Development of Workflow Efficiencies for *Mycobacterium tuberculosis* complex Processing and Testing Algorithms

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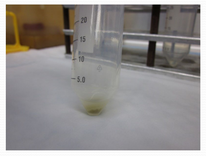
Introduction

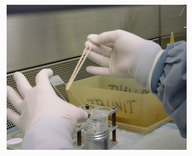
A Mycobacteriology laboratory is always looking for ways to improve efficiency when it comes to specimen processing, nucleic acid amplification (NAAT), acid-fast bacilli (AFB) isolation and identification, and susceptibility testing. The purpose of this poster is to share *Mycobacterium tuberculosis* complex (MTBC) algorithms and process improvements within the Michigan Department of Health and Human Services (MDHHS) laboratory. MDHHS has implemented techniques over the years that have mainstreamed our TB processing, maintained turnaround time (TAT) for identification using MALDI-TOF, and created various job aides to monitor cross contamination and TAT for TB specimen receipt.

TB specimen processing

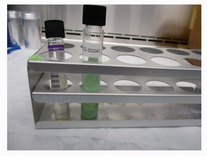
In 2009, MDHHS was involved in an evaluation regarding the length of incubation time to negative of TB cultures in commercial broth systems. During the study, it was brought to our attention that MDHHS had a slightly higher TAT to positive on instrument when compared to other sites, especially on slide positives that also had MTD testing. This was due to the fact that media was not inoculated until after slides were read and 0.5 ml was removed for MTD before media inoculation. MDHHS continues to make the smear prior to diluting for media inoculation, however, based on the results of the study, the MGIT tube is inoculated prior to removing specimen for NAAT. Solid media is inoculated last.



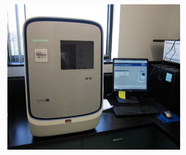
After digestion and decontamination



AFB slide inoculation from pellet



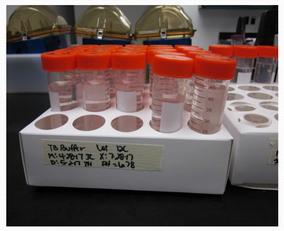
MGIT and solid media



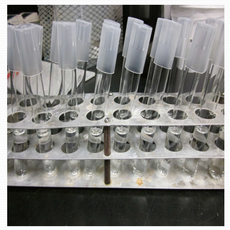
NAAT for MTBC and MAC

Tips for cross contamination prevention

MDHHS makes all reagents involved in TB processing in house. By using single dose volumes for buffer and digestant, we have not had any issues with cross contamination during processing. Buffer is made in batches and stored at 2-8º for 3 months. Digestant is made daily based on the number of specimens received; 7 ml is aliquoted in tubes for digestion and decontamination. Cross-contamination investigation paperwork (Figure 1) is completed when the daily batch is resulted after 6 weeks incubation.



TB Buffer, 50 ml
Single use aliquoted Buffer



Single use aliquoted digestant

Figure 1 Cross-Contamination Worksheet

Cross Contamination Investigation:

***M. tb* TMC 102 control strain: WG MLST MTBC000005**

New Isolate Specimen Numbers:

Any unresolved issues: Yes / No
Explanation:

Date completed / Initials:

Figure 2 Turn Around Time Monitoring Worksheet

Quality Assurance - Daily Delivery Time Date/Initials

Specimen Number	Date Collected	Date Received	Delivery Time	Delayed Submissio n >3days	Submitter	Phone Call Necessary Y/N

TAT improvement

MDHHS tracks the TAT for specimen receipt (Figure 2). The laboratory call submitters when there are delays greater than 3 days. Most of the time delays are due to specimen containers sent home with the patient, collecting 3 in a row, and sending them all at once.

Our average TAT between 2016-2020 was 42% within 24 hours. This is an improvement from the previous 5 years (2011-2015) when the average was 26.5%. MDHHS encouraged submitters to get specimens to a central location when there were couriers available to pick up other testing such as newborn screening.

