

## Purpose

In order to assist with the rapid identification of patients infected with drug-resistant strains of tuberculosis, the Washington State Public Health Laboratories (WAPHL) has developed in-house capacity to perform DNA sequencing-based screening for mutations associated with resistance to the most commonly-used, first-line drugs for TB treatment—isoniazid (INH), rifampin (RIF), and pyrazinamide (PZA). This document provides information about this new method and instructions for submitting specimens to WAPHL.

## Background

The Drug Resistant Screening by Sequencing (DRSS) developed at WAPHL combines 4 assays for the detection of common mutations located within specific genetic regions of TB genomic DNA that have been shown by previous studies, both internally and externally, to have a strong correlation with drug resistance. DRSS screens for mutations associated with RIF resistance by analyzing a region of the *rpoB* gene, which includes the RIF Resistance Determining Region where 95 % of all previously-reported mutations associated with RIF resistance are located. Another component of DRSS testing screens for mutations within a region of the *pncA* gene which have been reported in 72-92 % of PZA resistant isolates. Finally, two separate regions are analyzed to screen for mutations reported in 50 –75 % of INH-resistant TB strains. The first is a mutation in codon 315 of *katG*, contained in approximately 50-60 % of INH resistant strains, while mutations in the promoter region of the *inhA* gene may allow for the detection of an additional 10-15 % of resistant strains.

## Performance at WA PHL

In 2010-2011, an internal study of the DRSS method was conducted at the WAPHL in order to verify performance of the method for detection of resistant TB. A total of 173 TB isolates provided by WA State TB Laboratory were tested for RIF and INH resistance in addition to 131 specimens screened for PZA resistance. The results of the DRSS assay were compared to results obtained with gold-standard, culture-based drug susceptibility testing (DST) methods. Shown below are the DRSS performance results obtained during our internal study including data we have gathered since implementing DRSS (29 tests).

DRSS vs. traditional methods					
Drug	Gene	Sensitivity %	Specificity %	PPV %	NPV %
RIF	<i>rpoB</i>	97.6	99.4	97.6	99.4
INH	<i>inhA + katG</i>	78.2	98.4	96.8	87.8
PZA	<i>pncA</i>	79.3	98.5	92.0	95.6

## Important Note

Test results obtained with DRSS are primarily intended for epidemiologic surveillance purposes and should not form the sole basis for treatment decisions or for the assessment of a patient's health status. Given the high specificity of the assay, detection of mutations is highly indicative of resistance. However, the assay will not detect mutations associated with resistance other than those located in the regions of *M. tuberculosis* screened by the method. Since resistance could be due to other mutations not tested by the assay, the absence of mutations does not necessarily indicate a lack of resistance. All results obtained by the DRSS method should be considered preliminary pending final confirmation with traditional culture-based DST methods.



**M. tuberculosis Drug Resistance Screening by Sequencing (DRSS)  
Washington State Public Health Laboratories (WAPHL)  
Phone: 206-418-5473**

<p><b>Acceptable Specimens</b></p>	<p><b>Case by case basis (with prior approval):</b> AFB smear positive respiratory sediments <u>confirmed positive for MTB</u>. However, the inability to obtain sufficient quantities of quality TB genomic DNA template from original specimens will result in a delay of test results**</p> <p align="center">**DNA/nucleic acid quantities appropriate for further testing will be determined by TB NAAT screening assay. Samples will have to meet the minimum value necessary for sequencing established by the Molecular Lab of the WA-PHL. Specimens with insufficient quantities of DNA will require growth in culture prior to DRSS testing.</p> <p><b>Recommended:</b> MTB confirmed cultures</p>
<p><b>To request DRSS</b></p>	<p>Based on your patient’s county of residence, please contact one of the following TB Health Officers to determine if your patient’s scenario meets submission criteria for testing.</p> <p><b>Dr. Masa Narita</b>      206-744-4579 – Primary contact for King County  <b>Dr. Chris Spitters</b>      206-383-0474 – Contact for Snohomish, Yakima, Klickitat, or Island Counties; back-up for Dr. Narita in King County  <b>Dr. Scott Lindquist</b>      206-718-2664 – Contact for all other counties</p> <p><b>Complete the DRSS Approval form and fax it to the WA TB Lab 206-418-5545</b></p>
<p><b>Fee</b></p>	<p>Testing will be performed free of charge for specimens meeting acceptance criteria.</p>
<p><b>Turnaround times</b></p>	<p>Results from culture isolates and respiratory specimens containing sufficient amounts of TB DNA will be available within 3-4 business days of receiving the specimen. Results from respiratory specimens that require additional work up, such as growth in culture, will be delayed.</p>
<p><b>Reporting</b></p>	<p>Results will be faxed to the submitter.</p>
<p><b>Confirmation</b></p>	<p>All specimens screened for drug resistance using the DRSS assay will also undergo traditional culture-based drug susceptibility testing.</p>
<p><b>Additional Molecular Testing for Drug Resistance (MDDR)</b></p>	<p>The Centers for Disease Control and Prevention offers an MDDR screens for an extended array of mutations associated with resistance to INH, RIF, FQ, KAN, AMK, and CAP. To request this test, please contact the State TB Laboratory at 206-418-5473 for current details and instructions.</p>



Drug Resistance Screening by Sequencing Approval Form

Instructions: Please provide the following information and submit the completed form to the consulting TB Health Officer. Fax the completed form to WA PHL TB Lab 206-418-5545. The DRSS test will be performed after the sample is screened by NAAT. The samples must be received on Monday for Tuesday run and by Wednesday for Thursday run. Results will be available within 3-4 business days.

Section 1. Requestor Information

TB Health Officer Approving DRSS: \_\_\_\_\_

Date of the request: \_\_\_\_\_

Patient Name: \_\_\_\_\_

Person requesting DRSS: \_\_\_\_\_

Type of specimen: \_\_\_\_\_

Phone#: \_\_\_\_\_

Specimen Location: \_\_\_\_\_

Fax #: \_\_\_\_\_

Section 2. Submission Criteria

- Patient is at high risk of RIF-R, INH-R or MDR-TB, if any of the following applies:
- Previously treated for TB and the following are suspected/observed (please circle):
 \* relapse
 \* clinical treatment failure
 \* persistently culture-positive after 2-3 month of treatment
 \* reversion to smear and culture positivity
- MDR-TB contact
- From an area with a high rate of drug resistant TB
- Patient is a known Rif-R, INH-R
- High profile patient (e.g. daycare worker, nurse)
- Specimens from patients that do not meet current acceptance criteria may be considered on a case-by-case basis with prior approval from WA State TB Health Officers

Comments: \_\_\_\_\_

For WAPHL Use Only

Comments: \_\_\_\_\_