STANDARD DIAGNOSTIC METHODS

OPTIMAL USE AND ALGORITHMS
THE ENEMY.......... 

1/25,000_{th} OF AN INCH
ROLE(S) OF THE LABORATORY

- Primary Care – for better patient management

- Public Health – for appropriate implementation of infection control and public health measures to control the transmission of tuberculosis
THE EXPECTATIONS (CDC)

- Specimens reach the laboratory within 24 h of collection.
- Physician receive AFB stain results within 24 h of laboratory’s receipt of specimen.
- NAAT results ≤ 48 h of specimen collection
- Positive cultures detected within 14 days of specimen collection.
- MTB is identified ≤ 21 days of specimen receipt.
NONTUBERCULOUS MYCOBACTERIA (NTM)

- Increasing incidence of infections caused by these organisms
- Increasing number of species
  - > 1975: ~ 30 species
  - > 30 years later: >120 species
TASK AT HAND:

HIGHLIGHT IMPORTANT ASPECTS REGARDING THE LABORATORY DIAGNOSIS OF MYCOBACTERIAL INFECTIONS
Laboratory Detection and Identification of Mycobacteria; Approved Guideline

This document provides guidance to clinical mycobacteriology laboratories on the most optimum approach for the diagnosis of mycobacterial infections. A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

- Safety
- Levels of Laboratory and Referral Services
- Clinical Significance of *Mycobacterium* spp.
- Specimen Types, Collection, Transport and Storage
- Detection of Mycobacteria
- Identification Procedures
SAFETY - RISK ASSESSMENT

- Used to determine the type of practices to employ in the laboratory
- Who
- Factors to take into account
  - Level of TB diagnostic services offered
  - Laboratory design
  - TB prevalence
  - Rate of multi-drug resistant MTB
  - Whether or not aerosol-generating procedures are performed as well as their respective frequency

Ongoing evaluation for the risk of transmitting MTB
CLINICAL SIGNIFICANCE OF MYCOBACTERIUM SPP.

- *M. tuberculosis* complex
  > Most clinically significant, therefore its detection should be a primary focus of the laboratory
  > NOT in the environment
  > Isolation almost always signifies disease
CLINICAL SIGNIFICANCE

NTM - WHY MORE ATTENTION?

> Associated with AIDS
> Recognition that NTM lung disease is encountered with increasing frequency in the non-AIDS population
> Improvement in mycobacteriology laboratory methods
  » Enhanced isolation
  » More rapid and accurate identification of NTMs
NTMs

- Determining clinical significance when an NTM is isolated:
  - Clinical setting and host
  - Organism species and its pathogenic potential
  - Source of the culture isolate & likelihood for contamination
  - Quantification of organisms detected both in culture and AFB smears
  - Number of positive cultures
CLINICAL SIGNIFICANCE - NTM: LABORATORY INDICATORS

- **Bacterial burden**
  - Reflected by number of organisms seen in AFB smears
  - Culture
    - More sensitive than smear so will detect smaller numbers of organisms
    - ATS recommendation = quantitate mycobacterial growth on solid media cultures

- **Number of positive cultures**
  - Indicative of disease relevance
  - ATS recommendation = 3 sputum specimens to screen for NTM disease
CLINICAL SIGNIFICANCE - NTM: LABORATORY INDICATORS

- Microbiologic criteria for diagnosing NTM lung disease
  - Positive culture results from at least 2 expectorated sputum samples
  - Positive culture result from at least 1 BW or BAL
  - Transbronchial or other lung biopsy with mycobacterial histopathologic features and 1 positive culture OR biopsy showing mycobacterial histopathologic features and 1 or more sputum or BWs that are culture-positive for NTM

- Mixed cultures
SPECIMEN TYPES, COLLECTION, TRANSPORT, AND STORAGE
KEY POINTS TO ENSURE SPECIMENT QUALITY

- PROPER SPECIMEN COLLECTION.
- PROPER SPECIMEN COLLECTION.
- PROPER SPECIMEN COLLECTION.
KEY POINTS TO ENSURE SPECIMENT QUALITY (con’t)

