Nucleic Acid Amplification Testing (NAAT)

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MDHHS

Michigan Department of Health & Human Services

Disclosure

- I have no relevant financial relationships to disclose.

Nucleic Acid Amplification Testing(NAAT) for *Mycobacterium tuberculosis* complex

MDHHS Michigan Department of Health & Human Services
Bureau of Laboratories

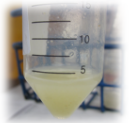
Guidelines for the use of NAAT for *M. tuberculosis* diagnosis

CDC recommends that NAAT testing be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary tuberculosis (TB) when:

- A diagnosis of TB is being considered but has not yet been established
- The test result would alter case management or TB prevention activities, like contact investigations

NAAT platform utilized by MDHHS: Real-time PCR (non-FDA approved)

- Detects *M. tuberculosis* complex (MTBC) and *M. avium* complex (MAC)



What can MDHHS Test?

Clinical Specimens:

- **Direct specimens** processed at MDHHS or **sediments** processed by hospital labs are acceptable
- 24-48 hour turn-around- time (TAT) from specimen receipt
- All sources, **except stool**, are acceptable for testing
- Specimens slide positive for acid-fast bacilli (AFB)
- Specimens slide negative for AFB on patients with high suspicion of MTBC

Culture Isolates:

- Broth cultures positive for AFB from any source
- Culture with low numbers of AFB
- Cultures mixed with non-mycobacterial organisms
- Rule in/out MTBC within 48 hours of culture receipt if other identification tests are not available

Remember: NAAT tests for DNA - therefore a 'DETECTED' result does NOT guarantee viability of organism

NAAT should NOT be used on the following patients:

- Patients culture confirmed TB disease within the last 12 months
- Patients currently receiving TB treatment

Nucleic Acid Amplification Testing(NAAT) Reporting

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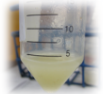

Clinical Specimen Report:

Real Time PCR

M. tuberculosis complex DNA – DETECTED/Not Detected/Indeterminant
M. avium complex DNA – DETECTED/ Not Detected

1. This test was developed and its performance characteristics determined by the Michigan Department of Health and Human Services. It has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary if the performance characteristics are verified at the testing laboratory.
2. All NAAT results should be confirmed by culture. A negative result does not rule out the possibility of isolating M. tuberculosis complex and/or M. avium complex from this specimen.
3. MYCOBACTERIUM CULTURE IN PROGRESS
   or
   SPECIMEN RECEIVED AS PROCESSED SEDIMENT
   SLIDE AND CULTURE EXAMINATION NOT PERFORMED

Cultural Isolate Report:

AFB Identification

PRELIMINARY REAL TIME PCR REPORT: M. tuberculosis complex DNA - DETECTED/Not Detected/Indeterminant
PRELIMINARY REAL TIME PCR REPORT: M. avium complex DNA - DETECTED/Not Detected

1. This test was developed and its performance characteristics determined by the Michigan Department of Health and Human Services. (see above example)
2. ADDITIONAL REPORT WILL FOLLOW

Any MTBC DNA DETECTED result from clinical specimen or culture isolate will have same day verbal notification

Importance of mycobacterial culture:

- Cultures should be performed even when NAAT is performed
- Culture remains the gold standard for diagnosis, is needed for **phenotypic susceptibility testing** and whole genome sequencing (WGS)
- A negative NAAT result does not exclude the possibility of MTBC growth in culture
- Negative NAAT results with a positive AFB smear is a good indicator of the presence of a nontuberculosis mycobacteria

NOTE: All laboratory results should be interpreted based on the clinical situation. A single negative NAAT test should not be used as a definitive result to exclude TB, especially when clinical signs and symptoms are suggestive of TB. Consultation with a TB expert should be considered if the clinician is not experienced in the interpretation of NAAT tests or the diagnosis and treatment of TB.

References:

- Centers for Disease Control and Prevention. Updated guideline for the use of nucleic acid amplification tests in the diagnosis of tuberculosis. MMWR Morb Mortal Wkly Rep.2009 Jan 16:58(1):7-10. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5801a3.htm
- Virginia Department of Health. DCLS Nucleic Acid Amplification Testing (NAAT) for M. tuberculosis. https://www.vdh.virginia.gov/content/uploads/sites/175/2022/04/DCLS-NAAT-MTBC-Flier.pdf

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- If you know PCR is needed for a patient, **prior** notification is needed
  - Phone call to lab
  - Written request on test requisition – far right column has AFB Nucleic Acid Amplification

NAAT platform utilized by MDHHS: Real-time PCR (non-FDA approved)

- Detects *M. tuberculosis* complex (MTBC) and *M. avium* complex (MAC)

- All specimen is used for processing of clinical specimens
- PCR requests must be made prior to specimen processing
- Only one specimen/patient will have NAAT performed:
  - Quality of specimen
  - Collection time

