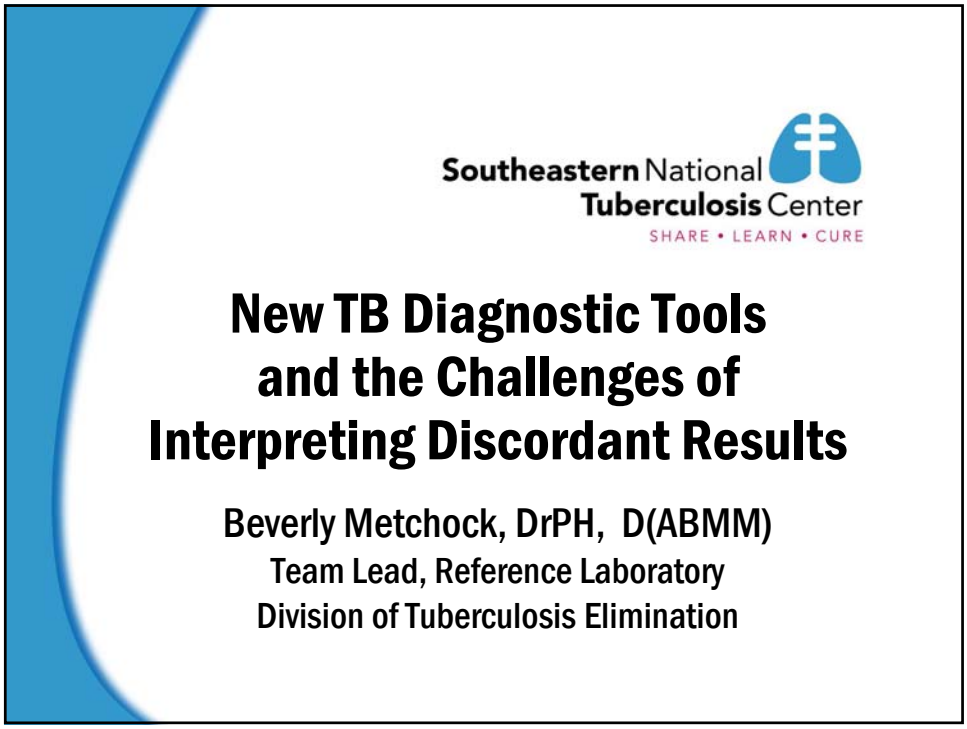

Southeastern National
Tuberculosis Center
SHARE • LEARN • CURE
New TB Diagnostic Tools
and the Challenges of
Interpreting Discordant Results
Beverly Metchock, DrPH, D(ABMM)
Team Lead, Reference Laboratory
Division of Tuberculosis Elimination


New TB Diagnostic Tools and the Challenges of Interpreting Discordant Results
Beverly Metchock, DrPH, D(ABMM)
Team Lead, Reference Laboratory/Division of Tuberculosis Elimination
July 2012
National Center for HIV/AIDS, Viral Hepatitis, STD & TB Prevention
Division of Tuberculosis Elimination
CDC

## 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"

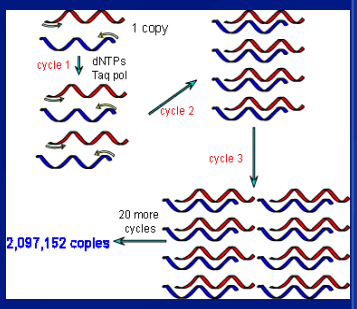
- No lab test  is perfect
- Do not order a lab test if you are not ready to deal with the result
- Treat the patient, not the lab test
- For TB—There is a lot we still need to learn about DST and molecular detection of drug resistance
  - Discordance

## Important Definitions

- Clinical specimen- material taken directly from the patient (e.g., sputum, CSF, pleural fluid); may be "raw" specimen or may be "processed" specimen (e.g., sediment)
- Isolate- organism isolated (i.e., grown) from culture of a clinical specimen (e.g., an LJ tube with MTBC growth)
- Direct detection- detection of RNA or DNA sequences of interest in organisms present in a clinical specimen; currently requires nucleic acid amplification (NAA)
- Probe - piece of DNA that hybridizes specifically to a target nucleic acid sequence

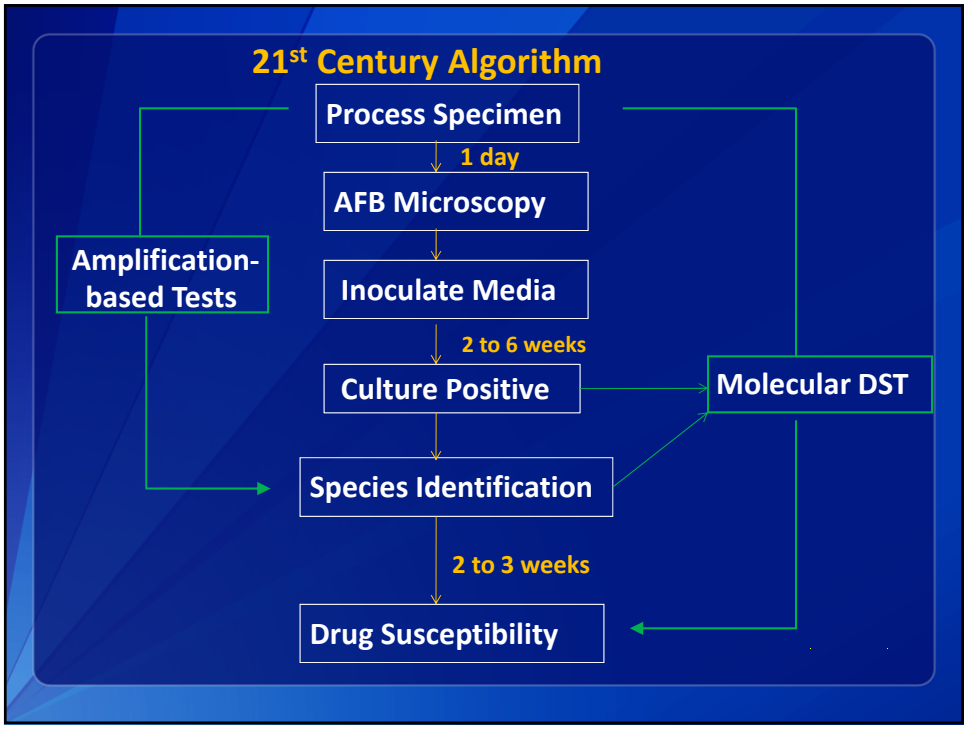
## What is Nucleic Acid Amplification (NAA) ?

