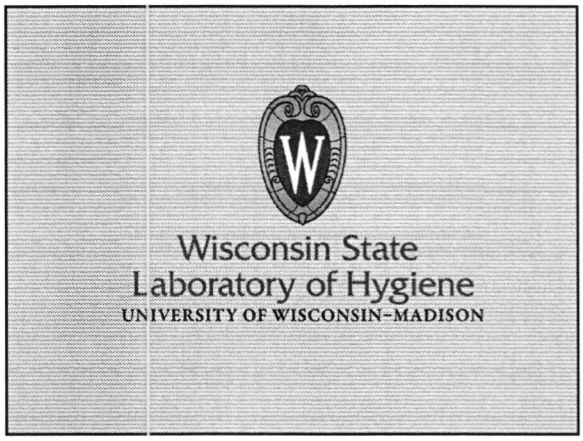
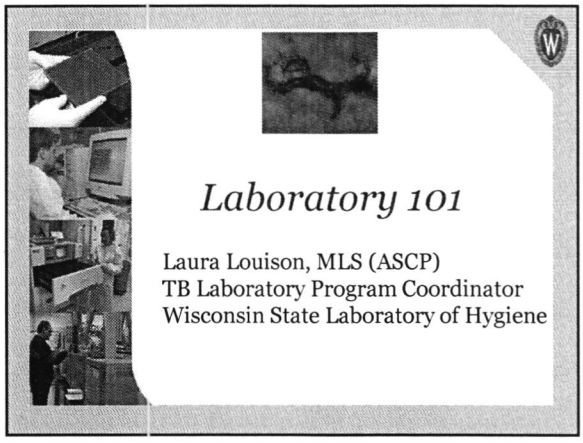
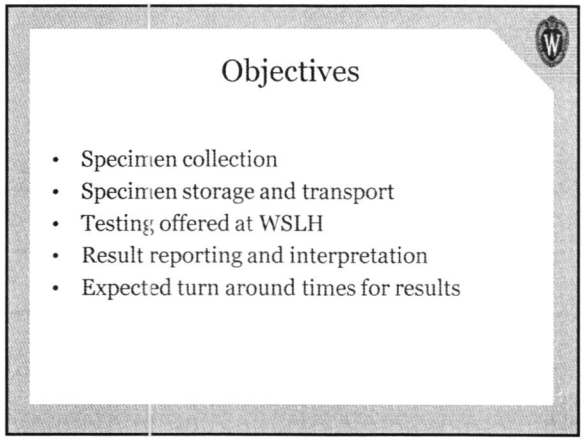

Wisconsin State
Laboratory of Hygiene
UNIVERSITY OF WISCONSIN–MADISON

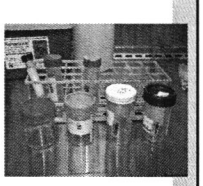

Laboratory 101
Laura Louison, MLS (ASCP)
TB Laboratory Program Coordinator
Wisconsin State Laboratory of Hygiene


Objectives
• Specimen collection
• Specimen storage and transport
• Testing offered at WSLH
• Result reporting and interpretation
• Expected turn around times for results

Specimen Types

Almost any source is acceptable for AFB culture and smear

- **Sputum**
  - **Induced**
  - **Expectorated**
- Bronchial washing/BAL
- Gastric aspirate
- Fresh tissue
- Bone
- Blood
- Bone Marrow
- CSF
- Body fluids
- Abscess
- Stool
- Urine
- Skin



Sputum

- Recently discharged material from the bronchial tree, with minimal amounts of oral or nasal material
- Expectorated: from deep productive cough
- Induced: use of nebulization to increase fluid in the airway and ease clearance of sputum

Indications for sputum collection:

- To establish an initial diagnosis of TB
- To monitor the infectiousness of the patient
- To determine the effectiveness of treatment

Image Credit: WHO

Specimen collection

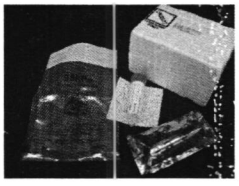
- Supervise patient for at least the first specimen, until ability to properly collect the specimen has been demonstrated
- Patient should be in a negative pressure room
- Anyone in the room should wear a fit tested N-95 respirator
- All specimens are collected into sealed leak proof containers
- Label specimen with two patient identifiers, collection date/time and specimen type.

