GeneXpert MTB/RIF Performance Characteristics in a State Public Health Laboratory Context
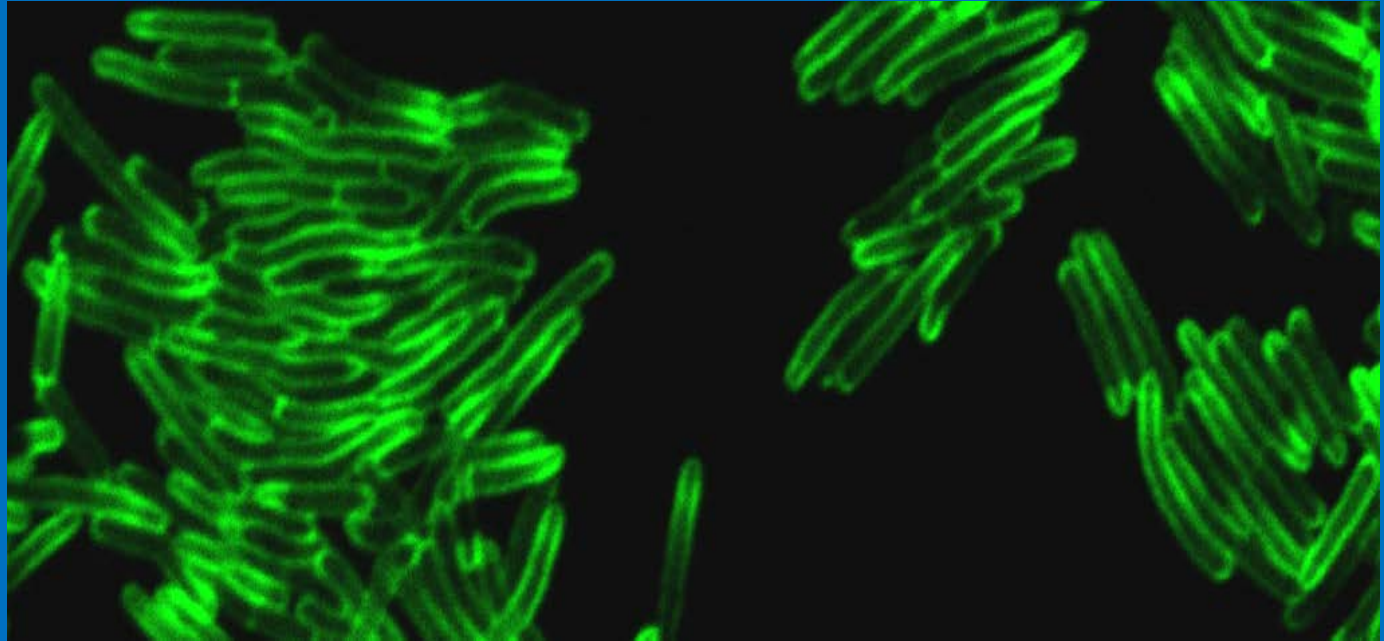

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Texas Dept. of State Health Services

June 9, 2015

APHL 9th National Conference on Laboratory Aspects of TB

Outline

- Rifampin Resistance Background
- Xpert Mechanism
- Texas Public Health TB Lab Overview
- Xpert Performance
  - Detection of Mtb complex
  - Detection of Rif resistance
  - Effects of specific mutations