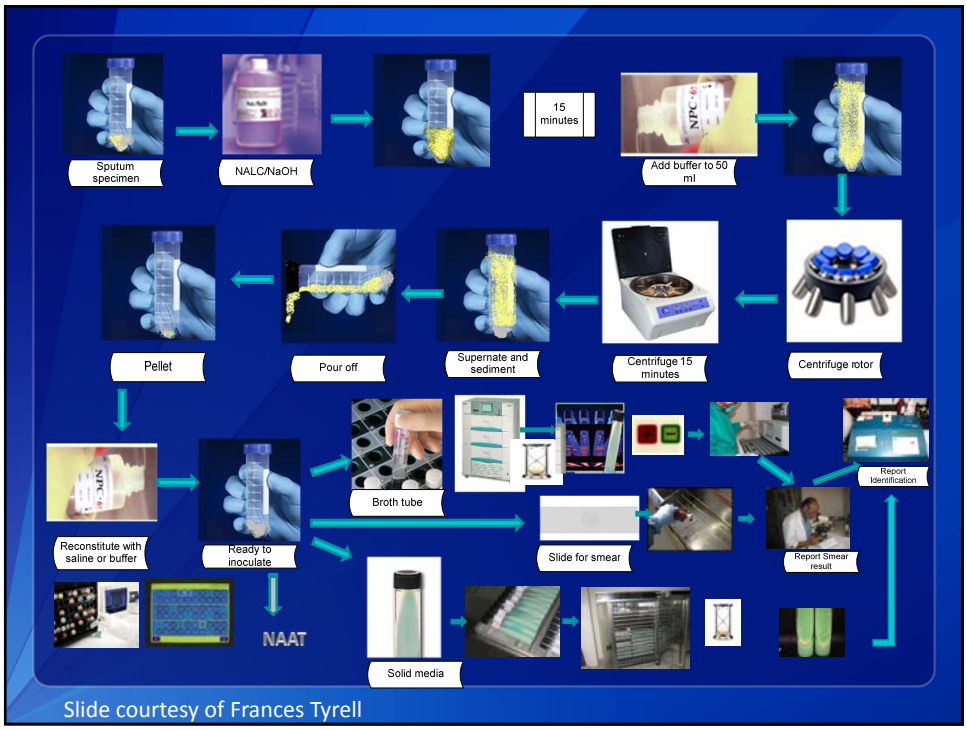
- Exponential amplification of a specific sequence of nucleic acid
- NAA helps to increase the sensitivity of the assay especially when only a few organisms may be present




- Two most common types
  - Polymerase Chain Reaction (PCR)
  - Transcription Mediated Amplification (TMA)
- Amplified nucleic acid product (amplicon) detected by specific DNA probe or analyzed by DNA sequence analysis

http://bitesizebio.com/wp-content/uploads/2007/10/pcr.gif


21st Century Algorithm
Process Specimen
1 day
AFB Microscopy
Inoculate Media
2 to 6 weeks
Culture Positive
Species Identification
2 to 3 weeks
Drug Susceptibility
Amplification-based Tests
Molecular DST

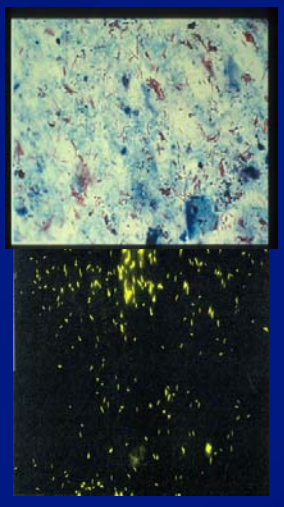

Sputum specimen
NALC/NaOH
15 minutes
Add buffer to 50 ml
Centrifuge rotor
Centrifuge 15 minutes
Supernate and sediment
Pour off
Pellet
Reconstitute with saline or buffer
Ready to inoculate
NAAT
Broth tube
Slide for smear
Report Smear result
Report Identification
Solid media
Slide courtesy of Frances Tyrell

## 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
  - 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)

## Culture Methods—Solid Media

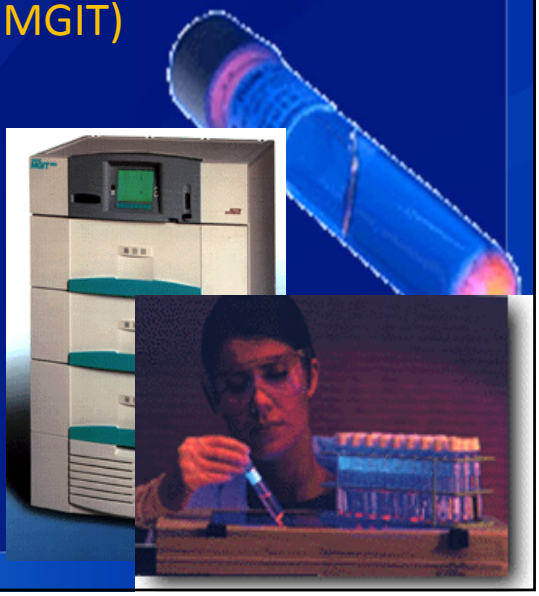
- Middlebrook agar
- Lowenstein-Jensen media
- Advantage – can see colonies on surface of media
- Incubate 6 to 8 weeks

