

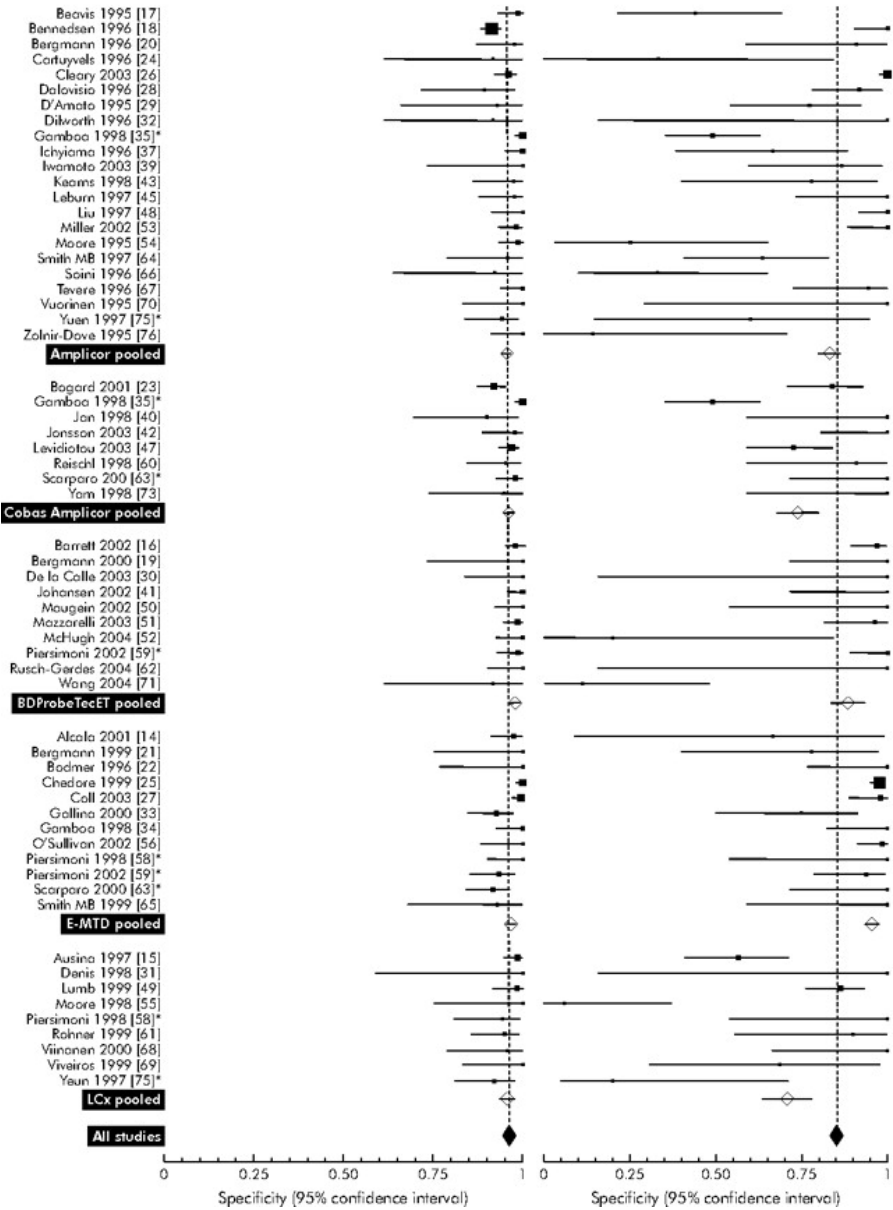
# Xpert MTB/RIF

Automated molecular detection of TB and MDR at point  
of treatment