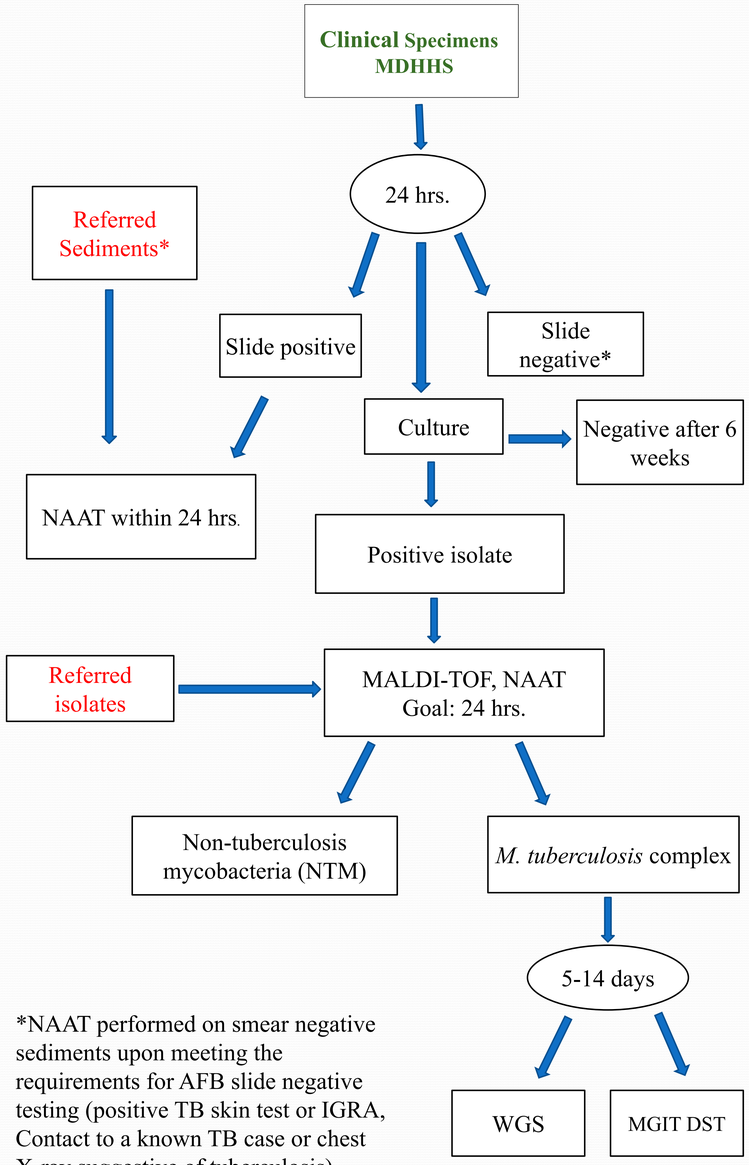
Improved TAT for MALDI-TOF Identification

Table 1 Average Turn Around Time for MALDI-TOF Identification

Calendar Year 2022	
***Mycobacterium* Species**	**# Days**
M. avium	3
M. chelonae	1
M. chimaera-intracellulare group	3
M. fortuitum	1
M. gordonae /paragordonae	3
M. kansasii	1
M. tuberculosis cpx	3
M. xenopi	2

MDHHS receives a variety of broth cultures (MGIT, BacT Alert, etc.) for identification from hospitals throughout the state. 7H9 broth produced in house is used as the starting point for all MALDI-TOF (Bruker platform) identifications. **The average TAT in Table 1 is based on the day it was received in the laboratory, inoculated into 5 ml of 7H9 broth, and identified on MALDI-TOF using the RUO library.** When the turbidity of the broth is approximately a 1 McFarland, 1ml is removed and centrifuged for 5 minutes at 13,200 RPM. The supernatant is discarded, 300 µl HPLC grade water is added, proceed with Bruker MycoEx procedure. The TAT for MTBC is based on patients with multiple cultures, NAAT is performed on all new suspected patients.

MDHHS Workflow



*NAAT performed on smear negative sediments upon meeting the requirements for AFB slide negative testing (positive TB skin test or IGRA, Contact to a known TB case or chest X-ray suggestive of tuberculosis)

Acknowledgments-MDHHS receives support thorough the National TB Elimination Cooperative Agreement