

New Tools in the Diagnosis of TB: Principles, Indications and Limitations (Understanding TB Laboratory Results and Challenges of Reporting Results)

Beverly Metchock, DrPH, D(ABMM)
Team Lead, Reference Laboratory
Division of TB Elimination

November 6, 2014

When Worlds Collide



Objectives

- ❑ Briefly review mycobacteriology testing practices in US with emphasis on potential “problems”
- ❑ Describe the new molecular tests available for detecting drug resistance in MTBC
- ❑ Use case-based scenarios to explain the use of molecular test results and the benefits and limitations of these tests

“Rules of the Lab”

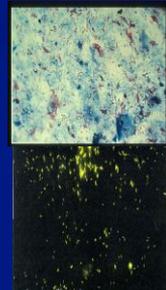
- ❑ No lab test is perfect
- ❑ Do not order a lab test if you are not ready to deal with the result
- ❑ Treat the patient, not the lab test
- ❑ If you don’t know what something means, please call the lab
- ❑ For TB—We are still learning about DST and molecular detection of drug resistance
 - Discordance will happen
 - Things are not always black and white

TB testing/mycobacteriology in U.S.

- ❑ Types of laboratories (not mutually exclusive):
 - ❑ Hospital/medical center laboratories
 - ❑ Public health laboratories (e.g., State, county, city)
 - ❑ Commercial laboratories (e.g., LabCorp, Quest, ARUP)
 - ❑ Reference Laboratories (Nat. Jewish, CDC, Mayo)
- ❑ Mycobacteriology laboratory services are often dispersed
 - Work is often piecemeal – specimens or isolates referred from one lab to another
 - Difficult cases may have drug susceptibility results from several laboratories (discordance not uncommon)
 - Communication between labs may be a problem
- ❑ Communication with care-giver/TB program a problem especially when testing becomes further removed from originating lab

AFB Microscopy

- ❑ Not very sensitive
 - 50-70% for pulmonary TB
- ❑ Not specific for MTBC
- ❑ Value for TB
 - Inexpensive and rapid; 1st bacteriologic evidence of TB
 - Infectiousness; follow therapy
 - Determine need for additional testing (e.g., NAAT)
- ❑ Primary method for TB diagnosis in developing countries



Microscopy vs. Culture

- 5,000 to 10,000 AFB/mL for smear
- 10 to 100 AFB/mL for culture
- Significance of culture
 - Confirm TB/ mycobacteriosis; obtain isolate for DST, genotyping; evaluate therapy
 - Only 85-90% cases of pulmonary TB are culture-positive (culture-negative TB; clinical diagnosis)



Identification of Mycobacteria from Growth in Culture

- Conventional biochemical tests
- HPLC of cell wall mycolic acids
- DNA probes (AccuProbe®; Gen-Probe, Inc.)
 - Does not require Nucleic Acid Amplification
- “in-house” or laboratory developed tests (LDT) such as PCR/RE analysis/genetic sequencing

Direct Detection of MTBC in Clinical Specimens; Nucleic Acid Amplification Tests (NAAT)

- Objective is to detect/identify MTBC directly from clinical specimens and avoid the weeks required for culture
 - Rapid turnaround time of 24 to 48 hours after specimen receipt
- Positive result demonstrates the presence of MTBC
 - Does not distinguish live and dead bacilli
- Negative result does not necessarily mean the absence of MTBC
 - Inhibition of amplification
 - Target below the limit of detection

NAAT for Direct Detection of MTBC

- FDA-approved for use with respiratory specimens
 - Amplified MTD® (*Mycobacterium tuberculosis* Direct) Test: Gen-Probe, Inc.
- FDA market authorization for use with raw sputum or sputum sediments
 - GeneXpert® MTB/RIF: Cepheid
- Non-FDA approved tests (RUO; Research Use Only)
 - Hain Lifescience Genotype® MTBDRplus and MTBDRsl
- Laboratory developed tests or LDT (e.g., DNA sequencing, and real-time PCR assays)