Genetics of Rifampin Resistance in *M. tuberculosis*

81 base pair rpoB Hot Spot

S. Ramaswamy & J. Musser 1998. Tubercle and Lung Dis. 79:3-29
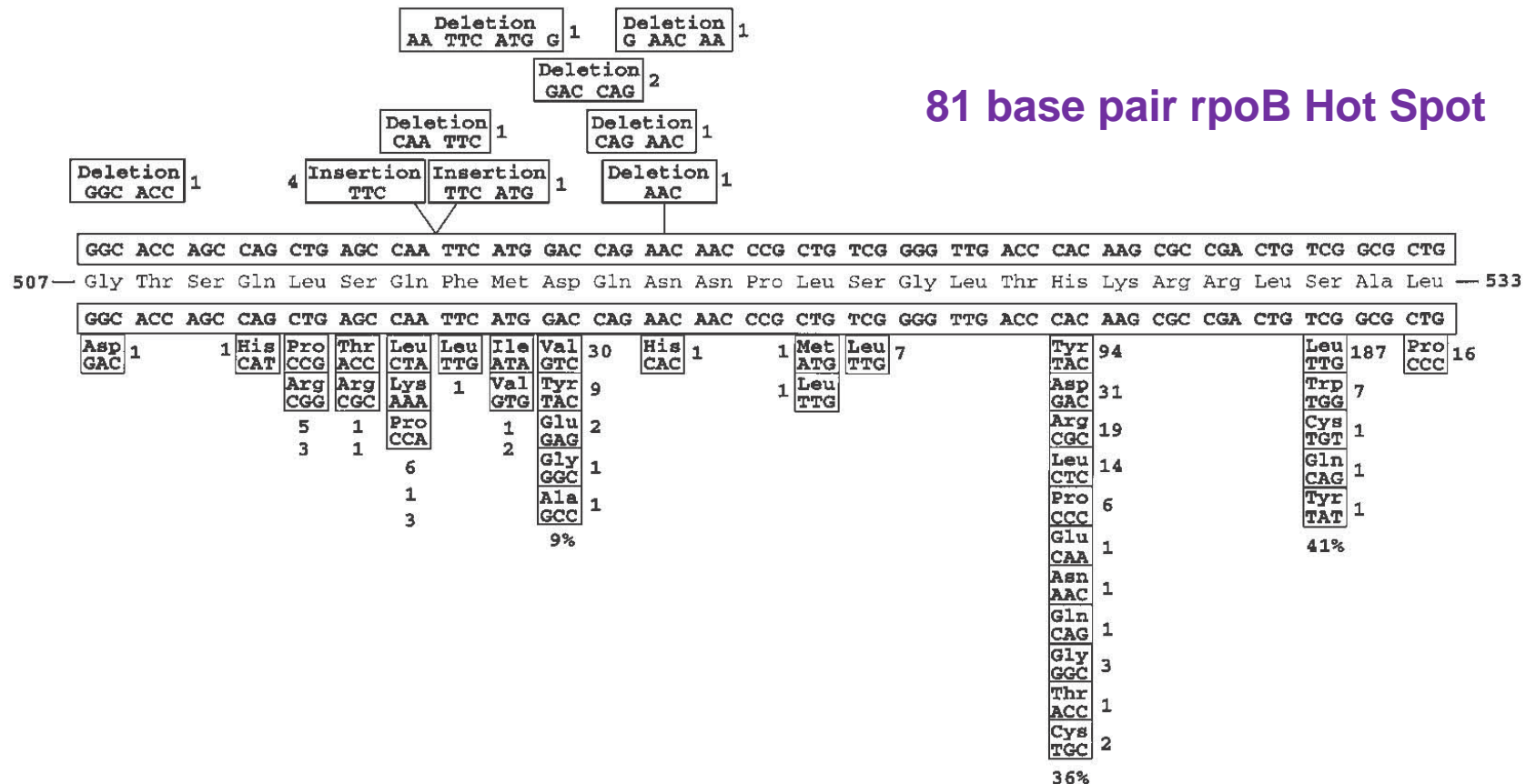
rpoB Mutations

- Synonomous or Silent Mutation
  - Change in the nucleotide sequence
  - No change in the amino acid or protein
  - Not associated with drug resistance
  - Not common but neither is rifampin-R in U.S.

