



The Secret Life of Sputum: In the Laboratory

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Objectives

- Storage, transport, and submission of sputum specimens
- Assessing sputum quality
- Sputum processing in the lab: AFB smear and culture
- 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



Storage and Transport

- Sputum samples should be refrigerated if they cannot be transported immediately
 - Recommended: Include a cold pack during specimen transport (included in WSLH kits!)
- Deliver specimens to the laboratory as soon as possible—try not to batch!
 - Recommended: Deliver specimens each day collected, if possible

Storage and Transport

Why is refrigeration important?

- Minimize overgrowth of normal flora
 - Doubling time 15-20 minutes (respiratory flora) vs. 12-24 hours (MTBC)
- Viability of AFB

Why is rapid delivery important?

- Viability of AFB
- Rapid turn-around times
 - Isolation precautions
 - Start/Stop treatment

WSLH 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

Submission of Specimens to WSLH

Laboratory of Hygiene UNIVERSITY OF WISCONSIN-MADISON 2601 Agriculture Drive Madison, WI 53718 Kits a			://www.sli D Custome ne: 800-86 : 844-390-	1.wise er Sei 52-10 6233	vice 13	CDD Requisiti				-1-17	
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- Requisition form A
- Order: 1-800-862-1088
- Preprinted with account information
- 2 unique patient identifiers MUST be included on BOTH specimen and requisition form to prevent specimen rejection!
- Please submit one form for each specimen submitted!

Submission of Specimens to WSLH

Mycobacteriology:					
MM00250	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				

*AFB smear and culture test includes mycobacteria ID

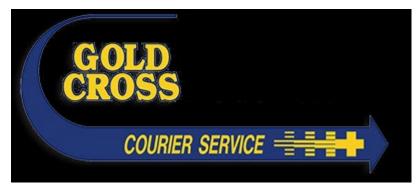
*Positive AFB smears will automatically have TB/MAC PCR performed

Courier information

Service is offered at no charge to submitters

Call to set up an account and schedule a pickup: 1-715-839-9989





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

Sputum Quality

- High-quality specimens are thick and contain mucopurulent material
- 3-5 ml in volume, but smaller quantities accepted if the quality is satisfactory
 - <1 ml sputum will be rejected
- Poor quality specimens are thin and watery— Saliva and nasal secretions are unacceptable and will be rejected
- Induced sputum should be indicated on requisition form to avoid rejection



Assessing Specimen Quality- Why is this important?



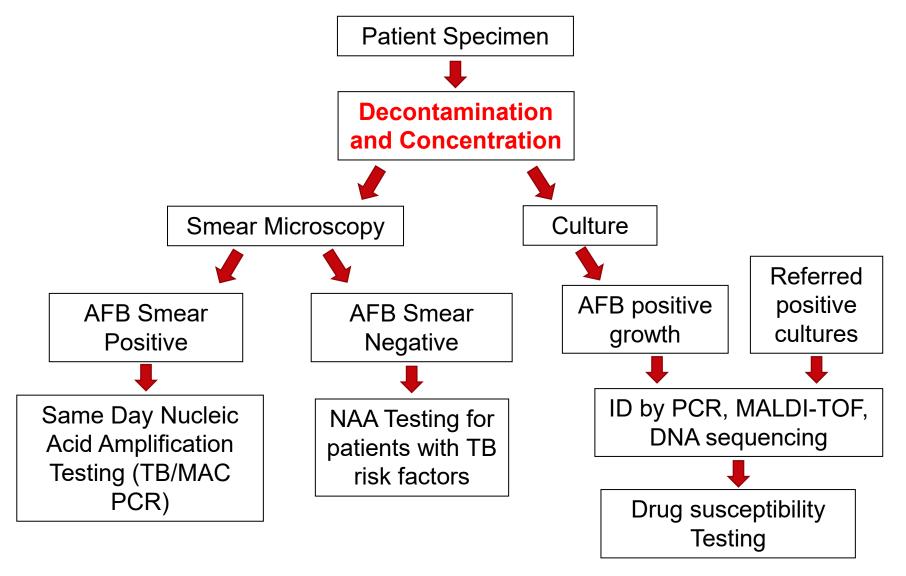
- BY SPUTUM MICROSCOPY
- Processing of poor quality specimens can be a burden on financial and personnel resources
- Results from poor quality specimen may be unreliable
- If rejected, WSLH will add a comment to testing results stating the reason for rejection

Sputum processing

 Once a sputum has been deemed acceptable, what happens in the lab (and why does it take so long to receive test results)?