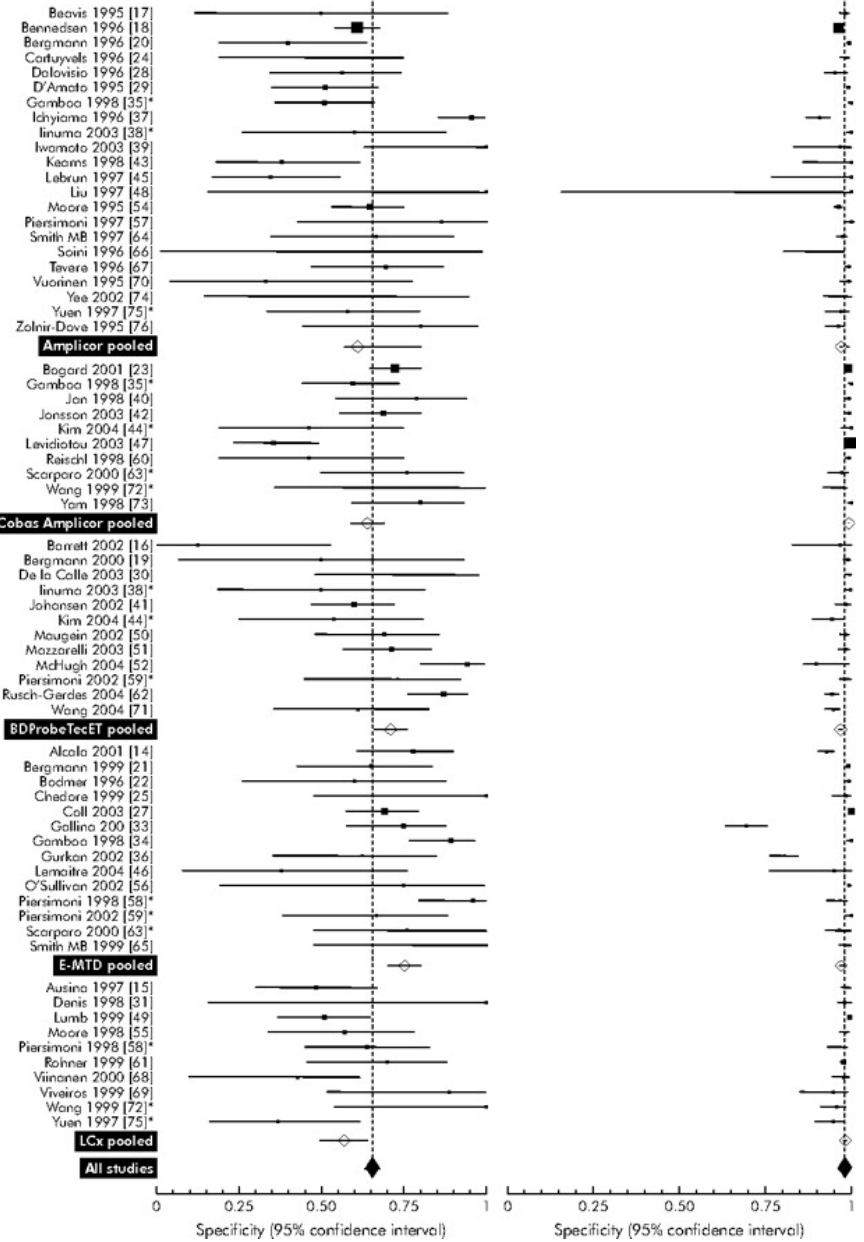
Mark Perkins, MD  
CSO FIND

# Performance of commercial NAAT for AFB+ and AFB- pulmonary TB

A

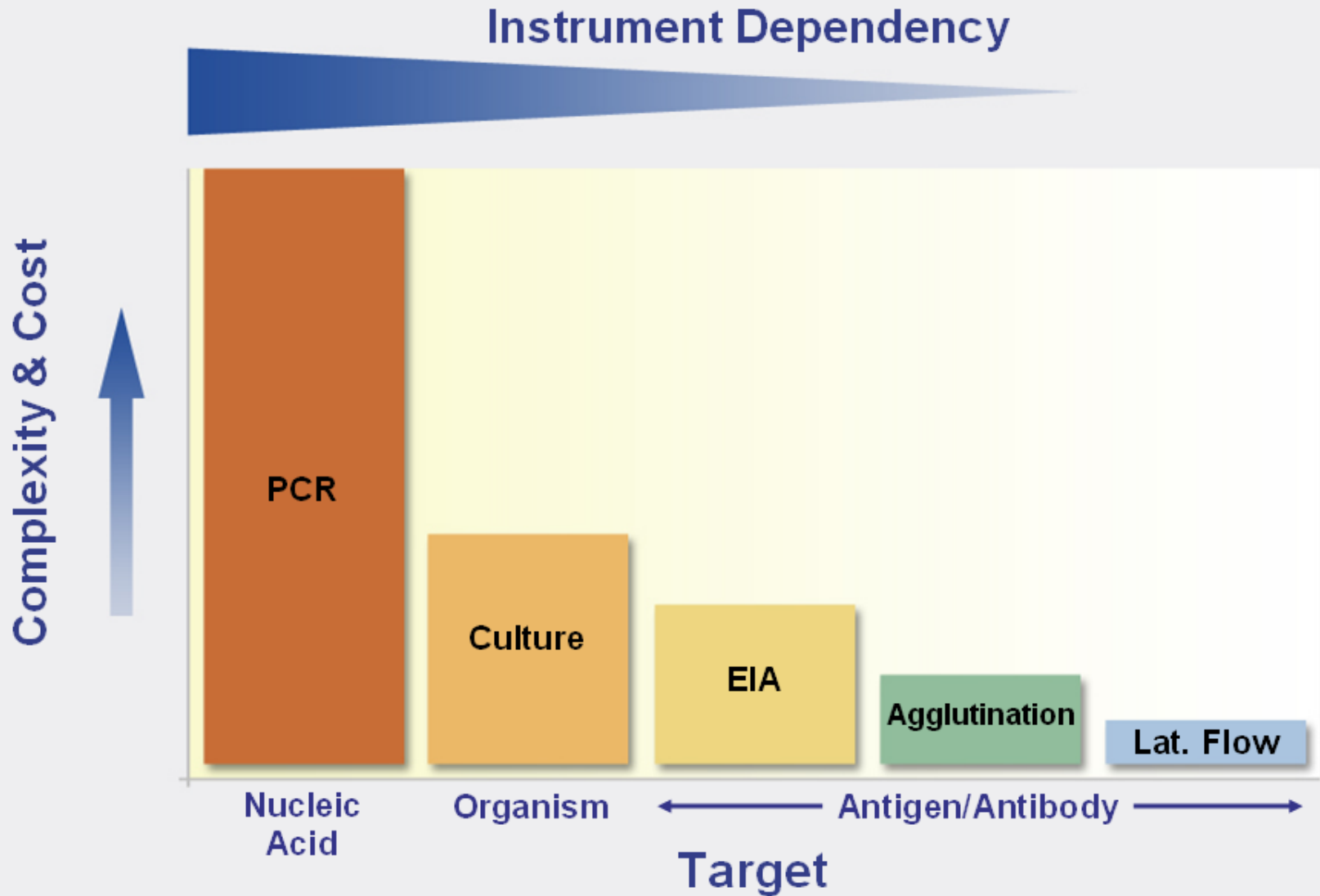