Limitations and Considerations

- Sensitivity
 - Reduced for smear negative specimens and some specimen types?
 - Do you want “rule in” or “rule out”?
 - Platform dependent
- Specificity
 - Platform dependent
- Does not replace need for culture
 - Culture still needed for conventional DST, genotyping
- Amplicon cross contamination in open systems
- Cost and sustainability
 - Expense can limit utilization

Drug Susceptibility Testing (DST) of MTBC

Current recommendations (Clinical and Laboratory Standards Institute [CLSI] M24-A2)

- Initial isolate should be tested against primary or first-line drugs (FLD)
 - INH, RMP, PZA, EMB
- For RMP-resistant isolates, or resistance to any 2 FLD, test second-line drugs (SLD)
 - To include FQ, AMK, KAN, CAP

Possible Definitions of Drug Resistance

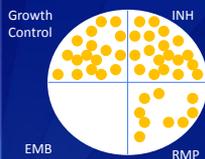
- MIC that is beyond or at the extremes of the MIC distribution of presumed wild-type isolates
- Comparison of the MIC to a PK parameter (e.g., peak/MIC or AUC/MIC)
- MIC that is associated with an increased risk of failure/relapse (clinical validation)
- Change in MIC from baseline to time of treatment failure
- Change in genotype (i.e., wild type to mutation) from baseline to time of treatment failure

Bill Burman, June 2011

Current Practice for DST

- For FLD, FDA-cleared broth-based methods are routine and widely available
 - Results generally available within 28 days of specimen receipt in laboratory
- Molecular assays (RMP, INH) are available in some jurisdictions – Cepheid GeneXpert® MTB/RIF; LDT or RUO tests
 - Performed directly on clinical specimens or on culture isolates and results available within 1–2 days
- For SLD, testing often performed in piecemeal fashion through referrals; few laboratories with technical expertise / capacity
 - Slow turn-around-time; Indirect agar proportion takes ~28 days after isolation from culture
 - Some laboratories have validated methods for broth-based testing
 - Some laboratories offer molecular assays (LDT or RUO tests)

Agar Proportion Method for MTBC DST



- The method of proportion using Middlebrook 7H10 agar has been considered the “gold standard” method in the U.S. for several decades – used at CDC
- Plate bacteria onto media containing
 - no drugs (growth control)
 - critical concentrations of a drug
- Incubate for 3 weeks
- Count colonies
- Isolate is resistant if the number of colonies on drug-containing media is $\geq 1\%$ of the colonies on drug-free media

Critical Concentration

- DST of MTBC typically involves testing the susceptibility of the organism against the critical concentration of a drug
- Critical concentrations were adopted by international convention
 - Ideally the critical concentration is the lowest concentration of a drug that discriminates between susceptible and resistant strains of MTBC
 - That means, it inhibits growth of all susceptible strains AND allows growth of all resistant strains
- It is difficult to find a drug concentration that precisely meets this definition; we settle for the concentration that **BEST DISCRIMINATES** between susceptibility and resistance

Equivalent Critical Concentrations

- Critical concentrations were originally determined in Lowenstein-Jensen medium
- Equivalent concentrations of drugs later established in Middlebrook 7H10 and 7H11 for agar proportion method and in media used in commercial DST systems

	LJ	7H10 Agar	7H11 Agar	Bactec 460*	MGIT	Versa-TREK
INH	0.2	0.2	0.2	0.1	0.1	0.1
RMP	40	1.0	1.0	2.0	1.0	1.0
EMB	2.0	5.0	7.5	2.5	5.0	5.0

*No longer manufactured

What about MIC testing?



Sensititre (ThermoScientific Trek Diagnostic Systems)
Dry Microdilution Plate for Mycobacterium spp.

