



PUBLIC HEALTH

**ALWAYS WORKING FOR A SAFER AND
HEALTHIER WASHINGTON**

DRSS

Drug Resistance Screening by Sequencing

WA Public Health Laboratories
Tuberculosis Lab
2012

MAC NAAT is HERE!

- Detection of *M. avium* complex (MAC) DNA in respiratory clinical samples has been completely validated and is offered as part of our routine NAAT testing as of June 25th 2012
- The MAC NAAT will be reported in conjunction with the TB NAAT

MAC NAAT – Review

- Target for MAC is 16S rRNA region of the DNA
- Inhibition control is present in the same tube
 - Some specimen will contain inhibitors that will interfere with amplification
 - Test for inhibition at the same time as the sample, no need for extra repeats
- All the samples are tested in duplicates to assure concordance of the results
 - Tests are performed on Tuesday and Thursday starting first thing in the morning
 - The specimen must be received by Monday for Tuesday run or by Wednesday for Thursday run
 - Results will be reported 24 hours from the test

NAAT for MAC Sensitivity/Specificity

- Results from the Prospective Study for the Validation of NAAT for MAC

Sputum vs Culture		Total # Samples	Sensitivity	Specificity	PPV	NPV
Sputum	TB	259	84.2%	98.8%	84.2%	98.8%
Sputum	MAC	259	42.9%	100%	100%	98.4%

PPV = Positive Predictive Value

NPV = Negative Predictive Value

DRSS WEBINAR

- How does the DRSS work
- How to order the test
- Acceptable samples
- Results interpretation

DRSS Background

- WA PHL has developed and validated the method to screen for mutations on *M. tuberculosis* complex DNA that could indicate resistance to Rifampin (RIF), Isoniazid (INH), and Pyrazinamide (PZA)
- This test has been offered at the WA PHL as of June 18th, 2012

Advantages of DRSS Test

- Rapid identification of Multidrug Resistant (MDR) cases
- Quicker initiation of appropriate treatment regimen
- Results of preliminary drug resistance are available within 1 week instead of 2-3 weeks of traditional Drug Susceptibility Testing (DST)
- May be performed on clinical specimens, if sufficient DNA is available
 - AFB smear positive of 2+ or higher

Disadvantages of DRSS

- Only the most common mutations are screened
- Isolates may still show resistance if no mutations are found
- Isolates with mutations may be sensitive by traditional DST
- Consult with the TB Physicians if you have any questions

Resistance in WA State 2010 to Present

- 8 patients were MDR cases
- Monoresistance to INH is the most common
 - Approximately 8% of TB isolates in WA
- Monoresistance to RIF is very rare
- Monoresistance to PZA
 - Possible *M. bovis* infection should be considered

Drug Resistant Isolates WA State cont. 2010-Present

County	RIF only	INH only	INH & RIF *	PZA only	INH & PZA	INH, RIF & PZA *
Benton				1		
King	2	18	1	9	5	1
Kitsap						1
Pierce		4	1			
Skagit				1		
Snohomish		5	1	1		2
Spokane		2		1	1	
Thurston		2		1		
Whatcom		1	1			
Yakima		1				
TOTAL	2	33	4	14	6	4

*MDR cases

- From 01/2010 to present there are 431 cases counted in WA with a case verification of “Positive Culture”
- 64 isolates were resistant to at least one of the drugs considered
- During this time period, about 15% of TB patients required modified treatment due to resistance

Streptomycin and Ethambutol Resistance was not considered for this data

How Does DRSS Work?

- DNA mutations occur spontaneously in any living organism
- Certain mutations alter the structure of the functional protein
 - The protein is no longer able to interact with the drug

