TB Laboratory Testing and Case Studies

September 26, 2018

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Learning Objectives

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

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Disclosures

- None

TB Testing - What Do All the Words Mean?

NAA culture Amplification MGIT Genotypic WGS

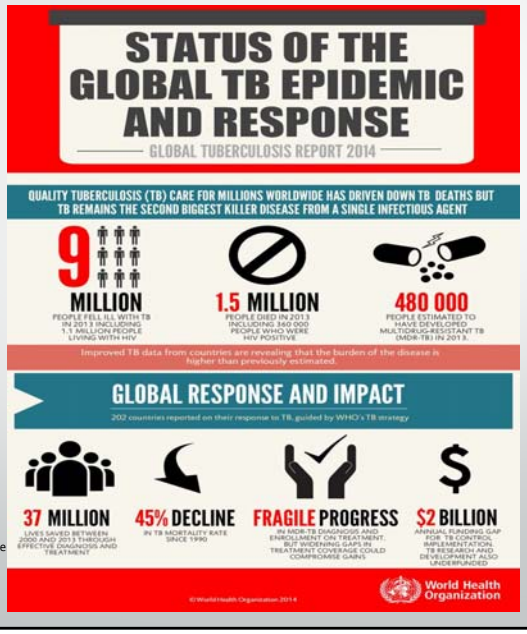
MTD 16 S Sequencing Susceptibility

Smear Phenotypic Molecular mutation

HPLC MALDI-Tof Gene Xpert

MDDR NAAT Genotyping

Status of the Tuberculosis Problem in 2014



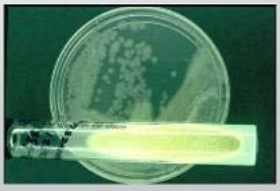
Does this patient have TB disease?

Clinical Clues

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

Laboratory Tests

- PPD
- IGRA
- Sputum studies:
  - AFB Cultures
  - Molecular studies
- X-rays
- Biopsies



Mycobacterial Examination

- ***See the "Bugs"***

  Specimen collection, decontamination, acid-fast bacilli (AFB) smears

- ***Multiply the "Bugs"***

  Nucleic acid amplification test

- ***Grow the "Bugs"***

  Mycobacterial culture, identification, and drug susceptibility testing

  TB genotyping / WGS

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TB is Difficult to Diagnose

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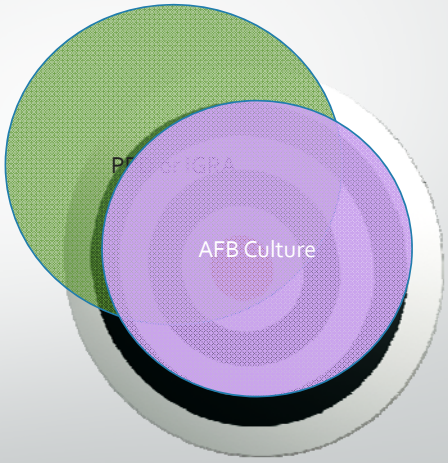
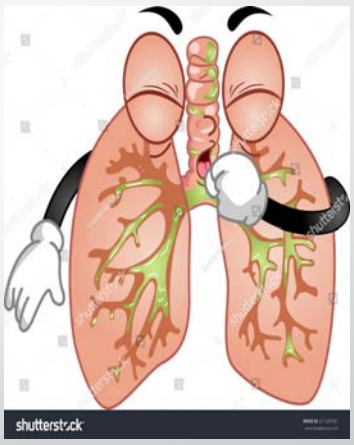
High Accuracy for Diagnosis of HIV in Contrast to TB DISEASE

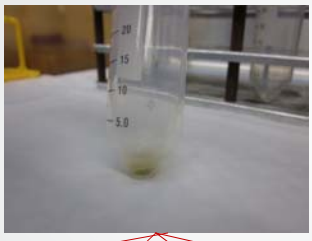


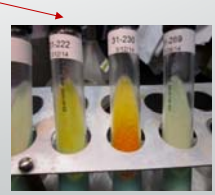
TB Specimens

- Sputum, Pulmonary aspiration
- Tissues, Body fluids (CSF, pleural, peritoneal), etc.
- All persons suspected of TB disease should have sputum cultured
- Early morning specimens have the highest yield of AFB
- Collect at least 3 consecutive specimens at 8-24 hour intervals
- Recommended volume for testing is 3-7 ml, less may compromise the recovery of AFB



How Many Tests!





