

# TB Laboratory Testing and Case Studies

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

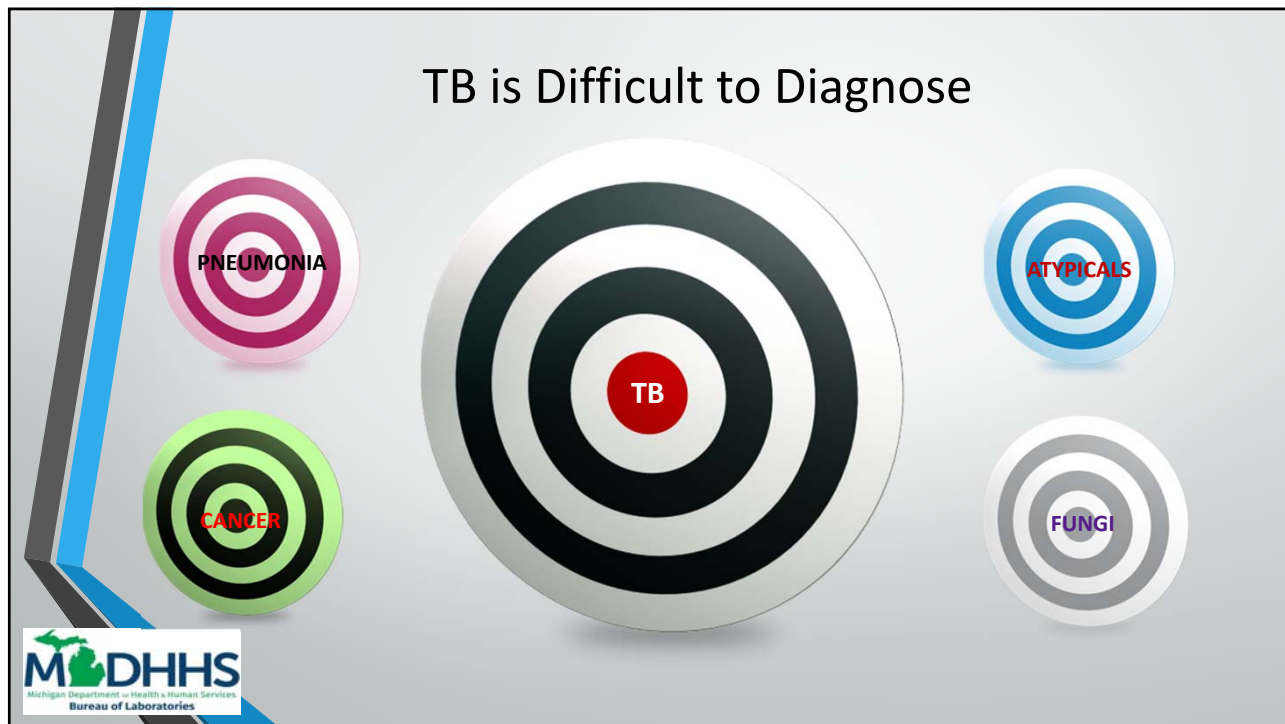


### Laboratory Tests

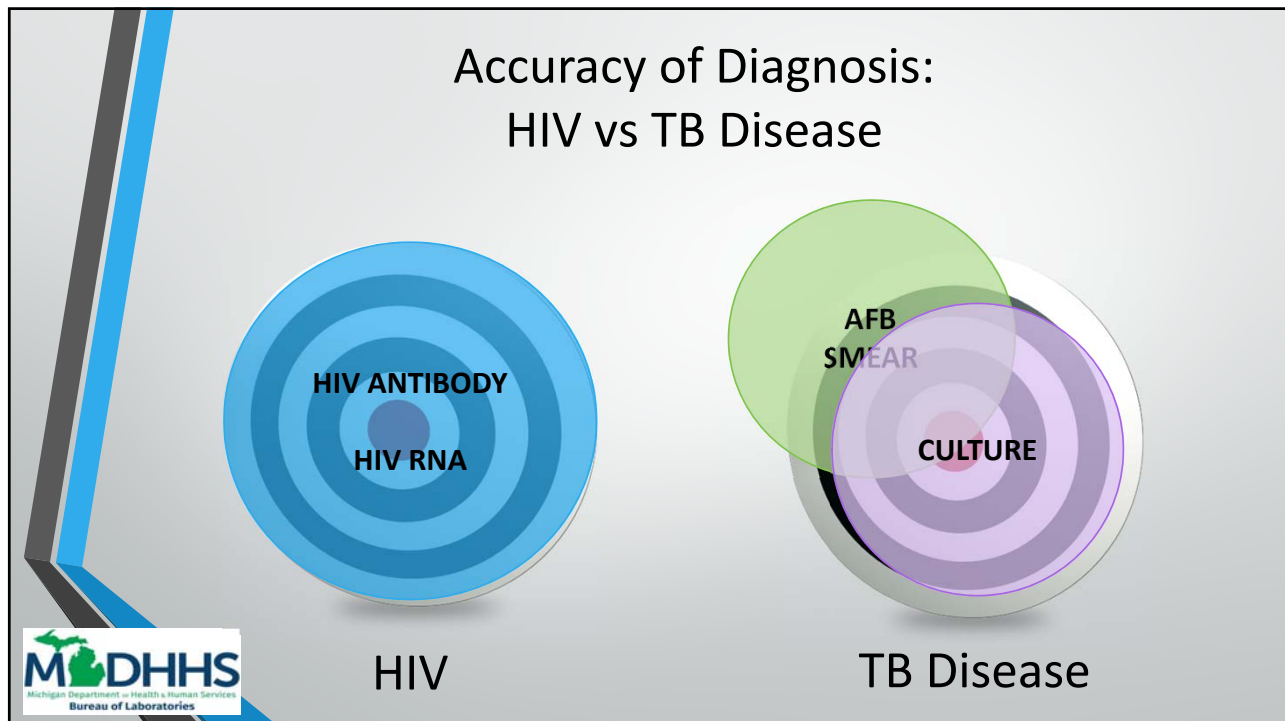
- PPD
- IGRA
- Sputum studies:
  - AFB Cultures
  - Molecular studies
- X-rays
- Biopsies



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## Acid Fast Bacilli Examination

### 1. **See**

- Specimen decontamination and concentration, acid-fast bacilli (AFB)

### 2. **Amplify**

- Nucleic acid amplification test (NAAT) aka PCR



### 3. **Grow**

- Mycobacterial culture, identification, drug susceptibility testing
- TB genotyping / WGS



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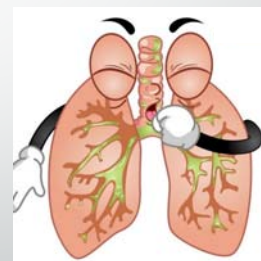
## Specimen Collection

### • **Respiratory**

- Sputum
  - ✓ All persons suspected of TB disease should have sputum cultured
  - ✓ Collect at least 3 consecutive specimens at 8-24-hour intervals with at least one early morning specimen
- Bronchial
- Tracheal aspirates

### • **Non-Respiratory**

- Body fluids (CSF, pleural, peritoneal, etc.)
- Tissues
- Blood
- Abscess



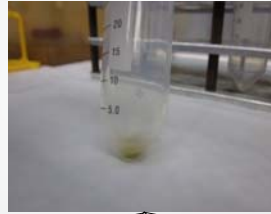
- Recommended volume for all samples is 3-7 ml, less may compromise the recovery of AFB



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## Specimen Processing

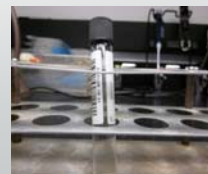
- Digest / Decontaminate
- Concentrate
- All of specimen used for processing