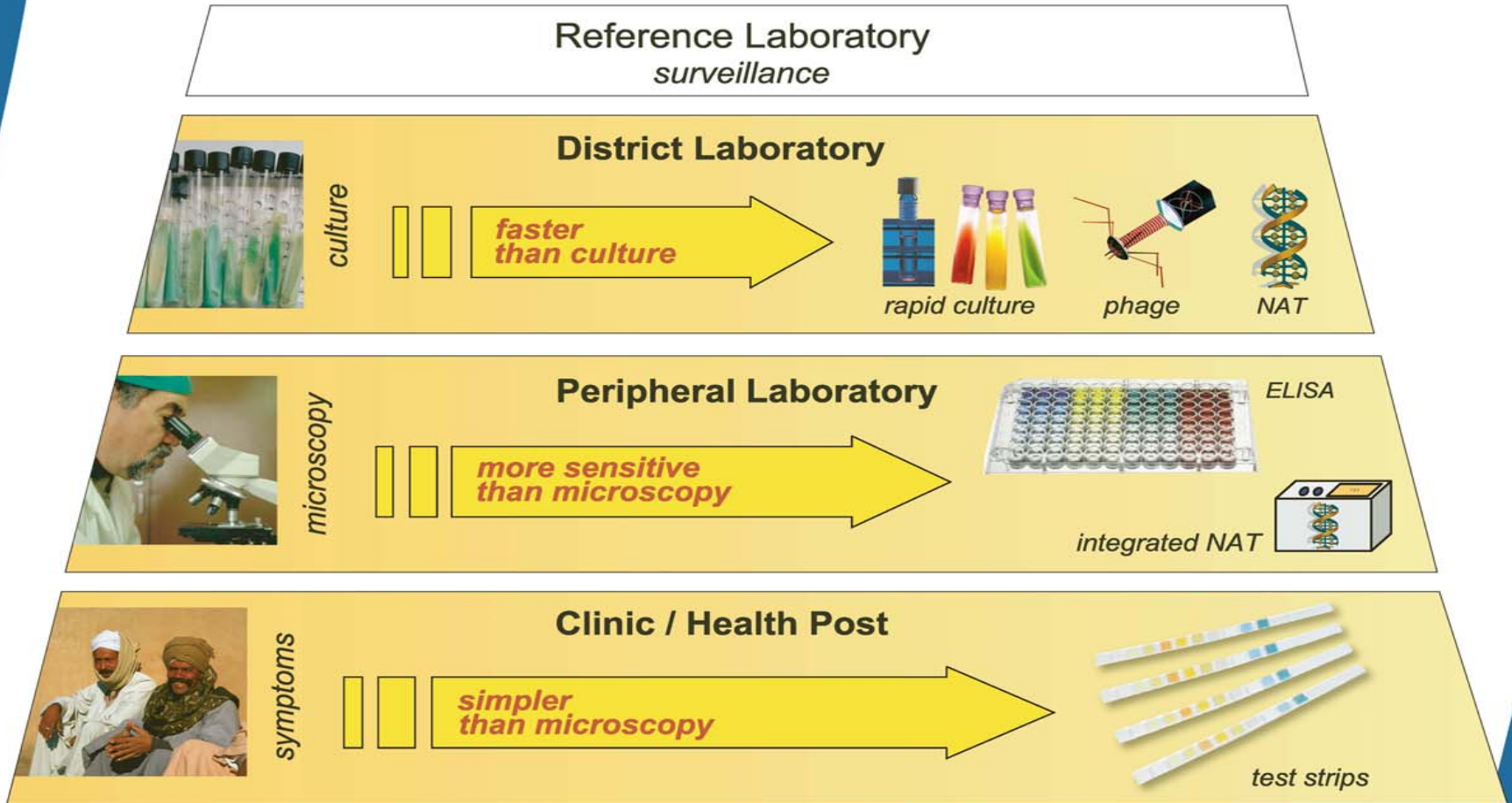
B





# Complexity and cost of routine technologies





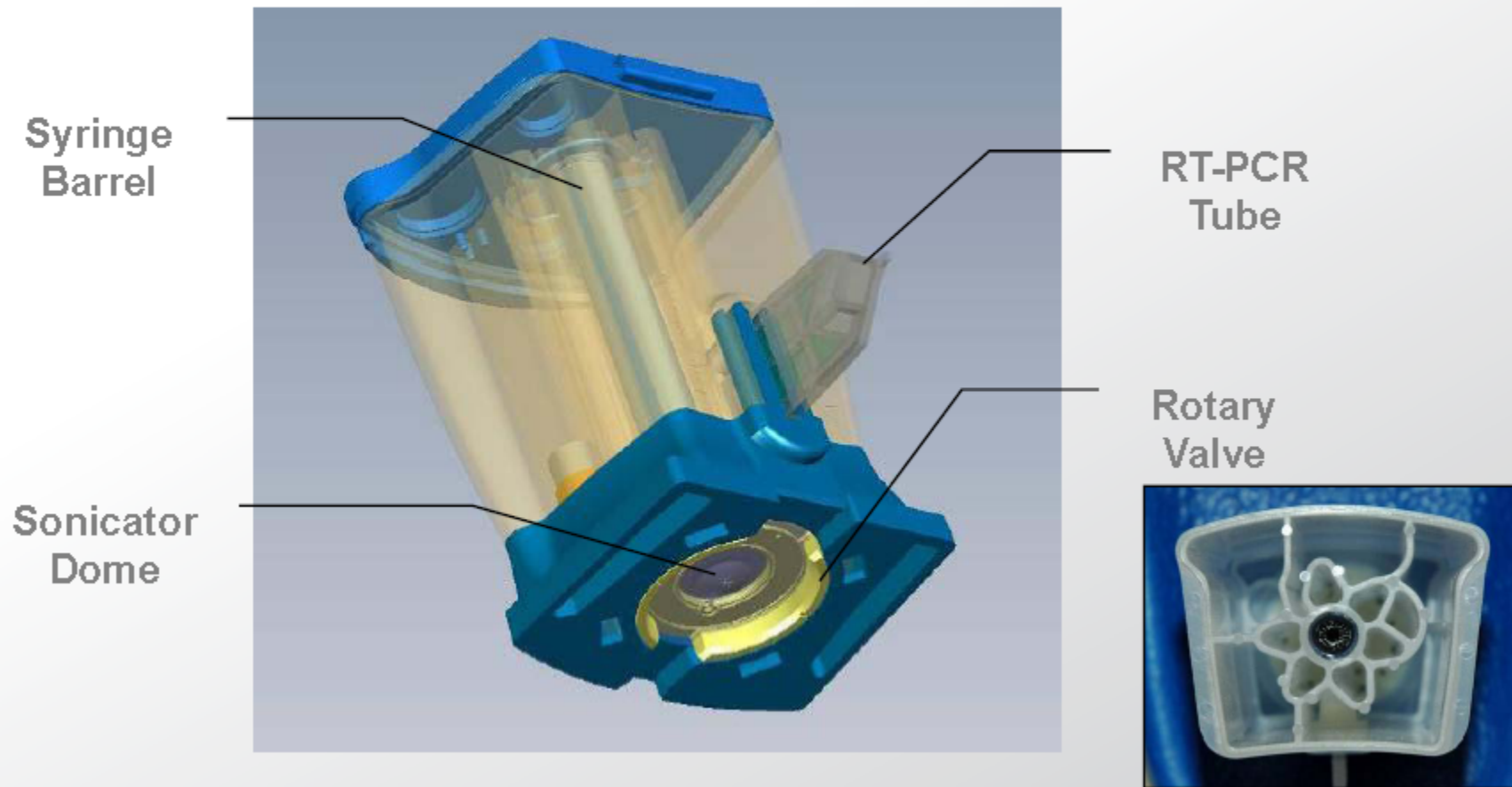
