Washington State Department of Health

TB SEQUENCING FOR ANTIBIOTIC RESISTANCE GENES: SAILING NEW WATERS

Washington State Public Health Laboratories

Whole Genome Sequencing (WGS) Requests

Laboratory Branch / Division of TB Elimination/ CDC

Section 1. Laboratory Contact Information

Section 2. TB Program Contact Information

Section 3. Type of specimen

Section 4. Submission Criteria (check all that apply)

Pros:

1. **Faster turnaround time (TAT)?**
2. **New WGS workflow**
3. **Potential in a few years to include genotyping reports**

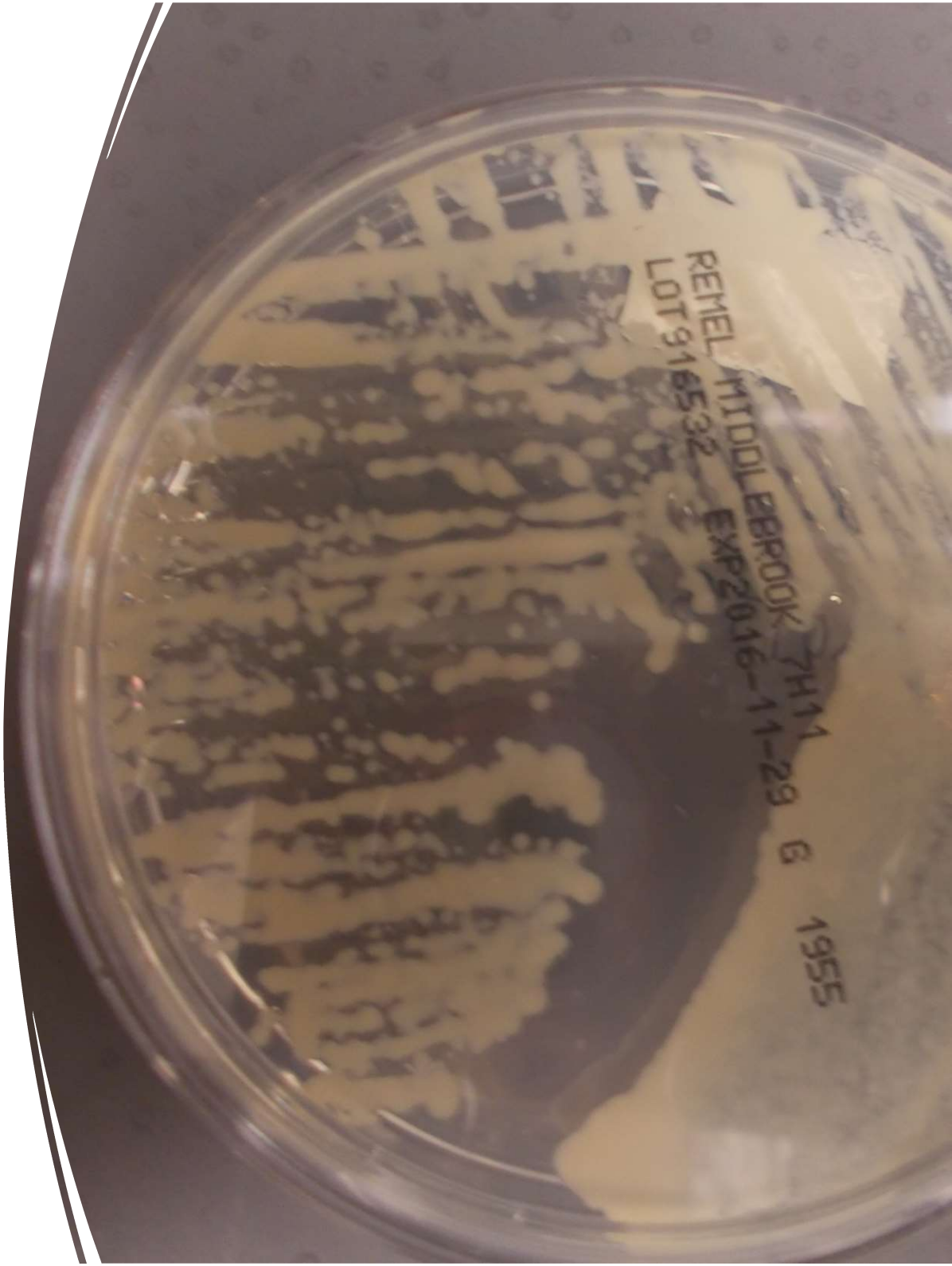
Cons:

1. **Increased cost**

+/- : CAP Regulations for WGS and Diagnostic Results

Validation: Sample types

1. **Culture isolates:** purified and grown in liquid broth
   - ➢Long TAT
   - ➢Pure WGS reads



Validation: Sample types

1. Culture isolates: purified and grown in liquid broth
   - ➢ Long TAT
   - ➢ Pure WGS reads
2. **MGIT primary culture**
   - ➢ Intermediate TAT
   - ➢ Potential contaminations

Validation: Sample types

1. Culture isolates: purified and grown in liquid broth
   - Long TAT
   - Pure WGS reads
2. MGIT primary culture
   - Intermediate TAT
   - Potential contaminations
3. **Processed Sputums**
   - Short TAT
   - Metagenomic: multiple genomes present

Past Report

Patient Name: **Wil E Coyote**

Sex: **??** Birthdate: **01/01/0001** Age: **ANCIENT**

Specimen ID: **WA0999999**

CDC Specimen ID: **123456789**

Public Health Submitter:

Material Submitted: *M. tuberculosis complex* isolate
Specimen Source: Sputum
Medium: Middlebrook 7H9 broth

Washington State Department of Health
1610 NE 150th St
Shoreline, WA 98155-9701

Date Collected: **12/12/1212**
Date Received: **01/17/1212**
Date Reported: **01/19/1212**

Results for Molecular Detection of Drug Resistance (Pyrosequencing; INH and RMP only); Conventional Drug Susceptibility Test in progress.

Drug	Locus *	Result	Interpretation
Rifampin	rpoB	No mutation	Probably Rifampin susceptible. (97% of RMP-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at this locus.)
Isoniazid	InhA	No mutation	Isoniazid resistant. (100% of isolates in our in-house evaluation of 550 clinical isolates with this mutation are INH-R.)
	katG	**Mutation:** AGC>ACC, Ser315Thr	
	fabG1	No mutation	

*A negative result (e.g., no mutation) does not rule out contributory mutations present elsewhere in the genome.

NOTE: MDDR testing (Sanger sequencing, complete panel) will not be performed because mutations associated with RMP resistance were not detected. Contact laboratory if this testing is required for clinical reasons.

Report

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Rifampin	rpoB	No mutation	Probably Rifampin susceptible. (97% of RMP-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at this locus.)

Report

Interpretations Summary:

Drug	Variant	Interpretation
INH	katG_p.Arg463Leu	INH-S
RIF	rpoB_p.His445Tyr	RIF-R
PZA	No reportable variant detected	PZA-S
FQ	gyrA_p.Asp94His	FQ-R
EMB	No reportable variant detected	EMB-S

Report

Interpretations Summary:

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DISCLAIMER: This test was adopted, and its performance characteristics determined by the WA PHL. It has not been approved by the FDA. Results are for infection control and epidemiological purposes and should not be used as the sole means for clinical diagnosis or patient management.

WHO Disclaimer: the molecular basis of resistance to these drugs is not fully understood, and therefore phenotypic DST methods are currently recommended to detect clinically relevant resistance

Report

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		Cannot rule out EMB resistance.
embB	No mutation	

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	

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