CDC's Approach to Fast Track Laboratory Diagnosis for Persons at Risk of Drug Resistant TB: Molecular Detection of Drug Resistance (MDDR) Service

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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

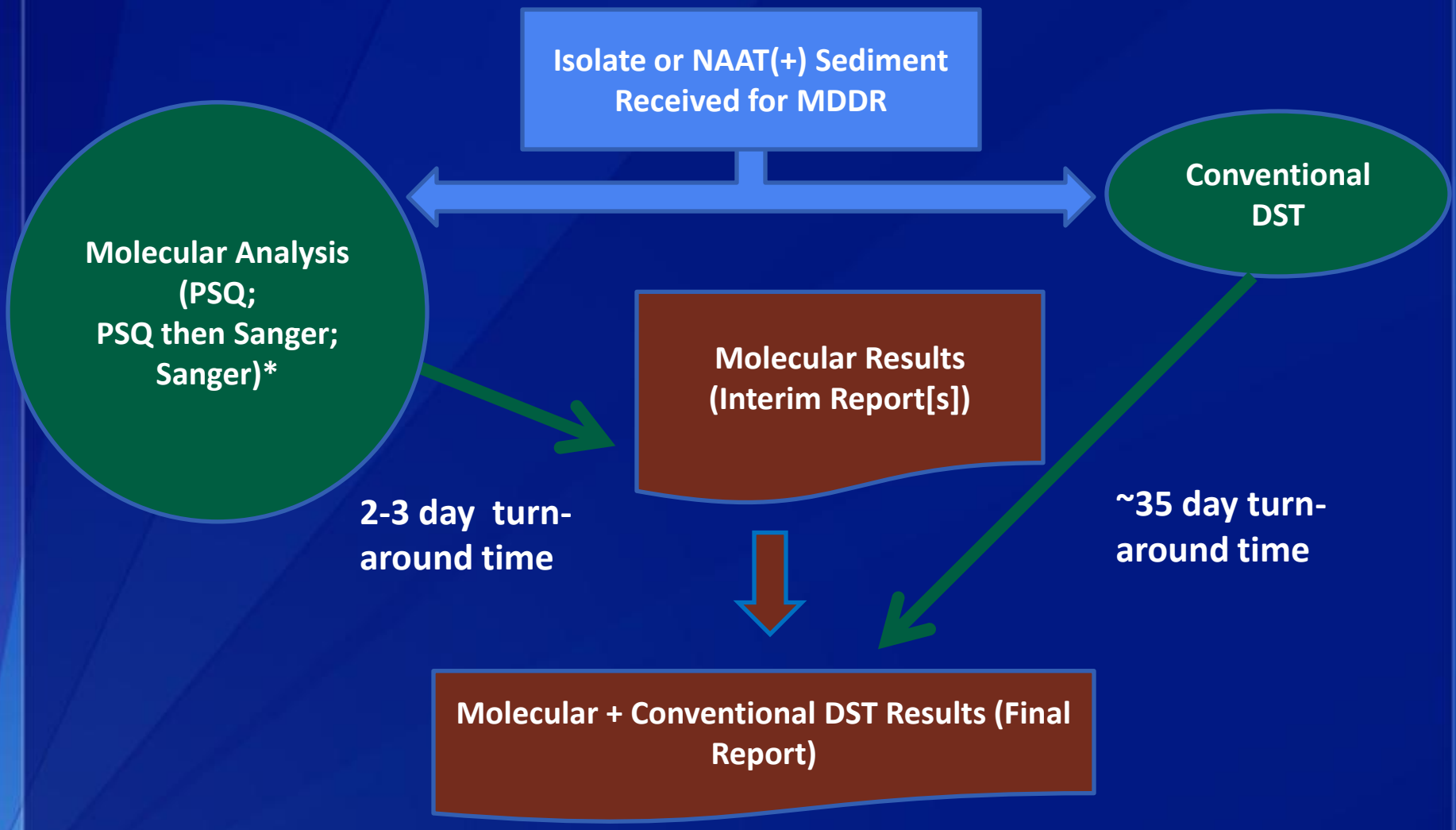
MDDR Service: Sanger Sequencing Drugs and Genes for Panel