Mycobacteriology Testing at WSLH



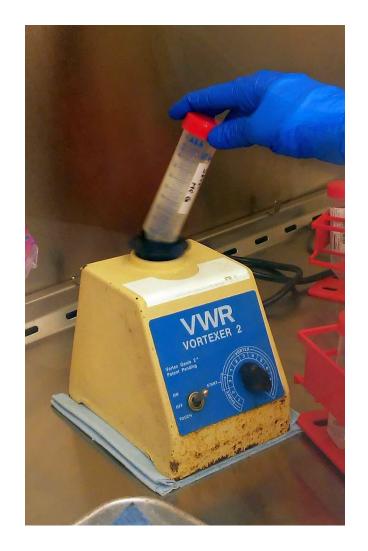
Sputum processing



Sputum processing: Digestion and Decontamination

 Digestion: 0.5% NALC; agent used to liquefy sputum by breaking down mucus and freeing bacilli from cells or tissue

 Decontamination: 2% NaOH; agent used to kill normal respiratory flora that grow faster than MTBC and would otherwise overgrow cultures



Sputum processing: NaOH neutralization

 Add buffer to neutralize specimen—don't want to decontaminate too long and kill off MTBC!



Sputum processing: Centrifugation

Aerosol-tight rotor bucket

Refrigerated centrifuge

- Centrifugation concentrates mycobacteria in specimen for improved detection by smear or culture
- 3-50ml of specimen → <1ml concentrated sediment





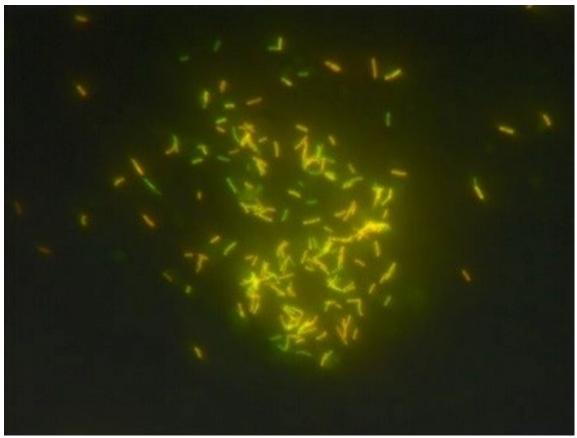
Sputum processing: AFB Smear

Make smears

- Small amount of concentrated specimen is placed on slide
- 1 specimen per slide
- Smears are stained with Auramine O fluorescent stain



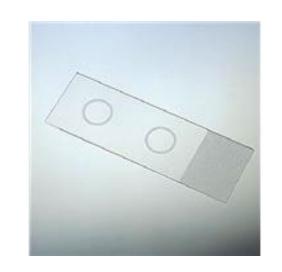
Auramine O Smear



laboratoryinfo.com

AFB Smear Microscopy: Advantages

- Rapid and inexpensive screening tool
- All types of specimens can be examined
- First confirmation of mycobacterial infection and possible TB disease
 - Isolation decisions if suspect TB case
 - Antibiotic treatment decisions
- However, must be accompanied by additional testing, including culture for confirmatory diagnosis



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
Moderate (1-9 AFB per field)	3+	Positive	infectious
Many (>9 AFB per field)	4+	Positive	Highly infectious

AFB 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
 - Local prevalence of MTB and NTM determine the predictive values of a positive smear for MTB
- ***Should not be done without a culture!***

AFB Smear Microscopy: Follow-up

- TB/MAC PCR will be run on sediment if smear is positive or if requested by WI TB Program
- Sediment is acceptable for other molecular testing
- Remaining sediment material is held for 2 weeks

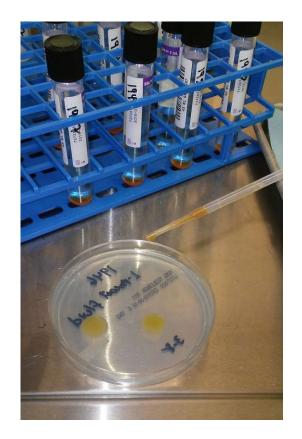
WSLH MTBC NAAT Testing

- Detect M. tuberculosis complex and M. avium complex directly from processed sediment (LDT)
- Automatically performed on all new smear-positive specimens
 - Respiratory and non-respiratory sources
 - Fee-exempt testing for smear-positive or patients suspected of having active TB (pre-approved by WI TB Program)
- Smear-negative respiratory specimens tested with submitter charge
- Sensitivity
 - >95% for AFB smear-positive, culture-confirmed TB patients
 - LOD: <1 MTBC bacillus/reaction (≈140 AFB/ml)