today

tomorrow

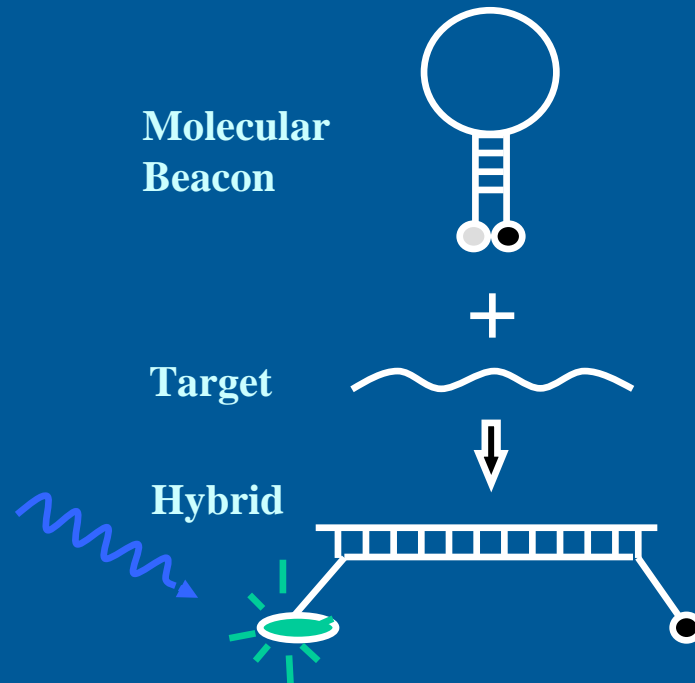
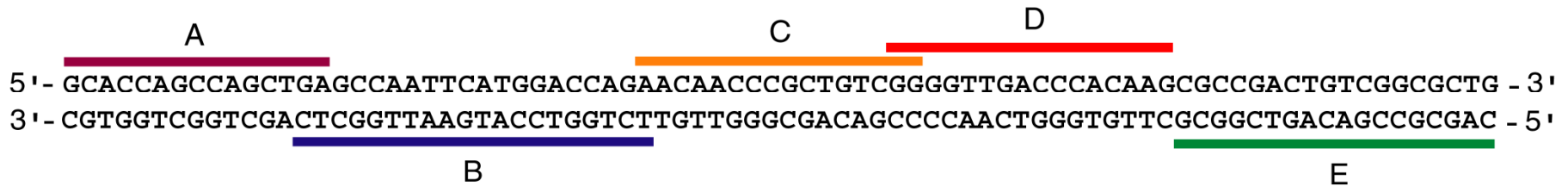
## Automated solution: Cepheid



