

TB Laboratory Testing & Case Studies

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Angie Schooley, MT

James Sunstrum, M.D.

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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

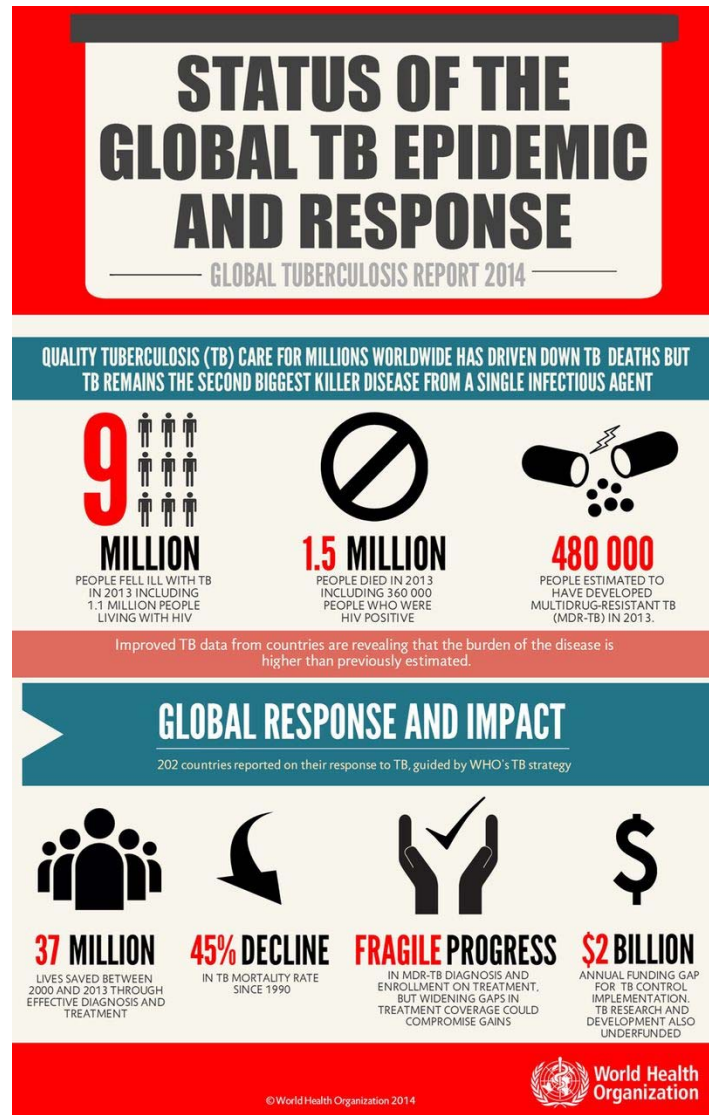


16 S Sequencing

◦ 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

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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



Recommended diagnostic options for pulmonary TB

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

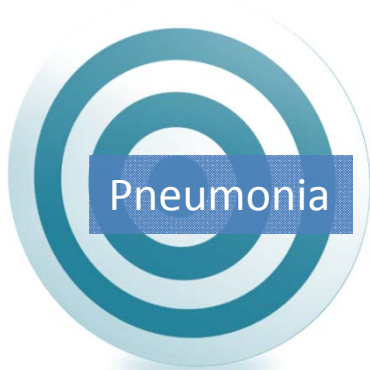
- Courtesy of Prof. Madhukar Pai, MD, PhD Mayo TB Center Webinar March 2016