## Culture Methods—Broth-based Systems

- (BACTEC 460); MGIT; TREK; MB/BacT
- More rapid recovery than solid media
- Current recommendations are to use at least one piece of solid media with the broth (mixed culture detection; increased sensitivity)

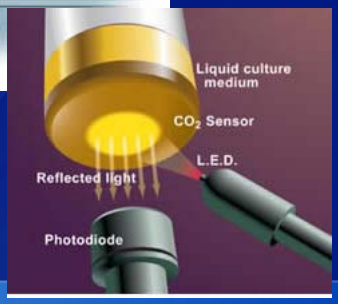
## Mycobacteria Growth Indicator Tube (MGIT)

- Fluorescence quenched by $O_2$ in $O_2$-rich media
- If mycobacteria present, $O_2$ used up, no quench, and fluoresces under UV light
- DST for INH, RMP, EMB, STR, PZA



BacT/Alert 3D

bioMerieux




VersaTrek

TREK Diagnostic Systems


## 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" assays such as PCR/RE analysis/genetic sequencing

## What does "probe positive for MTBC" mean?

1. A DNA probe was used to identify MTBC growing in culture — 0%
2. A nucleic acid amplification test was used to detect MTBC directly in a clinical specimen — 0%
3. I don't know — 0%
4. I don't care how the lab identifies it, just let me know if it is TB or not — 0%

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

- 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

## NAA Tests for Direct Detection of MTBC

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

## Limitations and Considerations

- Sensitivity
  - Reduced for smear negative specimens and some specimen types?
  - Do you want to "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

## Accuracy problems in the Mycobacteriology Lab; False-negative and False-positive results

- False-negative cultures – over-decontamination; improper collection/transport; overheating during transport/centrifugation; media not inoculated
- False-positive results - Test result on a patient's specimen (smear and/or culture) that is positive for a species of mycobacteria that in reality is not infecting the patient
  - Occur sporadically or as outbreaks
  - May result in misdiagnosis, unnecessary and costly therapy and medical treatment, unnecessary public health interventions

## False Positive Cultures

- Cross-contamination—Source may be another patient's specimen/isolate, PT specimen/isolate, QC isolate; splashes, transfer on tools, aerosols during processing; contaminated reagents
- Specimen problem—Improper specimen collection; mislabeling; specimen mix-up (not necessarily in the lab); AFB in water
- Clerical errors
- Lab should have protocol in place to detect
- Rapid genotyping can help but genotyping cannot prove it!!
- Can cause a lot of problems!!!!!

## Drug Susceptibility Testing (DST) of MTBC

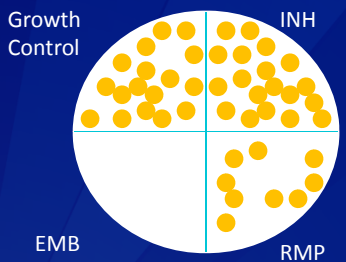
Current recommendations (Clinical and Laboratory Standards Institute [CLSI] M24-A2)

- 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

## Current Practice for DST

- For FLD, 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 a few jurisdictions – Laboratory developed tests or research use only tests
  - Performed directly on clinical specimens or on culture isolates and results available within 1–2 days
- For SLD, testing is often is performed in piecemeal fashion through referral algorithms; few laboratories with technical expertise and capacity
  - Slow turn-around-time
    - Indirect agar proportion takes ~28 days after isolation from culture
  - Some laboratories have verified and validated methods for broth-based testing

## 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 in RLT
- 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 ≥1% of the colonies on drug-free media

## DST in Broth Systems

- Selection of critical (testing) concentrations based on comparison of results with agar proportion = "equivalent critical concentrations"
- Much more rapid results (5-7 days) than agar proportion (21-28 days)
- FDA cleared for first-line drugs
  - MGIT – IRES, Z
  - TREK – IRE, Z
- Published evaluations of second-line drugs

## Problems/Concerns with Current DST Practices

- Most testing algorithms based on referrals of specimens/isolates
- Lack of confidence/reluctance of labs to report resistance prior to confirmation
- Discordant results – inter- and intra-lab, different methods, etc.
- Manpower/training issues

## Molecular Detection of Drug Resistance (Molecular DST)

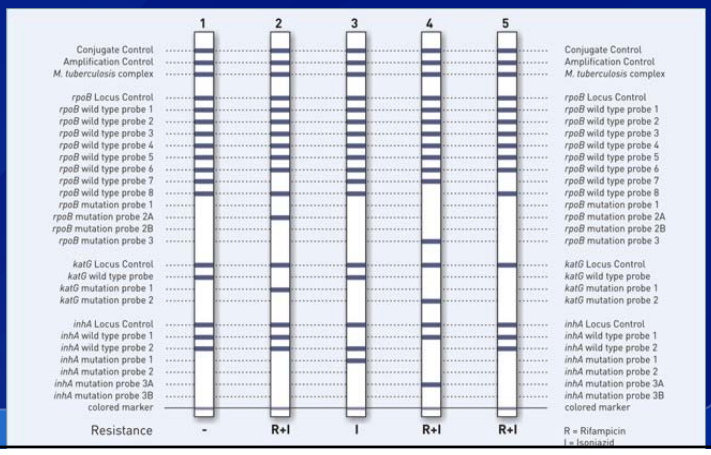
- Examining DNA of specific genes for mutations known to be associated with phenotypic resistance
  - Mutations in what genes are associated with resistance?
  - Where are the mutations within the gene?
  - Some areas are "hot spots"—resistance determining regions
- DNA sequence examined may be important for protein expression, code for the protein itself, or code for rRNA