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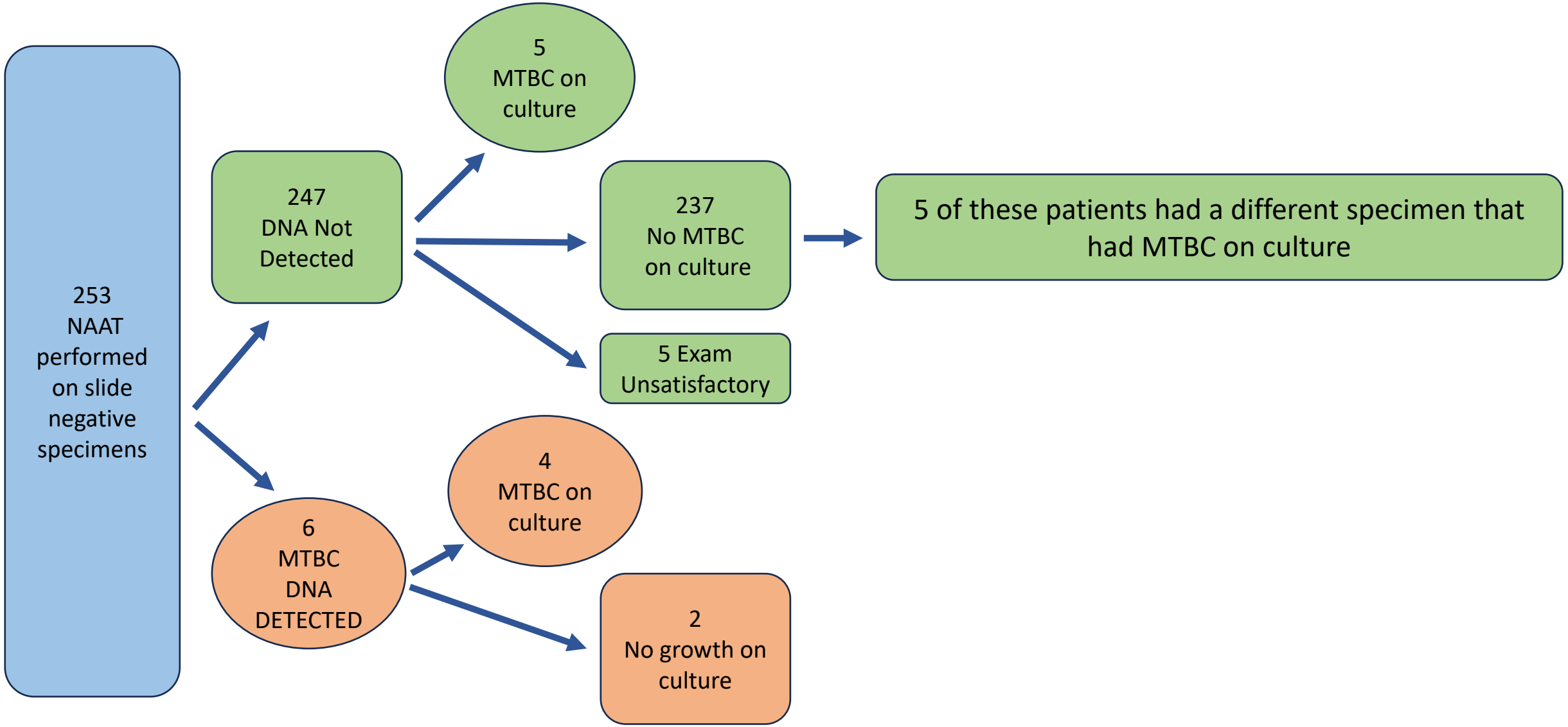
- Phenotypic susceptibility always done on first isolate from a patient
  - Begin test as soon as possible once culture is available
- Repeat testing at 12 weeks is still culture positive

NAAT on Slide Negative Specimens Processed at MDHHS CY 2022-2023



Molecular Detection of Drug Resistance (MDDR) Testing

Rifampin (RIF)	Result	Interpretation
RIF interpretation		RIF resistant
rpoB*	Ser450Leu	

Comments and Disclaimers

* DTBE Reference Laboratory has transitioned from the E. coli to the M. tuberculosis numbering system for reporting rpoB gene mutations.

Isoniazid (INH)	Result	Interpretation
INH interpretation		INH resistant
inhA	No mutation	
fabG1	No mutation	
katG	Ser315Thr	

Ethambutol (EMB)	Result	Interpretation
EMB interpretation		Likely EMB resistant
embB	Met306Ile	

Pyrazinamide (PZA)	Result	Interpretation
PZA interpretation		Cannot rule out PZA resistance.
pncA	No mutation	

Fluoroquinolones (FQ)	Result	Interpretation
FQ interpretation		Cannot rule out FQ resistance.
gyrA	No mutation	
gyrB	No mutation	

- Prediction of potential drug resistance by testing certain loci for mutations
  - These loci are known to have roles in drug susceptibility
- Centers for Disease Control and Prevention performs by request only
  - Approval, sample send out, and reports are through MDHHS only
  - New targeted next generation sequencing (tNGS) platform has caused delays in testing
- Performance on clinical specimens is problematic
  - Need enough DNA for sequencing

MDHHS is currently validating an in-house tNGS

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Any questions, please call the TB Lab

517-335-9636 or 517-335-9367