Critical Concentration Differs from Minimum Inhibitory Concentration

- The minimum inhibitory concentration (MIC) is defined as the lowest concentration of a series of drug dilutions (usually serial two-fold dilutions) that prevents visible / detectable growth of MTBC
 - Can perform in agar or broth system
 - Interpretive criteria needed to define categorical result of resistant or susceptible
- MIC testing differs from testing using critical concentrations which uses single drug concentrations and provides a categorical result of resistant or susceptible

Limitations of MIC Testing for MTBC

- Procedures are not standardized
- Assays not FDA-cleared
- No universally established breakpoints or interpretive criteria
- Few studies on how MIC correlates with clinical presentation or patient outcome
- MIC results may not correlate with results obtained by critical concentration methods
- Additional research is needed to understand how DST results using different methods correlate with treatment efficacy

What is the Gold Standard for DST?

- Traditionally believed to be the result obtained by growth-based testing
 - BUT, discordance between methods is not rare
 - Equivalent critical concentrations (different media)
- Lack of clinical validation of drug resistance (especially 2nd and 3rd line drugs)
- Introduction of genotypic tests –
 - Significant mutation detected but S by growth-based test
- Multidrug therapy (challenge to get clinical validation)

Molecular Detection of Drug Resistance (Molecular DST)

- Examining DNA of specific genes for mutations known to be associated with phenotypic resistance
 - Mutations in what genes are associated with resistance?
 - Where are the mutations within the gene?
 - Some areas are “hot spots”—resistance determining regions

Molecular-based Tests for Detecting Mutations Associated with Drug Resistance

	Method			
	GenoType [®] MTB/RIF	HAIN GenoType [®] MTDRbL4	Anger Sequencing	Pyrosequencing
Company	Cepheid	HAIN Lifescience	Not Applicable (N/A) (laboratory developed test)	N/A (laboratory developed test)
Format	Semi-automated real-time PCR	Line probe assay	DNA sequencing	DNA sequencing
FDA approved	Market authorization	No	N/A (laboratory developed test)	N/A (laboratory developed test)
Expected turn-around time	1-2 working days	1-2 working days	1-2 working days	1-2 working days

- Output from the assay depends on the platform (e.g., DNA sequencing, molecular beacons, line probe assay)
- Important to understand platform to understand limitations

Cepheid Xpert MTB/RIF Assay

The screenshot displays the software interface for the Cepheid Xpert MTB/RIF Assay. It features a graph showing fluorescence intensity over time for different drugs. Below the graph, there is a table of results for various drugs, including Isoniazid, Rifampin, Fluoroquinolones, and Trimethoprim-sulfamethoxazole. The results are categorized as Susceptible (S) or Resistant (R).

Drug	Result
Isoniazid	S
Rifampin	S
Fluoroquinolones	S
Trimethoprim-sulfamethoxazole	S

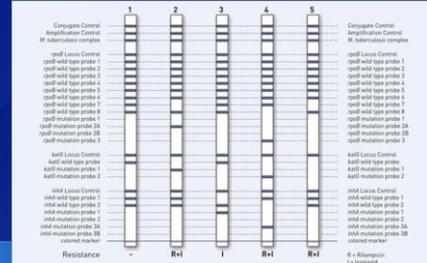
Below the software interface, there are images of the physical assay components, including a cartridge with a QR code and the Xpert MTB/RIF instrument.

Cepheid Xpert MTB/RIF Assay

- Automated commercial system for identification of *M. tuberculosis* complex and mutations in *rpoB*
- Uses real-time PCR with molecular beacons
 - 5 probes for wild-type RRDR in *rpoB* and 1 probe for amplification control (*B. globigii*)
- Decontamination, digestion, DNA extraction, amplification, and detection in same cartridge; Limited biosafety requirements
- Results in ~2 hours
- Minimal hands on manipulation- technically simple
- Platform is random access