## What tests are being used for molecular detection of drug resistance?

- Laboratory developed tests (LDT)
  - DNA sequencing
  - Real-time PCR assays
- Non-FDA approved tests (Research Use Only [RUO])
  - Genotype® MTBDRplus and MTBDRsl- *Hain* Lifescience
  - Cepheid GeneXpert® Xpert MTB/RIF

## Genotype MTBDR*plus*

- 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



## 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

## Cepheid Xpert MTB/RIF Assay

## CDC's MDDR Service (Molecular Detection of Drug Resistance)

- Implemented in September 2009 (CLIA compliant)
- Comprehensive clinical service to domestic TB control programs and clinicians
  - Rapid confirmation of RMP-resistant and MDR TB
  - Laboratory testing data available about SLD resistance in cases of RMP-resistant or MDR TB
- New technologies may fill the role in the future but demand exists now

## Criteria for MDDR Testing Version 2.0*

- Isolate or NAAT (+) sediments (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

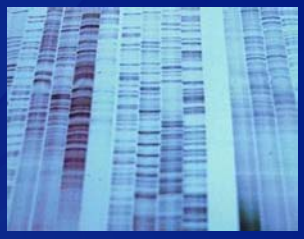
## MDDR Service Description

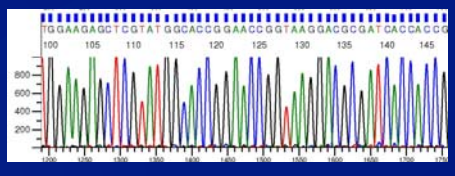
- Pyrosequencing
  - RMP (*rpoB*) and INH (*katG*, *inhA*)
- Sanger Sequencing*
- Conventional DS T performed in parallel

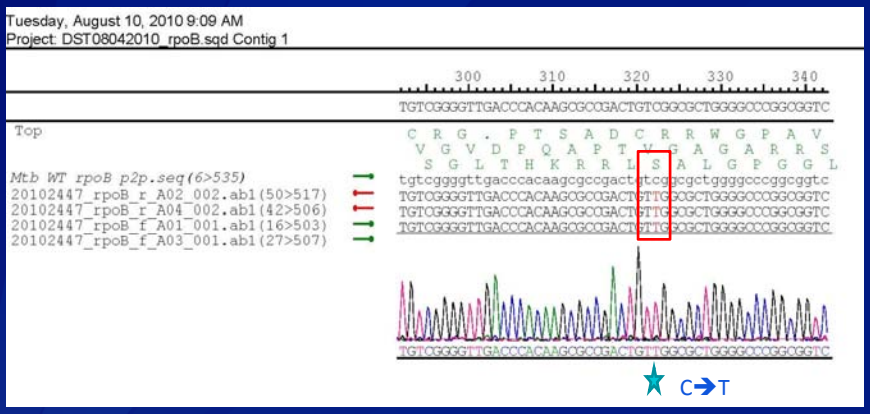
* Campbell, PJ, et al. 2011. Antimicrob Agents Chemother 55:2032-2041.

## Conventional (Sanger) DNA Sequencing

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








Most commonly observed *rpoB* mutation:

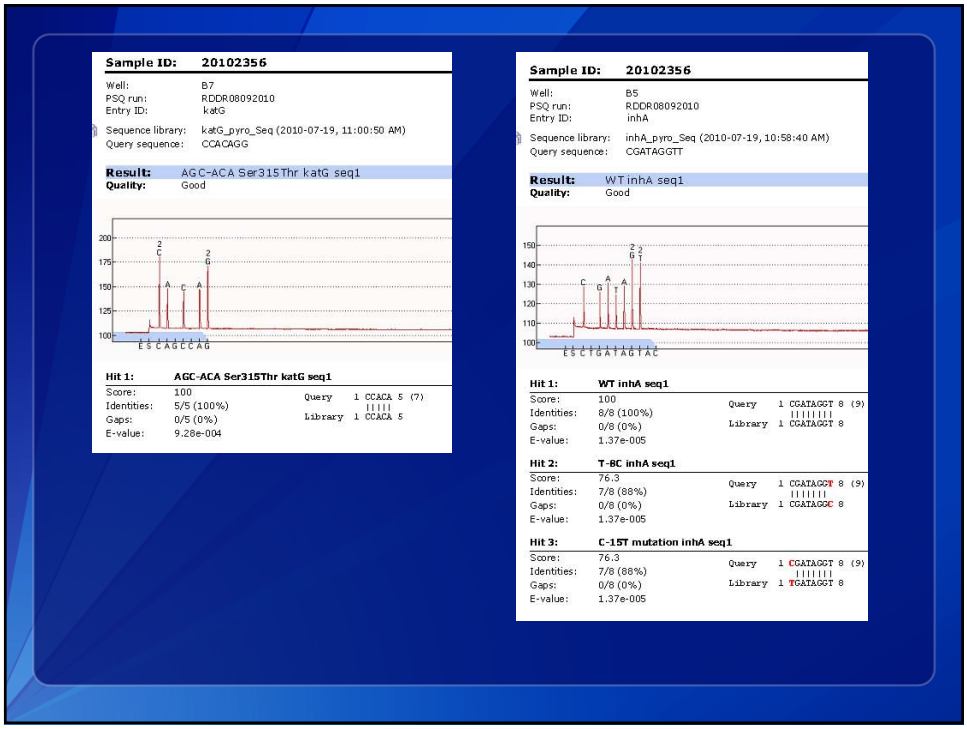
TCG>TTG Ser531Leu

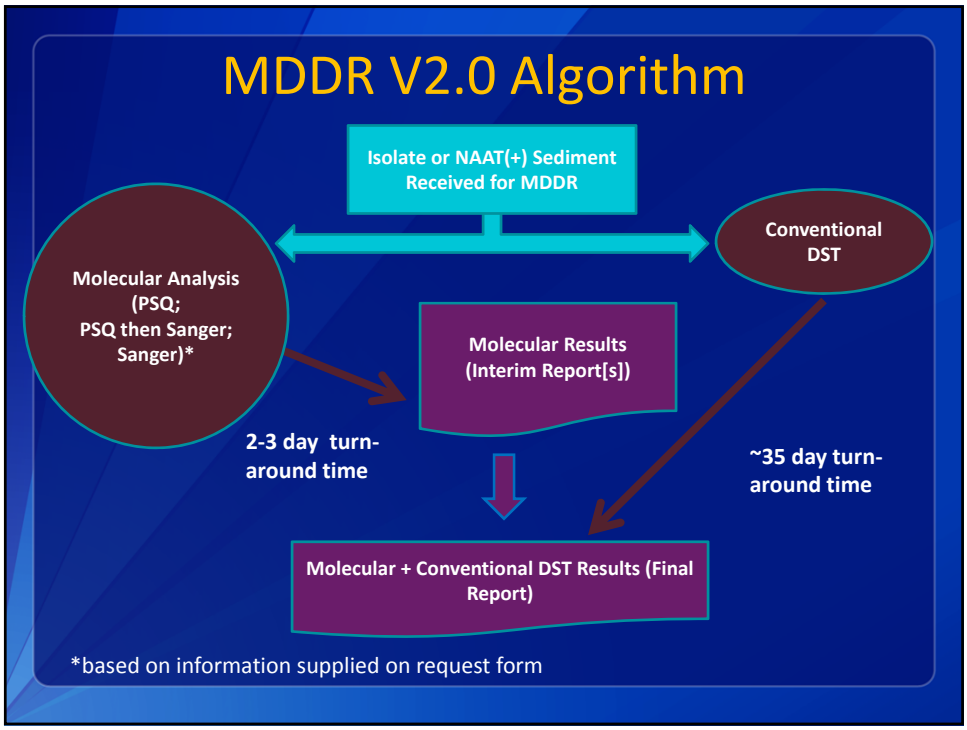
## Pyrosequencing

Direct DNA sequencing of PCR products

- Unique chemistry - detection of released pyrophosphate
- Visible light is generated that is proportional to the number of incorporated nucleotides

- Instrument carries out DNA sequencing reaction and analysis <2 hrs


Sample ID: 20102356
Well: B7
PSQ run: RDDR08092010
Entry ID: katG
Sequence library: katG_pyro_Seq (2010-07-19, 11:00:50 AM)
Query sequence: CCACAGG
Result: AGC-ACA Ser315Thr katG seq1
Quality: Good
Hit 1: AGC-ACA Ser315Thr katG seq1
Score: 100
Identities: 5/5 (100%)
Gaps: 0/5 (0%)
E-value: 9.28e-004
Query 1 CCACA 5 (7)
Library 1 CCACA 5
Sample ID: 20102356
Well: B5
PSQ run: RDDR08092010
Entry ID: inhA
Sequence library: inhA_pyro_Seq (2010-07-19, 10:58:40 AM)
Query sequence: CGATAGGTT
Result: WT inhA seq1
Quality: Good
Hit 1: WT inhA seq1
Score: 100
Identities: 8/8 (100%)
Gaps: 0/8 (0%)
E-value: 1.37e-005
Query 1 CGATAGGT 8 (9)
Library 1 CGATAGGT 8
Hit 2: T-8C inhA seq1
Score: 76.3
Identities: 7/8 (88%)
Gaps: 0/8 (0%)
E-value: 1.37e-005
Query 1 CGATAGGT 8 (9)
Library 1 CGATAGGC 8
Hit 3: C-15T mutation inhA seq1
Score: 76.3
Identities: 7/8 (88%)
Gaps: 0/8 (0%)
E-value: 1.37e-005
Query 1 CGATAGGT 8 (9)
Library 1 TGATAGGT 8


MDDR V2.0 Algorithm
Isolate or NAAT(+) Sediment Received for MDDR
Molecular Analysis (PSQ; PSQ then Sanger; Sanger)*
Conventional DST
Molecular Results (Interim Report[s])
2-3 day turn-around time
~35 day turn-around time
Molecular + Conventional DST Results (Final Report)
*based on information supplied on request form

## MDDR Service: Drugs and Genes for Panel

PSQ

MDR TB

- Rifampin — *rpoB* (81bp region)
- Isoniazid — *inhA* (-15)
- Isoniazid — *katG* (Ser315)

XDR TB

- Ethambutol — *embB* (Met306, Gly406)
- Pyrazinamide — *pncA* (promoter and coding regions)
- Fluoroquinolones — *gyrA* (coding region)
- Amikacin, Kanamycin, Capreomycin — *rrs* (nt1401/1402,1484)
- Kanamycin — *eis* (promoter region)
- Capreomycin — *tlyA* (coding region)