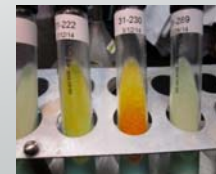
*See*



*Amplify*



*Grow*

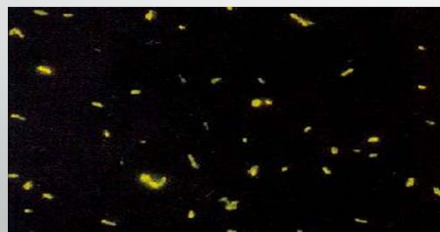


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## Acid Fast Microscopy

*"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 prior history of mycobacterial disease

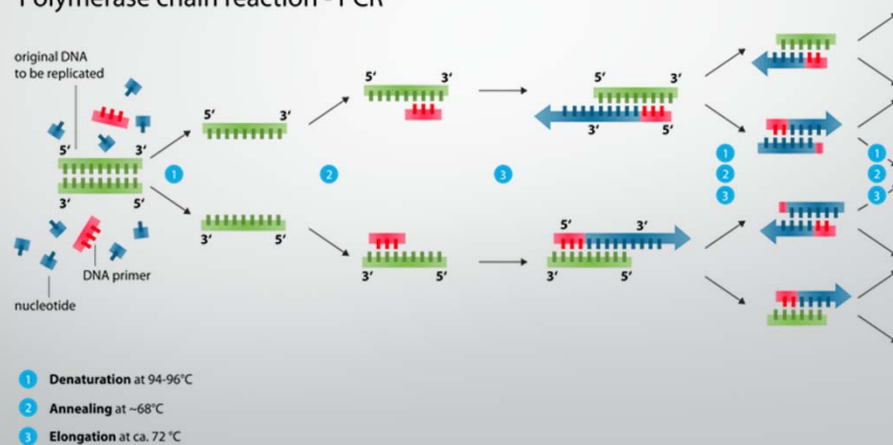
Slide negative patients that are highly suspect for Mycobacterium tuberculosis complex (MTC) (let the lab know in advance)



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

### Polymerase chain reaction - PCR



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## Available NAAT Testing Systems

### GenExpert

- FDA approved
- Detects MTBC DNA
- Detects resistance to rifampin
- Sputum specimens only



### Real Time PCR (lab developed)

- Not FDA approved (developed by Wadsworth, validated at MDHHS)
- Detects *M. tuberculosis* complex (MTBC) and *M. avium* complex (MAC) DNA
- Respiratory and non-respiratory specimens



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## NAAT at MDHHS



- Used to rule-in/-out MTBC
- Extraction and set-up: ~1.5 hours
- Run-time: ~1 hour

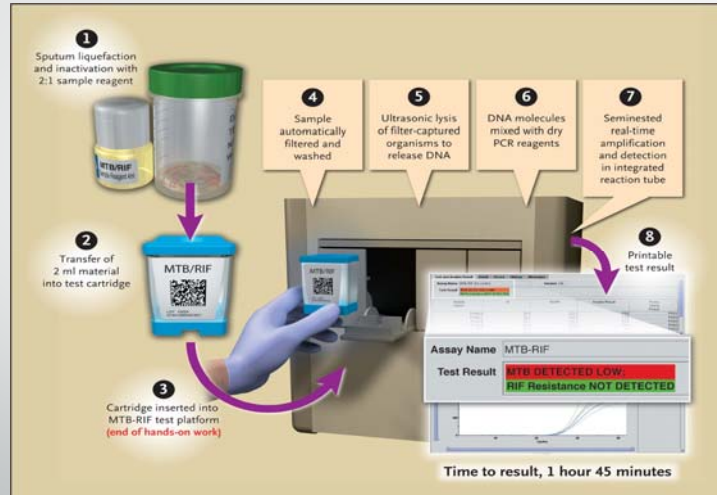
REMEMBER: Detection of DNA of organisms from the *M. tuberculosis* complex\*



\* *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



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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



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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



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## AFB Culture Identification Methods *(Which tools to use)*