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

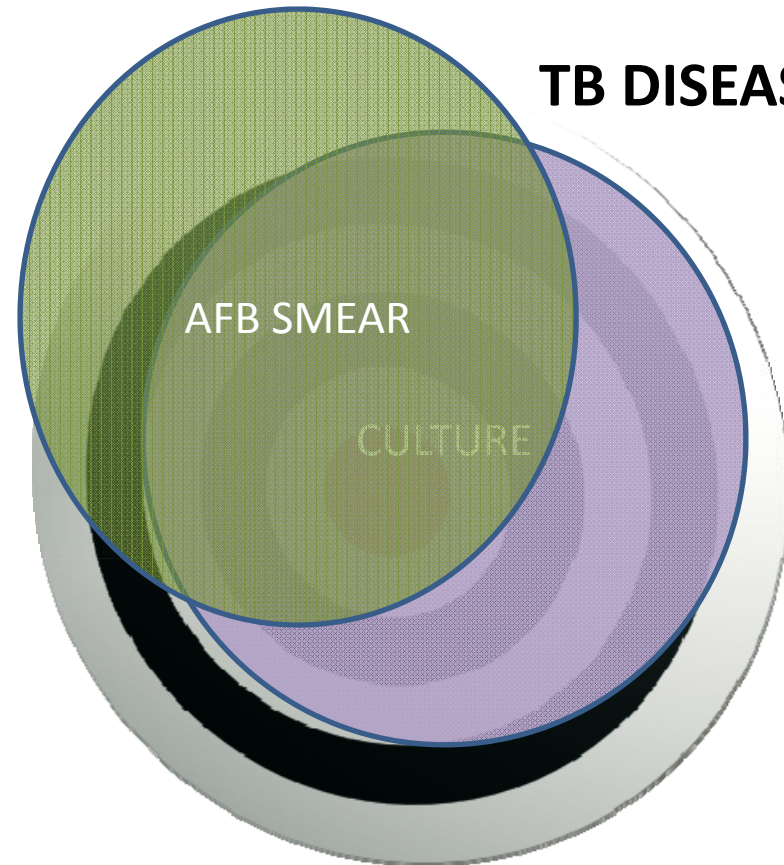


High Accuracy for Diagnosis of HIV in Contrast to TB DISEASE

HIV



TB DISEASE



Studies Michigan 2015 pulmonary TB cases....N= 87

Test	% POSITIVE	COMMENT
AFB smear	44%	Negative smear does not rule out TB
NAAT on AFB+ smear	84%	May be performed on AFB smear negative sputums
AFB culture confirms <i>M. tb</i>	78%	Gold standard, not always positive
IGRA	89%	May be negative even with positive cultures!

Specimen Sources

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

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
- Infection control precautions during specimen collection
- 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

Specimen Collection

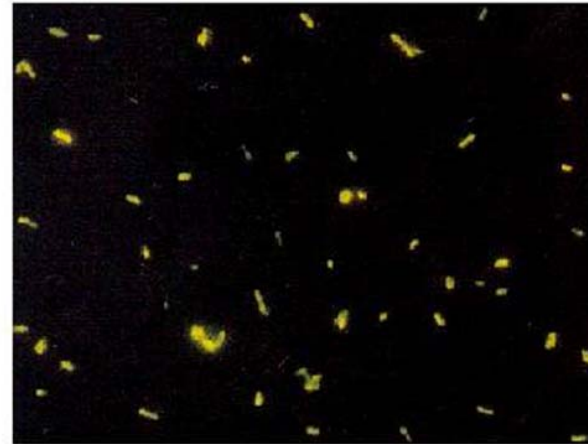
- Collect in sterile, leak proof containers
- Seal with tape
- Refrigerate specimen to reduce overgrowth of contaminating bacteria during transit to lab
- Deliver specimen to TB lab within 24 hrs
- Always include patient name on both test request form and the specimen container

Acid-fast Bacilli (AFB) smear

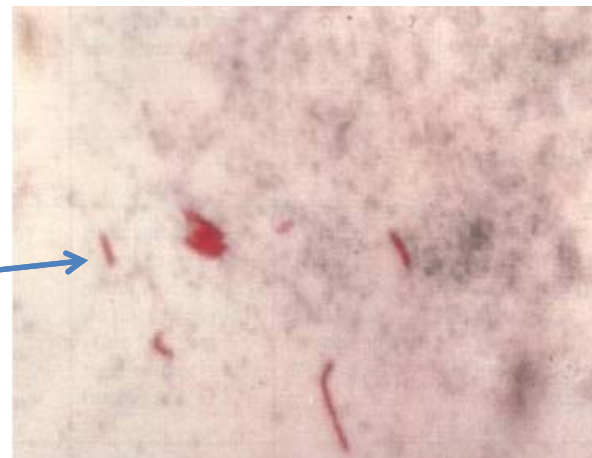
- Least sensitive of all AFB Tests (20-75% positivity)
- Requires 10,000 AFB/ml to be positive
- Positive slide does not differentiate TB from atypical mycobacteria (i.e. *M. avium*)
- Reported within 24 hours of receiving the specimen in the laboratory