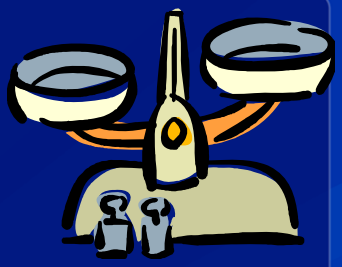
## Sensitivity and Specificity of Loci*

| Drug | Gene(s) | Sensitivity | Specificity |
|---|---|---|---|
| RMP | *rpoB* | 97 | 97 |
| INH | *inhA, katG* | 86 | 99 |
| FQ | *gyrA* | 79 | 99 |
| KAN | *rrs, eis* | 87 | 99 |
| AMK | *rrs* | 91 | 98 |
| CAP | *rrs, tlyA* | 55 | 91 |
| EMB | *embB* | 79 | 94 |
| PZA | *pncA* | 86 | 96 |

*Analysis of 550 clinical isolates (2000-2012); compared to AP results (MGIT 960 for PZA)

# How to report results?

## Weighing Genotypic versus Phenotypic Results



- The term 'Gold Standard' can be misleading.
- New, previously uncharacterized or poorly characterized mutations
  - Reported as clinical significance unknown
  - Anecdotal information may be reported
- Functional genetic analysis is necessary to definitely determine effect of mutation on resistance
- Need to develop standardized reporting language

National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention (NCHHSTP)
Division of Tuberculosis Elimination (DTBE) Laboratory Branch
Reference Laboratory

CDC
Centers for Disease Control and Prevention

**Report Status: Interim**

**CLIA ID # 11D0668319**

*Original Submitter:*

*Submitter to CDC:*

*CDC Specimen ID:* 2012
*Specimen:* *M. tuberculosis* complex isolate
*Medium:*

*Date Collected:* //2012
*Date Received:* //2012
*Date Reported:* //2012

*Patient:* *Submitter Specimen Identifiers:*

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

| Locus (region) examined* | Result | Interpretation (based on in-house evaluation of 550 clinical isolates) |
|---|---|---|
| rpoB (RRDR) | **Mutation:** TCG>TTG; Ser531Leu | Rifampin resistant. (100% of isolates in our in-house evaluation of 550 clinical isolates with this mutation are RMP-R.) |
| inhA (promoter) | No mutation | 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.) |
| katG (ser315 codon) | No mutation | |

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

**MDDR assays were developed and the performance characteristics determined by the DTBE Reference Laboratory. They have not been cleared or approved by the Food and Drug Administration.**

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

## Benefits of Molecular Detection of Drug Resistance

- Rapid results within days as compared to weeks for conventional testing
- Expedite further conventional testing (e.g., second-line drug susceptibility testing)
- High throughput
- Some assays are "closed systems"—reduces potential for cross contamination
- Development of technologies requiring limited biosafety infrastructure; does not require BSL-3 once DNA is extracted
- Information provided by some platforms may be used to enhance accuracy of conventional DST

## Limitations and Considerations (review)

- Not all mechanisms of resistance are known and the lack of a mutation ≠ susceptibility
- Limited genes and sites are targeted
- Emerging resistance (mixed populations) may not be detected; limit of detection
- Not all mutations are associated with phenotypic resistance
  - Silent (synonymous) mutations—no change in protein
  - Neutral polymorphisms (e.g., *gyr*A codon 95 may be Ser or Thr )
  - Output is platform dependent

## Limitations and Considerations (review 2)

- Still filling in gaps in knowledge about drug resistance (phenotypic and genotypic testing)
- "Gold-standard" DST may not be perfect
  - Mutations resulting in elevated MICs but S at critical concentration (e.g., Leu511Pro in *rpo*B)
- Clinical utility- Do results impact patient care? Will clinicians "trust" these results or "wait for the conventional DST result?"
- Expertise of staff
  - Output from the assay depends on the platform; Need to understand platform to understand limitations
- Educational partnerships (laboratory, program, and clinicians) need to be developed

## Understanding Discordance

- Can have discordant results
  - Between different phenotypic DST results (e.g., MGIT 960 and agar proportion)
  - Between phenotypic and genotypic (molecular) results
  - Between different genotypic results (e.g., GeneXpert and Sanger sequencing)
- Can occur within a lab, between labs, between different methods, and with in the same method
- Which is correct?
  - Both, only one, neither

## What causes discordant DST results?

- "Human error/lab error"
  - Transcription, labeling errors
  - Cross contamination/specimen mix-up
- Different "inoculum"/bacterial population
  - e.g., isolates from different specimens; sampling from same specimen; original isolate vs. subculture
  - Size of inoculum/clumps
  - Different growth kinetics
- Different method or media
  - "equivalent" critical concentrations
  - "calling" result too soon
- The "bug" - MIC is close to the critical concentration
  - Evaluations performed with "highly resistant" bugs

## What causes discordance between molecular and phenotypic DST results?

- "Human error/lab error"
- Not all mechanisms of resistance are known
  - the lack of a mutation ≠ susceptibility
- Limited genes and sites are targeted
- Emerging resistance (mixed populations)
  - may not be detected; limit of detection
- Not all mutations are associated with phenotypic resistance
  - Silent (synonymous) mutations—no change in protein
  - Neutral polymorphisms (e.g., *gyr*A codon 95 may be Ser or Thr)
- Output is "platform dependent"
- "Gold-standard" DST may not be perfect
  - Mutations resulting in elevated MICs but S at critical concentration (e.g., Leu511Pro in *rpo*B)