- MALDI-TOF (Matrix-Assisted Laser Desorption Ionization - Time of Flight)
- Accuprobe
- 16S Sequencing
- Conventional biochemical testing



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## MALDI-TOF



- Extraction time: ~2 hours
- Run-time: ~1 minute

## Accuprobe



- MTBC, MAC, *M. kansasii*, *M. goodii*
- Run-time: ~1.5 hours



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## 1<sup>st</sup> Line (Primary) *M. tuberculosis* Antibiotics

- Isoniazid (INH)
- Rifampin (RIF)
- Ethambutol (EMB)
- Pyrazinamide (PZA)



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



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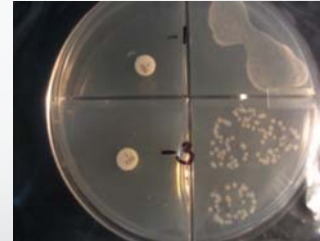
## 2<sup>nd</sup> Line *M. tuberculosis* Antibiotics

### Fluoroquinolones

Ofloxacin  
Ciprofloxacin  
Moxifloxacin  
Levofloxacin

Para-aminosalicylic acid (PAS)

Ethionamide  
Cycloserine  
Bedaquiline (special request)  
Second concentration of INH  
Ethambutol



### Secondary Injectables

Amikacin  
Kanamycin  
Capreomycin



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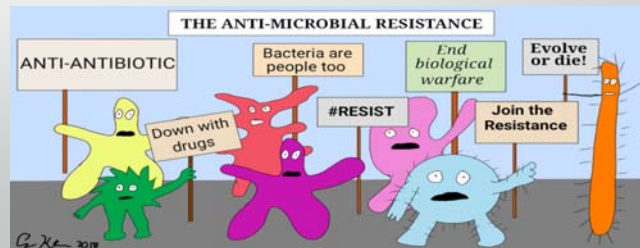
## Drug Resistant TB Definitions

### Multi-drug Resistant tuberculosis (MDR)

- Resistant to as least Rifampin (RIF) and Isoniazid (INH)

### Extensively Drug Resistant tuberculosis (XDR TB)

- MDR TB plus resistance to at least 1 fluoroquinolone and 1 second-line injectable drug



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## Molecular Detection of TB Drug Resistance (MDDR)

- Performed by CDC
- Rapid testing for drug resistance, results in 2-4 days
- Testing for the common mutations associated with drug resistance
- Request must come from your state lab
  - CDC has submission criteria – tell us your story
    - ✓ Known MDR
    - ✓ Contact to MDR
    - ✓ Country with high incidence of drug resistance
  - Specimens must be NAAT positive (DNA) or culture positive (MGIT)
  - Isolates identified as MTBC
- Primary (INH/RIF only) & second line drug testing



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## Why Genotyping?

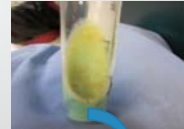
- Confirm epidemiologic links
- Detect unsuspected transmission
- Detect and control outbreaks earlier
- Detect or confirm false-positive cultures
- With WGS data, detect drug resistance/susceptibility



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## Isolate Receipt & DNA Extraction

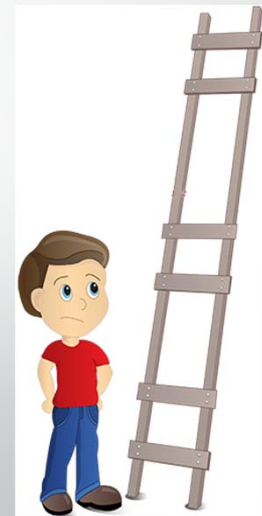
- Confirmed cultures are received and opened in a BSL3 lab
- Specimens are heat inactivated before removal from the BSL3
- DNA extracted via Bead Beater Homogenizer



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## Genotyping Methods *Spoligotyping*