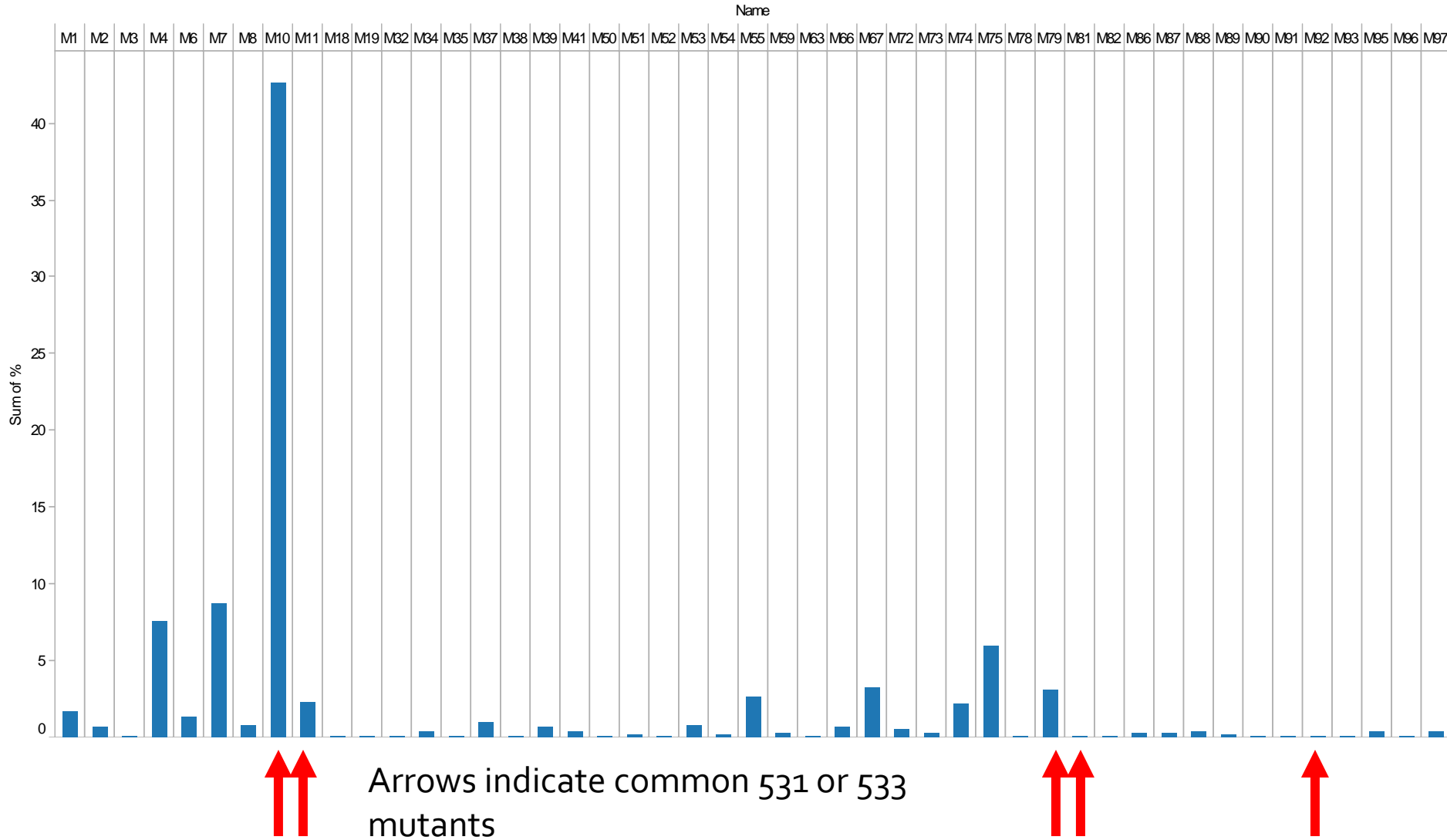
# Cartridge Design and Operating Principle



