TB Laboratory Testing & Case Studies

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Objectives

- Review the cascade of laboratory tests a clinician may order to diagnose TB disease
- Integrate molecular assays with culture results
- Demonstrate the proper use of TB diagnostic tests using 3 sample cases of TB disease (*easy, medium & difficult*)

Disclosures

- None

NAA Amplification MGIT MTD

PCR Pyrosequencing Molecular

mutation HPLC MALDI-Tof Gene Xpert

MDDR NAAT Genotyping

What do all the words mean?

WGS 16 S Sequencing

◦ Prevent Disease ◦ Promote Wellness ◦ Improve Quality of Life ◦

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Status of the tuberculosis problem in 2014.

Madhukar Pai, and Marco Schito J Infect Dis. 2015;211:S21-S28

The Journal of Infectious Diseases

Does this patient have TB disease?

CLINICAL CLUES

- Cough > 2 weeks
- Fever > 2weeks
- Exposure to TB
- Chronic immune suppression
- Endemic country
- Abnormal physical exam

LABORATORY TESTS

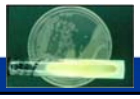
- PPD
- IGRA
- Sputum studies:

AFB Cultures

Molecular studies

- X-rays
- Biopsies





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Recommended diagnostic options for pulmonary TB

- ***See*** the bugs [AFB microscopy]
- ***Multiply*** the bugs [NAATs]
- ***Grow*** the bugs [cultures]
- Courtesy of Prof. Madhukar Pai, MD, PhD Mayo TB Center Webinar March 2016

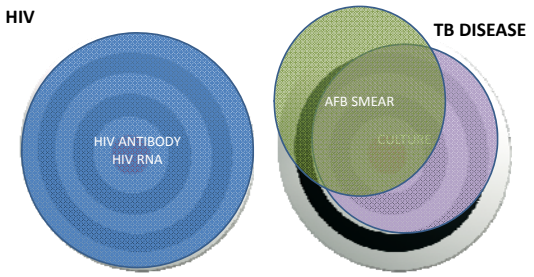
Mycobacterial Examination

Mycobacterial examination has 6 stages:

1. Proper specimen collection
2. Examination of acid-fast bacilli (AFB) smears
3. Direct identification (NAAT-nucleic acid amplification test)
4. Specimen culturing and final identification
5. Drug susceptibility testing
6. TB genotyping

TB is difficult to diagnose

High Accuracy for Diagnosis of HIV in Contrast to TB DISEASE



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Sputum studies Michigan 2016

Test	% POSITIVE	COMMENT
AFB sputum smear	41%	Negative smear does not rule out TB
NAAT on AFB+ sputum smear	91%	May be performed on AFB smear negative sputums
AFB culture confirms ***M. tb***	68%	Gold standard, not always positive

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Specimen Sources

- **Sputum** (primary)
- Pulmonary aspiration (secondary)
- Body fluids (CSF, pleural, peritoneal, etc)
- Tissue biopsy
- Blood
- Urine
- Gastric aspirate
- Stool (special request)
- Other

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Sputum and AFB smears

"*See* the bugs"

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Specimen Collection

Pulmonary Specimen (sputum)

- Early morning specimens = highest yield of AFB
- Collect at least three consecutive specimens at 8-24 hr intervals (at least 1 early morning specimen)
- Recommended volume for testing is 5-10 ml, less may compromise recovery of AFB
- If patient cannot produce sputum by coughing, consider other methods: sputum induction, bronchoscopy, or gastric aspiration
- All persons suspected of TB disease should have sputum cultured

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Specimen Collection

- Collect in sterile, leak proof containers
- Seal with tape or parafilm
- Refrigerate specimen to reduce overgrowth of contaminating bacteria during transit to lab / Do NOT refrigerate blood
- Deliver specimen to TB lab within 24 hrs
- Always include patient name on both test request form and the specimen container

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Acid-fast Bacilli (AFB) smear

- Least sensitive of all AFB Tests (20-75% positivity)
- Requires 10,000 AFB/ml to be positive
- Positive slide does not differentiate *Mycobacterium tuberculosis* from Non-tuberculosis mycobacteria (i.e. *M. avium*)
- Reported within 24 hours of receiving the specimen in the laboratory