- **Avoid swabs**
- **Refrigerate specimens until processed (except for blood specimens)**
- **Reject the following specimens**
  - Dry swabs
  - Gastric washing **not** neutralized and > 1 h old
SPECIMEN PROCESSING
SPECIMEN DECONTAMINATION

Viable AFB

Dead bacteria
**SPECIMEN PROCESSING**

- **Contamination rates**
  - Expected: 2 to 5%
    - If <2%, processing method may be killing many AFB
    - If >5%, processing may be inadequate.
  - Useful to monitor the recovery rate of *M. gordonae*

- **Digestion/ decontamination methods**
  - No clear consensus
  - Number of methods – appendices
  - NTMs and cystic fibrosis patients – specific decontamination methods employed
    - Two-step NALC-NaOH-oxalic method
    - Chlorhexidine method
DECONTAMINATING AGENTS

- Decontaminating reagents - examples
  - N-acetyl-L-cysteine (NALC) with NaOH (commonly used in US)
  - Sodium lauryl sulfate (commonly used in Europe)
  - Whole host of others: e.g. zephiran-trisodium phosphate

- After decontamination, adding buffered solution helps not only to neutralize the base but also
  - Dilutes toxic substances
  - Decrease specific gravity so that AFB can be readily concentrated by centrifugation
SPECIMEN PROCESSING (con’t)

- Concentration
  - $\text{RCF} = 1.12 \text{ Rmax (rpm/1000)}^2$
  - AFB smear and culture sensitivity depend on optimum RCF
  - Minimum recommended RCF $= 3000 \times g$ for 15 minutes

- Measures to prevent cross-contamination of specimens
DETECTION OF MYCOBACTERIA

- Microscopy
- Nucleic acid amplification tests for direct detection of *M. tuberculosis* in clinical specimens
# Sensitivity and Specificity of AFB Sputum Smears

<table>
<thead>
<tr>
<th>Reference</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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<tbody>
<tr>
<td>Alluoch et al</td>
<td>39</td>
<td>99.8</td>
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<tr>
<td>Boyd, Marr et al</td>
<td>22</td>
<td>99.3</td>
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<tr>
<td>Burdash et al</td>
<td>43</td>
<td>99.9</td>
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<tr>
<td>Levy et al</td>
<td>53</td>
<td>99.8</td>
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<tr>
<td>Lipsky et al</td>
<td>33</td>
<td>99.8</td>
</tr>
<tr>
<td>Strumpf et al</td>
<td>78</td>
<td>99.7</td>
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FACTORS INFLUENCING AFB SMEAR

- **SENSITIVITY:** Specimen type, species of infecting mycobacteria, efficiency of decontamination & concentration procedures, smear thickness, type of stain, extent of decolorization or the type of counterstain used, training & experience of staff.

- **SPECIFICITY:** Not using of ‘bulk’ staining trays/containers, experience of microscopist reagents for performing the stain (as well as all other components of digestion) are not properly filtered or replaced. Common sources of AFB: tap water, infrequently cleaned distilled water reservoirs.
• Use of the fluorochrome stain strongly recommended because of simplicity, sensitivity & speed.

• False + stains should occur at a rate of <1% of all stained specimens.

• What about screening for quality of sputum? NO
  > Lower respiratory tract secretions preferred
  > BUT, specimens with significant oropharyngeal secretions can still grow MTB
EXAMINATION & REPORTING

- Smear size = 1 x 2 cm
- Examine the equivalent of 300 OIFs
- Smear results must be quantified to be meaningful...important that magnification used be taken into consideration so that results are equivalent to 300 OIFs seen under fuchsin stains examined at 1000X.
DETECTION OF AFB BY CULTURE

- **Key to successful cultures**
  > Combination of different culture media is required to optimize the sensitivity of culture.
    » For example: an agar-based or egg-based medium, selective or plain **AND** a liquid medium.
  > Depending on whether suspecting a particular AFB infection, duplicate cultures on primary media should be inoculated at 30-33 degrees C.
  > Certain mycobacterial species have special nutritional requirements: *M. genavense*, *M. haemophilum* and *M. paratuberculosis*

- **Advantages and disadvantages of media agar-, egg-based and liquid media**
AFB CULTURE (con’t)