Genotype MTBDRplus

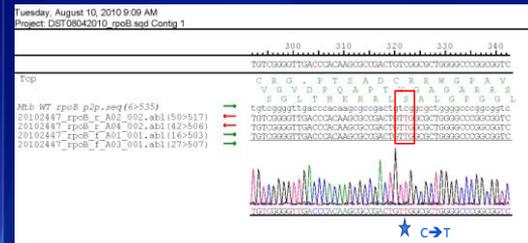
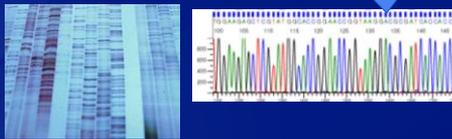
- NAA and hybridization-based test use immobilized DNA probes on nitrocellulose membranes (line probe assay [LPA])
- Colorimetric change indicates hybridization
- “Read” the bands to determine MTBC or not and to detect resistance-associated mutations for RMP and INH



Conventional DNA Sequencing (Sanger sequencing)



- PCR Amplification of target regions
- DNA Sequencing
- Sequence Analysis



Most commonly observed *rpoB* mutation:
TGT>TGG Ser531Leu

Actual DNA sequence detected

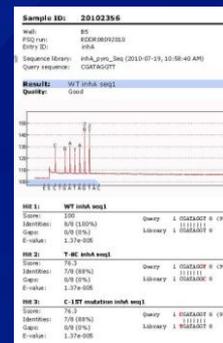
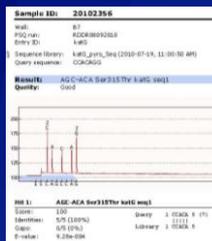
Pyrosequencing

Direct DNA sequencing of PCR products

Unique chemistry



- Biotin labeled PCR product (1 strand)
- Biotinylated DNA strand captured on beads
- Beads hybridized with sequencing primer
- Instrument carries out DNA sequencing reaction and analysis <2 hrs



Actual DNA sequence detected

“Differences” in Testing Platforms (simplified version)

- ❑ Sanger sequencing
 - Identifies actual mutations
 - Can examine long sequences
- ❑ Pyrosequencing
 - Identifies actual mutations
 - Short sequence reads
 - Minor or mixed populations are harder to detect
- ❑ GeneXpert MTB/RIF
 - Detects wild type sequence and thus may miss mixed populations
- ❑ Hain
 - Interpretation of banding pattern may give indication of actual mutation

Advanced Molecular Diagnostics (AMD)

Next Generation Sequencing
High-throughput sequencing
'Whole Genome' sequencing

High throughput, multi-use platforms – need to batch
Massive amount of data generated

DTBE using next generation sequencing tools and bioinformatics to identify outbreaks so TB control programs will be able to focus their investigations, conserve scarce resources, and identify and respond more quickly to outbreaks to interrupt transmission.

“Real World” Considerations

- ❑ Silent mutations
 - Base changes; amino acid does not change
 - No alteration in protein sequence
- ❑ Missense mutations
 - Base changes; amino acid changes
 - Protein sequence changes
 - Not all result in phenotypic resistance
 - Neutral polymorphisms (possible lineage markers, not associated with resistance)
 - “border-line” or low-level resistance
- ❑ ‘New’ mutations
 - Not previously observed, not reported in the literature, not yet shown to be associated with resistance
- ❑ Mixed wild type / mutant populations
 - Emerging resistance
 - >1 strain of TB in patient
 - Limit of detection
- ❑ Lack of a mutation does not confirm susceptibility
- ❑ Our “gold standard” DST is not perfect

MDDR Service at CDC: Rationale (2008-2009)

- ❑ Clinical/Program
 - Make rapid confirmation of MDR TB available
 - Make laboratory testing data available to clinicians about SLD resistance in cases of RMP- R or MDR TB
 - New technologies may fill the role in the future but demand exists now
- ❑ Development
 - Continuous correlation of molecular (genotypic) results and DST (phenotypic) results
 - Addition of new drugs and alleles
- ❑ Research
 - Determination of mechanisms of resistance
 - “Fine tune” DST

MDDR Service History

- ❑ Implemented in September 2009 (CLIA compliant)*
 - DNA sequencing , ABI 3130xl
 - MTBC isolates
 - Anticipated workload - conservative estimate, 1-2 isolates/week
 - Loci examined for INH, RMP, FQ, and injectables
- ❑ Loci for EMB and PZA incorporated October 2010
- ❑ MDDR V 2.0 implemented in June 2012
 - Incorporation of pyrosequencing screen (INH and RMP only)
 - MTBC isolates and NAAT(+) sediments (not raw specimens)