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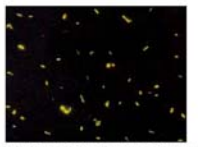
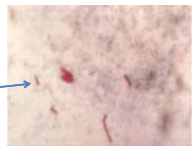
Fluorescent AFB Smear Using Auramine-O Staining

- Very sensitive, takes minutes to read
- Not all that is fluorescent is AFB (need a careful eye)



Auramine-O staining of AFB under Fluorescence Microscopy

- Chemical fluorescence, **not** an immune stain or Direct Fluorescent Antibody
- Can be confirmed with Ziehl-Neelson (ZN) smear



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Nucleic Acid Amplification Test (NAAT) or PCR

"*Multiply* the bugs"

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New CDC Guidelines of Use of NAA MMWR January 16, 2009

- "NAA testing should be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management or TB control activities."
- NAAT should be performed on all new AFB+ sputum specimens

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MTD-Hologic and Gene Xpert-Cepheid are the only FDA approved methods

Gene XPERT

This is a cartridge based NAAT that can detect the presence of M. tuberculosis complex DNA and resistance to Rifampin.

ABI 7500 FastDX

NAA tests are available that are not FDA approved, such as real time PCR assays

MDHHS performs a real time lab developed NAA test to detect Mtb and MAC using the ABI 7500 Fast DX

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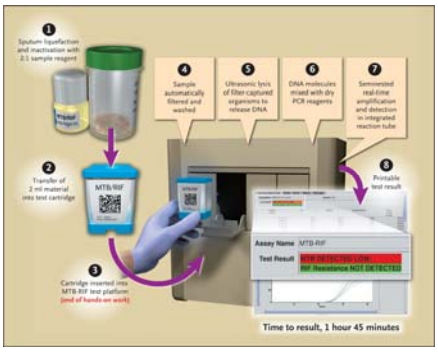
GenExpert Assay Procedure for the MTB/RIF Test.



Boehme CC et al. N Engl J Med 2010;363:1005-1015.

THE NEW ENGLAND JOURNAL of MEDICINE

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AFB Cultures

"*Grow* the bugs"

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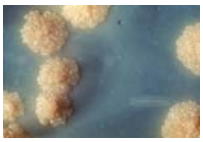
AFB Culture Test

- More sensitive than AFB smear
- 10 AFB/ml can produce a positive result **vs** AFB smear 10,000 AFB/ml
- Culture may be AFB positive even if smear was negative



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Tests Performed on Growth in Mycobacteria Culture

- *Accuprobe* DNA test (***not*** amplified)
- HPLC (high performance liquid chromatography)
- MALDI-TOF
- Biochemical Identification Confirmation
- Drug Susceptibility

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MALDI-TOF / HPLC / Accuprobe



- Matrix-Assisted Laser Desorption Ionization - Time of Flight
- Extraction time ~2 hour
- Run time on the instrument approx. 1 minute

- **High Performance Liquid Chromatography**
- Extraction time ~2 hours
- Run time per specimen is ~15 minutes

- M. *tuberculosis* complex
- M. *avium* complex
- M. *kansasii*
- M. *gordonae*
- Results in ~2 hours

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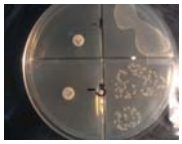
Susceptibility Testing of M. *tuberculosis*

When to test

- All new *M. tb* isolates
- Repeat after 90 days of therapy, if specimens continue to produce *M. tb*
- Relapse or failed therapy





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Additional Molecular Tests for TB

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CDC – Molecular Detection of TB Drug Resistance (MDDR)

- Rapid testing for DNA mutations associated with drug resistance
- NAAT (+) sputum specimens or culture isolates (prior approval)
- Must meet the following criteria:
  - Known Rifampin resistance
  - Known MDR
  - High risk of Rifampin resistance or MDR-TB
  - High profile patient (e.g. daycare worker, nurse)
  - Mixed or non-viable culture
  - Drug Adverse reaction (e.g. Rifampin allergy)

