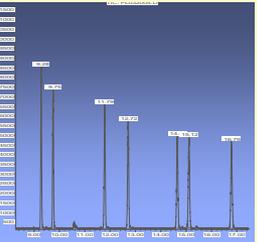
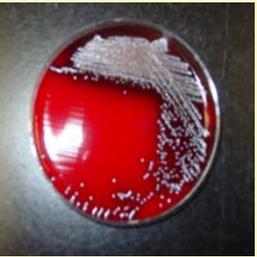




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*Healthy*



# ***TB Laboratory Overview***

Angie Schooley  
Michigan Department of  
Community Health  
Mycobacteriology Unit

# 2011 MDCH TB LAB TECHNOLOGY

- Chemical specimen decontamination
- Fluorescent acid-fast microscopy
- TB Nucleic Acid Amplification Probe Testing (NAAT)
- Rapid broth culture (MGIT)
- HPLC AFB identification
- Genetic probe TB identification
- Rapid broth based susceptibility testing
- Molecular Detection of Drug Resistance (CDC)

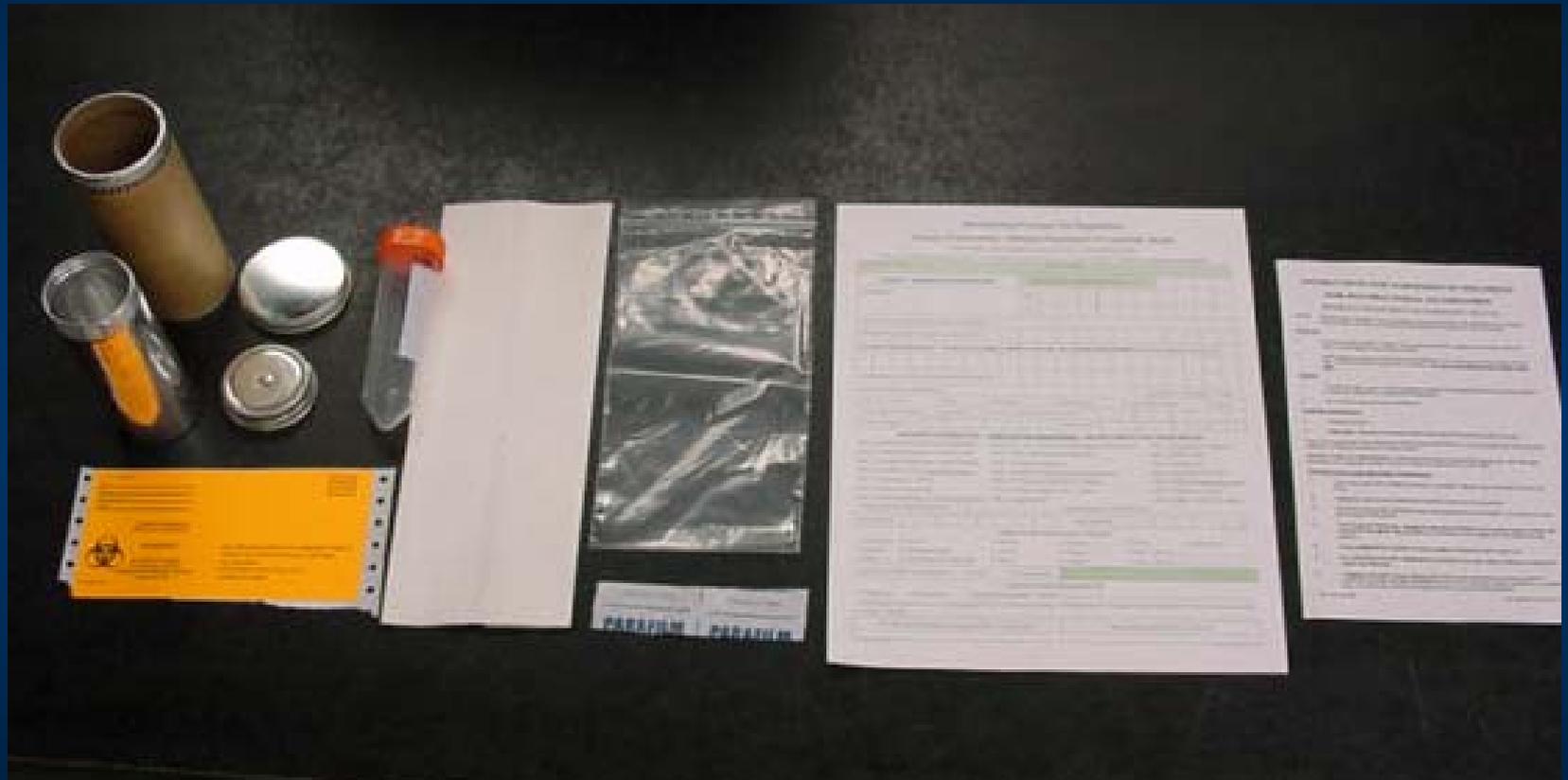


# ***TB SPECIMEN SOURCES***

- Sputum (primary)
- Pulmonary aspiration (secondary)
- Gastric aspiration
- Body fluids
- Tissues
- Blood
- Stool
- Other