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

MDR TB

XDR TB

CDC's Molecular Detection of Drug Resistance Service



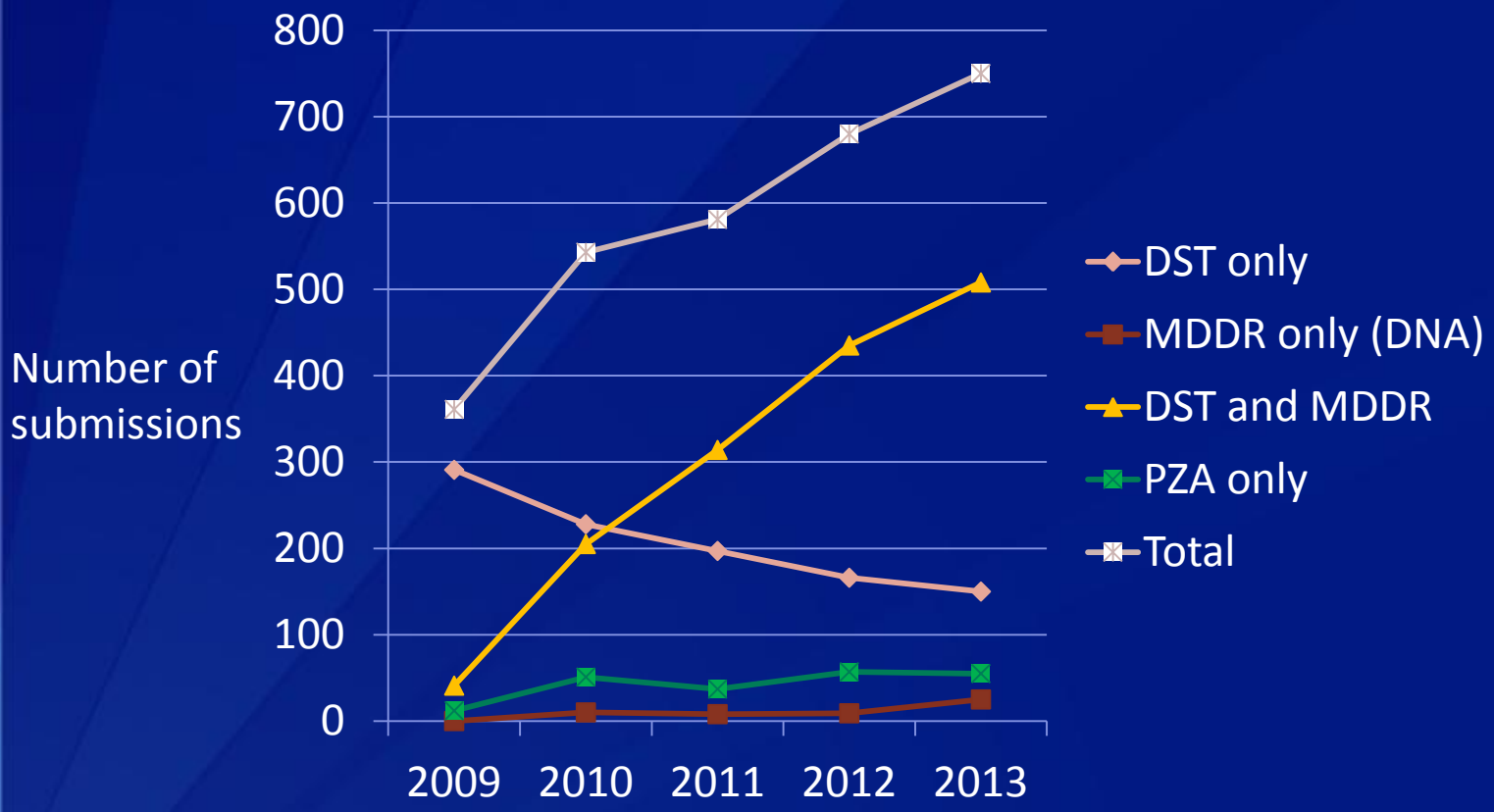
*based on information supplied on request form

http://www.cdc.gov/tb/topic/laboratory/MDDRUsersGuide.pdf

"Differences" in Testing Platforms (simplified version)