• Media Selection
  > Liquid vs solid media
  > Depends on the type of clinical specimen
    » Specimens from patients on anti-TB therapy
    » Extrapulmonary specimens – inoculate in duplicate (one w/ and one w/o antibiotics) due to generally low numbers of organisms present
    » Skin, bone and joints: inoculate to media supplemented with an additional iron source
      • Hemin
      • Ferric ammonium citrate
      • Commercial X-strips
AFB CULTURE (con’t)

- Examination of growth
  - Solid media
    - Necessary to incubate a minimum of 4 weeks
    - CDC-recommended criteria for colony counts:
      
      | Quantitation          | Report       |
      |-----------------------|--------------|
      | No colonies           | No AFB growth|
      | <50 colonies          | Actual count |
      | 50-100 colonies       | 1+           |
      | 100-200 colonies      | 2+           |
      | 200-500 colonies      | 3+           |
      | >500 colonies         | 4+           |

  - Liquid media
    - Confirmation for presence of AFB
    - Visual checks
Contamination

- Sources: specimen, contaminated reagents or the environment

- Negative control for each batch of specimens processed = sterile water or buffer

  » If + for AFB, results of all processed specimens in the batch become invalid

  » Recommendations for bacterial contamination
RECOMMENDATIONS:

a. Review all the procedures thoroughly.

b. Check all the reagents for sterility or prepare fresh reagents with confirmed sterility.

c. Disinfect the biosafety cabinet.

d. If enrichments or antimicrobial agents are added to the medium, add them inside the BSC. Do not open several tubes at a time, and open tubes for as short a time as possible. For addition of a reagent to several tubes, use a repeat pipettor if possible. Caps should be placed right side up (i.e. the inside of the cap facing the cabinet surface) on a 10% bleach-soaked towel.

e. Make all the additions prior to inoculation of the processed specimen.

f. Aliquot the reagents; discard unused reagents or resterilize the remainder of the reagents not used that day.

g. Open one tube at a time when adding reagents to the specimen tube, and close it immediately.

h. Inoculate the processed specimen by opening one tube at a time and closing after the inoculation.
**Contamination**

> Cross-contamination

» Indications

» Investigate episodes thoroughly and take corrective measures:

a. Make fresh reagents.

b. Aliquot reagents in small quantities.

c. Do not keep specimen tubes very close to each other in a test tube rack.

d. Open the specimen tubes one at a time and close after the addition of the reagent or after specimen inoculation.

e. Do not touch the reagent container or pipette with the lip of the specimen tube during the addition of reagents or manipulations.

f. Avoid splashing while adding reagents to the tube.

g. After mixing/vortexing, wait for about five minutes before opening the tube.
Recommended that important clinical isolates be frozen at -70 degrees C and saved for a minimum of 1 year

> Prepare suspensions in water, saline or 7H9 broth; homogenize, and adjust to McFarland No. 1 turbidity standard

> Positive cultures in liquid media may be stored directly in small aliquots by freezing at -70 degrees C

• Reporting
AFB CULTURE (con’t)

- **Quality Control**
  - **Media:** recommendations for prepared media including QC organisms
  - **Specimen Processing and Isolation of Mycobacteria**
    - Analyze data for 3-6 months to determine an overall average or a normal trend for the individual laboratory
    - Once parameters established, should remain uniform with minor fluctuations
PARAMETERS

- Total specimens processed
- Total and % AFB smear-positive and smear-negative
- Total and % AFB culture-positive from smear-negative and smear-positive specimens
- Total and % cultures positive for MTB and NTMs
- Average time to detection of AFB-positive cultures (smear-negative and smear-positive), TB and NTM
- Bacterial contamination rate
- Records of processing: technologist, all specimens in batch, negative control results, incubation temperature
Differentiation of *M. tuberculosis* from other mycobacteria represents an important public health issue.
IDENTIFICATION

- Phenotypic:
  - Conventional biochemical tests
  - HPLC

- Genotypic
  - Hybridization assays
  - Amplification followed by sequencing or REA
“TB or not TB, that is congestion. Consumption be done about it? Of cough, of cough. But it takes a lung, lung time.”