# ***MDCH Collection Kit***



# ***Specimen Collection***

- Collect in sterile, leak proof containers
- Refrigeration of specimen is recommended to reduce overgrowth during transit to lab
- Deliver specimen to TB lab within 24 hrs
- Goal: to process specimens ASAP after collection to minimize effects of contaminating bacteria
- Always include patient name on both test request form and the specimen container (CLIA requirement)



# ***Specimen Collection***

- Pulmonary Specimen (usually sputum)
  - Early morning specimens = highest yield of AFB
  - Recommended 3 consecutive specimens in 18-24 hrs (at least 1 early morning specimen)
  - Recommended volume for testing is 5-10 ml, low volume specimens may compromise test results
  - If patient cannot produce sputum, then induce sputum, perform bronchial or gastric washing (less preferred).
  - 24 hour collections (pooled specimens) are not acceptable for testing



# *AFB Tests Performed Directly from specimen*

- AFB Slide Examination: 24 hrs (M-F)
  - The first and most rapid result
  - Determine presence of acid-fast organisms in specimen
  - Assess infectivity of patient, i.e. AFB load
- TB Nucleic Acid Amplification Test (NAAT): 24-48 hrs (M-F)
- Culture Inoculation-incubation time up to 8 weeks

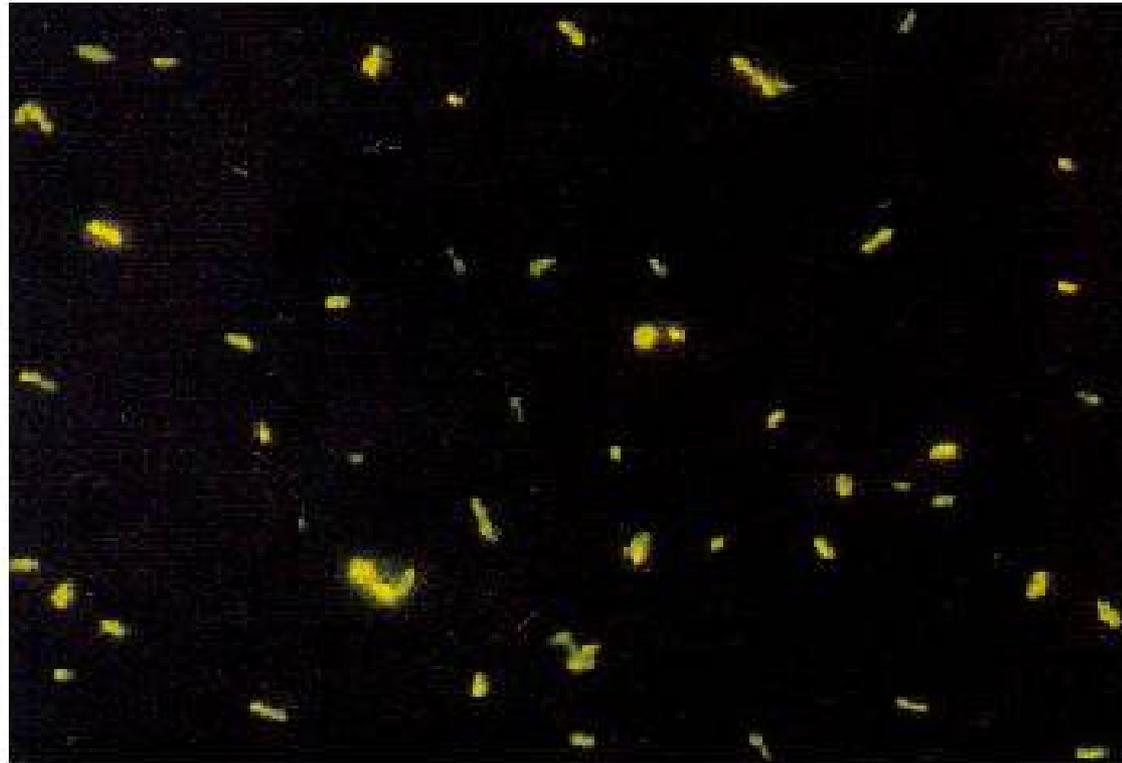


# ***AFB Slide Test***

- Least sensitive of all AFB Tests (20-75%).
- Requires 10,000 AFB/ml for a slide to be positive
- If positive the patient can infect others
- Positive slide cannot determine AFB viability
- Positive slide does not determine whether TB or MOTT
- To be reported within 24 hours of receiving the specimen in the laboratory



