

# Prediction of Mutations Associated with Drug Resistance for *Mycobacterium tuberculosis* complex using Next Generation Targeted Sequencing

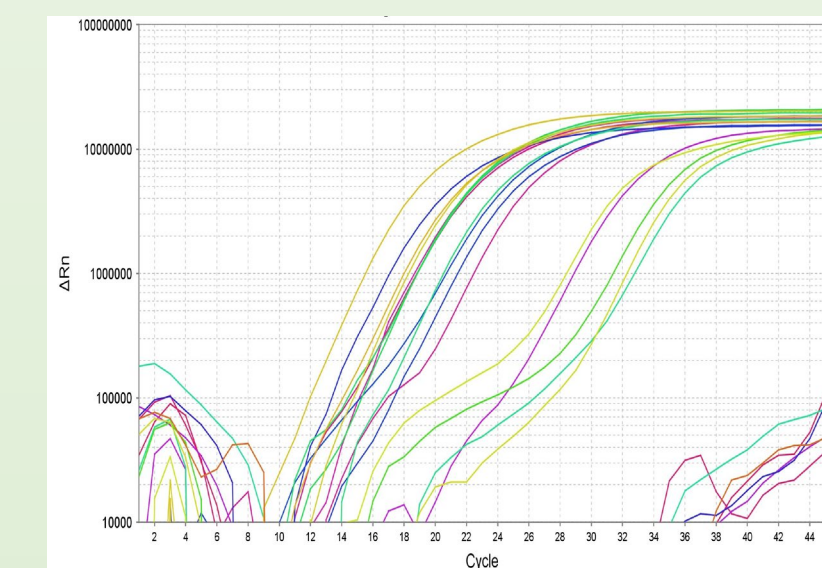
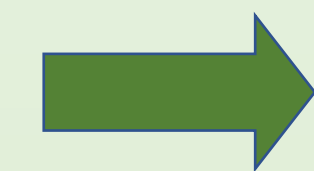
J. Vanneste, R. Kramer, H. Blankenship, F. Nindo, A. Schooley, K. McCullor  
Michigan Department of Health and Human Services, Bureau of Laboratories

**Goal:** Prediction of drug resistance utilizing next generation targeted sequencing (NGS) from clinical specimens to assist clinicians with appropriate drug regimen.

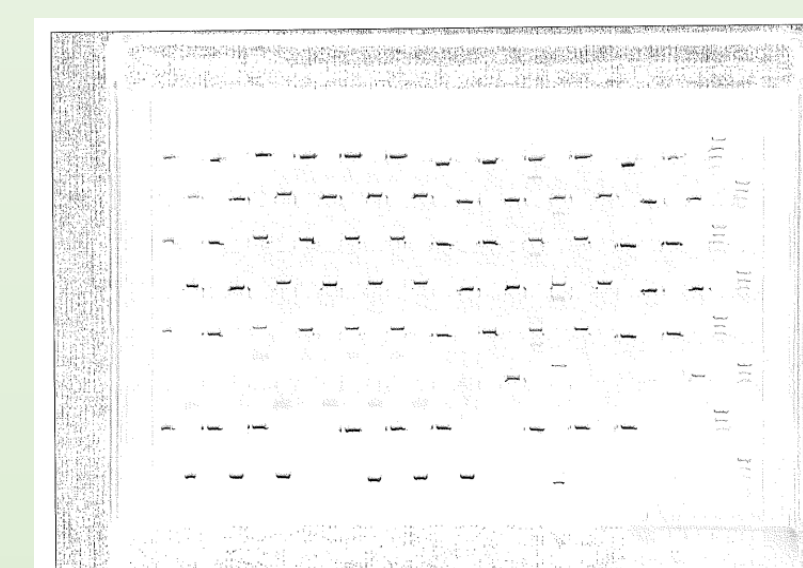
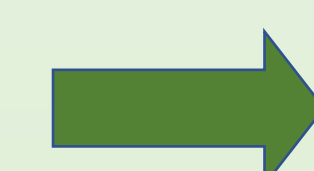
**Projected Algorithm**