## What causes discordance between different molecular platform results?

- "Human error/lab error"
- Not necessarily looking at the same segment of DNA
  - looking for a particular single nucleotide polymorphism (SNP) in one codon versus looking at 30 codons
- Limited genes and sites within genes are targeted
  - *katG* only versus *katG+inhA*
- Emerging resistance (mixed populations)
  - may not be detected; limit of detection

# Cases

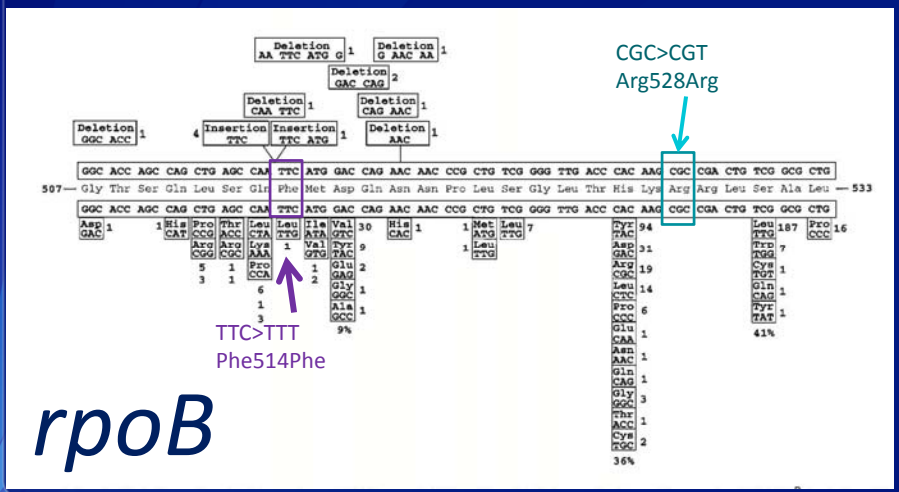
# Case # 1—Is it RMP-R?
# (RMP Discordance between molecular and conventional results)

- 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, *rpo*B DNA sequence – Phe514Phe

# SILENT MUTATIONS

Nucleotide changes that do not result in a change in amino acid sequence



## 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

## What is a possible explanation for the RMP discordance between the 2 tests at the PHL?

1. Emergence of RMP-R 0%
2. The 460 "missed" RMP-R 0%
3. AP is wrong (false-R) 0%
4. Results can be different since you are testing at different drug concentrations 0%
5. Any one of the above 0%

## How do you interpret the RMP results?

1. S to RMP
2. R to RMP
3. I don't know

## Recap

- State PHL—discordant RMP DST results
  - Bactec 460—S to RMP (2 µg/ml)
  - AP (7H10)—80% R to RMP (1 µg/ml)
- CDC—RMP-R according to the mutation detected by *rpoB* sequencing

- CDC— 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
- *rpo*B DNA sequence — Asp516Tyr; RMP resistant
- CDC AP — RMP-S

## How do you interpret the RMP results?

1. S to RMP
2. R to RMP
3. I don't know

## rpoB mutations associated with highly discordant DST results

(van Deun, A. et al. 2009, J Clin Microbiol. 47:3501-3506)

- "Low–level" or "borderline" resistance
- 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

## Clinical failures associated with *rpoB* mutations in phenotypically occult MDR TB

(Williamson, DA, et al. 2012, Int J Tuberc Lung Dis, 16:216-220)

- Significant association between the presence of *rpoB* mutations that are not detected in DST and treatment failure
- 94 patients INH-R, RMP-S by MGIT DST (2004-2010)
  - 4 had *rpoB* mutations (GeneXpert)
    - RRDR sequenced—Leu511Pro/Met515Ile, His526Asn/Ala532Val, Asp516Tyr, His526Leu
    - 3 of 4 were treatment failures; other was unknown

# Asp516Tyr (CDC MDDR) (cases #2 and 3)

| | RMP-R by DST # with mutation/Total | RMP-S by DST # with mutation/Total |
|---|---|---|
| Original (retrospective) validation | 2/152 | 0/102 |
| Prospective validation | 0/17 | 1/63 |
| Service (9/2009—2/2011) | 2/84 | 3/143 |
| Total | 4/253 | 4/308 |

# Case #4—Persistently smear (+) patient with drug susceptible MTBC

- MDDR
  - *rpoB* , *inhA*, *katG* all wildtype (no mutations)
  - pncA – mutation with unknown significance
- *AP DST*
  - R to INH, S to RMP
- MGIT PZA—S

## How do you interpret the INH results?

1. S to INH; the DST is incorrect
2. R to INH; ~10% of INH-R MTBC do not have mutation in *inhA* or *katG*
3. I don't know

## Case #5—Discordant results for INH and RMP between 2 laboratories

- Lab A; INH-R and RMP-R (MGIT)>>MDR
- Lab B; INH-S and RMP-S (MGIT and AP)
- MDDR
  - *rpoB* , *inhA*, *katG* all wildtype (no mutations)
- *AP DST*
  - S to INH, S to RMP

## What are the possible reasons for the discordant results?

1. Specimen mix-up 0%
2. Technical error in lab A 0%
3. Lab B and CDC did not test same bug as labA 0%
4. All of the above 0%
5. I don't know 0%

## Case # 6—Is it RMP-R?

- Isolate submitted for MDDR
  - HIV+, prison, Mexico, intermittent therapy, "funky" RMP on Bactec 460
- CDC *rpo*B —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—His536Tyr (100% of isolates with this mutation are RMP-R)

## What is a possible explanation for the RMP discordance?

1. Emergence of RMP-R 0%
2. Proportion of MTBC resistant to RMP below the limit of detection of the MDDR assay 0%
3. Sampling problem 0%
4. Any of the above 0%