# *Fluorescent AFB Using Auramine-O*



Auramine-O staining of AFB under Fluorescence Microscopy

# *Ziehl Neelsen Acid Fast Staining (ZN)*



# ***AFB Smear ZN Interpretation***

## **AFB Seen (1000X)**

0

1-2 / 300 fields

1-9 / 100 fields

1-9 / 10 fields

1-9 / field

>9 / field

## **Report**

No AFB observed

Doubtful; repeat

Rare (1+) AFB

Few (2+) AFB

Moderate (3+) AFB

Numerous (4+) AFB



# ***GEN-PROBE<sup>®</sup> MTD – Direct Amplified Probe for M.tb complex***

- Rapid Test (24-48 hrs)
- Sensitivity – smear neg 72%/smear pos 97%
- Specific – smear neg 99.3%/smear pos 100%
- Non-Bloody Pulmonary Specimens only
- Specimen processing may cause inhibition
- Reduces subsequent test sensitivity
- Delays culture & susceptibility results
- Not a test of viability or effective therapy
- Not available in most laboratories
- Is available at MDCH



# ***CDC Guidelines for Use of MTD***

## ***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.”



# '09 Guidelines (continued)

Interpret NAA test results in correlation with AFB smear results

1. If NAA + and AFB smear +, presume patient has TB
2. If NAA + and AFB smear -, use clinical judgment, retest, i.e. 2 NAA+'s and smear -, presume patient has TB
3. If NAA – and AFB smear +, test for inhibition. If inhibition, then no result. If no inhibition, presume MOTT
4. If NAA- and AFB smear-, then await culture and use clinical judgment. Test not sensitive enough on smear- to exclude the diagnosis of TB

