  - Detection of Drug Resistance

- Test turnaround time measured in hours

Courtesy of Denise Dunbar

Nucleic Acid Amplification Test (NAAT)

- Detects *M. tuberculosis* complex nucleic acids; does not distinguish between live and dead bacilli
  - For initial diagnosis only
  - Not suitable for follow-up specimen or monitoring; cured patients may be NAAT + for years!
- Sensitivity compared to TB culture
  - >95% for AFB smear-positive
  - Only 55-75% for AFB smear-negative
- Does not replace culture for bacteriological Dx

Courtesy of Denise Dunbar

Laboratory Examination

Cultures

Used to confirm diagnosis

- Perform on ALL specimens regardless of AFB smear results
- Results available in 10 to 14 days (on liquid media, e.g. BACTEC)

TB may be diagnosed on the basis of
signs and symptoms in the absence of a + culture

AFB Culture

- More sensitive than smear
  - 5,000 to 10,000 AFB/ml for smear
  - ~10 viable AFB/ml for culture
- Positive for only ~85% of Pulmonary TB
  - Requires a quality specimen
  - May be invalid due to contamination
- Used to monitor patient response to treatment (like smear)
- Required for drug susceptibilities & genotype
- Lengthy
  - 1-6 weeks by liquid media
  - 2-8 weeks by solid media

Courtesy of Denise Dunbar

Drug Susceptibility Testing (DST) of *M. tuberculosis* complex

Current CDC Recommendations

- Initial isolate should be tested against first-line drugs (FLD)
  - Isoniazid, Rifampin, Ethambutol, Pyrazinamide
  - Repeat test if patient cult+ after 3 mo. Rx
- For isolates resistant to Rifampin or to any 2 FLDs: **test second-line drug panel**
  - Minimum: Fluoroquinolone (Ofloxacin), Ethionamide, & Injectable (Amikacin, Capreomycin, Kanamycin)

Current DSHS DST Testing

- Includes all of the above

Courtesy of Denise Dunbar

Molecular Detection of Drug Resistance

- Examining DNA of specific genes for mutations known to be associated with phenotypic resistance
- Rapid - analysis takes less than 1 day
- Can be done on culture isolates or directly on NAAT+ specimens

Courtesy of Denise Dunbar

CDC Molecular Detection of Drug Resistance (MDDR)

- Test Indications
  - Known/suspect DR case or contact to DR case
  - Previous TB Treatment
  - Patient from area with high rate of DR TB
  - Large public health impact
  - Mixed or nonviable culture

Courtesy of Denise Dunbar

CDC Molecular Detection of Drug Resistance (MDDR)

- Provides 2-3 day DNA sequence analysis for drug resistance prediction
  - 7 classes of anti-TB drugs sequenced
- MDDR complements conventional DST
  - Used alone, MDDR and conventional DST are imperfect
  - Used together, accuracy of drug resistance or susceptibility detection can be improved.
- Conventional DST results are still needed to confirm susceptibility to individual drugs.

Courtesy of Denise Dunbar

Summary

- Make friends with the laboratory that processes you specimens. Often if you can tell them what you are trying to do, they will help you get there
- Like most things we do, quality matters. That goes for the specimens that are sent to the laboratory
- Molecular tests are one of the biggest jumps forward in information informing patient decisions

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Questions?

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