- Nonsynonomous Mutation
  - High Rifamycin MIC
    - Abandon all hope to use rifamycin
  - Van Deun or Disputed mutation
    - low rifamycin MIC increase
    - Rifampin may test susceptible, rifabutin may be active
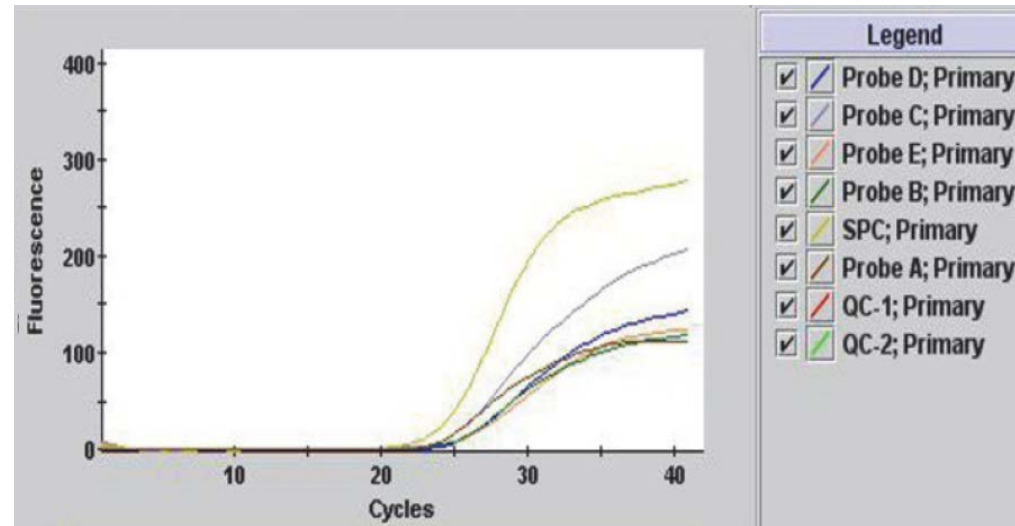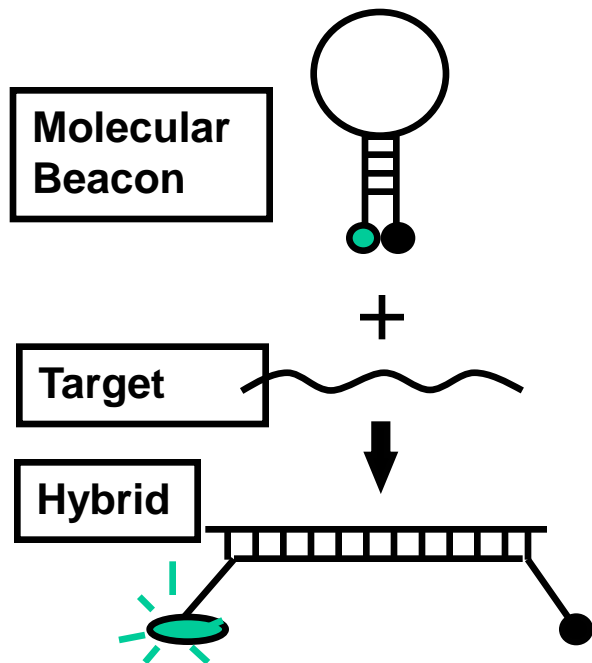
GeneXpert MTB/Rif Assay Design

A   C   D

```
5'- GCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTGTCGGCGCTG - 3'
3'- CGTGGTCGGTCGACTCGGTTAAGTACCTGGTCTTGTTGGGCGACAGCCCCAACTGGGTGTTCGCGGCTGACAGCCGCGAC - 5'
```

B   E

The MTB assay target is the 81 bp rifampin resistance determination region of the rpoB gene.