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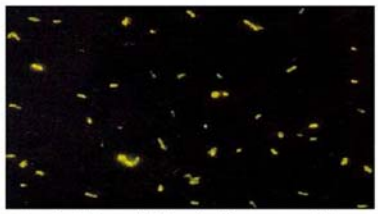
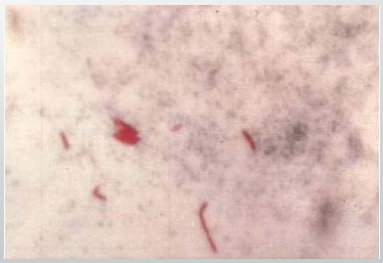
Acid Fast Microscopy-"*See* the Bugs"

- Least sensitive of all AFB Tests / first result available
- Requires 10,000 AFB/ml for a slide to be positive
- Positive slide cannot determine AFB viability or TB vs NTM (Non tuberculosis Mycobacterium)



Auramine-O staining of AFB under Fluorescence Microscopy



Ziehl-Neelson (ZN) smear

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Nucleic Acid Amplification Test (NAAT) or PCR

"*Multiply* the Bugs"

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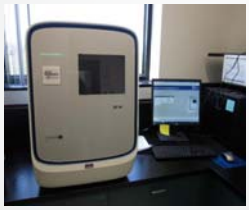
Available NAA Testing Systems

- Gene XPERT

FDA approved cartridge based NAAT that can detect the presence of *M. tuberculosis* complex DNA and resistance to Rifampicin from sputum

- Quant Studio



Non FDA approved real time PCR that can detect *M. tuberculosis* complex and *M. avium* complex from both respiratory and non-respiratory specimens (developed by Wadsworth, validated at MDHHS)

- MTD

FDA approved kit by Hologic that detects the presence of *M. tuberculosis* complex rRNA from respiratory specimens

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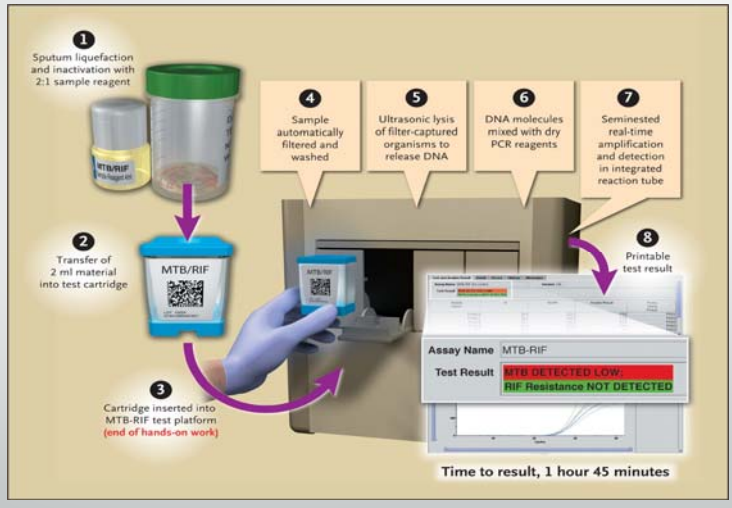
GenExpert Assay Procedure for the MTB/RIF Test



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

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Limitations of NAAT

- NAAT are intended for <u>initial diagnosis only</u>
- A negative test does not exclude the possibility of culturing MTBC
- May remain positive during and after TB treatment
- Inhibitors may be present that affect amplification

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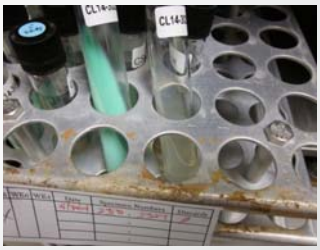
AFB Culture "*Grow* the Bugs "

- More sensitive than AFB smear, only 10 AFB/ml can produce a positive result
- Culture may be positive if the smear was negative
- Rapid broth system normally positive within 1-2 weeks
- Requires 6 weeks to report culture as negative



Mycobacterium Identification by Culture Based Methods (Which Tools to Use)

- HPLC: High Performance Liquid Chromatography
- MALDI-TOF: Matrix-Assisted Laser Desorption Ionization - Time of Flight
- Accuprobe: *M. tuberculosis* cplx., *M. avium* cplx., *M. kansasii*, *M. gordonae*
- 16S sequencing
- Conventional biochemical testing

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*

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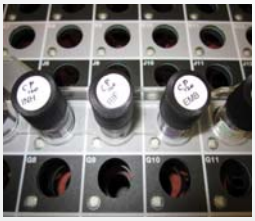
Primary TB Antibiotics

Most results are available within 7-14 days of *M. tuberculosis* complex Identification