Sputum Processing: Mycobacterial Culture

- Inoculate culture
 - All specimens inoculated into liquid media (Mycobacterial Growth Indicator Tube)
 - Limited specimens inoculated onto solid media
 - Sputum specimens from known MTBC patients





Mycobacterial Culture

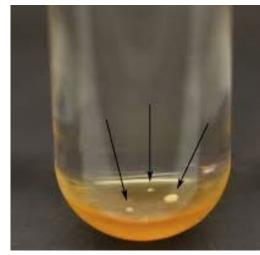
- Used to detect viable mycobacteria from patient specimens
- Most sensitive method for mycobacterial detection ("Gold Standard")
 - ~ 10 viable bacilli/ml for culture compared to >5000 bacilli/ml for microscopy
- Slowest method
 - Average time to detection for MTBC = 15 days
 - Range for detection of MTBC = 8-30+ days



BD BACTEC MGIT 960



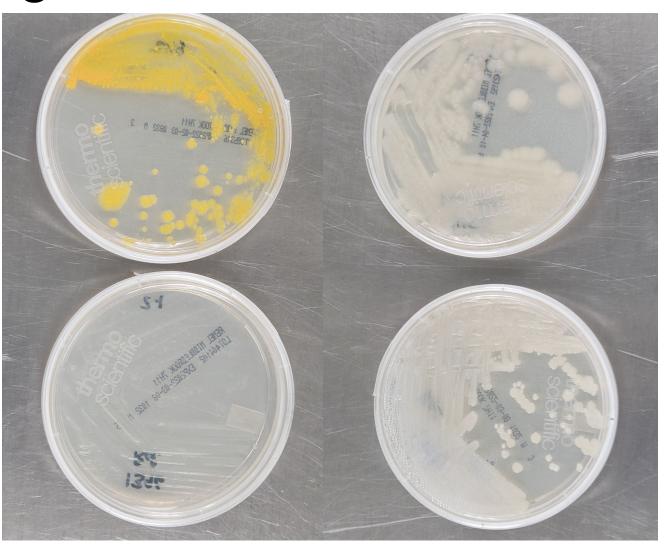






7H11 Middlebrook Agar





Mycobacterial Culture

Cultures are incubated for 42 days*





WSLH Report

Final report: Negative for mycobacteria

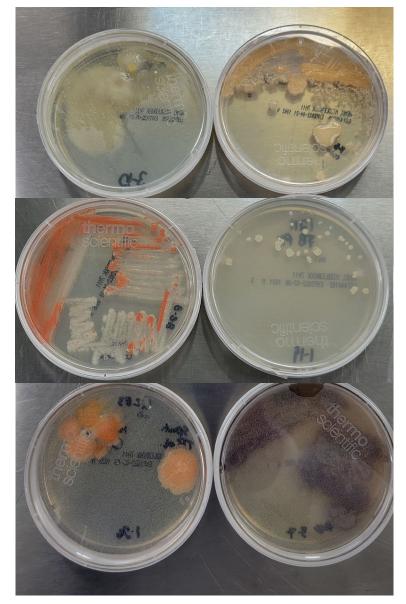
Mycobacteria identified to species level (if possible)

aerobic Actinomycetes; Nocardia sp.

Mycobacterial Culture: Re-decontamination

- Sputum is often contaminated with other respiratory flora
 - Contaminating organisms are reduced by decontamination process, but not always complete
- TB patients are susceptible to pulmonary infection or colonization of lung cavities during treatment
 - Normal flora
 - Other mycobacteria
 - aerobic Actinomycetes
- Liquid cultures are put back through the decontamination process and re-inoculated
 - **Restarts the 6 week culture incubation