Specimen collection

- Optimal: collect a diagnostic specimen before the initiation of drug therapy
  - Collect a series of three sputum specimens, 8-24 hours apart, at least one of which is an early morning specimen
- Monitoring of therapy: Obtain sputum specimens for culture at least monthly until cultures convert to negative for TB

Specimen collection kits

WSLH Kit #8: Sputum collection
Order: 1-800-862-1088
KITS ARE FREE!



- Sterile tube with label
- Absorbent pad
- Specimen transport bag
- Cold pack
- Instruction sheet
- Insulated mailer with labels

Storage and Transport

- Sputum samples should be refrigerated if they cannot be transported immediately
- Deliver specimens to the laboratory as soon as possible—try not to batch!
- Recommended: Include a cold pack during specimen transport.

Storage and Transport

WHY is this important?

- Minimize overgrowth of normal flora
- Viability of AFB
- Rapid turn-around times
  - Isolation precautions
  - Start/Stop treatment

Submission of Specimens to WSLH

- Requisition form A
- Order: 1-800-862-1088
- Preprinted with account information
- One form per specimen

Submission of Specimens to WSLH

Code		Test Description
MM00250	X	Mycobacteria (AFB) Smear and Culture
MM00253		Mycobacteria Isolate Identification
MM02881		Mycobacterium tuberculosis Isolate Genotyping
MM00204		Mycobacterium tuberculosis Susceptibility-1st Line Drugs
MM00202		Mycobacterium avium Complex (MAC) Susceptibility
MM00207		Mycobacteria Rapid Grower Susceptibility Organism ID _____
MM00260		Mycobacterium avium Complex PCR Decontaminated? Yes No Smear Result _____
MM00256		Mycobacterium tuberculosis PCR Decontaminated? Yes No Smear Result _____

Courier information

Service is offered at no charge to submitters

Call to set up an account and schedule a pickup:
763-233-0099

Assessing Sputum Quality

Test results are used as an aid in patient diagnosis and treatment.

Test results are directly related to the quality of the specimen.

Assessing Specimen Quality

Collection Date/Time:

- CDC MMWR 2005: 54, RR-17: "Persons requiring sputum collection for smear and culture should have at least three consecutive sputum specimens obtained, each collected in 8-24 hour intervals, with at least one being an early morning specimen"
- Minimize transport time to laboratory (no batching!)
- Specimens >7 days old are rejected

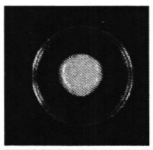
Assessing Specimen Quality

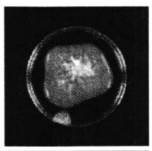
Sputum Quality

- Specimens are thick and contain mucopurulent material
- 3-5 ml in volume (ideal), but smaller quantities accepted if the quality is satisfactory
- Poor quality specimens are thin and watery—Saliva and nasal secretions are unacceptable
- Induced sputum should be indicated on requisition form to avoid rejection

Assessing Specimen Quality

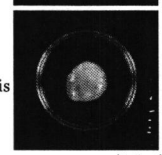
Thick Mucopurulent

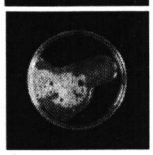




Watery (induced?)

