The Clinician, the Program, and the Mycobacteriology Laboratory

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Effective TB Control depends on an integrated system that includes clinicians, laboratories and TB Controllers

APHL Task Force: *The Future of TB Laboratory Services*, 2003

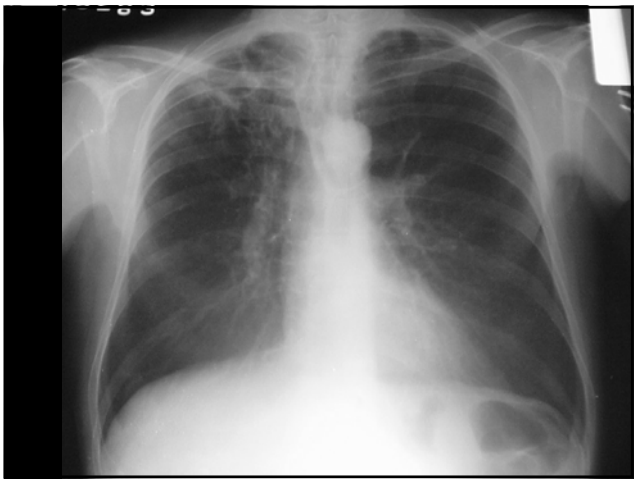
Objectives

- To review the role of the Mycobacteriology Laboratory in the Diagnosis and Management of Tuberculosis
  - How the Lab works
  - What the Lab does
  - How to interpret results
- Discuss the potential of new tools

Will not discuss: Molecular Epidemiology
Interferon-gamma Release Assays

TB Among the Hob**os**

- 52 y/o gentleman, traveling (recently to WI) street person
- History of ROH abuse, heavy smoking
- Presents in 4/09 with 2 mos increasing cough, purulent sputum, wt loss
- Questions???
- CXR??



TB – or *NOT* TB?

- Admitted to MGH
  - Sputum smears AFB-Positive
  - TST: 16mm induration
  - Started 4 drugs: *TB Suspect*
  - Reported to *1-888-MASS MTB*
- *Contacts???*
- NAAT (MTD™) negative for MTb complex
  - Cultures subsequently grew *M.* avium
    - Negative for MTb at 8 wk (final)
- Treatment changed to Clarithromycin + Ethambutol
- Patient's symptoms resolved rapidly

Approach TB Diagnosis

- Clinical presentation
  - History
  - Signs and Symptoms: Site of disease
- Clinical Suspicion is key
  - Personal risk factors for TB
    - Most disease represents reactivation (US)
  - Prevalence of TB disease in population
    - Affects predictive values of diagnostic tests
  - Patient's level of immune competence
    - Medical Risk Factors
    - Presentation varies with degree of immunosuppression

TB is a Clinical Diagnosis
most of the time

- Most clinicians will initiate multi-drug therapy *if the disease is suspected on clinical grounds*
  - But many cases go undiagnosed until a laboratory reports a positive culture
- How is that diagnosis confirmed?
  - *In the Laboratory*

Role of Mycobacteriology Lab

- *Target: Mycobacterium tuberculosis* Complex (MtbC)
  - Use rapid methods to detect, identify (ID), and perform drug susceptibility testing (DST)
    - *TB vs. not TB*
- Non-tuberculous mycobacteria (NTM)
  - Provide accurate / clinically relevant information (accurate ID IF clinically relevant; appropriate DST IF clinically relevant)
- Issue rapid, clinically useful, and reliable reports
- Evaluate testing and reporting algorithms as necessary
- Develop and maintain 2-way communication - clinicians, care-givers, TB program, referring laboratories, etc.