* Campbell, PJ, et al. 2011. Antimicrob Agents Chemother 55:2032-2041

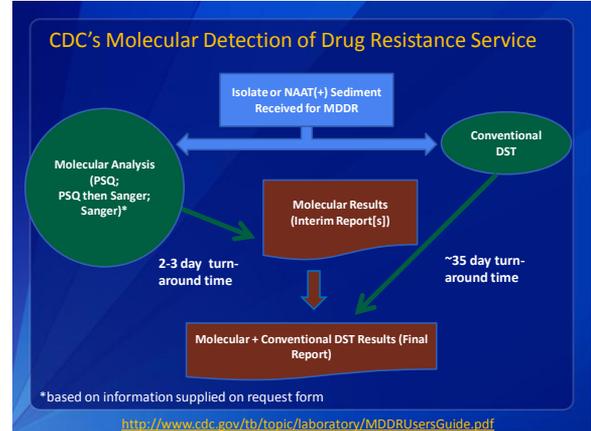
Criteria for MDDR Testing Version 2.0* (Expanded MDDR)

- ❑ Isolate or NAAT (+) sediment (not raw specimen)
- ❑ High-risk patients (RMP-R, MDR TB)
 - From population with high rates of drug resistance
 - Exposed to DR case
 - Failing therapy
- ❑ Cases of public health importance
 - Impact on public health measures & public health response
- ❑ Known RMP Resistance
 - Conventional or molecular test by submitter
- ❑ Mixed or non-viable cultures
- ❑ Other Reasons

*June 2012

MDDR Service: Sanger Sequencing Drugs and Genes for Panel

<ul style="list-style-type: none"> • Rifampin • Isoniazid • Isoniazid • Ethambutol • Pyrazinamide • Fluoroquinolones • Amikacin, Kanamycin, Capreomycin • Kanamycin • Capreomycin 	MDR TB	<ul style="list-style-type: none"> • <i>rpoB</i> (81bp region) • <i>inhA</i> (-15) • <i>katG</i> (Ser315) • <i>embB</i> (Met306, Gly406) • <i>pncA</i> (promoter and coding regions) • <i>gyrA</i> (coding region) • <i>rrs</i> (nt1401/1402,1484) • <i>eis</i> (promoter region) • <i>tlyA</i> (coding region)
	XDR TB	



How should we report the mutation detected?

rpoB TCG>TTG; Ser531Leu

761155CT =
Ser450Leu =
S450L
Ser531Leu (E. coli) =
S531L (E. coli) =
531TTG

What about tests which do not determine actual genetic mutation / provide actual genetic mutation as output?

Hain Test Report (lab 1)

HAIN Test GenoType-MTBDRplus

rpoB point mutation detected

katG point mutation detected

No inhA point mutation detected

--As with any DNA-based assay, this test only screens the nucleic acid sequence and not the amino acid sequence. Therefore, it is possible that mutations that do not cause an amino acid exchange (silent mutations) will still produce the absence of one of the wild type probes.

The GenoType MTBDRplus test only indicates those resistances of the *M. tuberculosis* complex that have their origins in the *rpoB*, *katG*, and *inhA* regions examined here. Resistances originating from mutations of other genes or gene regions as well as other rifampin and isoniazid resistance mechanisms will not be detected by the test.

Theoretically, a resistance can exist in spite of a wild type pattern. If, at investigation, the sample contains a strain that has developed only a partial resistance that is not covered by the mutation probes, the wild type pattern will appear. If the sample contains more than one *M. tuberculosis* strain (due to mixed culture or contamination) and one of these harbors a mutation that is not covered by the mutation probes, the wild type pattern will appear.