# *rpoB* Molecular Beacon Assay



# Mutation Frequency



Frequency of mutation based on review of 4000+ strains

# Rifampin Susceptible Sample

**Module Name** B1

**Sample ID** uganda sputum.6

**Assay** MTB Beta

**Assay Version** 2

**Assay Type** Research Use Only

**Reagent Lot ID** 00502

**Cartridge S/N** 0

**Expiration Date** <None>

**Test Type** Specimen

---

**Notes**

**Start Time** 2008/4/16 15:50:33

**End Time** 2008/4/16 17:15:31

**Status** Done

**Error Status** OK

**User** support

**S/W Version** 2.1

**Instrument/Module S/N** 702266/600013

**Views**

- Result View
- MTB Beta Assay
- Temperature-Sampl
- Optic-All Options
- Optic-Primary**
- Pressure
- Optic-Primary-Thres
- 2nd Derivative

**Legend**

- Probe D; Primary
- Probe C; Primary
- Probe E; Primary
- Probe B; Primary
- Bg; Primary
- Probe A; Primary

---

**Views**

- Result View**
- MTB Beta Assay
- Temperature-Sampl
- Optic-All Options
- Optic-Primary
- Pressure
- Optic-Primary-Thres
- 2nd Derivative

**Test and Analyte Result** | Detail | Errors | History | Messages

**Assay Name** MTB Beta | **Version** 2

**Test Result** **MTB POSITIVE MEDIUM;**  
**Rif Resistance NOT DETECTED**

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
Probe D	19.1	240.0	POS	PASS
Probe C	17.7	279.0	POS	PASS
Probe E	18.4	174.0	POS	PASS
Probe B	18.8	214.0	POS	PASS
Bg	27.4	240.0	NA	PASS
Probe A	17.3	220.0	POS	PASS

# Rifampin Resistant Sample

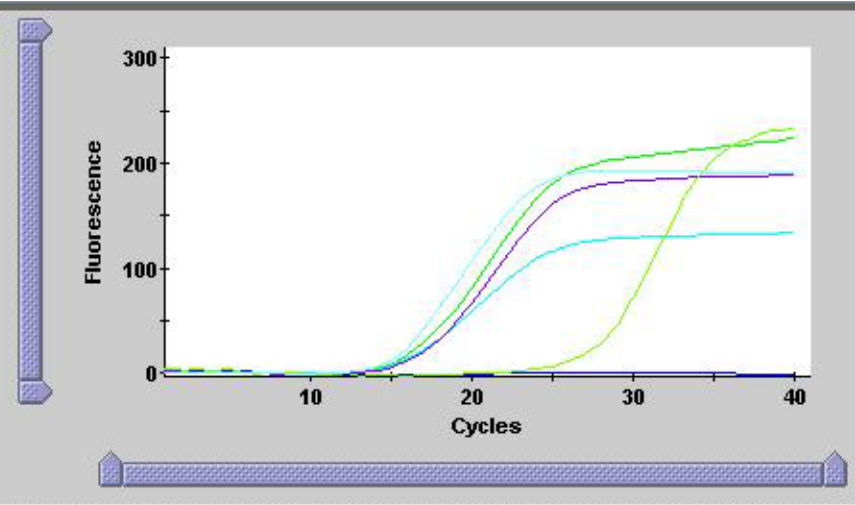
**Module Name** A3  
**Sample ID** uganda sputum.78  
**Assay** MTB Beta  
**Assay Version** 2  
**Assay Type** Research Use Only  
**Reagent Lot ID** 00502  
**Cartridge S/N** 0  
**Expiration Date** <None>  
**Test Type** Specimen

---

**Notes**  
 Start Time 4/21/2008 10:51:12  
 End Time 4/21/2008 12:16:08  
 Status Done  
 Error Status OK  
 User support  
 S/W Version 2.1  
 Instrument/Module S/N 703759/600943

Views

- Result View
- Primary Curve



Legend

- Probe D; Primary
- Probe C; Primary
- Probe E; Primary
- Probe B; Primary
- Bg; Primary
- Probe A; Primary