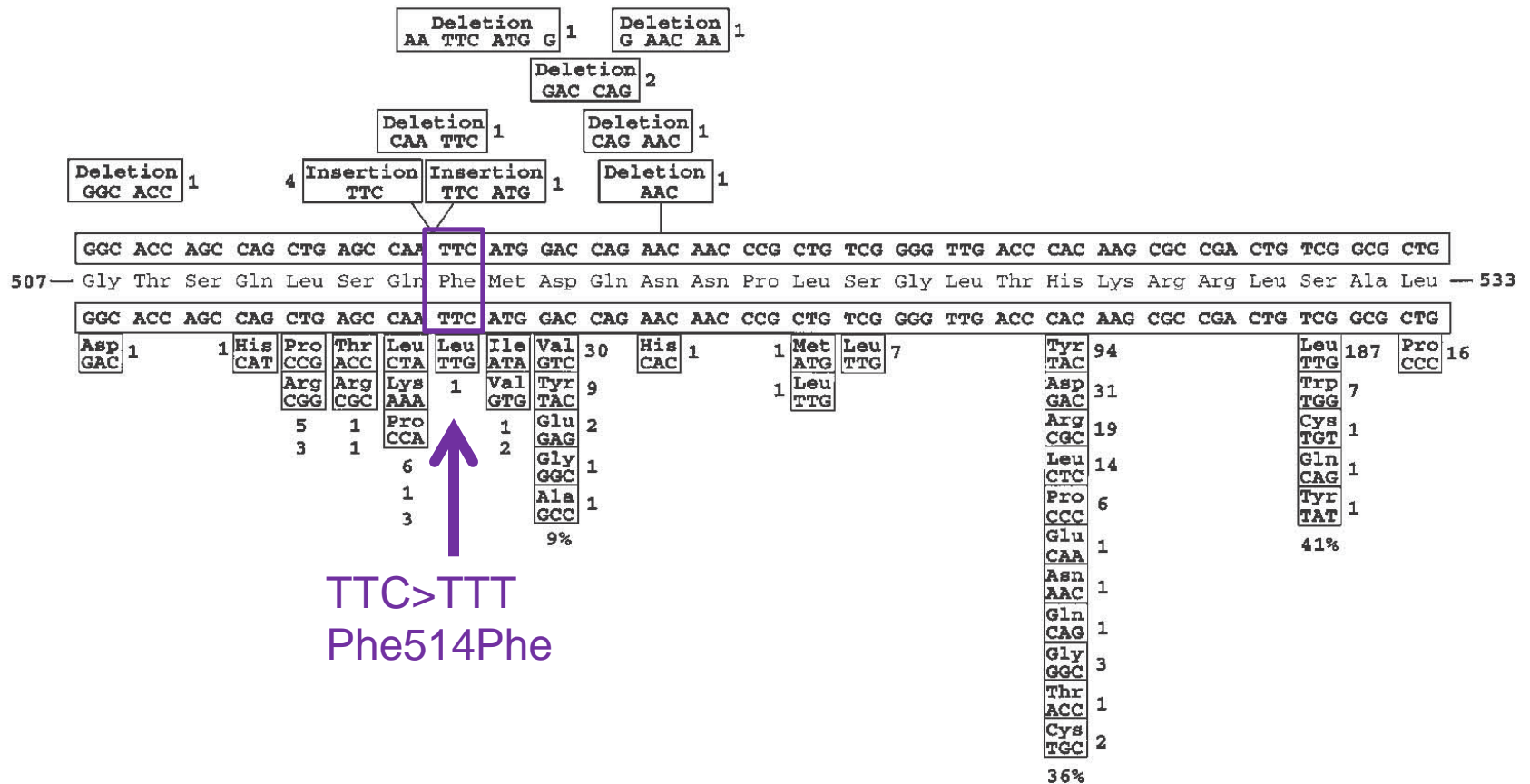
Molecular Beacon

+

Target

Hybrid

Each probe is labeled with a different fluorophore, permitting simultaneous detection of the presence of wild type.

Example of Rif-Susceptible Profile – all 5 probes and SPC are positive

Legend
- Probe D; Primary
- Probe C; Primary
- Probe E; Primary
- Probe B; Primary
- SPC; Primary
- Probe A; Primary
- QC-1; Primary
- QC-2; Primary

Xpert Algorithm

- "MTB DETECTED" is reported when at least two probes result in Ct values within the valid range and a delta Ct min (the smallest Ct difference between any pair of probes) of less than 2.0.

- "Rif Resistance NOT DETECTED" is reported if the delta Ct max (the Ct difference between the earliest and latest probe) is ≤4.0.

- "Rif Resistance DETECTED" is reported if the delta Ct max is >4.0.

Ct	Mtb Amount Detected	Typical AFB Smear Result
<16	High	3+ or 4+
16-22	Medium	2+
22-28	Low	1+
>28	Very Low	Negative