- Isoniazid
- Rifampin
- Ethambutol
- Pyrazinamide



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Molecular Detection of TB Drug Resistance (MDDR) CDC

- Rapid testing for DNA sequences associated with 1st and 2nd line drug resistance, results in 2-4 days
- NAAT (+) sputum sediment or growth based culture isolates, only requested by state health lab
- Submission criteria:
  - ✓ Known Rifampin resistance
  - ✓ Known MDR
  - ✓ High risk of Rifampin resistance or MDR-TB (e.g. previous TB, MDR-TB contact, foreign born)
  - ✓ High profile patient (e.g. daycare worker, nurse)
  - ✓ Mixed or non-viable culture
  - ✓ Adverse reaction (e.g. RIF allergy)

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Why Genotyping?

- Confirm epidemiologic links
- Detect unsuspected transmission
- Outbreaks detected earlier; controlled more rapidly
- Detect or confirm false positive cultures
- With WGS data, detect drug resistance/susceptibility

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TB Genotyping and Michigan

- 2003 & 2008 - Michigan & California awarded contract for genotyping on 4500 isolates each
- 2013 - Michigan awarded sole contract for 9000 isolates
- 2018 - Michigan awarded contract for genotyping and WGS on 9000 isolates

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Isolate Receipt & DNA Extraction

- Confirmed cultures are received and opened in a BSL3 lab
- Specimens are heat inactivated before removal from the BSL3
- DNA extracted via Bead Beater Homogenizer

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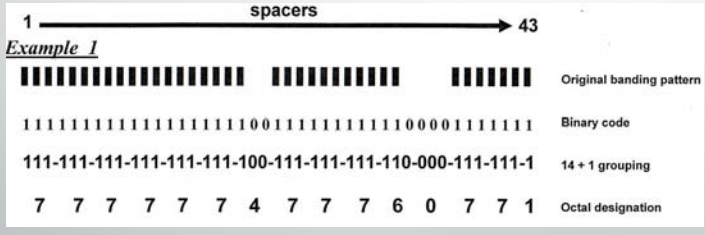
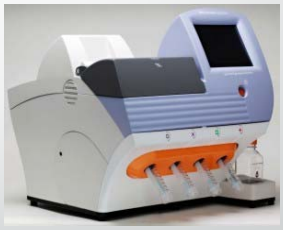
Genotyping Methods - Spoligotyping

- Variability in direct repeat region
- Direct repeats separated by unique "spacer" sequences
- 43 spacer sequences





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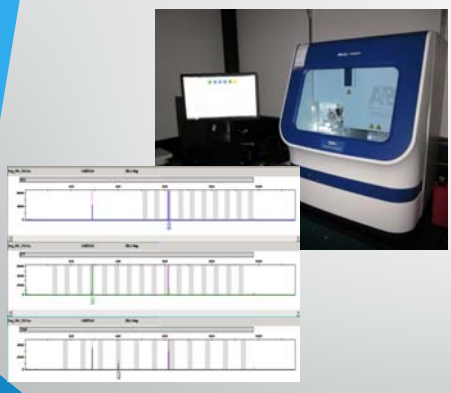
Genotyping Methods – MIRU-VNTR



- PCR reactions amplify DNA at 24 different loci
- Capillary Electrophoresis
- Electropherograms analyzed
- Results uploaded to National TB Website

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Genotype Reporting

- Results uploaded to TBGIMS National Database, clustered and compared to other isolates both within states and across the US

SpoligoType	MIRU	MIRU2	State Cluster	State Cluster Name2	GENType	Genotyping Lineage
000000000003771	223325173533	445644423328	MI_0016	MI_0016_003	G00012	East Asian (L2)

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Whole Genome Sequencing

- Reveals complete genetic make-up of an organism at base-pair level
- Detect single nucleotide variants, insertions, deletions, structural variants, etc.
- TB Genome = 4.5 million base pairs