Fluorescent AFB Smear Using Auramine-O Staining

- Very sensitive, takes minutes to read
- Not all that is fluorescent is AFB (need a careful eye)
- Chemical fluorescence, **not** an immune stain or Direct Fluorescent Antibody
- Can be confirmed with Ziehl-Neelson (ZN) smear



Auramine-O staining of AFB under Fluorescence Microscopy



Nucleic Acid Amplification (NAA) 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

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

MTD



Gene XPERT

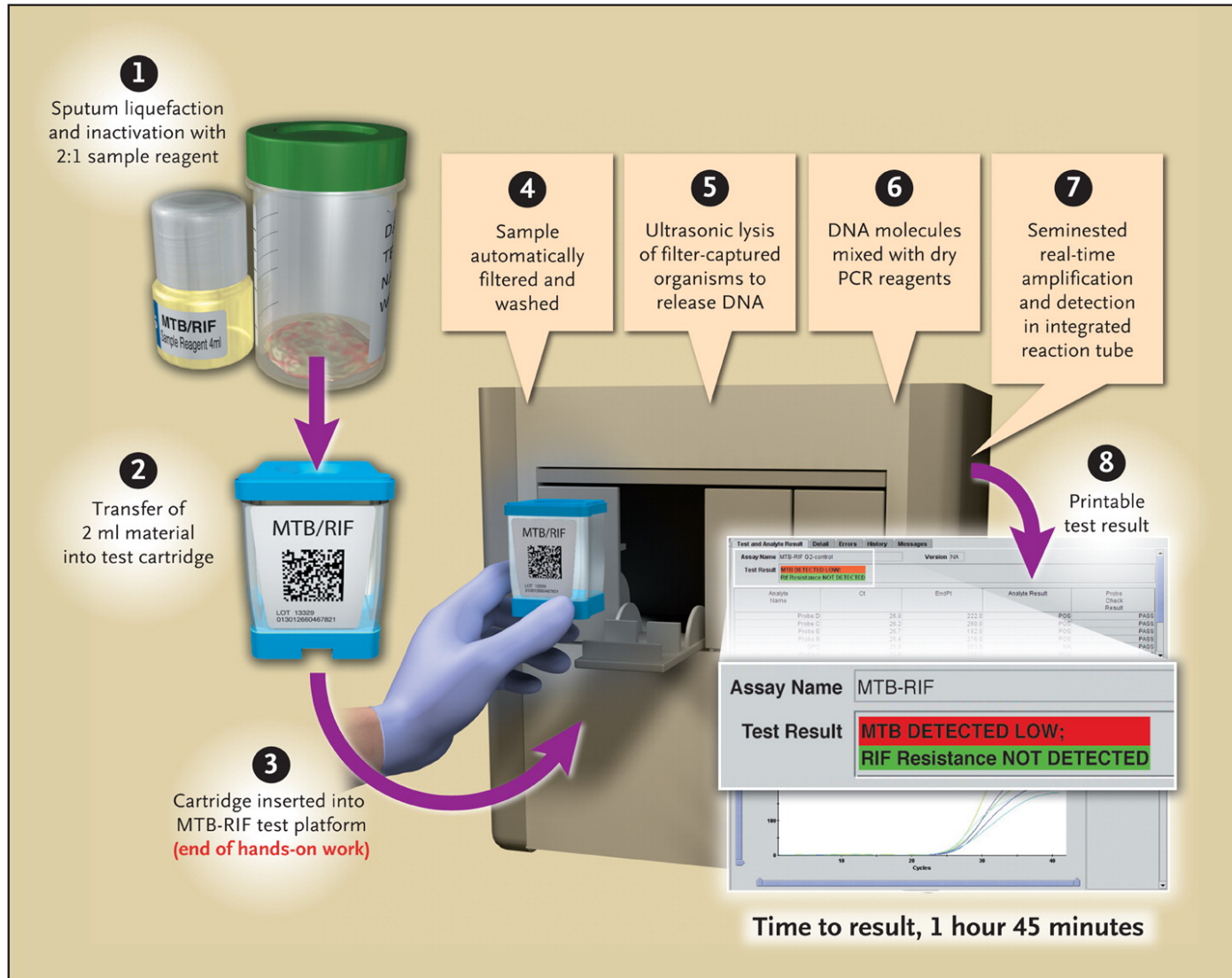


NAA tests are available that are not FDA approved, such as real time PCR assays

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