Views

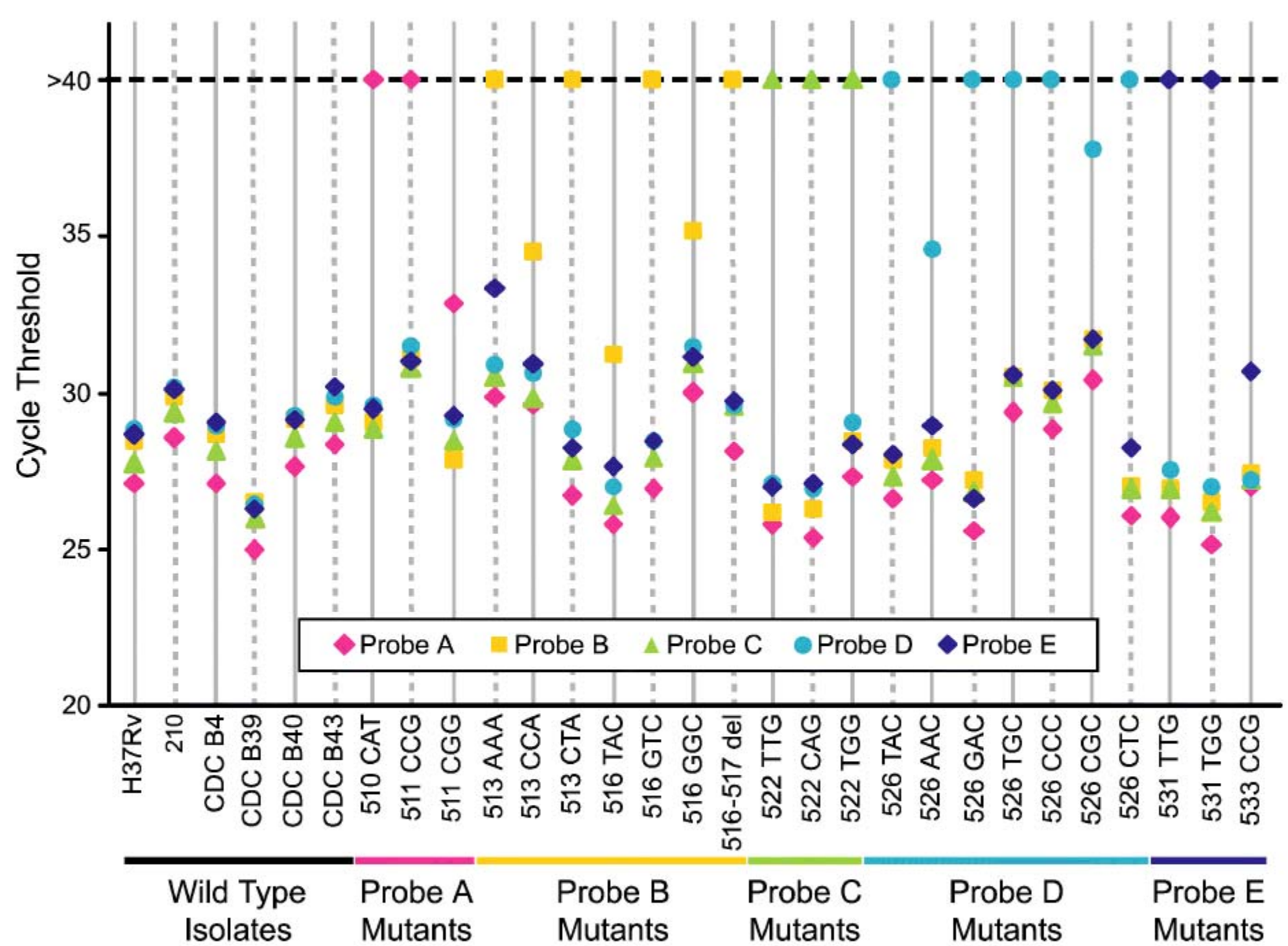
- Result View
- Primary Curve

Test and Analyte Result | Detail | Errors | History

**Assay Name** MTB Beta      **Version** 2

**Test Result** **MTB POSITIVE HIGH;**  
**Rif Resistance DETECTED**

Analyte Name	Ct	EndPt	Analyte Result	Probe Check Result
Probe D	0.0	-1.0	NEG	PASS
Probe C	16.3	223.0	POS	PASS
Probe E	16.8	134.0	POS	PASS
Probe B	17.0	189.0	POS	PASS
Bg	27.2	233.0	NA	PASS
Probe A	15.7	191.0	POS	PASS