Hain Test Report (lab 2)

RAPID ID OF MDR	
RIFAMPIN GENO	rpoB MUTATION DETECTED RESISTANT TO RIFAMPIN
ISONIAZID GENO	NO katG MUTATION DETECTED AND NO inhA MUTATION DETECTED SUSCEPTIBLE TO ISONIAZID
NOTE	The line probe assay only indicates those resistances of MTB complex that have their origins in the rpoB, katG, and inhA regions examined here. Resistances originating from mutations of other genes or gene regions will not be detected by this test. This test only screens the nucleic acid sequence and not the amino acid sequence. Therefore, it is possible that mutations that do not cause an amino acid exchange (silent mutations) will still produce the absence of one of the wild type probes.

Availability of an Assay for Detecting *Mycobacterium tuberculosis* Including Rifampin-Resistant Strains, and Considerations for Its Use (MMWR Oct. 18, 2013 / 62(41):821-824)

Interpretation and proposed minimum laboratory report language for results from the Cepheid Xpert MTB/RIF assay

Cepheid Instrument System Determined Response	Xpert MTB/RIF Assay Result Interpretation	Minimum Laboratory Report*
MTB DETECTED, RIF Resistance DETECTED	MTB target is detected within the sample. A mutation in the rpoB gene has been detected.	MTBC detected. A mutation in rpoB gene had been detected, indicating possible RMP resistance. Confirmatory testing should follow.
MTB DETECTED, RIF Resistance NOT DETECTED	MTB target is detected within the sample. A mutation in the rpoB gene has not been detected.	MTBC detected. No rpoB gene mutations detected; probably RMP susceptible.
MTB DETECTED, RIF Resistance INDETERMINATE	MTB target is detected within the sample. A mutation in the rpoB gene could not be determined because of insufficient signal detection.	MTBC detected. Presence of rpoB gene mutations cannot be determined.
MTB NOT DETECTED	MTB target is not detected within the sample.	MTBC not detected.

CDC suggested minimum language for the laboratory report. Laboratories are encouraged to enhance and customize this basic language in accordance with the capabilities or referral systems of their institution.

Interpretative Comments Considerations

- Will differ with platform
- Examples
 - “Resistant” vs. “Associated with resistance” vs. “Predicts resistance” vs. “Probably resistant” vs. “Likely resistant”
 - “Cannot rule out resistance” vs. “Suggests susceptibility” vs. “Likely susceptible” vs. “Susceptible”
- “Unknown” or “novel” mutation
 - Defer to DST?
- Discordance between molecular and DST results
 - Report both; no comment?
 - Defer to DST?
 - Defer to molecular result?
 - Report both; clarifying comment?

Discordant Results – CDC Example

- *rpoB* Leu533Pro
 - the Leu533Pro mutation detected in *rpoB* is associated with low-level, but probably clinically-relevant, RMP resistance. Isolates with this mutation often test as susceptible by conventional techniques.”
- RMP S by AP DST
 - RMP reported as “see comments”
 - EXCEPTION: rpoB —Leu533Pro mutation; RMP DST—RMP-S. Low-level but probably clinically relevant rifampin resistance has been linked to the Leu533Pro mutation detected in the rpoB locus; isolates with this mutation may test as susceptible by conventional techniques.

Discordant Results – CDC Example

- No mutations detected in *inhA* and *katG*
 - Cannot rule out INH resistance. (86% of INH-R isolates in our in-house evaluation of 550 clinical isolates have a mutation at one or both of these loci.)
- INH R by AP DST
 - INH reported as R
 - EXCEPTION: *inhA* and *katG*—no mutations; AP DST—INH-R. The absence of a mutation in *inhA* and *katG* does not rule out resistance to INH; ~14 % of INH-R isolates do not have a mutation at either of these loci.

Considerations for Standardization of Reporting Tuberculosis Molecular Diagnostic Results

- What is considered the “gold standard” for interpretation?
- What interpretive comments are provided for novel mutations?
- How are silent mutations described?
- How much information should be provided to aid in understanding discordant results?
- What is the threshold when the complexity of information provided challenges data accuracy for surveillance reporting?