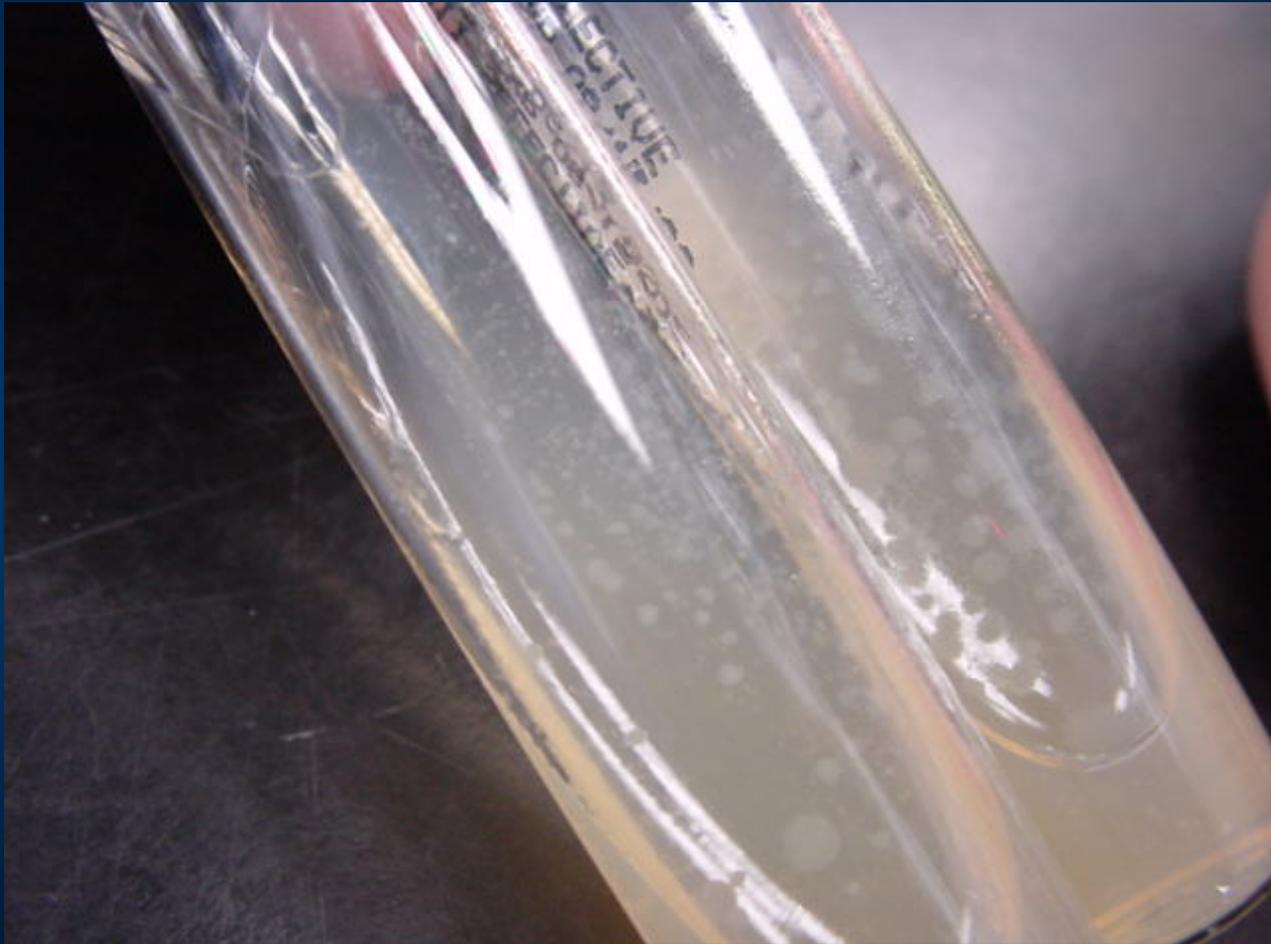


# ***AFB Culture Test***

- More sensitive than the AFB slide test.
- Only 10 AFB/ml of specimen can produce a positive culture result.
- Culture may be AFB positive even though the slide test was reported negative.
- MDCH uses 2 pieces of solid media and a broth system (MGIT).
- Rapid broth testing is normally positive within 1-2 weeks.
- Positive culture result may be either *M. tuberculosis* complex or MOTT
- Requires 6 weeks to report culture as negative
- Need AFB growth used for final identification, susceptibility and genotyping testing



# *M. tuberculosis* on solid media



# *AFB Tests Performed From Culture Growth*

- Genetic Probe ID
- HPLC ID
- Susceptibility
- Biochemical ID Confirmation
- DNA Fingerprinting
- MDDR-CDC



# ***AFB Identification Testing***

- Genetic Probe tests
  - DNA probe tests are species specific
  - M. tuberculosis tests done on Fridays-reported same day
  - Other probes available for M. avium complex, M. kansasii, M. gordonae (only used when HPLC fails and upon special request)
- High performance liquid chromatography (HPLC)
  - Test run on Mon. and Wed.- results reported on Tues. and Thurs.
  - HPLC uses a chromatography method to identify several mycobacterial species
  - This method is normally only available at AFB reference laboratories



# *Susceptibility Testing of* **M. tuberculosis**

- **When to test**
  - All new (initial) *M. tb* isolates
  - Suspected new drug resistance
  - Repeat after 90 days if specimens continue to produce *M.tb*
  - Relapse or failed therapy



# ***TB Susceptibility Testing***

- **Methods**

- “Rapid Broth” method for primary anti TB drugs ( results in 5-14 days)
- “Proportion Plate” method – “Gold Standard” for 1<sup>st</sup>, 2<sup>nd</sup> line drugs (final read @ 3 weeks)
- “Molecular PCR” assays (not FDA approved)
- Molecular Detection of Drug Resistance (MDDR)-CDC