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, whereas AFB smear needs 10,000 AFB/ml
- Culture may be AFB positive even if smear was negative for AFB



Tests Performed on Growth in Mycobacteria Culture

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

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

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)

CDC MDDR

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

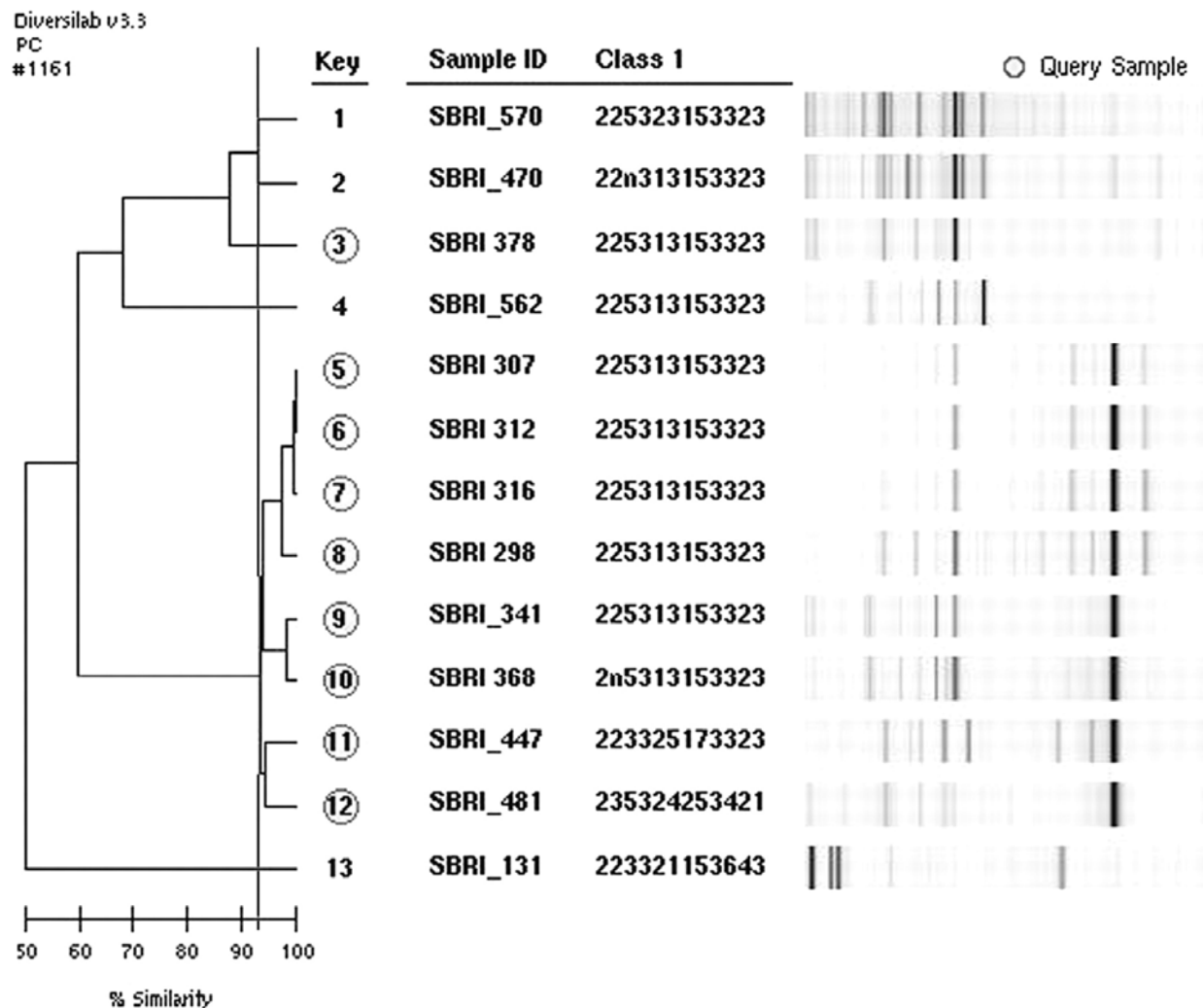
- Second-line MDDR to detect XDR-TB
 - *gyrA* (Fluoroquinolones)
 - *rrs* (Kanamycin, Amikacin, Capreomycin)
 - *eis* (Kanamycin)
 - *tlyA* (Capreomycin)
 - *pncA* (Pyrazinamide)
 - *embB*(Ethambutol)