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CDC MDDR

- First-line MDDR to detect MDR-TB
- *rpoB* (Rifampin)
- *inhA* and *katG* (Isoniazid)

- Second-line MDDR to detect XDR-TB
- *gyrA* (Fluoroquinolones)
- *rrs* (Kanamycin, Amikacin, Capreomycin)
- *eis* (Kanamycin)
- *tlyA* (Capreomycin)
- *pncA* (Pyrazinamide)
- *embB*(Ethambutol)

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TB DNA Genotyping Universally offered by CDC

Genotyping provides a fingerprint of each isolate
Michigan performs MIRU-VNTR testing , CDC performs the Spoligo testing:

Used with traditional investigations, genotyping can:

- Identify outbreaks not previously recognized
- Confirm/detect transmission
- Identify risk factors for recent infection
- Demonstrate re-infection with different strains
- Detect possible lab cross-contamination

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Demographics of Selected Genotype Clusters in Southeast Michigan, 2008 – 2012

	PCR00012 (MI_0002) n = 58	PCR00291 (MI_0008) n = 48	PCR04678 (MI_0047) n =23
Race	63% African-American 16% White	97% African-American	100% African-American
Ethnicity	11% Hispanic	3% Hispanic	0% Hispanic
Homeless	37%	44%	27%
Alcohol	32%	35%	27%
Drug	42%	29%	46%
Incarceration	0%	6%	9%
HIV positive	16%	15%	0%
MDR	0%	6%	0%

* All clusters were majority 45 – 64 yrs of age; male and US-born

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3 Sample Cases

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The JAMA Network

Case #1 EASY

From: **Current Approaches to Tuberculosis in the United States**

JAMA. 2012;308(3):283-289. doi:10.1001/jama.2012.7505

Figure Legend:

Admission chest radiograph showing bilateral lung infiltrates with prominence in the right upper lobe and lingula of the left lung.

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APRIL 2016 "EASY" CASE					1 TB suspected	2 Sputum PPD/IGRA
3 AFB smear positive	4 PPD 15 mm	5 NAAT positive	6 INH, RIF, PZA, EMB	7	8	9
10	11	12 AFB in broth DNA probe+	13	14	15	16
17	18	19	20	21	22 Drug susceptibility	23
24	25	26 DNA genotype	27	28	29	30

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#2 case MEDIUM

57 yr male

- Routine cultures negative
- No improvement
- Bronchoscopy AFB smear negative
- HIV +
- CD4 478 cells/mm^3

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APRIL 2016 "MEDIUM" CASE					1 HIV+ TB suspected	2 Sputum PPD/IGRA
3 AFB smear negative	4 PPD 0 mm 2nd smear negative	5	6 IGRA negative	7 NAAT positive	8 INH, RIF, PZA, EMB	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25AFB in broth, DNA probe +	26	27	28	29	30

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Case #3
Difficult

- Patient from Africa
- History of 3 prior episodes of pulmonary TB
- Coughing, sick again

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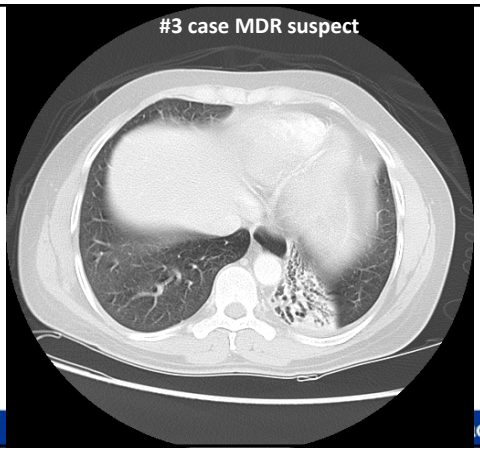