Silent Mutations

S. Ramaswamy & J. Musser 1998. Tubercle and Lung Dis. 79:3-29

Silent Mutation

PROBE A

5¹ - GCACCAGCCAGCTGAGCCAATTCATGGACCAG

507 508 509 510 511 512 513 514 515 516 517 s

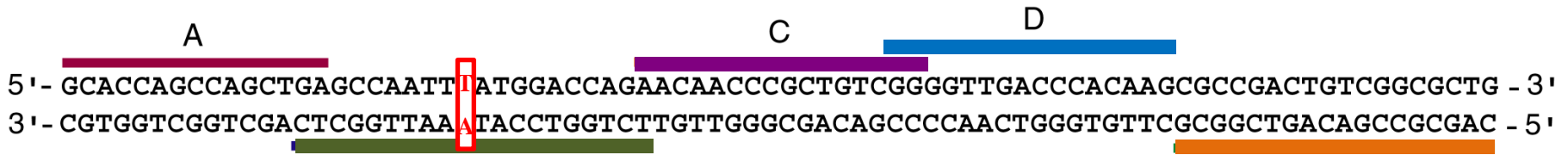3¹ - CGTGGTCGGTCGACTCGGTTAAGTACCTGGTC

PROBE B

Silent Mutation

PROBE A

5¹ - GCACCAGCCAGCTGAGCCAATT**T**ATGGACCAG

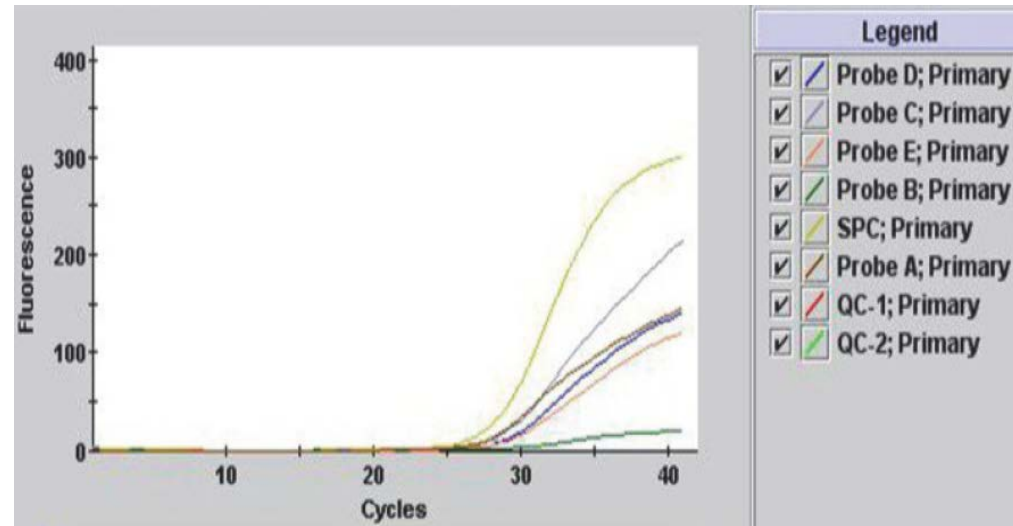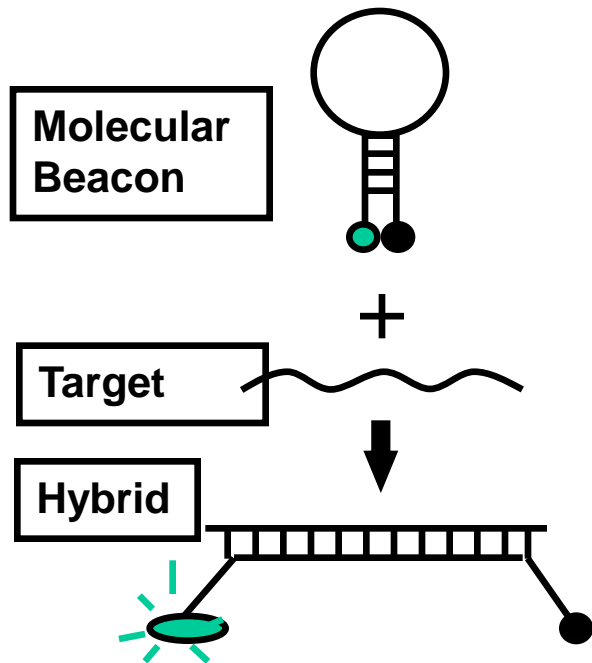507 508 509 510 511 512 513 **514** 515 516 517

3¹ - CGTGGTCGGTCGACTCGGTTAA**A**TACCTGGTC

PROBE B ✗

GeneXpert MTB/Rif Assay Design

```
                    A                                    C              D
5'- GCACCAGCCAGCTGAGCCAATTTATGGACCAGAACAACCCGCTGTCGGGGTTGACCCACAAGCGCCGACTGTCGGCGCTG - 3'
3'- CGTGGTCGGTCGACTCGGTTAAATACCTGGTCTTGTTGGGCGACAGCCCCAACTGGGTGTTCGCGGCTGACAGCCGCGAC - 5'
                         B                                                    E
```

The MTB assay target is the 81 bp rifampin resistance determination region of the rpoB gene.