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The TB Laboratory

- Types
  - Hospital-Clinical Laboratories
    - Process samples from within an institution and its affiliates
  - Private Laboratories
    - Process samples on contract basis (*e.g.* Quest, LabCorp, ARUP)
  - Network Laboratories
    - Process samples for organization (*e.g.* VA)
  - Public Health Laboratories
    - State/federally supported facilities: *Your State Lab*
  - Reference Laboratories
    - Provide specific services – culture confirmation, molec DST, drug level monitoring, … (*e.g.* CDC, National Jewish)
- Overall, *n* >1,932 (+ state labs)

Accommodating Escalating Complexity

- Varying *Levels of Service* Offered
  - Not all laboratories perform all tests
    - Most perform basic tests: smears, primary cultures
  - Ability to perform appropriate tests
    - Equipment, personnel
  - *Secondary and Reference Laboratories*
    - Receive/process samples for more complex tests
- Communication Challenges
  - Laboratory-to-laboratory
  - Provider-to-laboratory(*ies*)-to-provider-to- …
- Laboratory *Competence*
  - Determined locally
  - Centers for Medicare & Medicaid Services' Clinical Laboratory Improvement Amendments (CLIA) program
    - Proficiency testing

Diagnosis of TB: *Demonstration of M. tuberculosis*

- The *Gold Standard*
- Secretions or tissue
  - Subjected to laboratory techniques *to identify the organism*
- Ability to isolate organism varies with
  - Location of Disease
  - Density of organisms at disease site

Standard Mycobacteriology Laboratory Tests

- Smear/stain for *acid-fast* organisms
  - Sputum, sterile fluids, tissue
- Culture for identification of organism
  - Includes speciation
  - Drug susceptibility studies (DST)
- Nucleic Acid Amplification (NAA)
- Therapeutic Drug Monitoring

Step-By-Step "Typical" TB smear and culture (1)

- Specimen received in lab
- Specimen accessioned (assigned lab number; entered into lab computer/worklog, *etc.*)
- Specimen stored appropriately (refrigerated) until processed – usually 1x/workday
- Specimen processed (digested/ decontaminated) usually by NALC/NaOH method in batch with other specimens
- Smear prepared
- Culture media inoculated (usually 1 broth and 1 solid) and put into incubator/instrument

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Step-By-Step "Typical" TB smear and culture (2)

- Smear stained and examined and results reported same day as specimen processing
- Nucleic acid amplification (NAA) test set up if appropriate/if lab offers test; some labs also do "molecular DST"
- Culture media examined/monitored as prescribed by method (for 6-8 weeks)
- If growth detected, smear made and stained to confirm presence of AFB (acid fast bacilli)
- If AFB, go onto identification (*e.g.*, HPLC, nucleic acid probe)
- If TB, make appropriate notifications and perform DST as appropriate
- If no growth, keep 6-8 weeks and sign out as "negative for mycobacteria"

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Specimen Collection

- Sputum: Spontaneous or Induced
  - Initial: 3 *good* samples, 8-24hr apart (MMWR, 2005)
  - Monthly while on treatment until culture-negative
- Collect aseptically, avoid contamination
  - Sterile, leak-proof, disposable, non-breakable, appropriately-labeled lab-approved containers
  - No fixatives or preservatives
- Avoid contamination with tap water
  - NTM may be in water
- Collect initial samples prior to therapy if possible
- Transport immediately or refrigerate

Sputum Smears: *Definitions*

- Direct smear: stain performed on the submitted sample
- Concentrated smear: decontaminated-liquified (NaOH and NALC) and centrifuged (at 3,000xg)
  - Improves yield
  - Procedure kills >30% of mycobacteria
- Indirect smear: performed on growth from culture
  - Isolate from primary lab sent to *second lab*
    - For further identification (confirmation) and drug susceptibility studies
- Kinyoun or Ziehl-Neelsen (heat) stain: Light microscopy (1000x mag/oil)
  - *"Acid-fast":* Organisms retain red color following decolorization with acid-alcohol (the *Red Snapper*)
- Fluorochrome stain: Fluorescence microscopy (450x mag)
  - Auramine O
  - Recommended initial staining procedure (incr sensitivity, decr time)