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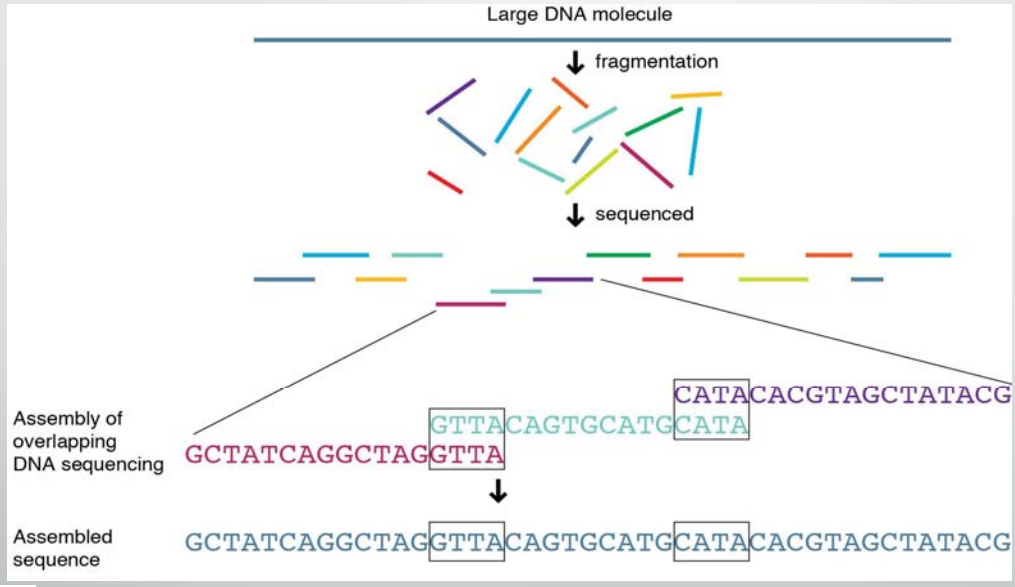


WGS Subtyping Analysis

- SNP (single nucleotide polymorphisms). Compares sequences to an established reference genome.

Reference genome: ACTTGCA

Isolate 1 ACTTGCA

Isolate 2 ACTTGCA

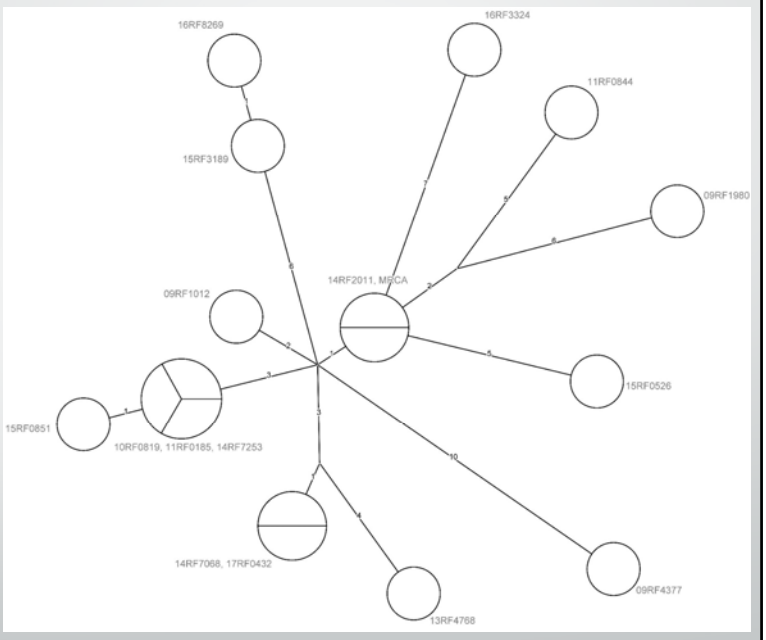
Isolate 3 ACTCGCA

Isolate 4 AGTTGCA

Isolate 5 GCTTGGA

- Results are displayed on dendrograms

- Bioinformatics needed to handle data
- Data is permanent, compatible with future technologies, will never be outdated



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WGS and the Future!

- Improved Epi Links
- Faster ID
- Drug Susceptibility

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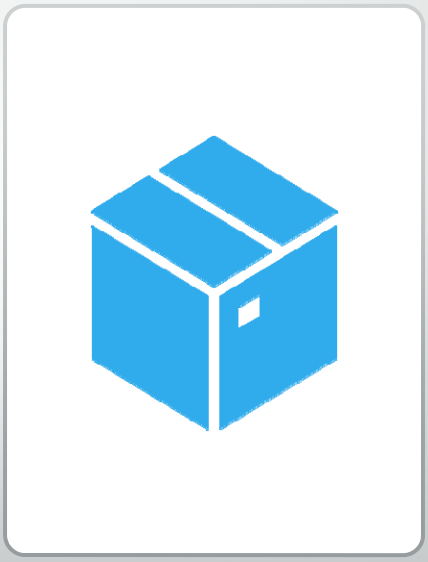
3 Sample Cases