Hemoptysis





Salivary

http://www.theunion.org

Mycobacteriology at WSLH

- Full-service mycobacteriology laboratory
- BSL-3 facility
- Roles:
  - Primary Diagnostic Facility
  - Reference laboratory
  - Public Health Laboratory

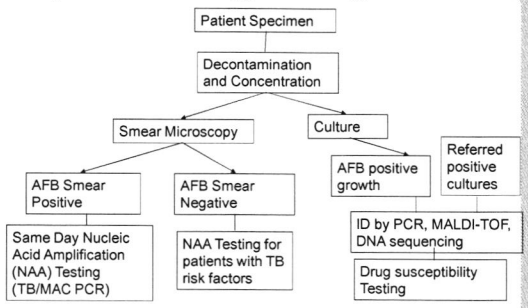
Mycobacteriology at WSLH

- 22 laboratories around the state perform smear and culture
  - 4 labs (other than WSLH) perform some level of identification
  - Most others send to WSLH for identification
- WSLH receives clinical specimens from:
  - 2 large local hospitals
  - Health Departments (state-wide)
    - Milwaukee City TB Clinic
    - Madison Dane County Public Health

Mycobacteriology at WSLH

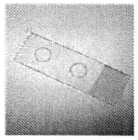
- Smear Microscopy
- PCR for direct detection (NAAT)
- Culture
- Identification
- Drug Susceptibility Testing
  - Molecular
  - Conventional

Mycobacteriology Testing at WSLH



Smear Microscopy

- Small amount of concentrated patient specimen is placed on slide and stained with Auramine O fluorescent stain
- Rapid and inexpensive screening tool
  - First indication of mycobacterial infection and possible TB disease
  - Must be accompanied by additional testing including culture for confirmatory diagnosis



Auramine O Smear

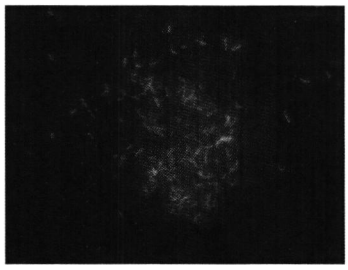


Photo Credit: laboratoryinfo.com

Smear Microscopy: Result Interpretation

WSLH Report	Graded Scale	Qualitative Scale	Interpretation
Negative	Negative	Negative	Potentially infectious
Rare (1-9 AFB per 100 fields)	1+	Positive	Low-level infectious
Few (1-9 AFB per 10 fields)	2+	Positive	Moderately infectious
Moderate (1-9 AFB per field)	3+	Positive	
Many (>9 AFB per field)	4+	Positive	Highly infectious

Smear Microscopy: Limitations

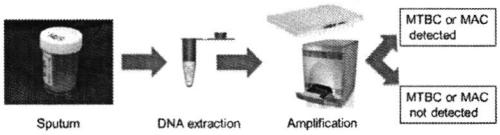
- Does not distinguish between viable and dead organisms
- Limited sensitivity
  - High bacterial load: 5,000-10,000 AFB/mL is required for detection
  - Misses >45% of U.S. TB cases
- Limited specificity
  - All mycobacteria are acid fast
  - Does not provide species identification
- Cannot be done without a culture

Microscopy Results Guide Decisions

- Clinical Management
  - Patient therapy may be initiated for TB
  - Changes in smear status important to monitor response to therapy
- Public health interventions
  - Smear status and grade useful for identifying the most infectious cases
  - Contact investigation priority based on smear result
  - Decisions regarding respiratory isolation

Direct Detection using PCR

- AKA: Nucleic Acid Amplification Testing (NAAT)



PCR testing

- Detect *M. tuberculosis* complex (MTBC) and *M. avium* complex (MAC) directly from sputum sediment
- PCR test developed at WSLH (not-FDA cleared)
  - 1 other laboratory in the state is performing NAAT (Cepheid GeneXpert)
- Testing takes about 2 hours
- Unable to distinguish live and dead bacilli

PCR testing (cont.)

- Automatically performed on new smear positive patients
- Fee-exempt testing for smear positive specimens and patients suspected of having active TB (approved by WI TB Program)
- Sensitivity
  - >95% for AFB smear-positive, culture-confirmed TB patients
  - 55-75% of AFB smear-negative, culture-confirmed TB patients