



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
1600 Clifton Road, Atlanta, GA 30333
Phone: 404-625-3423 FAX: 404-625-3451 TLElab@cdc.gov

Instructions: Please provide the following information and submit the completed form via email to TLElab@cdc.gov or fax at 404-625-3451. An email notification will be provided upon receipt with further instructions.

Action 1. Laboratory Contact Information

Washington Public Health Laboratories, TB Lab

Name of Requester: Submitting Laboratory
ID/Accession Number: 2064185473

Contact Name: Phone Number
Number: 206-433-1333 E-mail Address: j206@waphl.wa.gov

Action 2. TB Program Contact Information

Site/Practice Number: 805-236-3423
Contact Name: Phone Number
Number: 206-3405 E-mail Address: jw@waphl.wa.gov

Action 3. Type of specimen

Isolate; Specify medium: _____
NAAT+ sediment; Specify specimen source: _____

Action 4. Submission Criteria (check all that apply)

Known MDR; Test method: _____
Known RMP resistant; Test method: _____
Contact to known MDR Previously Treated for TB
From a country with a high rate of drug resistant TB; Specify: _____
Travel to / lived in a country with a high rate of drug resistant TB; Specify: _____
Rapid culture Non viable in culture No / poor growth in DST media
Other; Explain: _____

Is a sample from this patient been previously submitted to CDC? Yes No



Pros:

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

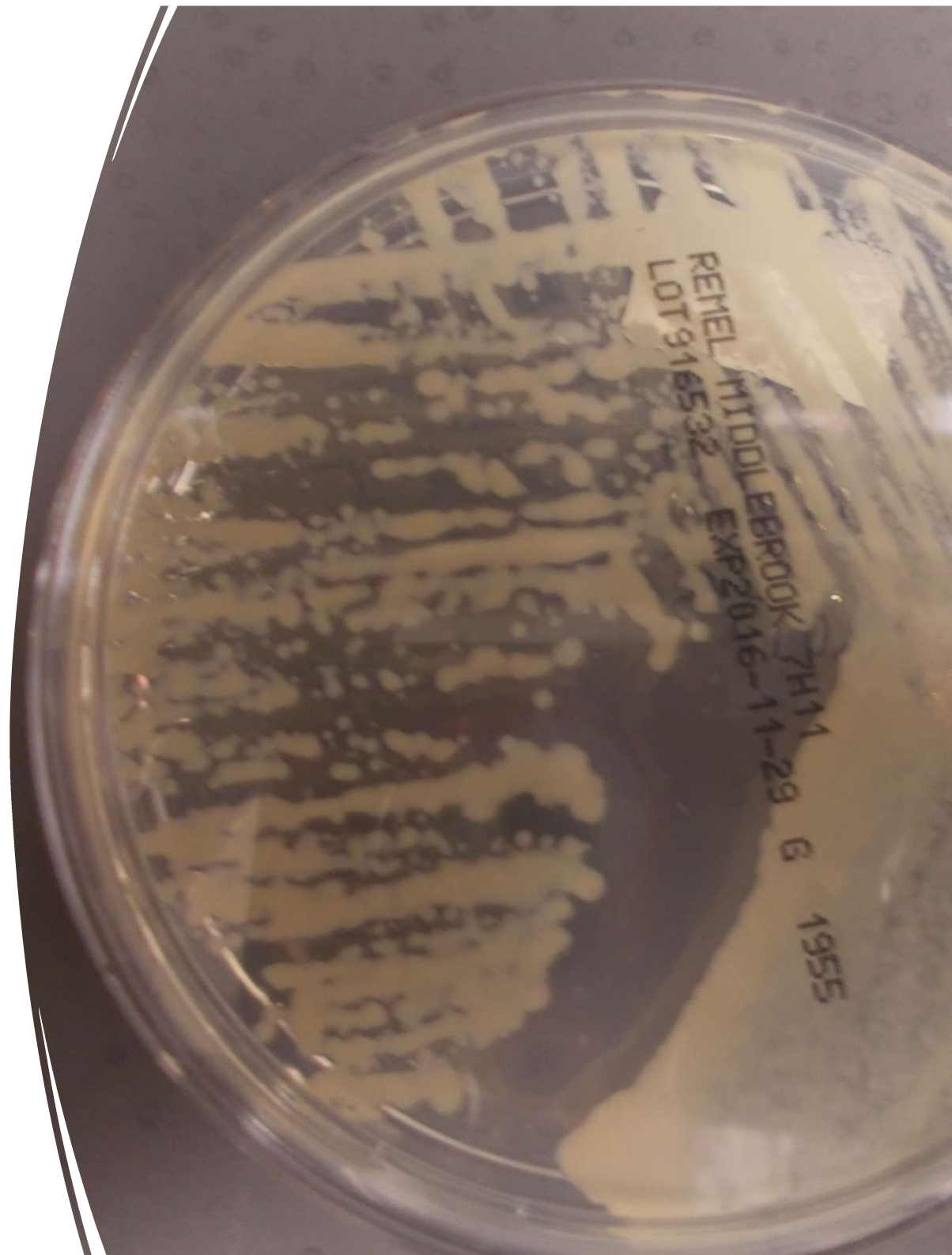
Cons:

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: Will E Coyote		Specimen ID: WAD999999
Sex: ?? Birthdate: 01/01/0001	Age: ANCIENT	
CDC Specimen ID: 123456789		Public Health Submitter:
Material Submitted: <i>M. tuberculosis</i> complex isolate Specimen Source: Sputum Medium: Middlebrook 7H9 broth		Washington State Department of Health 1610 NE 150 th 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	rrpB	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

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.)

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

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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)

RIF interpretation

rpoB*

Result

Ser450Leu

Interpretation

RIF resistant

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)

INH interpretation

inhA

fabG1

katG

Result

No mutation

No mutation

Ser315Thr

Interpretation

INH resistant

Ethambutol (EMB)

EMB interpretation

embB

Result

No mutation

Interpretation

Cannot rule out EMB resistance.

Pyrazinamide (PZA)

PZA interpretation

pncA

Result

No mutation

Interpretation

Cannot rule out PZA resistance.

Fluoroquinolones (FQ)

FQ interpretation

gyrA

gyrB

Result

No mutation

No mutation

Interpretation

Cannot rule out FQ resistance.



Washington State Department of Health is committed to providing customers with forms and publications in appropriate alternate formats. Requests can be made by calling 800-525-0127 or by email at civil.rights@doh.wa.gov. TTY users dial 711.