TB Case Diagnostics

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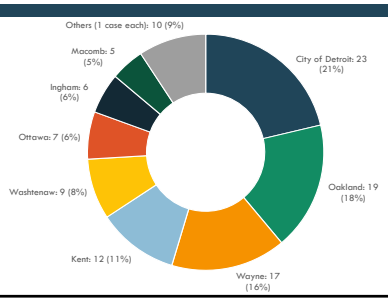
TB Cases by Local Health Jurisdiction

Michigan, 2018



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Classification of TB Cases

Michigan, 2018

- Verification Criteria
  - 62% Culture
  - 3% Smear/Nucleic Acid Amplification
  - 29% Clinical
  - 6% Provider Diagnosed
- 100% of culture positive cases were genotyped
- 13% in a genotype cluster

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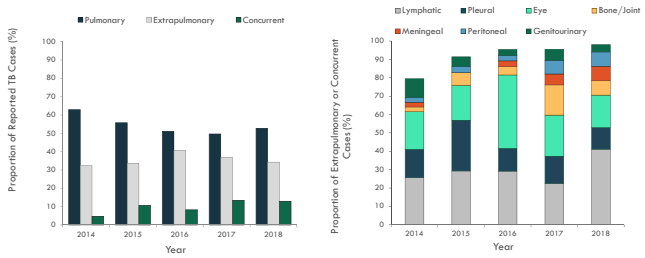
Tuberculosis Cases by Site of Disease

Michigan, 2014-2018



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TB Laboratory Testing and Case Studies

Following a TB Case Through the Diagnostic Pipeline

March 21, 2019

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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

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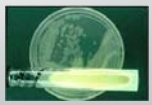
Does this patient have TB disease?

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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TB is difficult to diagnose

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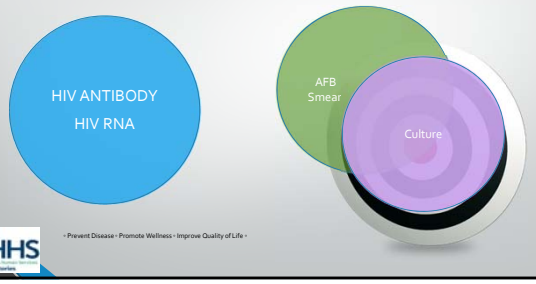
High Accuracy for Diagnosis of HIV in Contrast to TB DISEASE



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Acid Fast Bacilli Examination

- ***See***

  Specimens tested, decontamination, acid-fast bacilli (AFB) smears

- ***Amplify***

  Nucleic acid amplification test (NAAT aka PCR)

- ***Grow***

  Mycobacterial culture, identification, and drug susceptibility testing

  TB genotyping / WGS

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Specimen Collection

- Respiratory
  - Sputum
    - ✓ All persons suspected of TB disease should have sputum cultured
    - ✓ Early morning specimens generally yield greater number AFB
    - ✓ Collect at least 3 consecutive specimens at 8-24 hour intervals
  - Bronchial
  - Tracheal aspirates
- Non-Respiratory
  - Body fluids (CSF, pleural, peritoneal, etc.)
  - Tissues
  - Blood
  - Abscess
- Recommended volume for all samples is 3-7 ml, less may compromise the recovery of AFB

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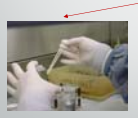
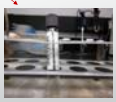
Specimen Processing

- Digest
- Decontaminate
- Concentrate

- All of specimen used during processing






See Amplify Grow

MDHHS Michigan Department of Health & Human Services Bureau of Laboratories

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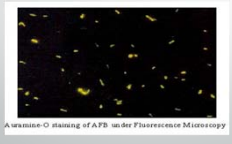
Acid Fast Microscopy - "*See*"

- 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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When is NAAT Performed on Clinical Specimens?

- Slide positive patients with no mycobacterial history or NTM history greater than 1 year
- Slide negative patients that are highly suspect for possible MTBC

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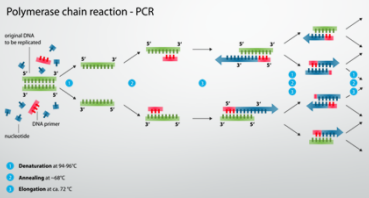
Nucleic Acid Amplification Test (NAAT) or PCR - *"Amplify DNA"*



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Available NAAT Testing Systems

- GeneXpert
  - FDA approved
  - Detects *M. tuberculosis* complex DNA
  - Detects resistance to rifampin
  - Sputum specimens only
- Real Time PCR
  - Not FDA approved (developed by Wadsworth, validated at MDHHS)
  - Detects *M. tuberculosis* complex and *M. avium* complex DNA
  - Respiratory and Non-respiratory specimens