Molecular Beacon

Target

Hybrid

Legend
- ✓ / Probe D; Primary
- ✓ / Probe C; Primary
- ✓ / Probe E; Primary
- ✓ / Probe B; Primary
- ✓ / SPC; Primary
- ✓ / Probe A; Primary
- ✓ / QC-1; Primary
- ✓ / QC-2; Primary

Fluorescence vs Cycles

Each probe is labeled with a different fluorophore, permitting simultaneous detection of the presence of wild type.

Example of Rif-Resistant Profile – 4 probes and SPC are positive; Probe B is negative

Public Health TB Lab

Binational Project

DSHS-Austin NAAT Indications

- No TB history in past year

- Smear positive reflex

- Provider request on submission form
  - If initial specimens are smear negative, & one is NAAT negative, subsequent specimens are ineligible for 2nd NAAT for 2 months post-collection date
  - 2nd NAAT performed upon special request
    - Typically for potential high profile case/susceptible population exposed
    - 1.1 NAAT performed per patient
  - Binational patients excluded

No. of Specimens Tested by Xpert at DSHS
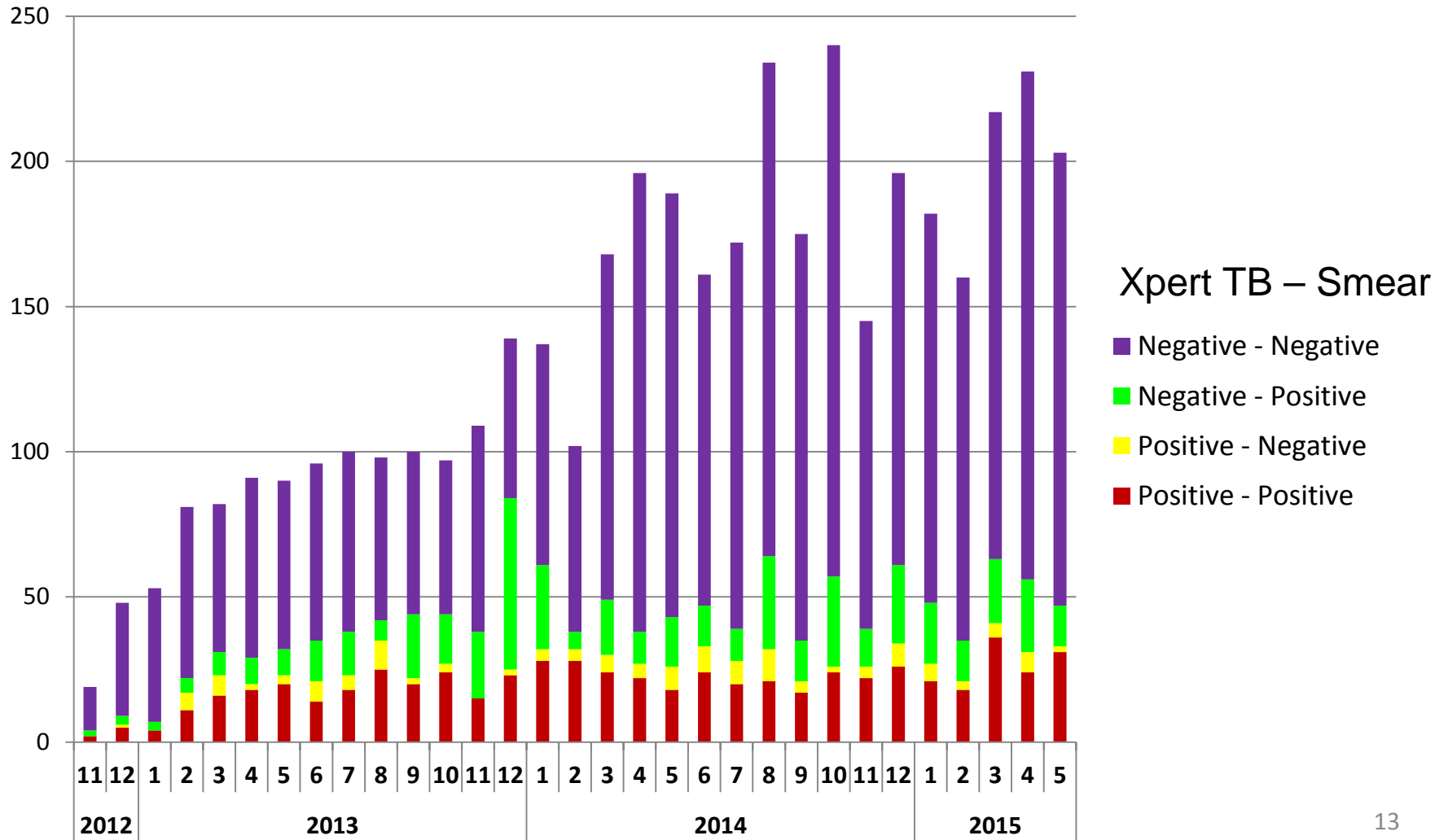
Correlation of AFB Smear & Xpert DNA Amount
435 Specimens with GeneXpert MTBC Positive Results at DSHS Austin Nov 2012- Aug 2014, 1.7 years, (2,545specimens)