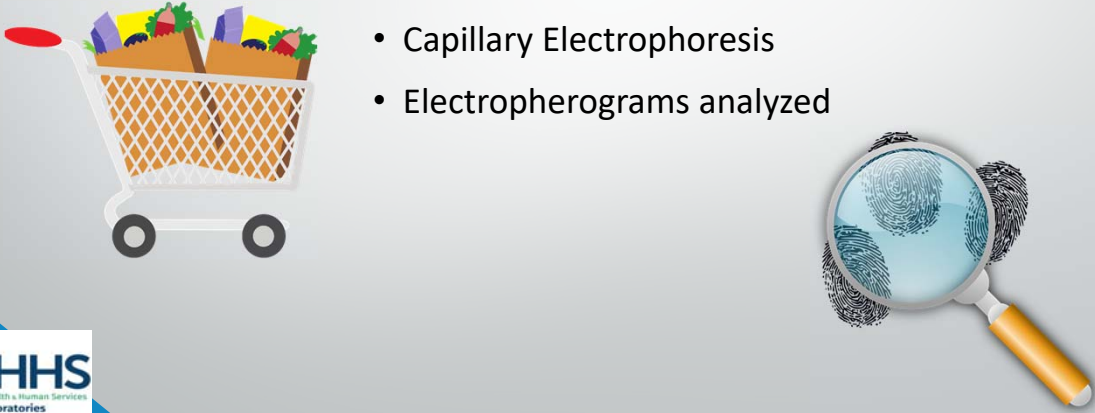
- Variability in direct repeat region
- Direct repeats separated by unique "spacers"
- 43 spacer sequences




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## Genotyping Methods *MIRU-VNTR*

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



The slide features a shopping cart on the left and a magnifying glass over a fingerprint on the right. The shopping cart is filled with various items like vegetables and bread. The magnifying glass is positioned over a blue fingerprint, symbolizing the analysis of genetic data.




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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	Genotyping GENType	Genotyping Lineage
000000000003771	223325173533	445644423328	MI_0016	MI_0016_003	G00012	East Asian (L2)



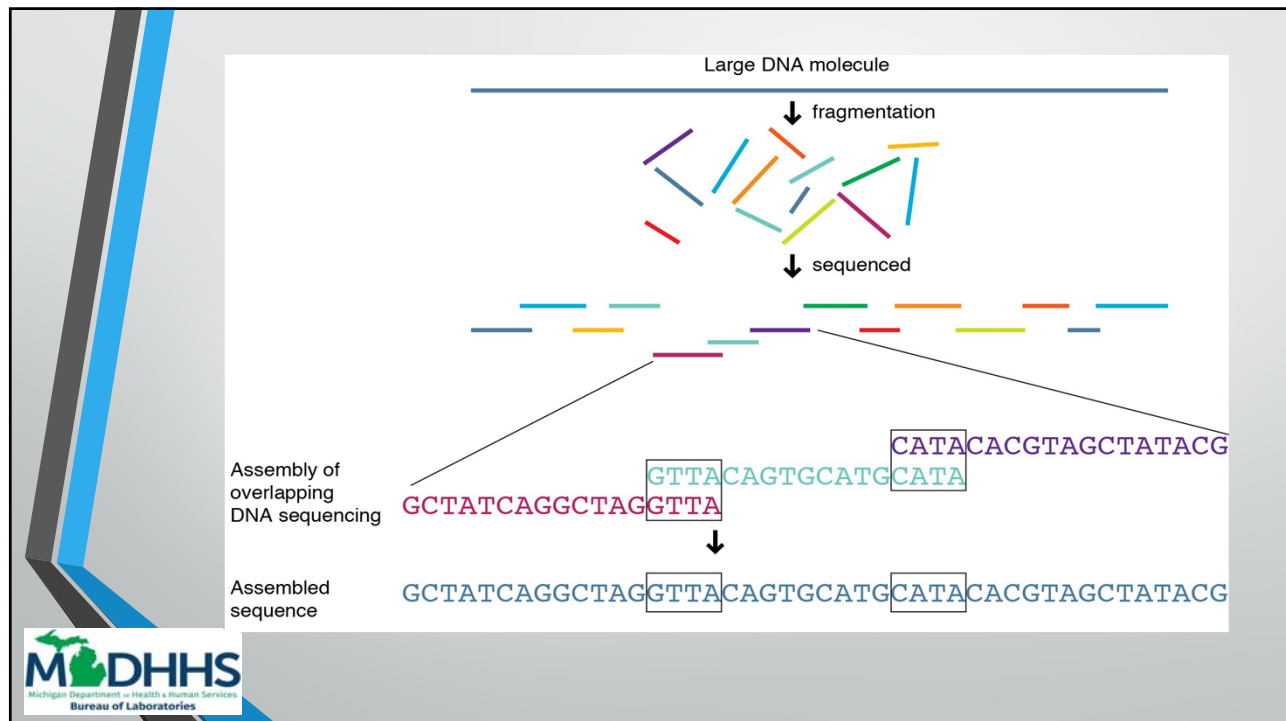
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## Whole Genome Sequencing