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NAAT at MDHHS

- Used to rule in/out MTBC
- Extraction time ~1 ½ hours
- Run time on instrument just over an hour
- Same testing platform for clinical specimens and broth cultures

- REMEMBER: Detection of DNA of organisms from the *M. tuberculosis* complex
- MTBC: *M. tuberculosis, M. bovis, M. bovis BCG, M. africanum, M. microti, M. canettii*

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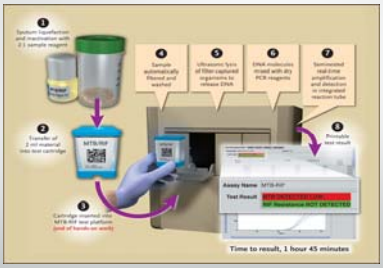
GeneXpert Assay Procedure for the MTB/RIF Test



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

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Limitations of NAAT

- NAAT detects DNA only
  - **NOT** a test of viability – a positive NAAT does not ensure growth on culture
  - Intended for **INITIAL** diagnosis only
- A negative NAAT does not exclude the possibility of culturing MTBC (or MAC)
- Clinical specimens may remain positive during and after TB treatment

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

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AFB Culture - "*Grow*"

- More sensitive than AFB slide test,
  - Only 10 AFB/ml can produce a positive result
- Culture may be positive if the initial slide was negative
- Rapid broth system: most positive within 1-3 weeks
- Requires 6 weeks to report culture as negative

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

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AFB Culture Identification Methods
(Which tools to use)

- NAAT (PCR): Detects DNA of *M. tuberculosis* cplx and/or *M. avium* cplx.
- MALDI-TOF: Matrix-Assisted Laser Desorption Ionization - Time of Flight
- Accuprobe: *M. tuberculosis* cplx., *M. avium* cplx., *M. kansasii*, *M. gordonae*
- Conventional biochemical testing

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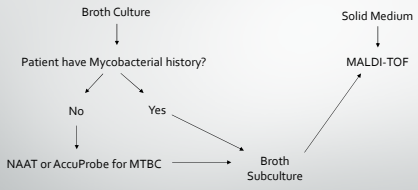
Identification Algorithm



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NAAT on AFB Cultures

- Same testing platform for clinical specimens and broth cultures
- Rule in/out MTBC

- REMEMBER: Detection of DNA of organisms from the *M. tuberculosis* complex
- MTBC: *M. tuberculosis, M. bovis, M. bovis BCG, M. africanum, M. microti, M. canettii*
- MAC: *M. avium, M. chimaera, M. intracellulare, M. marseillense, M. colombiense*

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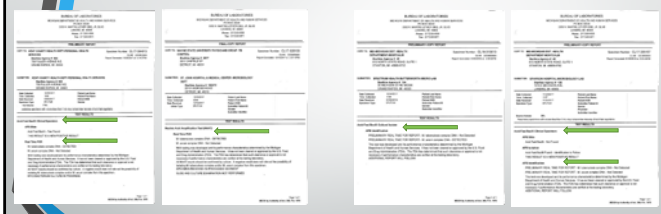
NAAT Report Comparison

NAAT on Clinical Specimen

NAAT on Culture



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MALDI-TOF / Accuprobe

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

- *M. tuberculosis* complex
- *M. avium* complex
- *M. kansasii*
- *M. gordonae*
- Run time approx. 1 ½ hours

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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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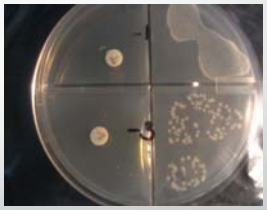
Secondary TB Antibiotics

- Isoniazid – 2nd concentration
- Ethambutol – 2nd concentration
- Amikacin
- Ciprofloxacin
- Ofloxacin
- Kanamycin
- Ethionamide
- Cycloserine
- Capreomycin
- Streptomycin



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Molecular Detection of TB Drug Resistance (MDDR) - Performed by CDC

- Rapid testing for drug resistance, results in 2-4 days
- Request must come from the State Health Lab (MDHHS)
  - CDC has submission criteria – tell us your story
  - Specimens that NAAT detected MTBC DNA
  - Isolates identified as MTBC
- Primary (INH/RIF only) & second line drug testing

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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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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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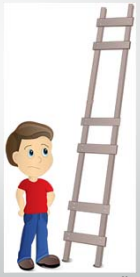
Genotyping Methods - Spoligotyping

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



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Genotyping Methods – MIRU-VNTR