TB DNA Genotyping

Universally Offered by CDC

- DNA “Fingerprint” of each isolate
- Michigan Department of Health & Human Services laboratory runs genotype on all TB cultures in United States and territories

Mycobacterium tuberculosis Genotyping To Prioritize Tuberculosis Outbreak Control Activities



Maegan Ashworth et al. J. Clin. Microbiol. 2008;46:856-862

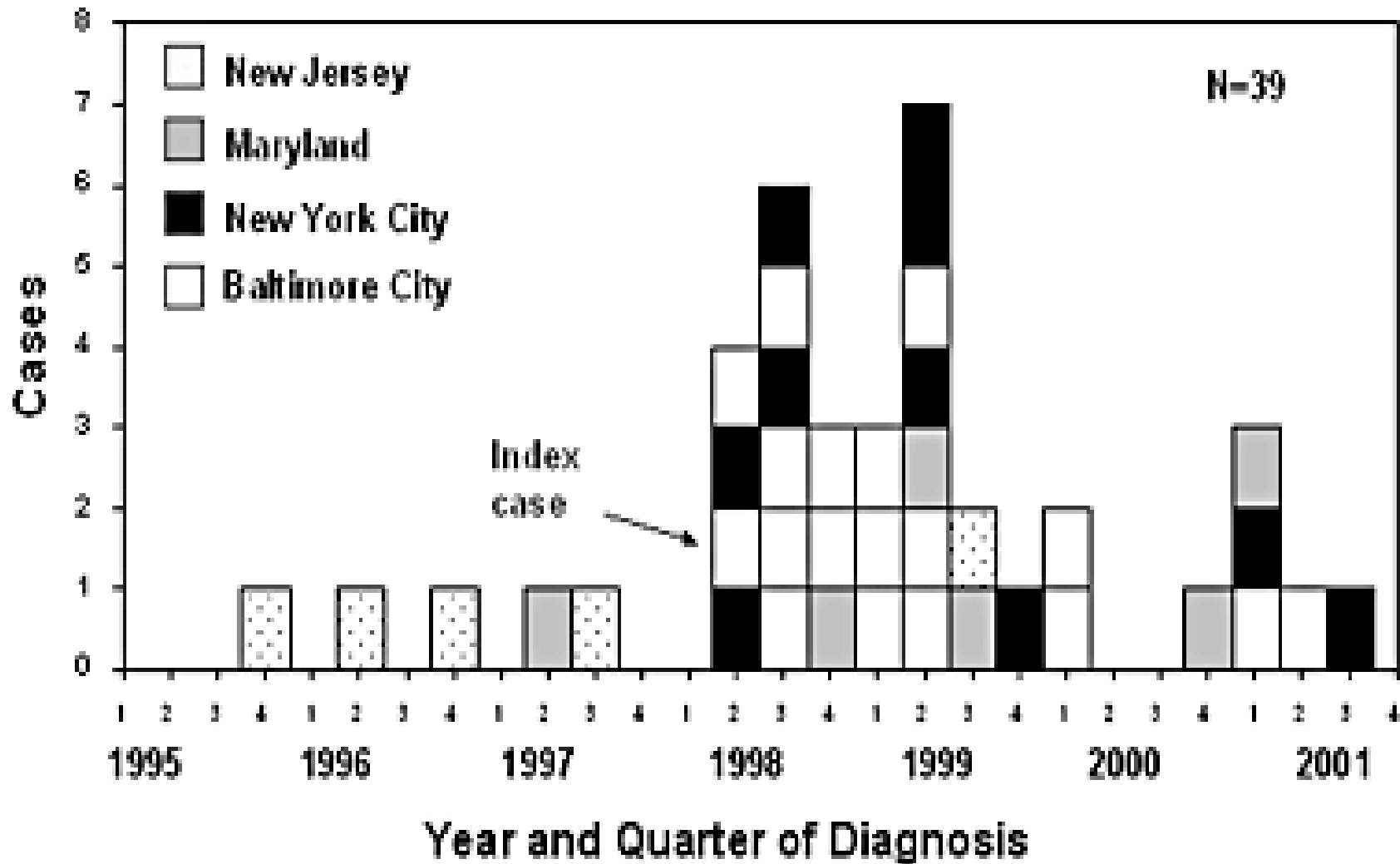
Journal of Clinical Microbiology

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.