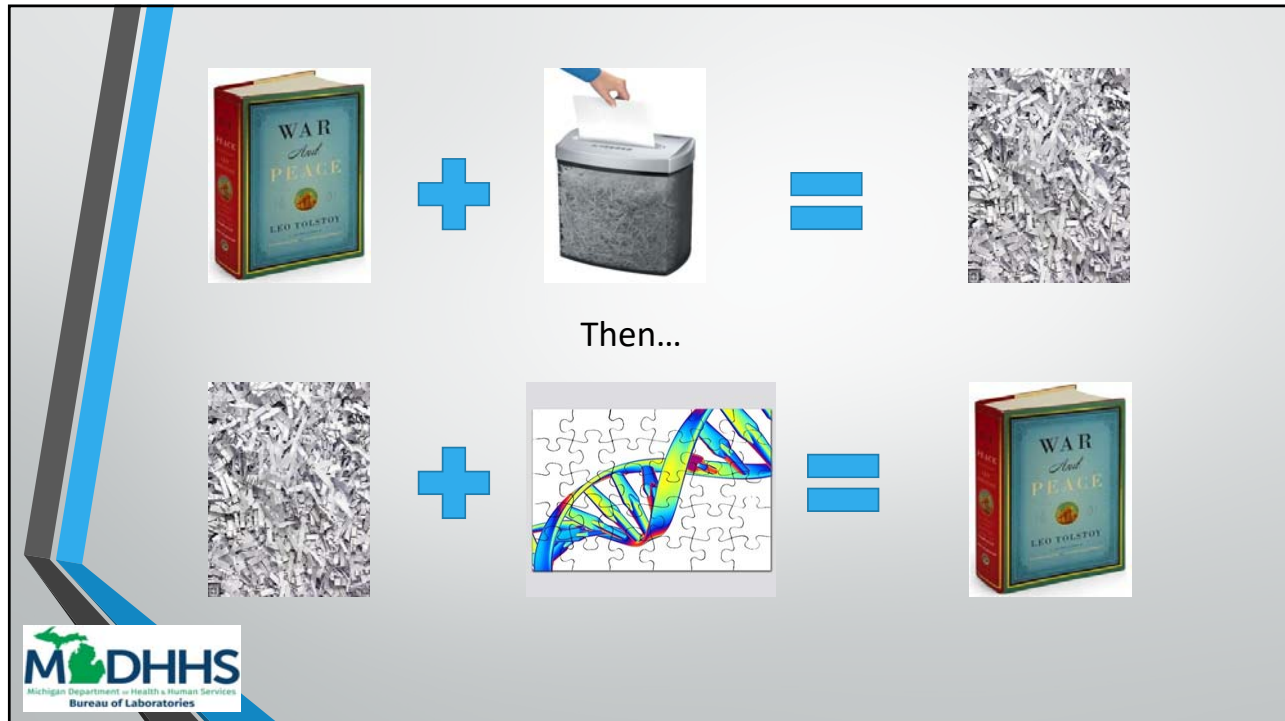
- Reveals complete genetic make-up of an organism at base-pair level
- Detect single nucleotide variants, insertions, deletions, structural variants, etc.
- TB Genome = 4.5 million base pairs