DNA Amount*	% of Smear Grade				
	>10 AFB	1-10 AFB	<1 AFB	1 or 2 AFB/smear	Negative
High	68%	31%	3%	0%	0%
Medium	30%	57%	47%	18%	5%
Low	2%	10%	42%	41%	48%
Very Low	0%	2%	8%	41%	48%
Total	100%	100%	100%	100%	100%

*DNA Amount	Ct
High	<16
Medium	16-22
Low	22-28
Very Low	>28

>=82% of each smear grade fell within 2 Xpert semi-quantitative categories

GeneXpert MTBC Detection Performance
DSHS-Austin Nov 2012 – Jan 2014, 1.7 Years
(1,178 sputum specimens)

AFB Smear Positive

	Mtbc Reference Result				
	Positive	Negative	Total	99%	Accuracy
Xpert Positive	212	2	214	99%	Sensitivity
Xpert Negative	3	185	188	99%	Specificity
Total	215	187	**402**	53%	Prevalence
				99%	PPV
				98%	NPV

AFB Smear Negative

	Mtbc Reference Result				
	Positive	Negative	Total	98%	Accuracy
Xpert Positive	41	5	46	79%	Sensitivity
Xpert Negative	11	719	730	99%	Specificity
Total	52	724	**776**	5%	Prevalence
				89%	PPV
				98%	NPV

GeneXpert Rifampin Resistance Performance
DSHS-Austin Nov 2012 – May 2015, 2.4 yr. (4,579 specimens)

	Rifampin Phenotype				
	Resistant	Susceptible	Total	99%	Accuracy
Xpert Rifampin-R	14	6	20	93%	Sensitivity
Xpert Rifampin-S	1	792	793	**99%**	**Specificity**
Total	15	798	813	**2%**	**Prevalence**
				70%	**PPV**
				99.9%	NPV

Xpert Rifampin Resistant Probes A, B, C DSHS-Austin Nov 2012 – May 2015

Patient	Xpert Probe	rpoB Mutation	DSHS Rifampin	CDC Rifampin	Comment
1	A	No Amplification	S	-	Xpert Ct =33 *
2	A	Leu511Pro	S	S	Van Deun mutation
3	B	Gin513Leu	R	Pending	
4	B	Phe514Phe	S	S	
5	B	Phe514Phe	S	S	
6	B	Phe514Phe	S	S	
7	B	Asp516Tyr	S	S	Van Deun mutation
8	C	Ser522Leu	R/S**	S	Van Deun mutation?

*Agar Proportion Resistant/MGIT 960 Susceptible

Xpert Rifampin Resistant Probes D & E DSHS-Austin Nov 2012 – May 2015

Patient	Xpert Probe	rpoB Mutation	DSHS Rifampin	CDC Rifampin	Comment
9	D	His526Asp	R	R	
10	D	His526Asp	R	R	
11	D	His526Arg/Cys/Tyr	R	Pending	
12	D	His526Tyr	R	R	
13	D	His526Tyr	R	Pending	
14	D	His526Tyr	R	R	
15	D	Ser531Leu	R	R	
16	E	Ser531Leu	R	R	
17	E	Ser531Leu	R	Not tested	
18	E	Ser531Leu	R	R	
19	E	Ser531Leu	R	Pending	
20	E	Leu533Pro	R/S*	S	Van Deun mutation