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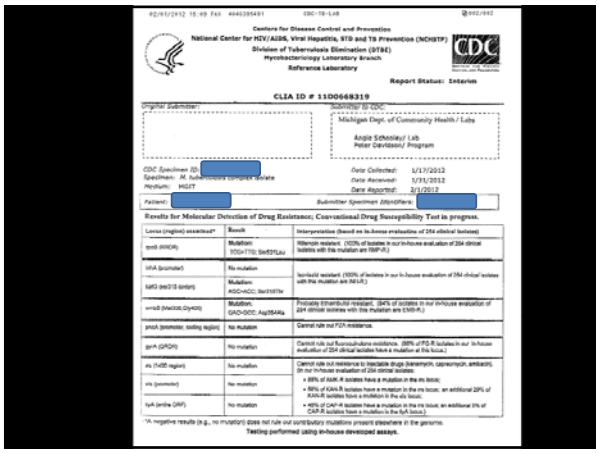
APRIL 2016	"DIFFICULT"	CASE		1 MDR-TB suspected	2 Sputum IGRA
3 AFB smear positive	4 IGRA positive	5 NAAT positive	6 **INH, RIF, PZA, EMB ???**	7	8 MDDR from CDC positive*	9
10	11 **MDR regimen started**	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30



Centers for Disease Control and Prevention
National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention (NCHSTP)
Division of Tuberculosis Elimination (DTBE)
Mycobacteriology Laboratory Branch
Reference Laboratory

Report Status: Interim

CLIA ID # 11D0648319

Submitter to CDC:
Michigan Dept. of Community Health / Labs
Angie Schooley/ Lab
Peter Davidson/ Program

CDC Specimen ID: [redacted]
Specimen: M. tuberculosis complex isolate
Medium: MGIT
Patient: [redacted]
Date Collected: 1/17/2012
Date Received: 1/31/2012
Date Reported: 2/1/2012
Submitter Specimen Identifiers: [redacted]

Results for Molecular Detection of Drug Resistance; Conventional Drug Susceptibility Test in progress.

Locus (region) examined*	Result	Interpretation (based on in-house evaluation of 254 clinical isolates)
rpoB (RRDR)	Mutation: TCG→TTG; Ser531Leu	Rifampin resistant. (100% of isolates in our in-house evaluation of 254 clinical isolates with this mutation are RMP-R.)
inhA (promoter)	No mutation	Isoniazid resistant. (100% of isolates in our in-house evaluation of 254 clinical isolates with this mutation are INH-R.)
katG (exon 2 hotspot)	Mutation: AGC→ACC; Ser315Thr	
embB (EmbCAB)	Mutation: GAC→GCC; Asp354Ala	Probably Ethambutol-resistant. (94% of isolates in our in-house evaluation of 254 clinical isolates with this mutation are EMB-R.)
pncA (promoter, coding region)	No mutation	Cannot rule out PZA resistance.
gyrA (QRDR)	No mutation	Cannot rule out fluoroquinolone resistance. (88% of FQ-R isolates in our in-house evaluation of 254 clinical isolates have a mutation at this locus.)
rrs (16S region)	No mutation	Cannot rule out resistance to injectable drugs (kanamycin, capreomycin, amikacin). (In our in-house evaluation of 254 clinical isolates: • 88% of AMK-R isolates have a mutation in the rrs locus; • 89% of KAN-R isolates have a mutation in the rrs locus; an additional 29% of KAN-R isolates have a mutation in the eis locus; • 40% of CAP-R isolates have a mutation in the rrs locus; an additional 5% of CAP-R isolates have a mutation in the tlyA locus.)
eis (promoter)	No mutation	
tlyA (entire ORF)	No mutation	

*A negative results (e.g., no mutation) does not rule out contributory mutations present elsewhere in the genome.
Testing performed using in-house developed assays.

MDHHS Lab Confirmation of 2nd Line Drugs

INH	R
Rifampin	R
PZA	R
Ethambutol	R
Ofloxacin	S
Ethionamide	R
Streptomycin	S
Kanamycin	S
Amikacin	S
Capreomycin	S
Cycloserine	S
PAS	S

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IN CONCLUSION

- ***See*** the bugs [AFB microscopy]
- ***Multiply*** the bugs [NAATs]
- ***Grow*** the bugs [cultures]
- ***Kill*** the bugs