AFB Smear Microscopy

- Variable sensitivity
  - 40-70% for pulmonary TB (less in miliary TB, late HIV, children)
  - LOD >10^4 AFB/ml by Ziehl-Neelsen; >10^3/ml fluorochrome
  - Correlates with disease severity and infectiousness
- Not specific for MTb Complex
  - *Red snappers*
- Inexpensive and quick
  - Turnaround time (TAT) <24hr
- Value
  - Usually provides the 1st evidence of TB
    - Direct smear light microscopy is the primary diagnostic method in developing world
  - Used to guide therapy (AFB in smear are quantified)
  - May guide additional testing (*e.g.,* NAA)

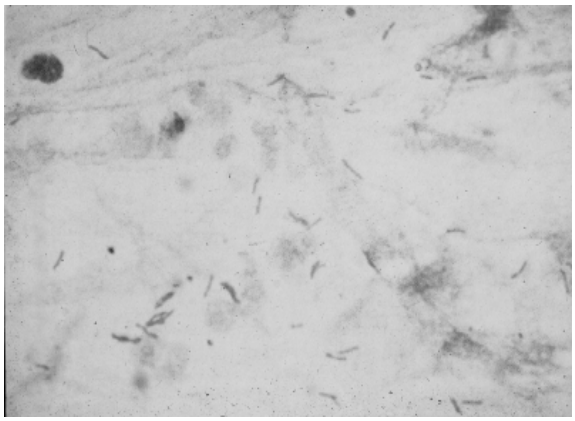
International Guidelines for Examining and Reporting Acid-Fast Smears: Organism Count at Specific Magnifications

	Number of AFB Observed	
Report	**200x, 250x**	**400x, 450x**
No AFB seen	0	0
Doubtful: repeat	1-2/30F*	1-2/70F
1+	1-9/10F	2-18/50F
2+	1-9/F	4-36/10F
3+	10-90/F	4-36/F
4+	>90/F	>36/F

*** number of acid-fast bacilli observed per microscopic field**

CDC

400x
Fluorochrome stain
Increases sensitivity
Decreases performance time



AFB Smears: *Rule Out TB?*

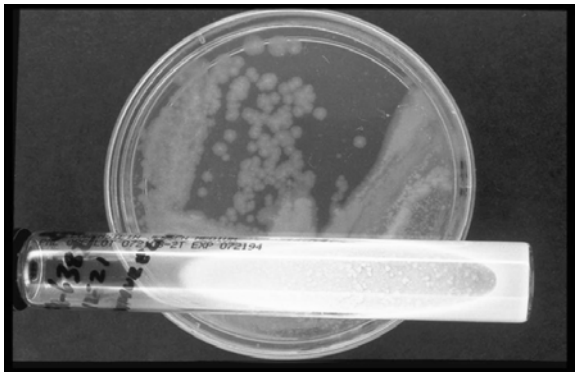
- *A positive smear does not establish dx*
- *A negative smear does not exclude TB*

Culture Isolation of *M. tuberculosis:* The *Gold Standard*

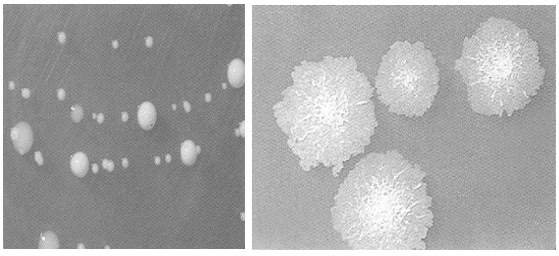
- Requires appropriate laboratory equipment & trained staff: *Competence*
- Allows for identification and speciation, drug susceptibility testing
- Performed on secretions or tissue
- Sensitive
  - Limits of Detection (LOD) 10 to 100 AFB/ml
    - 10,000 AFB/ml for smear (Z-N) - more specimen goes into culture