Epidemic Curve of Investigation of a Multistate TB Outbreak



3 Sample Cases

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

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

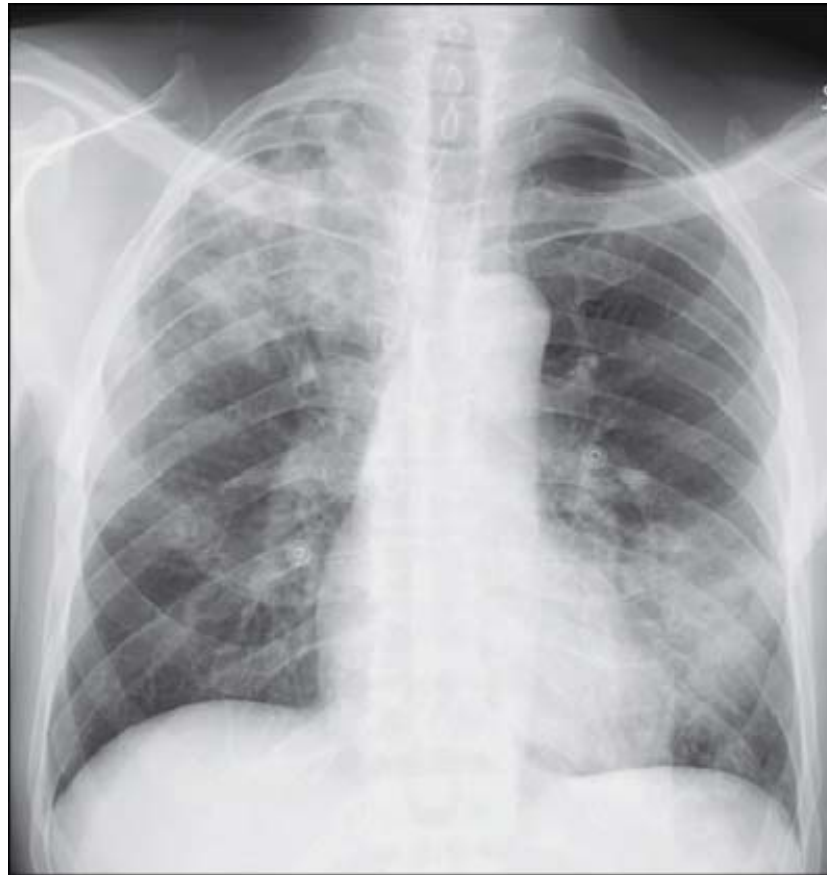


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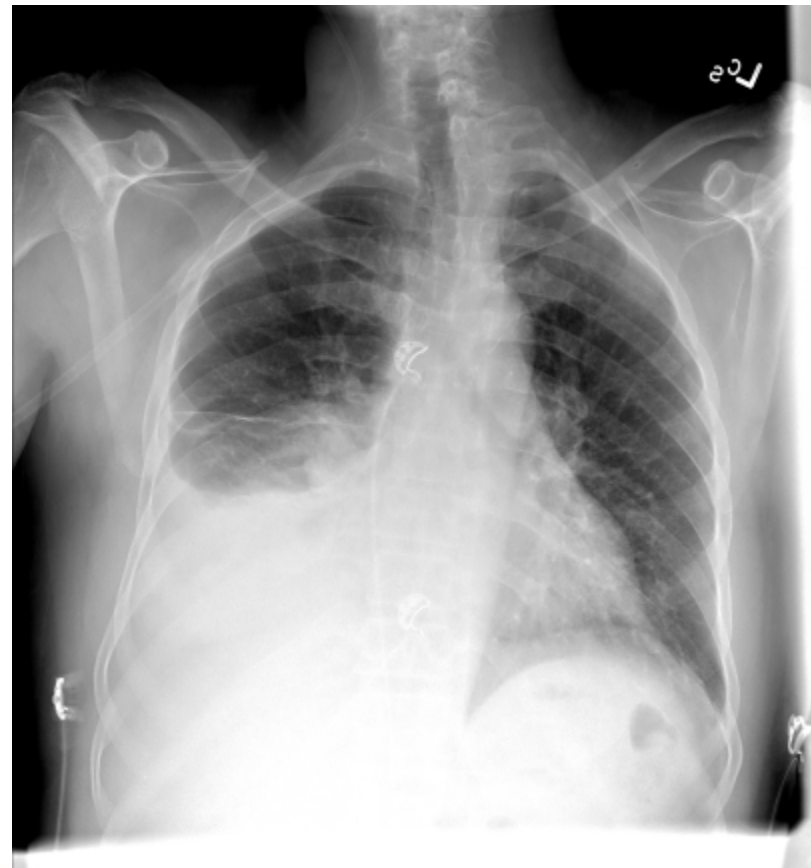
Admission chest radiograph showing bilateral lung infiltrates with prominence in the right upper lobe and lingula of the left lung.

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

#2 case MEDIUM

57 yr male

- Routine cultures negative
- No improvement
- Bronchoscopy AFB smear negative
- HIV +
- CD4 478 cells/mm³