Need to Know



Nice to Know

Must keep this complexity in mind with the ultimate goal of improving patient outcomes and public health

CASES

(Illustrate rapid turn-around time)

MGIT broth
Previous TB Treatment
From a country with a high rate of drug resistance
(China)
University Student

Collection Date: 1/17/2014 (Friday)
CDC contacted: 2/6/2014 (Thursday)
Date sent to CDC: 2/6/2014 (Thursday)
Date received at CDC: 2/7/2014 (Friday)

Pyrosequencing Report issued 2/10/2014

Locus (region) examined	Result	Interpretation
rpoB (RRDR)	No mutation	Probably Rifampin susceptible. (97% of RMP-R isolates have a mutation at this locus.)
inhA (promotor)	No mutation	Cannot rule out INH resistance. (86% of INH-R isolates have a mutation at one or both of these loci.)
katG (Ser315 codon)	No mutation	

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.

TAT from specimen collection date: 24 days
TAT within CDC: 3 days

Rapidly ruled out resistance in a case of public health importance

NAAT+ sputum sediment
Previous TB Treatment
From a country with a high rate of drug resistance
(PERU)

Collection Date: 12/29/2013 (Sunday)
CDC contacted: 12/30/2013 (Monday)
Date sent to CDC: 1/2/2014 (Thursday)
Date received at CDC: 1/3/2014 (Friday)

Pyrosequencing Report issued 1/6/2014

Locus (region) examined	Result	Interpretation
rpoB (RRDR)	Mutation: TCG>TTG; Ser531Leu	Rifampin resistant
inhA (promotor)	No mutation	Isoniazid resistant
katG (Ser315 codon)	Mutation: AGC>ACC; Ser315Thr	

MDDR testing (Sanger sequencing, complete panel) is in progress because a mutation associated with RMP resistance was detected. Report to follow.

TAT from specimen collection date: 7 days
TAT within CDC: 3 days

CDC Sanger Sequencing Report issued 1/7/2014

Locus (region) examined	Result	Interpretation
rpoB (RRDR)	Mutation: TCG>TTG; Ser531Leu	Rifampin resistant
inhA (promotor)	No mutation	Isoniazid resistant
katG (Ser315 codon)	Mutation: AGC>ACC; Ser315Thr	
embB (Met306, Gly406, other)	Mutation: ATG>ATC; Met306Ile	Ethambutol resistant
pncA (promotor, coding region)	Mutation: GGC>GAG; Ala146Glu	Likely PZA resistant
gyrA (QRDR)	Mutation: GAC>GGC; Asp94Gly	Ofloxacin resistant
rrs (1400 region)	Mutations: A1401G and C1402T	Amikacin and Kanamycin resistant; Possibly Capreomycin resistant
eis (promotor)	Mutation: C-14T	
tlyA (entire ORF)	Frameshift mutation	

Rapidly identified MDR / XDR TB

CASES

(Illustrate discordance)

Case # 1—Is it RMP-R? (RMP Discordance between molecular and conventional results)

- ❑ Smear (+) pulmonary TB; prisoner
- ❑ At hospital
 - Xpert (X2) — RMP Resistance Detected
 - DST (MGIT) — INH-R and RMP-S
- ❑ AP DST pending at State lab

- ❑ At CDC, *rpoB* DNA sequence — TTC>TTT; Phe514Phe

Case # 2 - Is it RMP R? (RMP discordance between broth and AP)

- ❑ State PHL DST results:
 - Bactec 460—R to INH; S to RMP (2 µg/ml)
 - AP (7H10)—100% R to INH; 80% R to RMP (1 µg/ml)
- ❑ MDDR:
 - *rpoB*—Asp516Tyr; RMP resistant
 - *inhA*—C(-15)T; INH resistant
 - 40% R to RMP by AP

Case # 3—Is it RMP-R? (RMP Discordance between molecular and conventional results)