Culture Methods

- Solid media
  - Agar (Middlebrooks) and egg-based (Lowenstein-Jensen) platforms
  - Require up to 6 - 8 weeks
  - Advantage: Can identify colonies (pigmentation, morphology)
- Broth – some are highly automated
  - BACTEC 460; MGIT; TREK; MB/BacT
  - More rapid recovery than solid media: 7-21 days
- Current recommendations are to use at least one type solid media *and* broth (mixed culture detection; increased sensitivity)



Colony Morphology



Broth (Liquid Media) BACTEC 460 Instrument

- Semi-automated; needles
- Laboratory work-horse
- 12B media
- Radiometric
- Detects CO_2 production by mycobacteria
- DST for INH, RMP, EMB, STR, PZA

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Mycobacteria Growth Indicator Tube (MGIT; Broth)

- Fluorescence quenched by O_2 in O_2-rich liquid media
- If mycobacteria present, O_2 used up, no quench, fluoresces under UV light
- DST for INH, RMP, EMB, STR, PZA

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MTB Culture Isolation

- Negative cultures do not exclude infectious TB
  - Sampling error, contamination, dead organisms, *etc.*
- False positive: cross-contamination?
  - Interpretation contextual
  - Depends on clinical suspicion of disease
    - *e.g.* smear negative, low probability patient
- Cultures guide management
  - Declining # colonies correlate with response to therapy
  - Monitor sputum monthly until culture conversion
  - If culture-pos at 3 mos, look for reason (malabsorption, drug resistance, *etc*)
- *Rule Out TB?*
  - *A positive culture can establish dx*
  - *A negative culture does not exclude TB*

Identification of Mycobacteria

- MTb *vs* NTM: *Treatment and Public Health implications*
- Preliminary ID based on growth characteristics solid media
  - Colony morphology, pigment, rate of growth (REQUIRES GROWTH)
- Conventional biochemical tests (all mycobacteria)
  - 2-21 d (may not necessarily be accurate for NTMs)
- HPLC of cell wall mycolic acids ("all" mycobacteria)
  - 2 h – usually by reference labs
- Commercially available genetic probes
  - ACCUPROBE, GenProbe, San Diego, CA (www.genprobe.com) probes for Mtb Complex, MAC, *M. kansasii, M. gordonae*
  - 2-4 h – many clinical labs
- "In-house" PCR/genetic sequencing/*etc*.
  - 1-2 d – reference labs/clinical labs

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Direct Detection of MTB Complex : Nucleic Acid Amplification Testing (NAAT)

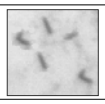
- *NAA assays*
  - *Amplicor®-Roche*: DNA
  - *MTD®-GenProbe*: r-RNA
- Advantages
  - Excellent sensitivity (10-100 organisms/ml) & specificity for MTb
  - TAT generally ≤ 48hr
  - Can affect treatment decisions, including isolation and other public health interventions, invasive procedures
- Disadvantages
  - $$ Costly $$
  - No indication of viability of organism or of susceptibility
- *Still requires culture for confirmation, DST*
- FDA-approved ONLY for respiratory secretions (sputum, bronchial)
  - smear +/- patients, ≤ 7 days therapy (*Amplicor:* smear + only)
- "Off-label" use
  - Physician education is important

Nucleic Acid Amplification Testing for Respiratory Specimens

- Becoming standard of care … *but do not test everyone*
  - Base testing on suspicion and communication with laboratory
    - Do not test smear positives when classic TB symptoms and history are present
    - Do test smear negatives when clinical suspicion of TB is high
- CDC* – "Test at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered (*i.e. Real suspects*) but has not yet been established, *and* for whom the test result would alter case management and TB control activities"

* MMWR (58:7-10), 2009

NAAT: Application and Interpretation

Sputum, Respiratory Secretions	NAA Positive	NAA Negative
	Dx of TB established; *cult. still required*	Consider clinical picture and repeat testing
	Consider clinical picture and repeat testing	Unlikely that *Mtb* will be grown from sample *(if controlled for inhibitor)*

Cannot replace clinical judgment

Remove from Isolation?