- Sanger sequencing
  - Identifies actual mutations
- Pyrosequencing
  - Identifies actual mutations
  - Minor or mixed populations are harder to detect
  - Sequence small regions of DNA
- GeneXpert MTB/RIF
  - Detects wild type sequence and thus may miss mixed populations; cannot distinguish synonymous mutations from clinically relevant mutations
- Hain
  - Interpretation of banding pattern may give indication of actual mutation

State Public Health Laboratory Submissions to RLT



MDDR TAT 2013

Indicator	Goal	JAN-MAR	APR-JUN	JUL-SEP	OCT-NOV
N		111	130	135	159
Range (d)		1-5	1-4	1-8	1-7
Mean (d)	<=3 d	2	2	2	2
Median (d)		1	1	1	1
80% by:	4 d	3	2	3	3
%<=4 d	80%	99.1%	100%	99.3%	89.3%

MDDR results for INH and RMP, 2013*

	No.	%
INH-S, RMP-S	303	56.7
INH-R, RMP-S	60	11.2
INH-S, RMP-R	18	3.4
INH-R, RMP-R	90	16.8
No amplification	47	8.8
Not done / outbreak	17	3.2
Total	535	

~50% PSQ (INH and RMP) only; of these, 5% reflexed to Sanger (full panel) due to detection of RMP=R

~30% processed sediments; 80% successfully amplified

5% DNA

*provisional data

CASES

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

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: GCG>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	

LJ slant
Known MDR (MGIT DST X3)

Collection Date: 11/8/2013
CDC contacted: 2/5/2014 (Wednesday)
Date sent to CDC: 2/6/2014 (Thursday)
Date received at CDC: 2/7/2014 (Friday)

Sanger Sequencing Report issued 2/10/2014

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)	Silent and Neutral mutations	Cannot rule out Ethambutol resistance.(79% of isolates have a mutation at this locus.)
pncA (promotor, coding region)	Mutation: ATT>ACT; Ile133Thr	Cannot rule out PZA resistance; significance of this mutation is unknown
gyrA (QRDR)	No mutation	Cannot rule out FQ resistance. (80% of FQ-R isolates have a mutation at this locus.)
rrs (1400 region)	No mutation	Cannot rule out resistance to injectable drugs (KAN, AMK, CAP).
eis (promotor)	No mutation	
tlyA (entire ORF)	No mutation	

TAT from specimen collection date: 94 days

TAT within CDC: 3 days

Neck aspirate sediment
Known RMP-R (GeneXpert, probe B)

Collection Date: 8/28/2013 (Wednesday)
CDC contacted: 9/6/2013 (Friday)
Date sent to CDC: 9/9/2013 (Monday)
Date received at CDC: 9/11/2013 (Wednesday)

Sanger Sequencing Report issued 9/13/2013

Locus (region) examined	Result	Interpretation
rpoB (RRDR)	Silent Mutation: TTC>TTT; Phe514Phe	Probably RMP susceptible. (96% of RMP-R isolates have a mutation, other than the one detected, 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	
embB (Met306, Gly406, other)	No mutation	Cannot rule out EMB resistance.(79% of EMB-R isolates have a mutation at this locus.)
pncA (promotor, coding region)	No mutation	Cannot rule out PZA resistance.
gyrA (QRDR)	No mutation	Cannot rule out FQ resistance. (80% of FQ-R isolates have a mutation at this locus.)
rrs (1400 region)	No mutation	Cannot rule out resistance to injectable drugs (KAN, AMK, CAP).
eis (promotor)	No mutation	
tlyA (entire ORF)	No mutation	

TAT from specimen collection date: 16 days

TAT within CDC: 2 days

Review of Cases

- Rapidly rule out resistance in a case of public health importance
- Rapidly identify MDR / XDR TB
- Rapidly confirm MDR TB
- Rapidly rule out RMP-R

Acknowledgements

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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.