- ❑ Pulmonary TB; Burma (Nepal camp)
- ❑ State Lab DST (MGIT) — INH-R and RMP-S

- ❑ *rpoB* DNA sequence — Asp516Tyr; RMP resistant
- ❑ CDC AP — RMP-S

rpoB mutations associated with highly discordant DST results

- ❑ “Low-level” or “borderline” resistance or “disputed” mutations
- ❑ Probably clinically relevant resistance
- ❑ Resistance often missed by standard, growth-based systems, especially automated broth systems
 - Critical concentration may be too high to cover all clinically relevant resistance, or
 - Maybe the methods need modification (e.g., prolonged incubation, larger inoculum size) to detect resistance
- ❑ Frequency of these strains unknown
- ❑ Mutations : Asp516Tyr, Leu511Pro, Leu533Pro, His526Leu, His526Ser, Ile572Phe

van Deun, J Clin Microbiol 2009; Williamson, Diagn Microbiol Inf Dis 2012; Rigouts, J Clin Microbiol 2013; van Deun, J Clin Microbiol 2013

Rifampin CDC MPEP Strain H (6/2008) and Strain T (5/2010)*

Method	H (6/2008)		T (5/2010)	
	No. RMP-R/ No. results	%	No. RMP-R/ No. results	%
LI Proportion	7/7	100	n/a	n/a
Agar Proportion	19/27	70	15/23	65
BACTEC 460	15/36	41	7/19	37
MGIT	13/69	18	9/61	15
VersaTREK	0/3	0	0/5	0
Total	54/139	38	31/108	29

*His526Leu mutation in *rpoB*

Case # 4—Is it RMP-R?

- Isolate submitted for MDDR
 - HIV+, prison, Mexico, intermittent therapy, “funky” RMP on Bactec 460
 - CDC *rpoB* —wildtype; probably RMP-S
 - CDC AP — contaminated
 - Resubmit isolate (A) and a newer isolate (B)
 - AP (A)—RMP-R (5%)
 - AP (B)—RMP-R (12%)
 - *rpoB* on colonies—His526Tyr (100% of isolates with this mutation are RMP-R)

Delayed Test Results Man-Made and Unexpected Delays

- Recent submissions to CDC
 - 4 month TAT
 - Collection date 9/26/2013
 - CDC receives and approves request 1/22/2014 (Wednesday); “delay on sensitivity; patient already on treatment over 2 months”
 - Isolate received 1/24/2014 (Friday)
 - MDDR results 1/27/2014 (Monday)
 - 9 month TAT
 - Collection date 4/30/2013
 - CDC receives and approves request 1/23/2014 (Thursday); “Somalia”
 - Isolate received 1/27/2014 (Monday)
 - MDDR results 1/31/2014 (Friday)

Does it make a difference?

- Survey of TB Programs:
 - “We had two specimens R to rifampin by GX.....with the “silent” *rpoB* mutation, After medical consultation to make recommendations for the providers both cases were treated as MDR-TB.”
- MDDR request:
 - MDDR results: *katG* mutation (Ser315Thr) with interpretation as INH resistant; no mutation in *rpoB*
 - “while the *katG* mutation does suggest high level resistance, I hesitate to recommend stopping the INH until there are susceptibility results to confirm this.”

Conclusions

- Paradigm shift in laboratory diagnosis of TB and detection of drug resistance in MTBC
 - Molecular tests for diagnosis do not replace culture
 - Molecular tests do not replace conventional DST
 - Need to develop cost-effective algorithms for incorporating new technology; timely referral
- Results from genotypic and phenotypic tests for drug resistance need to be used in conjunction with one another (may depend on drug and genetic locus). The use of both may involve “trade-offs.”
- molecular (genotypic) tests may
 - Elucidate “truth” in certain cases
 - Add to confusion in certain cases
 - Help us “fine-tune” conventional DST
- Communication is essential

Acknowledgements

DTBE Laboratory Branch
Angela Starks, PhD
Jeff Driscoll, PhD

BMetchcock@cdc.gov
(404) 639-1285

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.



National Center for HIV/AIDS, Viral Hepatitis, STD & TB Prevention
Division of Tuberculosis Elimination