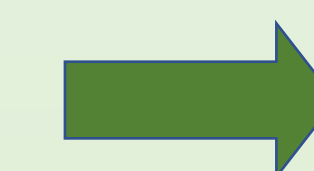
AFB Slide Positive Clinical Specimen



Real-time PCR: MTBC DNA Detected



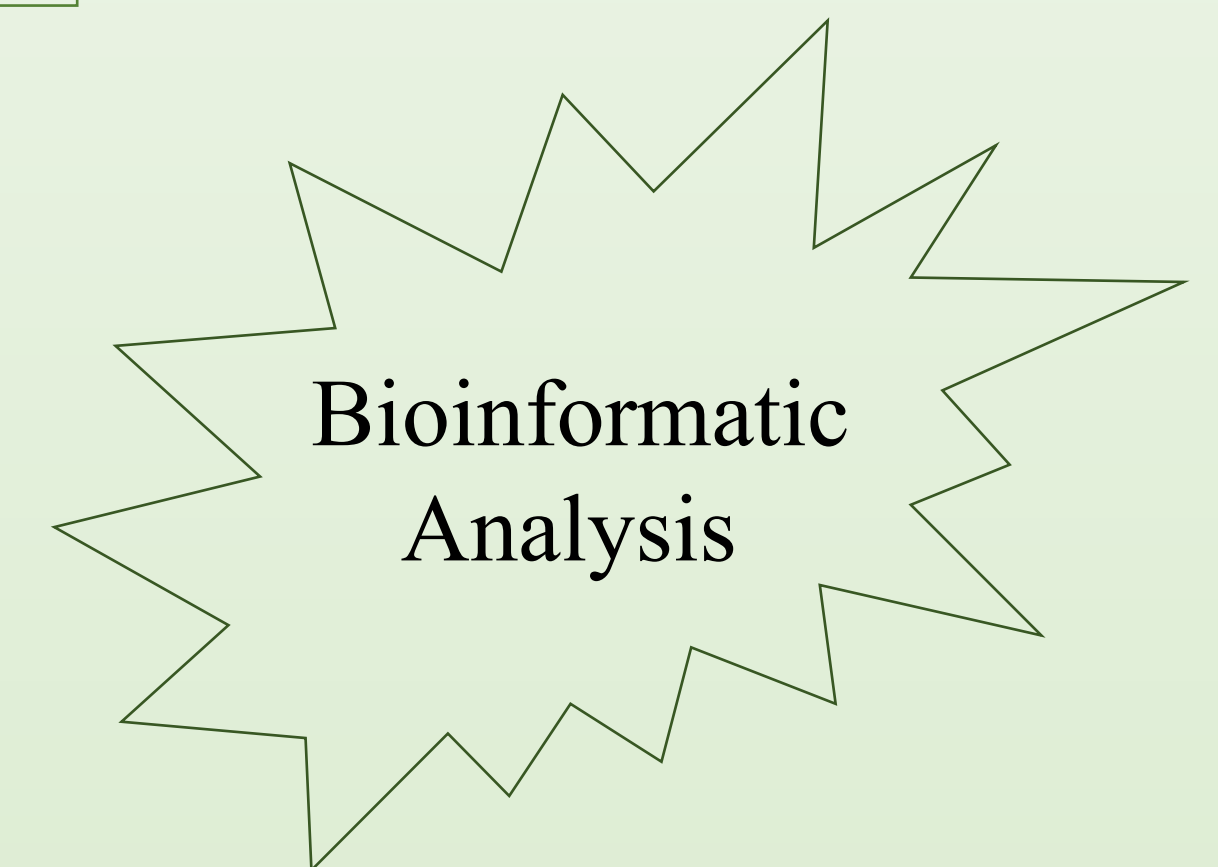
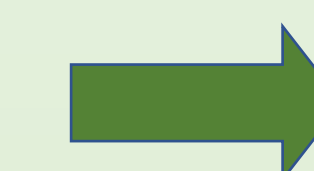
Loci PCR performed for 15 different Loci



Library prep on pooled Loci PCR amplicon



Sequencing on iSeq 100



Loci PCR is performed to produce amplicon of 15 different loci in the MTBC genome from the same extract used for real-time PCR. Each locus is known to be involved in drug resistance. The following loci and the drugs they are associated with are under validation at MDHHS: *rpoB170* & *rpoB* rifampin resistance determining region (rifampin), *katG1*, *katG2*, *katG3*, *katG4*, *inhA-fabG1* promoter, *aphC* promoter & *fabG1* (isoniazid), *embB* (ethambutol), *pncA* promoter (pyrazinamide), quinolone resistance determining region *gyrA* & *gyrB* (fluoroquinolones), and *rrs* & *eis* promoter (second-line injectables).

**PHASE 1:** Twenty isolates of MTBC were grown in 7H9 broth and tested using the above algorithm starting at the real-time PCR step. Susceptibility results from Molecular Detection of Drug Resistance (MDDR) testing by the Centers for Disease Control (CDC) and Prevention, MGIT susceptibility, and/or agar proportion susceptibility are used as the growth-based standards for comparison. Non-tuberculosis mycobacteria and other common oral flora were included to assess assay specificity.

Agreement Between Growth Based Testing/MDDR and Targeted Sequencing	
Drug	% Agreement
Rifampin	100
Isoniazid	100
Ethambutol	90*
Pyrazinamide	90**
Fluoroquinolones	95***
Second Line Injectables	100

\*Ethambutol: Two isolates produced mutations that are known to cause resistance and were susceptible by growth-based testing.

\*\*Pyrazinamide: Two isolates did not detect any mutations, however, were resistant with growth-based testing.

\*\*\*Fluoroquinolones: One isolate did not have any mutations associated with resistance, however, was resistant by agar proportion testing.

Note: Fluoroquinolone and Second Line Injectable agreement was calculated with 19 isolates. One isolate did not have growth-based testing for comparison.

Resistance Comparison between Growth Based and NGS Testing		
Drug	# Isolates with Resistance by Growth-Based Test	# Isolates with Mutations Present at Loci Known to Cause Resistance <sup>+</sup>
Rifampin	6	6
Isoniazid	7	7
Ethambutol	4	6
Pyrazinamide	5	6
Fluoroquinolones	1	2
Second Line Injectables	1	1

<sup>+</sup>The 'Catalogue of mutations in *Mycobacterium tuberculosis* complex and their association with drug resistance' by the World Health Organization was used for mutation analysis.

**PHASE 2:** Twenty sputum aliquots will be seeded with the 20 isolates used in Phase 1 and decontaminated/digested as sputum specimens. An aliquot of the sediment will be tested with the proposed algorithm. A subset will include low, medium, and high MTBC seeding concentrations to assess assay sensitivity. Reproducibility will also be assessed.

**PHASE 3:** Parallel testing with clinical specimens to begin after completion of Phase 2.