## Case # 7—Is it MDR?

- Born in Philippines; previously treated for TB in 2006
- CDC received request for MDDR 3/29
- Isolate also sent to another laboratory for "molecular beacons"-based laboratory developed test (LDT)

## Molecular Results

- Molecular Beacons:
  - A mutation associated with rifampin resistance has been detected
  - Mutations associated with INH resistance have NOT been detected in katG and inhA promoter

## Is this MDR TB?

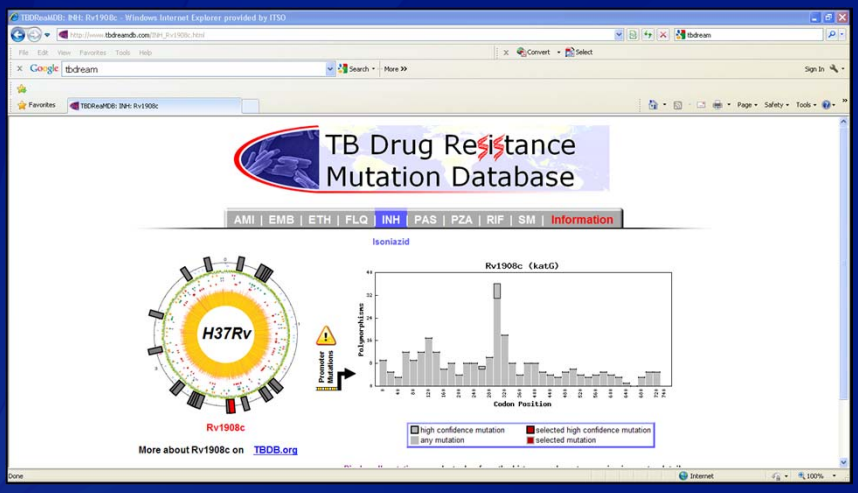
1. No, only “RMP mono R” 0%
2. Maybe, molecular beacon assay not 100% sensitive for detection of INH resistance 0%
3. Yes, RMP resistance absolutely indicates MDR 0%
4. It might <u>not</u> even be resistant to RMP 0%

## Molecular Results

- CDC MDDR 3/30:

| Locus | Result | Interpretation |
|---|---|---|
| rpoB (RRDR) | Ser531Leu | RMP resistant |
| inhA (promoter)<br>katG (ser315codon) | No mutation<br>Thr380Ile | Clinical significance of katG mutation is unknown. Cannot rule out INH resistance. |
| embB | Met306Val<br>(Leu355Leu; Glu378Ala) | EMB resistant |
| pncA | Ala134Val | Clinical significance of pncA mutation is unknown. Cannot rule out PZA resistance |
| gyrA | No mutation | Cannot rule out fluoroquinolone resistance. |
| rrs<br>eis<br>tlyA | No mutation<br>No mutation<br>No mutation | Cannot rule out injectable resistance. |

## TBDream and Literature Search



## Actual Interpretive comments for INH

The clinical significance of this katG mutation for prediction of INH resistance is unknown.

Cannot rule out INH resistance. (89% of INH-R isolates in our in-house evaluation of 254 clinical isolates have a mutation, other than the one detected, at one or both of these loci.)

## Is this MDR TB?

1. No, only "RMP mono R"
2. Maybe, need to wait for INH DST
3. Yes, the katG mutation definitely means it is resistant to INH

## Recap

- Molecular testing results
  - Patient has RMP-R TB;  also R to EMB and maybe PZA
  - "conflicting" results for INH
    - Molecular beacons – no mutations
    - MDDR – mutation in *katG*; unknown clinical significance

## Would you use INH?

1. Yes
2. No
3. Yes, but not "count on it"

## How do you interpret conflicting *katG* results?

1. Molecular beacons right and MDDR wrong — 0%
2. MDDR right and molecular beacons wrong — 0%
3. Both correct; assays are not necessarily comparable — 0%
4. Don't know — 0%

## "Differences" in Testing Platforms

- Molecular Beacons
  - Target codons 312-317
  - Detecting wild type
- MDDR
  - Covers codons 258-408
  - Identifies actual mutations
- Mutations listed in TBDream cover codons 1-735 and the promoter region

## MDDR Data (through 2/2011)

- Of 335 INH-R MTBC,
  - 47 (14.0%) are wild-type *inhA* and *katG*
  - 38 (11.3%) have *inhA* mutation
  - 27 (8.1%) have an *inhA* and *katG* mutation
  - 223 (66.6%) have *katG* mutation
- Of 250 with *katG* mutation,
  - 246 (98.4%) have mutation at codon 315
    - 241 (96.4%) are Ser315Thr

## What is more desirable? An assay with the problem of false-R or an assay with the problem of false-S?

[T]here are known knowns; there are things we know we know. We also know there are known unknowns; that is to say we know there are some things we do not know.
But there are also unknown unknowns – there are things we do not know we don't know.

—Former United States Secretary of Defense Donald Rumsfeld

## Case 7 Denouement—
## DST obtained from initial testing lab and reference lab

| | MGIT | AP |
|---|---|---|
| INH | R | R |
| RMP | R | R |
| EMB | R | R |
| STR | | S |
| PZA | R | R |
| AMK | | S |
| CAP | | S |
| CIP | | S |
| OFL | | S |
| ETH | | R |
| PAS | | S |

## 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)
- 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
Tracy Dalton, PhD
Jeff Driscoll, PhD
James Posey, PhD
Angela Starks, PhD

BMetchock@cdc.gov
(404) 639-1285



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