→ RESISTANCE

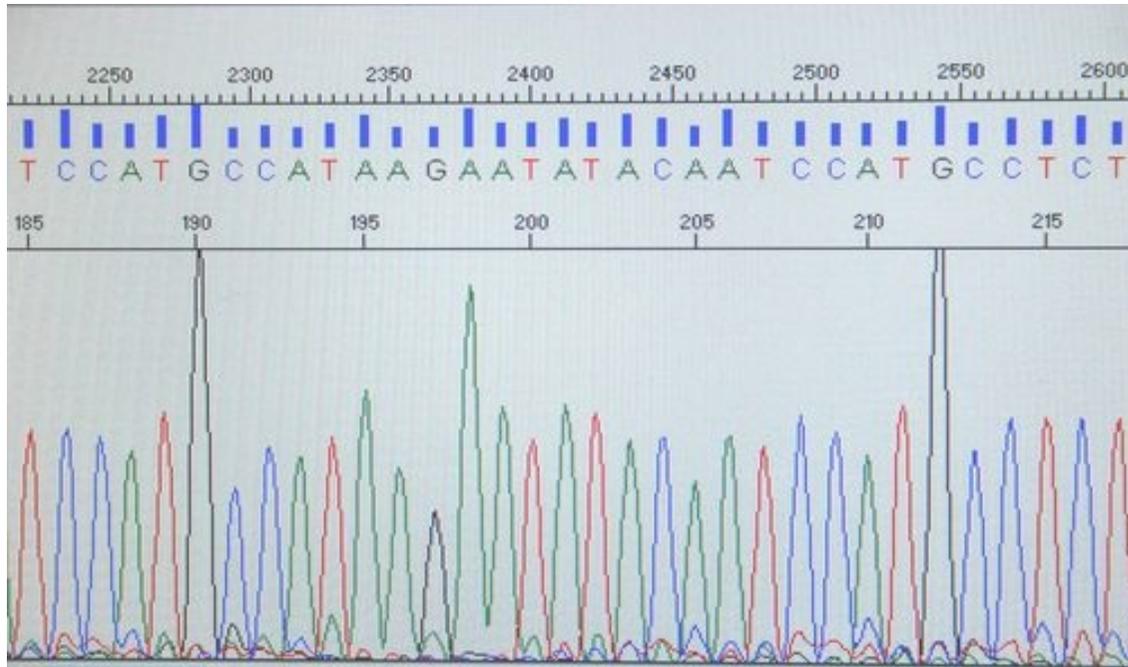
Molecular Basics for DRSS

- MTB DNA is amplified by a PCR process
 - Making more copies of the target DNA to increase detection level in clinical sample
- Target DNA region is sequenced
 - Nucleotides are building blocks of DNA
- The nucleotide sequences are compared with wild type sequences from a fully susceptible strain
 - If mutation is present, is it known to confer resistance?

Which MTB Genes are Screened?

- Rifampin
 - 95% of all rifampin resistance is associated with mutations found in *rpoB* gene
- Isoniazid
 - 50-60% of isoniazid resistance is associated with mutations in *katG* gene
 - Can achieve 83% by screening *inhA* gene in addition to *katG*
- Pyrazinamide
 - 72-97% of pyrazinamide resistance is associated with mutations in several locations within *pncA* gene

Example of Sequencing Results



Nucleotide Number

DNA Nucleotides

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The results from automatic DNA sequencing with the nucleotide bases, guanine (G), adenosine (A), tyrosine (T) and cytosine (C) shown in different colors

Examples of *rpoB* mutations

Codon #	Wild Type	Mutation
531	TCG	TTG
526	CAC	GAC
		CTC
		TAC
		TGC
		AAC
516	GAC	GTC
		TAC
513	CAA	AAA

Codon: three DNA nucleotides coding for one amino acid, building blocks of protein.



Ordering of DRSS

Due to the limited resources, the test requests need to be screened and approved by one of the TB Physician:

Dr. Narita, Dr. Spitters, or Dr. Lindquist

To obtain the Requisition Form, please call WA PHL TB Lab

ph: 206-418-5473

Complete the DRSS Approval form and fax it to the WA TB Lab

fax: 206-418-5545

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

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.

Section 1. Requestor Information

TB Physician Approving DRSS: _____

Date: _____ Patient Name: _____

Person completing the form: _____ Type of specimen: _____

Phone#: _____ Specimen Location: _____

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 (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

Date Request received in the WA State TB Laboratory: _____

Approved: Yes No By: _____ ETA to the WA PHL TB Lab: _____

Comments: _____



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Approval of DRSS

- Please contact one of the following TB Physicians to determine if your patient's scenario meets submission criteria for testing.
- **Dr. Masa Narita** 206-744-4579
Primary contact for King County
- **Dr. Chris Spitters** 206-383-0474
Contact for Snohomish, Yakima, Klickitat, or Island Counties; back-up for Dr. Narita in King County
- **Dr. Scott Lindquist** 206-718-2664
Contact for all other counties

Acceptable Samples for DRSS

- AFB smear positive respiratory sediments
 - AFB 2+ or higher
 - MTB DNA quantities will be determined by TB NAAT screening assay at WA PHL.
 - If sample contains insufficient quantities of DNA, it requires 5-7 days of incubation
 - Any delays in testing will be promptly communicated
- **Recommended:** MTB confirmed cultures

Sensitivity and Specificity

- During 2010-2011, the validation study of the DRSS assay was conducted at the WAPHL
- 173 TB isolates were tested for RIF and INH resistance
- 131 TB isolates were screened for PZA resistance
- The results of the DRSS assay were compared to culture-based drug susceptibility testing (DST)