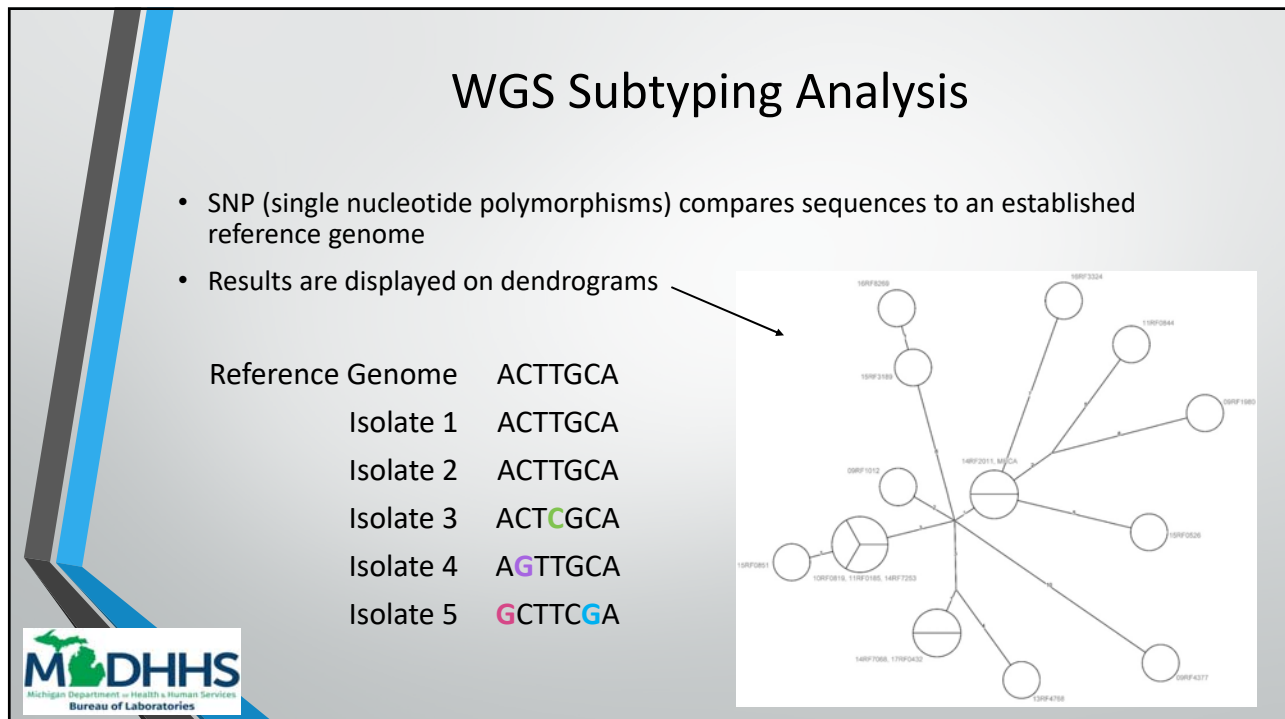
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## WGS and the Future!





- Improved Epi Links
- Faster ID
- Drug Susceptibility



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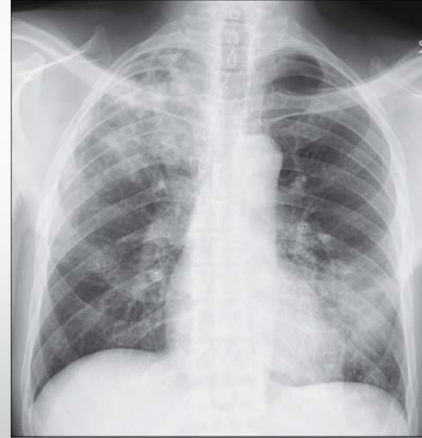
## 3 Sample Cases



