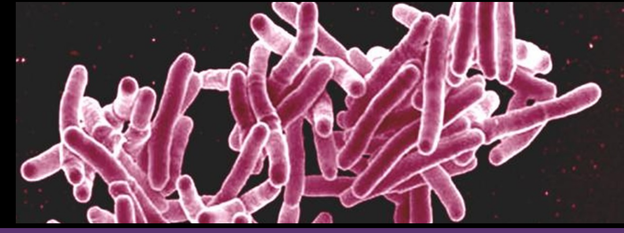
Detecting antimicrobial-resistant *Mycobacterium tuberculosis* directly from respiratory specimens using targeted Next Generation Sequencing

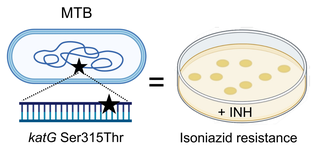


Shannon G. Murphy | Carol Smith | Tanya A. Halse | Vincent Escuyer | Marie-Claire Rowlinson | Pascal Lapierre | Kimberlee Musser

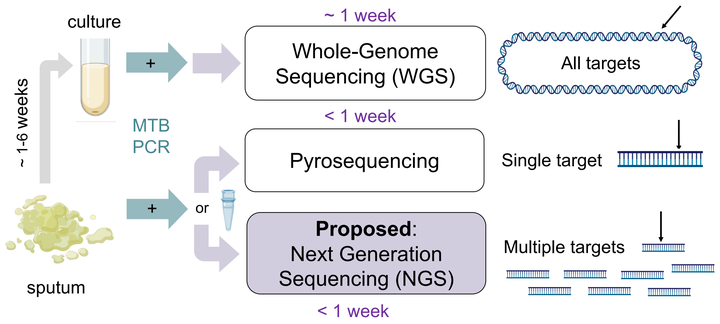
NEW YORK STATE | Department of Health | Wadsworth Center

M. tuberculosis Drug Susceptibility Testing

- *Mycobacterium tuberculosis* (MTB) infections are treated with combination antimicrobial therapies. Multi and extensively-drug resistant infections require altered antimicrobial regimens; inappropriate therapy can result in treatment failure and promote further resistance
- Phenotypic drug susceptibility testing requires many weeks to months to complete due to MTB's slow growth rate
- Sequencing-based assays predict antimicrobial resistance (AR) faster than phenotypic methods



Sequencing-based drug susceptibility testing in NYS



Objective: Design and validate a multiplexed, **targeted-Next Generation Sequencing (tNGS) assay** that can predict MTB AR directly from respiratory specimens

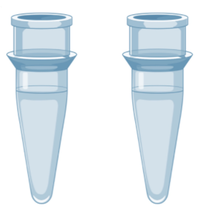
Targeted NGS (tNGS) Assay Design

- Primers designed to amplify 13 full-length genes or loci with mutations known to confer resistance to first-line[1] or second-line[2] antimicrobials
- PCRs optimized against a heat-killed MTB isolate (H37Rv)

Antibiotic	Loci
Isoniazid[1]	*katG*, mabA-inhA*, oxyR-ahpC*
Rifampin[1]	*rpoB**
Pyrazinamide[1]	*pncA*
Ethambutol[1]	*embB*
Fluoroquinolones[2]	*gyrA, gyrB*
Streptomycin[2]	*rrs, rpsL*
Kanamycin[2]	*eis, rrs*
Amikacin[2], Capreomycin[2]	*rrs*

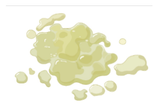
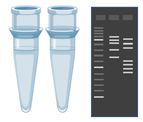
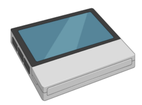
* Current pyrosequencing targets

- 13 targets amplified within two multiplexed PCR reactions
- Amplicon size: 900 – 3,700 bp



tNGS workflow in the NYS public health lab

- **Library Preparation Time**: ~ 2 days
- **Sequencing time**: 20 - 120 min, depending on run size
- **Reagent Cost**: ~$52/sample (extraction, amplification, and sequencing)
- **Flow Cell Cost**: variable, ~$25/sample