*Agar Proportion Resistant/MGIT 960 Susceptible

Xpert Rifampin Resistant DSHS-Austin Nov 2012 – May 2015

Xpert Probe	# Total	# No Amplification	# Synonomous (Silent)	# Nonsynonomous	
				# Van Deun	# High level RMP-R
A	2	1		1	
B	5		3	1	1
C	1			1	
D	6				6
E	6			1	5
Total # (%)	20 (100%)	1 (5%)	3 (15%)	4 (20%)	12 (60%)

GeneXpert MTB/Rif in Texas

- Negative Predictive Value for Rifampin resistance approx. 100%
  - Predicts RIPE is adequate until growth-based drug susceptibilities become available
    - INH-R common
    - INH & EMB-R rare

- Approx. 15% of rifampin resistant predictions are false due to Phe514Phe silent mutation
  - CDC rapidly (2 days!) confirms or refutes Xpert rifampin resistance prediction

Xpert False Rifampin Resistance

- Not enough DNA in specimen can lead to false positive resistance results

- Silent Mutations
  - Phe514Phe
  - Arg528Arg

- Van Deun or 'Disputed' Mutations
  - Codons 511, 516, 533

Xpert False Rifampin Susceptibility

- Mutations outside the *rpoB* 81 base pair 'hot spot"*
  - Ile572Phe, Val146Phe

- Heteroresistance
  - Xpert will not detect a minority resistant population when most of the population is susceptible
    - For Ser531Leu, 66% of population must be R, for Leu533Pro, 100% of population must be R **

- Siu et al. J Antimicrob Chemother. 2011; 66: 730–733
- Blakemore et al. J Clin Microbiol. 2010; 48: 2495–2501

Xpert Limit of Detection

- 131 CFU/mL commonly cited*
- DSHS-Austin Validation
  - 92 CFU/assay for Mtb H37Rv
  - 152 CFU/assay for CDC MPEP 2010 W (RMP-R)
- FDA-Authorized Package Insert:

Organism	Specimen Type	LOD Estimate	LOD Claimed
Mtb H37Rv	Sputum	414	600
	Sputum Sediment	2,046	3,000

* D. Helb et al. J Clin Microbiol. 2010; 48:229-237

Wisconsin State Laboratory of Hygiene TB NAAT Proficiency Testing

Year	Event #	Sample # & Mtb Concentration (CFU/ml)				
		1	**2**	**3**	**4**	**5**
2015	1	1.5×10^5	1.5×10^4	3×10^5	MAC	3×10^5
2014	2	3×10^5 + inhib.	3×10^4	MAC	3×10^4	3×10^5
2014	1	3×10^5	MAC	3×10^4	3×10^5	3×10^5
2013	2	3×10^5	MAC	3×10^5 + inhib.		
2013	1	3×10^5	3×10^4	MAC		
2012	2	1×10^5	MAC	1×10^5		
2012	1	1×10^5	1×10^3	MTB + inhib.		

Sample not scored due to lack of GX peer group consensus

13 (81%) of 16 Xpert labs missed; 18 (69%) of 26 MTD labs missed

Some Remaining Questions

- U.S. population-based distribution of rpoB mutations silent and low level RMP-R
  - CDC molecular surveillance pilot project
- Heteroresistance
  - What are the dynamics of transition from 0% resistant population to 100% resistant population?

Xpert Summary

- We like this test – a lot!
  - Changed NAAT from one of hardest to easiest assay we perform
- You will encounter discordant rifampin molecular & growth-based drug susceptibility results
- Discordant results may have clinical significance
- Rifampin resistance detected by Xpert must be confirmed by sequencing and growth-based drug susceptibility testing
- Consult experts at RMTCCs

That's all Folks!

MDR Relapse?

- 2006: 42 y.o. female w/sputum smear & culture positive MDR
  - 18 months Rx; Cured
- 2015: Recurrent disease, 4+ sputum smear positive
  - 1 y.o. daughter with pulmonary disease and irritability (? CNS disease)
    - Immediate Rx needed; MDR Rx considered
  - Mom's 2015 sputum was Xpert Mtb detected, RMP-R not detected
  - Mom's frozen isolate from 2006 case was Xpert RMP-R (Probe E)
  - Grandma had RIPE-S TB in 2011; mom & daughter were close contacts
- Based on history & Xpert, mom & daughter treated for susceptible TB
- Mom's 2015 isolate was RIPE-S
- Genotypes
  - Mom   2006   PCR01132
  - Mom   2015   PCR13947
  - Grandma 2011   PCR13947