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## Case #1: *Easy*

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



Current Approaches to Tuberculosis in the United States  
JAMA. 2012;308(3):283-289  
doi:10.1001/jama.2012.7505



Date of download: 8/22/2012  
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## “Easy” Case Timeline

APRIL 2016						
					1 TB suspected	2 Sputum & TST or IGRA ordered
3 AFB smear positive; NAAT positive	4 TST 15 mm; INH, RIF, PZA, EMB	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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## Case #2: *Medium*

- 57 y/o male
- Routine cultures negative
- No improvement
- Bronchoscopy AFB smear negative
- HIV +
- CD4 478 cells/mm<sup>3</sup>



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## “Medium” Case Timeline

APRIL 2016						
1	2					
HIV+; TB suspected	Sputum & TST or IGRA ordered					
3	4	5	6	7	8	9
AFB smear negative	TST 0 mm; 2 <sup>nd</sup> smear negative		IGRA negative	NAAT positive	INH, RIF, PZA, EMB	
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30
	AFB in broth; prelim. MTBC					



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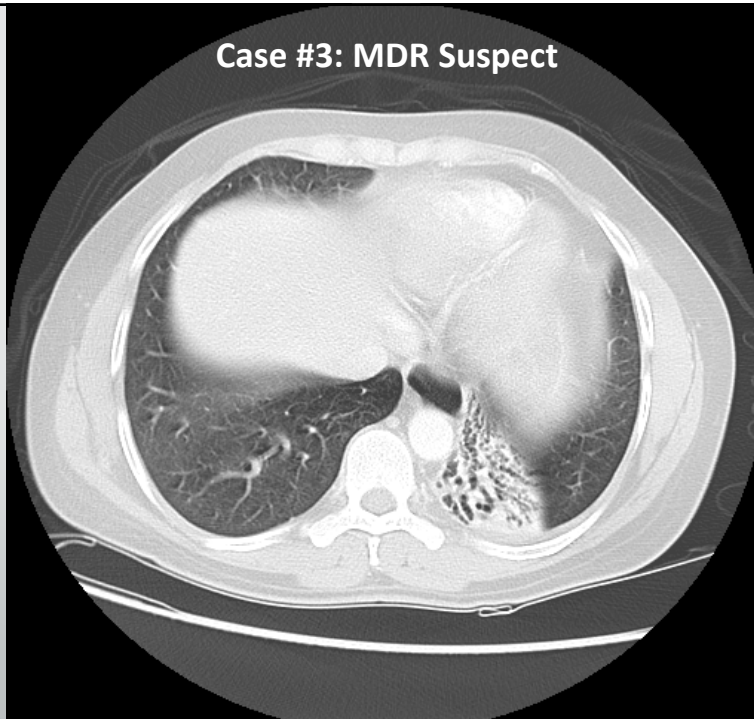
Case #3:  
*Difficult*

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



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Case #3: MDR Suspect



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## “Difficult” Case Timeline

**APRIL 2016**

							1 MDR-TB suspected	2 Sputum & IGRA
3	4	5	6	7	8	9	10	11
12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29
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759	760	761	762	763	764	765	766	767
768	769	770	771	772	773			

## MDHHS Lab Confirmation of 2<sup>nd</sup> Line Drugs

Isoniazid	<b>R</b>
Rifampin	<b>R</b>
Pyrazinamide	<b>R</b>
Ethambutol	<b>R</b>
Ofloxacin	<b>S</b>
Ethionamide	<b>R</b>
Streptomycin	<b>S</b>
Kanamycin	<b>S</b>
Amikacin	<b>S</b>
Capreomycin	<b>S</b>
Cycloserine	<b>S</b>
PAS	<b>S</b>



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## In Conclusion...

- **See** [AFB microscopy]
- **Amplify** [NAAT]
- **Grow** [cultures and susceptibility]
- **Track** [genotyping and WGS]



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