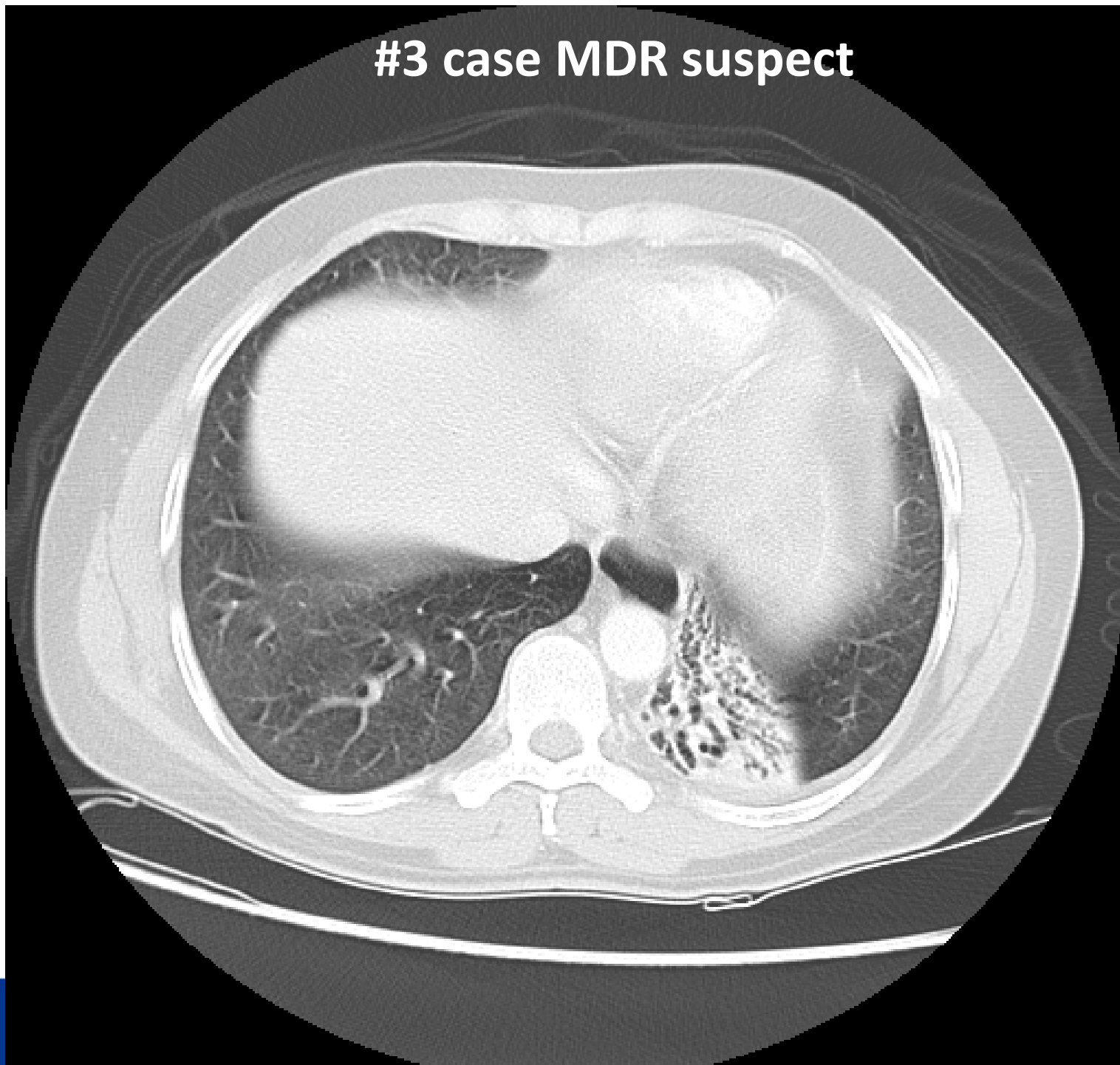
APRIL 2016 "MEDIUM" CASE					1 HIV+ TB suspected	2 Sputum PPD/IGRA
3 AFB smear negative	4 PPD 0 mm 2 nd smear negative	5	6 IGRA negative	7 NAAT positive	8 INH, RIF, PZA, EMB	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30

Case #3

Difficult

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

#3 case MDR suspect



ont

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 # 11D0668319

Original Submitter:

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

Date Collected: 1/17/2012
 Date Received: 1/31/2012
 Date Reported: 2/1/2012

Patient: [REDACTED]

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)
rrpB (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 (ser315 codon)	Mutation: AGC>ACC; Ser315Thr	
embB (Met306,Gly405)	Mutation: GAC>GCC; Asp354Ala	Probably Ethambutol resistant. (84% 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. (86% of FO-R isolates in our in-house evaluation of 254 clinical isolates have a mutation at this locus.)
rrs (1400 region)	No mutation	Cannot rule out resistance to injectable drugs (kanamycin, capreomycin, amikacin). (In our in-house evaluation of 254 clinical isolates: <ul style="list-style-type: none"> • 88% of AMK-R isolates have a mutation in the rrs locus; • 58% 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; • 46% of CAP-R isolates have a mutation in the rrs locus; an additional 5% of CAP-R isolates have a mutation in the tyA locus.)
eis (promoter)	No mutation	
tyA (entire ORF)	No mutation	

*A negative result (e.g., no mutation) does not rule out contributory mutations present elsewhere in the genome.

Testing performed using in-house developed assays.

MDCH 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

IN CONCLUSION

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