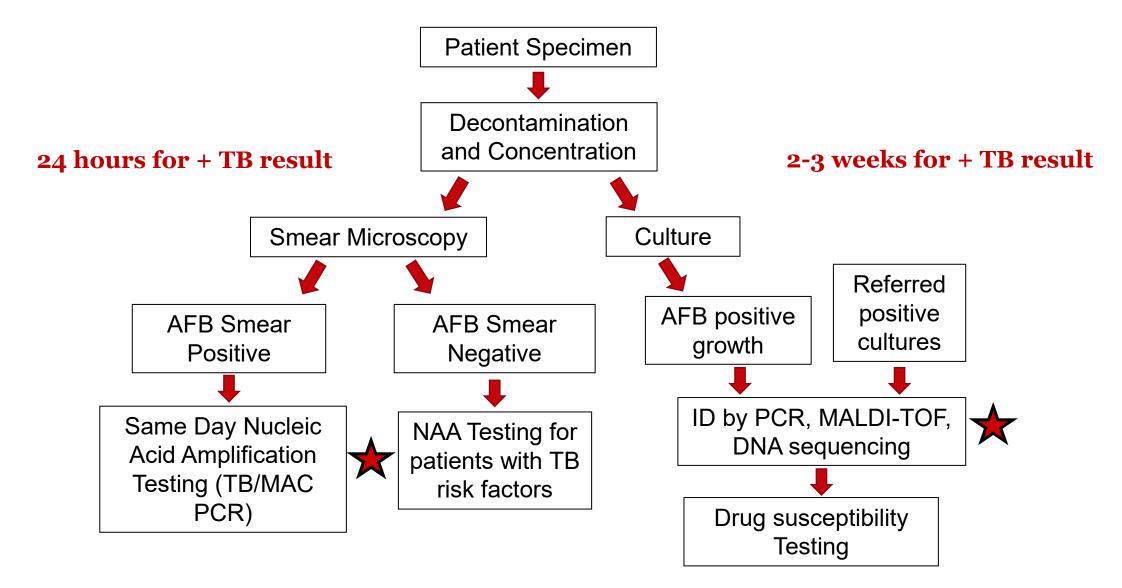
Mycobacterial Culture: Re-decontamination



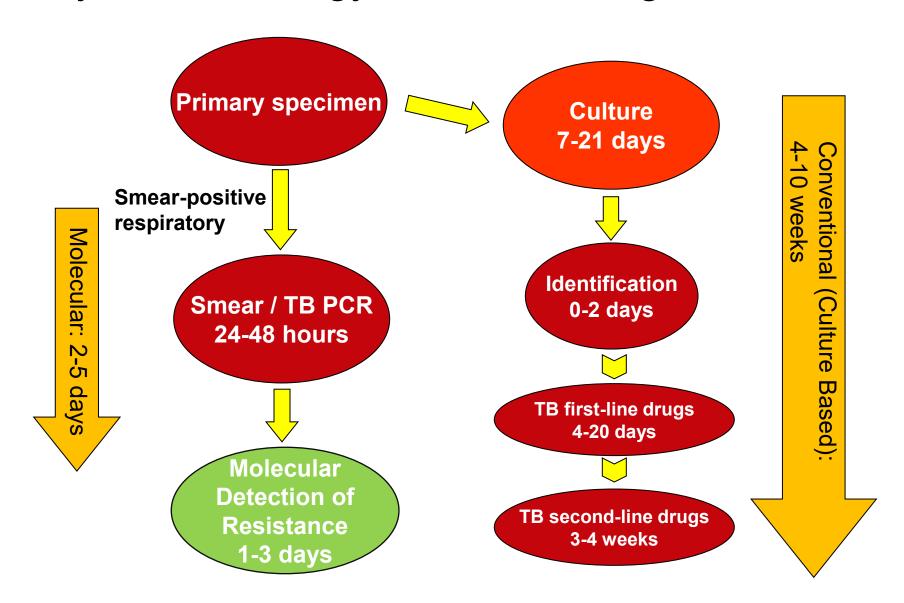




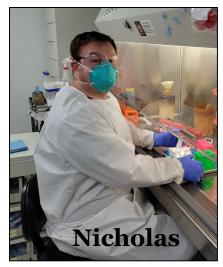
Mycobacteriology Testing at WSLH



WSLH Mycobacteriology: MTBC Testing Turn-Around Times



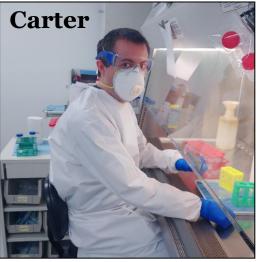
WSLH TB Laboratory Team











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- WSLH / WSLH Mycobacteriology Laboratory Staff





Questions?

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