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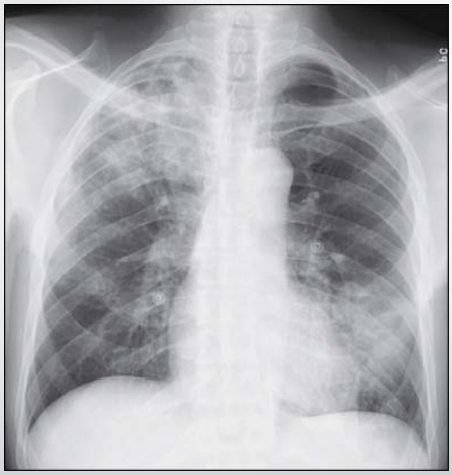
Case #1 EASY

The JAMA Network

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

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



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

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Case 1

- 50 yr male, cough for 2 months
- History of TB exposure 5 years ago. Never treated for latent TB
- HIV negative

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APRIL 2019 "EASY" CASE					1 TB suspected	2 Sputum PPD or 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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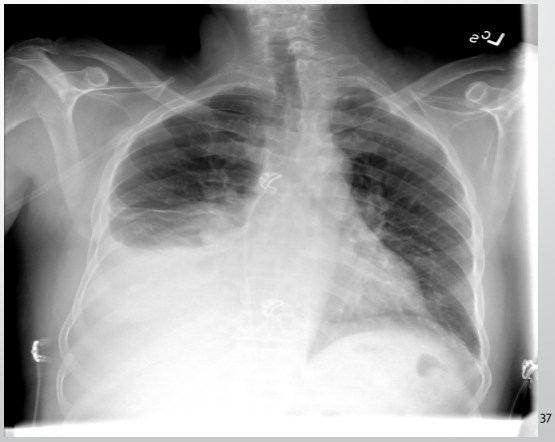
#2 Case MEDIUM

57 yr male sick for 2 months

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



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APRIL 2019 "MEDIUM" CASE					1 HIV+ TB suspected	2 Sputum PPD or 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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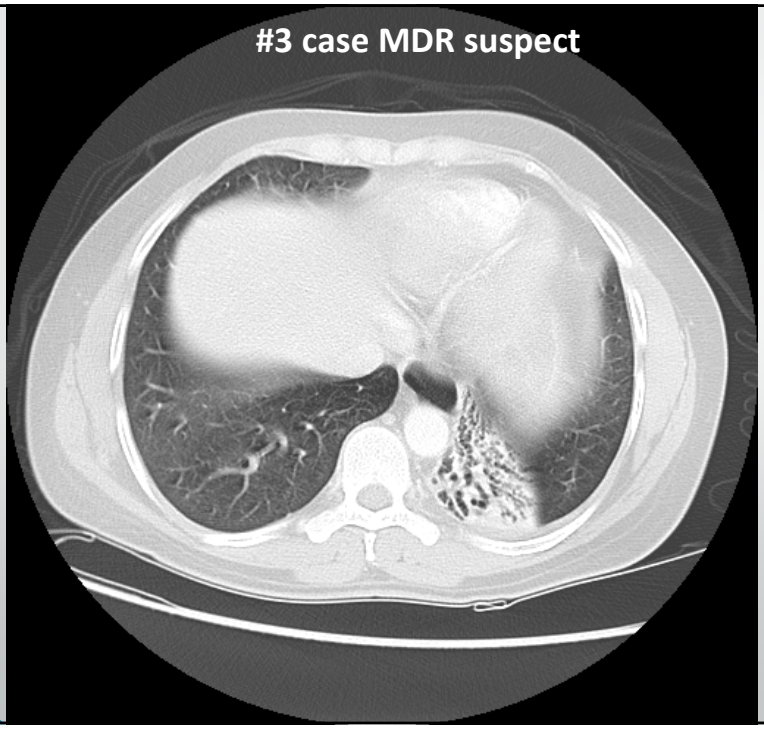

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APRIL 2019 "DIFFICULT" CASE					1 MDR-TB suspected	2 Sputum; IGRA
3 AFB smear positive	4 IGRA positive	5 NAAT positive	6 **INH, RIF, PZA, EMB ???**	7	8 MDDR from CDC positive*	9
10	11 **MDR regimen started**	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30

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02/01/2012 15:09 FAX 4046395491 CDC-TB-LAB 002/002

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)
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 (ser315 codon)	Mutation: AGC>ACC; Ser315Thr	
embB (Met306,Gly406)	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 FQ-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: • 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 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 / ***Kill*** the bugs[cultures]
- ***Track*** the bugs

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Used separately, Molecular (genotypic) and growth based testing (phenotypic) are imperfect, used together, the accuracy and speed of detection of *Mycobacterium tuberculosis* and drug resistance is greatly improved

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