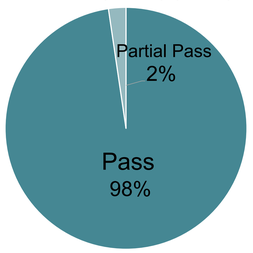
Extraction	Multiplex PCR	Library Prep	NGS
			
Lysis and purification (EZ1, Qiagen)	Two multiplexed PCR pools	End Repair, Barcoding, Adapter ligation	Oxford Nanopore long-read sequencing, In-house pipeline

tNGS accurately predicts drug susceptibility profiles

Blinded Retrospective Studies

- MTB+ isolates or respiratory specimens tested with tNGS
- Mutations predicting AR or susceptibility were compared to those identified via WGS of cultured isolates

Cultures (MGIT) (n=43)

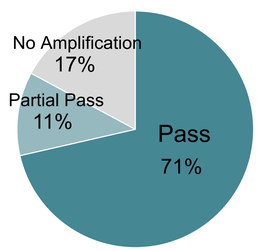


tNGS on MTB cultures (MGIT)
98% isolates pass QC on all targets
100% concordance with WGS profiles

13 of the 43 samples were positive for both MTB and *Mycobacterium avium* complex (MAC) via real-time PCR

tNGS works well on MTB cultures, even in the presence of MAC

Specimens (n=35)



tNGS on primary, respiratory specimens
82% of samples amplified
71% samples passed QC for all targets
11% samples passed QC for most targets

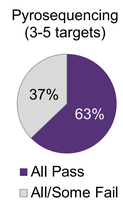
100% concordance with WGS profiles

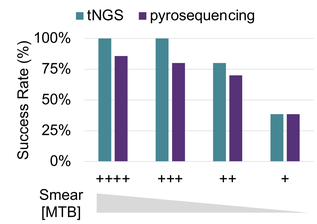
tNGS is accurate and can yield sufficient data from the majority of specimens tested

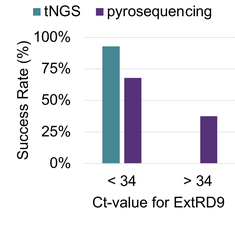
Sensitivity, specificity, reproducibility testing performed (data not shown)

Comparison with other direct methods

- tNGS is multiplexed and covers more loci (13 targets, 9 drugs) than pyrosequencing (3-5 targets, RIF/INH/FQ only)







- Success rate (all targets passing QC on first attempt) is greater for tNGS (71%) than for pyrosequencing (63%)
- tNGS works well on smear-positive samples with Ct-value < 34, pyrosequencing failure is more sporadic (possibly due to PCR inhibition, different extraction methods)

Conclusions

Targeted Next Generation Sequencing (tNGS)

- Is a viable way to predict MTB AR directly from respiratory specimens
- Correctly identifies high confidence mutations within 13 targets and predicts susceptibility for 9 antimicrobials (both first- and second-line)
- Generates AR predictions faster than culture-based WGS assays and has a higher success rate then other direct methods (pyrosequencing)

	WGS	Pyrosequencing	tNGS
Sample	MTB-pos Culture	Decontaminated, heat treated MTB-pos Respiratory specimen	Decontaminated, heat treated MTB-pos Respiratory specimen
Information	Genome wide or Entire genome	Single-plex (5 targets, 3 drugs)	Multi-plex (13 targets, 9 drugs)
Time to results from sample receipt	Culture: 1-6 weeks ~ 1 week	< 1 week	< 1 week

Next Steps

- Continue prospective sequencing of incoming MTB-positive specimens
- Submit tNGS for approval as a clinical diagnostic assay for initial AR predictions ahead of culture-based WGS results
- Implement an additional PCR pool targeting loci involved in resistance to linezolid, pretomanid, and bedaquiline/clofazimine

Works Cited

Shea J, Halse TA, Lapierre P, et al. 2017. Comprehensive Whole-Genome Sequencing and Reporting of Drug Resistance Profiles on Clinical Cases of *Mycobacterium tuberculosis* in New York State. J Clin Microbiol 55(6).

Halse TA, Edward J, Cunningham PL, et al. 2010. Combined real-time PCR and rpoB gene pyrosequencing for rapid identification of *Mycobacterium tuberculosis* and determination of rifampin resistance directly in clinical specimens. J Clin Microbiol. 2010;48(4):1182-1188. doi:10.1128/JCM.02149-09

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