- Airborne precautions can be discontinued when infectious TB disease is considered unlikely and either
  - another diagnosis is made that explains the clinical syndrome,
  - the patient has three negative AFB sputum smear results, or
  - the patient has a sputum specimen that has a negative NAA test result and two additional sputum specimens that are AFB-smear negative.*

*CDC Expert Panel on NAAT, MMWR 11/2008
Campos, M, *et al* AJRCCM 178:300-305, 2008

Drug Susceptibility Testing (DST)

- Mandatory on all new patients
  - 1st isolate from each site of disease; at 3mos if still cult pos.
  - Guides treatment decisions
    - Initially: for case and contacts
    - During treatment: determine reason for failure (emergence of resistance, absorption)
- Accurate and *timely* reporting of results is essential
  - Direct test; TAT 1 – 3 weeks
    - Smear positive cases; primary sample is tested
  - Indirect test; TAT 7+ weeks
    - Requires growth
    - Liquid media: 3 – 4 wk (can also do MIC for 1st line drugs)
- Direct agar proportion method: *Gold Standard*
    - Can test for multiple drugs, cheaply
    - Resolve agar/liquid media discrepancies

Susceptibility Testing of *M. tuberculosis Complex*

- Use rapid method (Broth-based)
  - Perform on all initial patient isolates
  - Test isolates from relapse or re-treatment cases; also if drug resistance suspected
- Test first-line drugs:
  - INH, Rif, EMB, PZA, SM*
- Test second-line drugs and higher conc. INH, EMB, SM:
  - if R to rifampin or any 2 primary drugs
- Second-Line DST
  - Technically difficult (*e.g.,* CS not recommended); not widely available
  - Cross-resistance
  - Methodologies not standardized – especially broth methods
  - Poor correlation with clinical response

NCCLS Standard M24-A, 2003

* M24A2 will drop SM, add AK and LQN

Discordance in DST

- Occurs between different labs, different methods, and within the same method
  - What do they mean?
  - Which is right?
- Many possible reasons …
  - Human
    - labeling, cross-contamination, …
  - Bacteria-specific
    - direct *vs* subculture, clumps, …
  - Methodology related
    - inoculation method, drug conc, media components, …

Laboratory Consortium (4 public health labs and CDC)

- Discordance INH (low level)
  - Within lab (BT vs. AP) – 2.4%
  - Interlab (BT) – 6.0%
  - Interlab (AP) – 12%
- Discordance EMB
  - Within lab (BT vs. AP) – 6.1%
  - Interlab (BT) – 20.2%
  - Interlab (AP) – 8.7%
- Discordance PZA
  - Interlab (BT) – 4%

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Molecular DST

- Molecular assays for INH, Rif most common
  - Detect polymorphisms associated with drug resistance
  - Performed on clinical specimens or culture isolates
  - Results available within 1-2 d
- In-house assays
  - Molecular beacons, pyrosequencing, RT-PCR
- Commercial assays
  - HAIN and INNO-LIPA line probe assays; Cepheid GeneXpert Rif
- Some Issues
  - Multiple mutations may confer resistance – not identified
  - Silent mutations – flagged but not really resistant
  - *None is FDA-approved (9/2011)*

Molecular Beacons

PHRI

GeneXpert MTB/RIF™ Cepheid

- Closed, self-contained and automated platform
- rt-PCR-based amplification of MTb DNA
  - 131 CFU/mL clinical LOD
- Boehme NEJM 9/9/10: *1730 pulmonary TB suspects*
  - Sensitivity: 551/561 sm-pos (98.2%); 124/171 sm-neg (72.5%)*
  - Specificity: 604/609 (99.2%)
  - Simultaneous detection of Rif resistance
    - 200/205 Rif-R (97%)
- Costly