# *Primary Anti-TB drugs*

- Isoniazid
- Rifampin
- Ethambutol
- Pyrazinamide



# *Bactec Susceptibility*

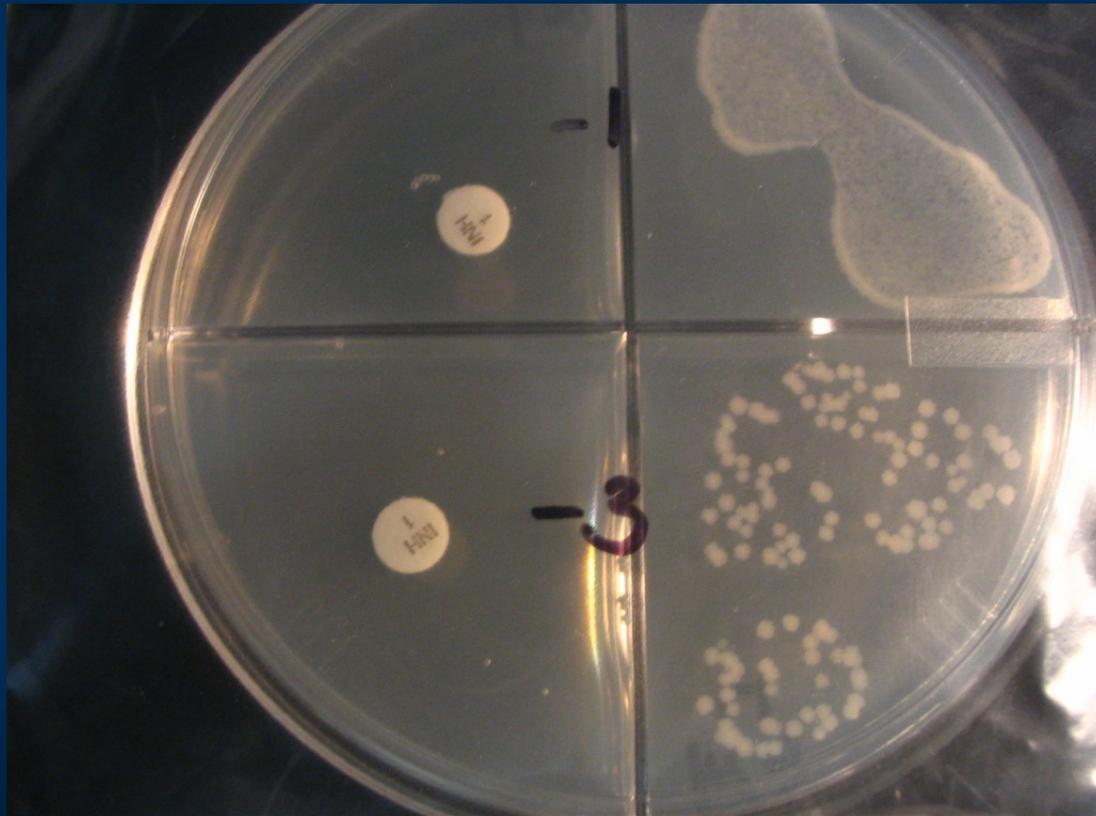


# ***Secondary Anti-TB Drugs***

- Fluoroquinolone (Cipro/Oflox,etc.)
- Kanamycin
- Ethionamide
- Cycloserine
- Capreomycin
- Amikacin
- PAS
- Streptomycin



# *Secondary Susceptibility*



# ***CDC – Molecular Detection of TB Drug Resistance (MDDR)***

- Testing for DNA sequences associated with 1<sup>st</sup> and 2<sup>nd</sup> line drug resistance
- ISOLATES Only
- 3-4 day TAT
- Must meet the following criteria:
  - Known Rifampin resistance
  - 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)
  - Others may be accepted on a case by case basis



# ***TB DNA Genotyping Universally Offered by CDC***

- CDC
- Michigan Department of Community Health – Eastern States
- California Department of Public Health – Western States



# *Genetic Typing*

- MIRU & Spoligo typing performed on all new culture-confirmed isolates
  - Isolates w/ same patterns for both tests are considered a clustered
  - Cluster may indicate transmission – need to interpret in conjunction with epidemiologic or contact investigation data
- RFLP typing may be performed in certain circumstances to differentiate clusters



# ***IGRA-Interferon Gamma Release Assay***

## ***Blood test for M. tuberculosis***

### ***Two FDA Approved Assays***

- Cellestis-QuantiFERON – TB Gold
- 
- Oxford Immunotec – T-SPOT



# IGRA

- Advantages
  - No boosting
  - One patient visit
  - No reader variability
  - Eliminates false positive due to BCG vaccination
  - Latent TB
- Disadvantages
  - Availability
  - Requires lab skills and instrumentation
  - Labs tendency to batch test
  - Cost
  - Need more studies on children, elderly, and HIV populations



# ***A Week in the life of Mycobacteriology***

- Process specimens M-F
- Read AFB smears M-F, results same day as specimen received in lab
- Amplified Probe done on slide positive specimens from MDCH and outside submitters M-F
- Clinical specimens inoculated M-F, broth cultures are continuously read on machine M-F, slanted media read once per week
- Identification of positive cultures is done by HPLC on Mon. and Wed., reported on Tues. and Thurs.
- Genetic Probes done on Fridays, reported same day
- Susceptibility tests set up on Fridays, results 5-14 days later. If resistance, secondary susceptibility results in 3 weeks.
- Genotyping M-F, results in about 14 days from positive culture