DRSS vs. traditional methods					
Drug	Gene	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
RIF	<i>rpoB</i>	97.5	98.5	95.1	99.2
INH	<i>inhA + katG</i>	79.7	98	96.7	86.6
PZA	<i>pncA</i>	79.2	98.1	90.5	95.5

PPV = Positive Predictive Value

NPV = Negative Predictive Value

Reporting DRSS Results

- Verbal
 - If mutations are detected, the ordering TB Physician will be called
- Fax
 - Reports are faxed to the submitter and the ordering TB Physician

Example of the Report

DRSS

	Result	Performed by	Date
RIF (rpoB)	Mutation NOT Detected	AXO	6/20/2012
303) Probably Rifampin susceptible. [97.5% of RIF-R isolates in our in-house evaluation of 173 clinical isolates have a mutation in this gene with a positive predictive value of 95.1%]			
INH (katG and inhA promoter)	Mutation NOT Detected	AXO	6/20/2012
304) Cannot rule out INH resistance. [79.7% of resistant isolates in our in-house evaluation of 173 clinical isolates have a mutation in the genetic regions evaluated with a positive predictive value of 96.7%]			
PZA (pncA)	Mutation NOT Detected	AXO	6/20/2012
305) Cannot rule out PZA resistance. [79.2% of resistant isolates in our in-house evaluation of 131 clinical isolates have a mutation in this gene with a positive predictive value of 90.5%]			
			6/20/2012
306) DRSS results are preliminary, pending confirmation with culture-based DST methods. This specimen was tested with a sequencing-based research procedure that is not cleared or approved for diagnostic use by the U.S. Food and Drug Administration (FDA). Results obtained with DRSS are primarily for epidemiologic surveillance purposes and should not form the sole basis for treatment decisions. The assay will not detect mutations associated with resistance other than those located in the regions of M. tuberculosis genome screened AND that also have a well-established strong correlation with resistance. Thus, the absence of mutations does not necessarily indicate a lack of resistance since other mutations including those located in genomic regions not tested by the assay could confer resistance. Improper specimen collection/handling, the presence of inhibitors, and/or the presence of multiple mycobacterial strains in a specimen may also contribute to a false result.			

Example of the Report cont

DRSS

	Result	Performed by	Date
RIF (rpoB)	Mutation Detected	AXO	6/21/2012
300) Probably resistant to Rifampin [97.5% of RIF-R isolates in our evaluation of 173 clinical isolates have a mutation in this gene with a positive predictive value of 95.1%]			6/21/2012
INH (katG and inhA promoter) Mutation Detected		AXO	6/21/2012
301) Probably resistant to isoniazid. [79.7% of resistant isolates in our evaluation of 173 clinical isolates have a mutation in the genetic regions evaluated with a positive predictive value of 96.7%]			6/21/2012
PZA (pncA)	Mutation Detected	AXO	6/21/2012
302) Probably resistant to pyrazinamide [79.2% of resistant isolates in our evaluation of 131 clinical isolates have a mutation in this gene with a positive predictive value of 90.5%]			6/21/2012
306) DRSS results are preliminary, pending confirmation with culture-based DST methods. This specimen was tested with a sequencing-based research procedure that is not cleared or approved for diagnostic use by the U.S. Food and Drug Administration (FDA). Results obtained with DRSS are primarily for epidemiologic surveillance purposes and should not form the sole basis for treatment decisions. The assay will not detect mutations associated with resistance other than those located in the regions of M. tuberculosis genome screened AND that also have a well-established strong correlation with resistance. Thus, the absence of mutations does not necessarily indicate a lack of resistance since other mutations including those located in genomic regions not tested by the assay could confer resistance. Improper specimen collection/handling, the presence of inhibitors, and/or the presence of multiple mycobacterial strains in a specimen may also contribute to a false result.			6/21/2012

Interpretation of the DRSS Results

- All DRSS results are preliminary and **must** be confirmed by traditional DST
- If mutation is present, most likely the isolate will have resistance
- If no mutations detected, can not rule out resistance because not all regions of DNA are tested for mutations

Alternate Molecular Testing

- The Centers for Disease Control and Prevention (CDC) offers an MDDR screens for an extended array of mutations associated with resistance to INH, RIF, EMB, PZA, FQ, KAN, AMK, and CAP. To request this test, please contact the State TB Laboratory for details and instructions.

WA PHL TB Lab Ph: 206-418-5473

THANKS TO:

TB Laboratory

- Karen Hiltbruner
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AND

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- Sherry Carlson
- Temple Parson

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Questions?



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