*increased to 90% with repeat testing

Molecular Detection of Drug Resistance (MDDR) Service at CDC: Rationale

- Clinical/Program: *available to providers*
  - Make rapid confirmation of MDR TB available
  - Make laboratory testing data available to clinicians about second-line drug resistance in cases of Rif-resistant or MDR TB
- Development
  - Continuous correlation of molecular (genotyping) results and DST (phenotypic) results
  - Addition of new drugs and alleles
- Research
  - Determination of mechanisms of resistance

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MDDR Service: Drugs and Genes for Panel

Drug	Gene(s)
RMP	*rpo*B
INH	*inh*A, *kat*G
KAN	*rrs, eis*
AMK	*rrs*
CPM	*rrs, tly*A
FQ	*gyrA*
PZA	*pnc*A
EMB	*emb*B

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False-negative and False-positive results

- False-negative cultures
  - Improper collection/transport; overheating during transport/centrifugation; over-decontamination; media not inoculated correctly; clerical (labeling, transcribing, etc.)
- False-positive results
  - Another patient's specimen or isolate; splashes; transfer on tools or aerosols during processing; contaminated reagents; AFB in water; clerical
- Clues
  - Increased number of sm +/ cult – detected by lab
  - Single positive cult among many submitted on patient
  - Delayed, scanty growth; multiple pos cultures on rack
  - Clinician: … *No Way this is TB*…
- Resolution
  - Lab must have process in-place
  - Molecular testing often helpful

Serum Drug Level Monitoring

- Useful in selected circumstances
- Helps determine therapeutic concentrations
  - Allows adjustments for variable drug absorptions
- Documents adherence to treatment
- May reduce toxicities

Serum Drug Level Monitoring

- Aminoglycosides
  - To reduce toxicity, achieve therapeutic levels
  - In-house (Amikacin) *vs* send-out (Kanamycin)
- Ethambutol
  - Useful in renal insufficiency to reduce toxicity?
- Rifampin
  - To determine malabsorption (*e.g.* in severe HIV)
- Cycloserine
  - To determine therapeutic levels

The TB Laboratory: Challenges

- Declining case rates
  - Reduced competencies in low-incidence areas
  - *Level of service*: small labs "farming out" tests
- Shifting public health priorities
  - Reduced categorical funding for TB labs
  - Increased support for "crisis" responses (Anthrax, BT)
- Increasingly complex technologies
  - Capital investments
  - Training/educational needs of staff, users of services
- Demand for high-quality services
  - Budget issues
  - Public *vs* private
- Erosion of Public Health Laboratory's key roles

2011

TABLE 3. Essential laboratory tests for tuberculosis control

Test	Maximum turnaround time
Microscopy for acid-fast bacilli	≤24 hours from specimen collection or, if test is performed offsite, ≤24 hours from receipt in laboratory; if latter, time from specimen collection to laboratory receipt should be ≤24 hours
Nucleic acid amplification assay	≤48 hours from date of specimen collection
Mycobacterial growth detection by culture	≤14 days from date of specimen collection
Identification of cultured mycobacteria	≤21 days from date of specimen collection
Drug susceptibility testing	≤30 days from date of specimen collection
Drug susceptibility testing of second-line drugs	≤4 weeks from date of request

CDC: MMWR 54:11/4/2005

Lab *vs* Clinician: Two Sides of the Same Coin

- Laboratory: many specimens; few +
  - Low specificity tests:
    - *e.g.* smears; some NAA tests
    - many *positives* will be *false*
- Clinician: few patients are real TB suspects
  - Some will have positive specimens
  - Interpretation of a test result depends on suspicion of disease
  - Choice of management may depend on interpretation of low-specificity tests