- PCR reactions amplify DNA at 24 different loci
- Capillary Electrophoresis
- Electropherograms analyzed

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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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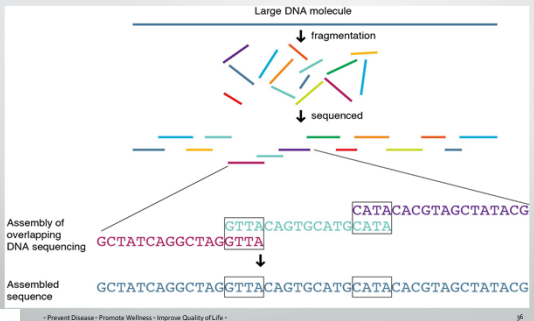

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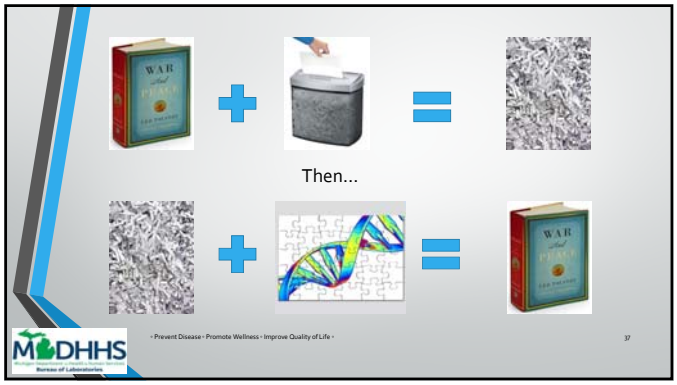

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WGS Subtyping Analysis

- 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	GCTTCGA

- Results are displayed on dendrograms

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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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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

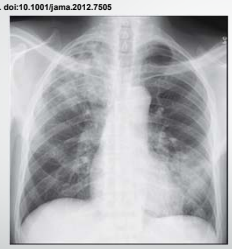

Figure Legend:

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; NAAT positive	4 **INH, RIF, PZA,EMB** PPD 15 mm	5	6	7	8	9
10	11	12 AFB in broth. Prelim. MTBC	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, prelim. MTBC	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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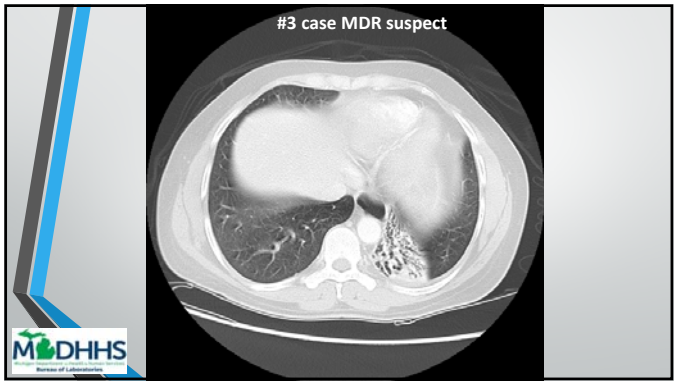

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APRIL	2016	"DIFFICULT"	CASE		1 MDR-TB suspected	2 Sputum IGRA
3 AFB smear positive. NAAT positive*	4 IGRA positive. Request MDDR	5	6 **INH, RIF, PZA, EMB ???**	7	8 MDDR report from CDC	9
10	11 **MDR regimen started**	12	13	14	15	16
17	18	19	20	21	22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

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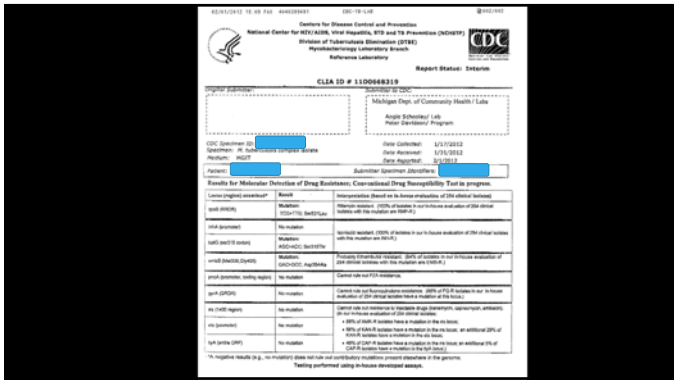

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MDHHS Lab Confirmation of 2nd Line Drugs

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*** [AFB microscopy]
- ***Amplify*** [NAATs]
- ***Grow*** [cultures]
- ***Kill*** [susceptibilities]
- ***Track*** [genotype]

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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

- Prevent Disease • Promote Wellness • Improve Quality of Life •

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