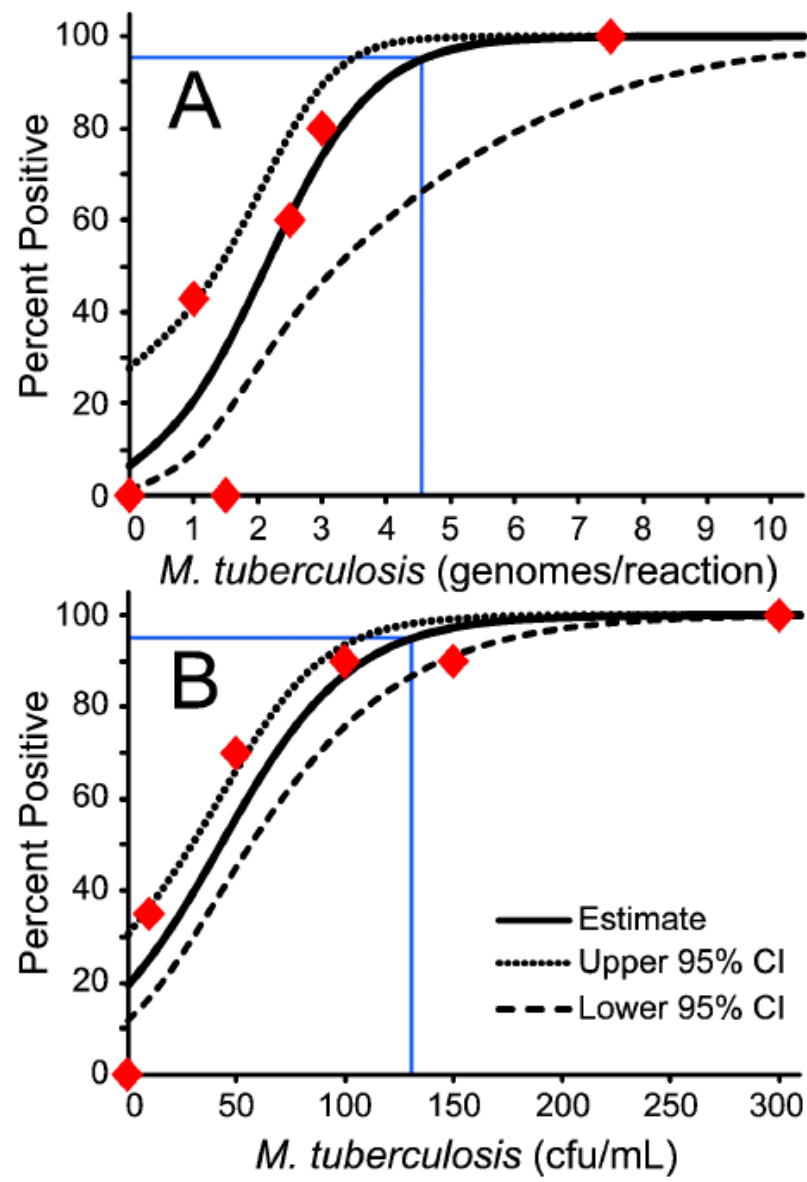
# Ubiquity Study

Origin	SCG								
	I	II	IIIa	IIIb	IIIc	IV	V	VIa	VIb
Unavailable	4	1			1		1		
Azerbaijan		1					1		
Bangladesh	2	3	4		1	1	1		
Belgium								1	
Burundi							2		
Camerun							1		
China		1							
Colombia						1			
Congo-Kinshasa							1	1	1
Georgia		1						1	
Germany							1	1	
Guinea				1					
Guinea Eq.							1		
India		1							
Kazakstan		1						2	
Korea		5	1	1					
Mexico					1	1			2
Morocco				1			1		
Nepal	1	1							
Nigeria							1		
Pakistan			1						
Peru				4	2	1	1		
Phillipines	1								
Portugal							1		
Rep. Domin.							1		
Rwanda								1	1
South Africa							2		1
Spain							1	2	
Tibet	1								
Ukraine								1	
USA						1			

- Tested dna from 80 strains from 30+ countries and 7 major clades.

- Included 37 rifampin resistant strains comprising 13 unique mutations.

Codon	Mutation	Mut. Name	Probe
511	ctg-ccg	M1	A
516	gac-gtc	M4	B
516	gac-tac	M55	B
526	cac-tac	M7	D
526	cac-cgc	M67	D
526	cac-agc	M70	D
526	cac-gac	M75	D
531	tcg-ttg	M10	E
531	tcg-tgg	M79	E
533	ctg-ccg	M11	E
Double	multiple	M79+un(512)	E, AB
Double	multiple	M55+un(515)	B, B
Double	multiple	M67+un(509)	A, D



# Exclusivity (Specificity) Study

- Testing GX with  $10^6$  copies of genomic DNA from common oral microorganisms and those causing tuberculosis like symptoms.
- DNA is spiked into PCR reagent resuspension buffer, simulating efficient capture, lysis and elution. (Unlikely)

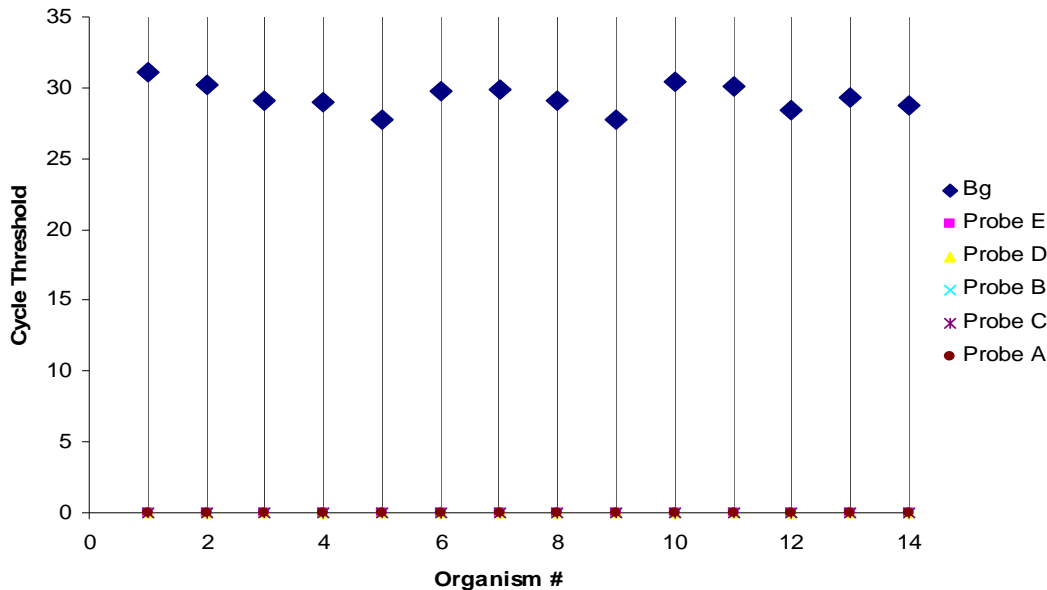
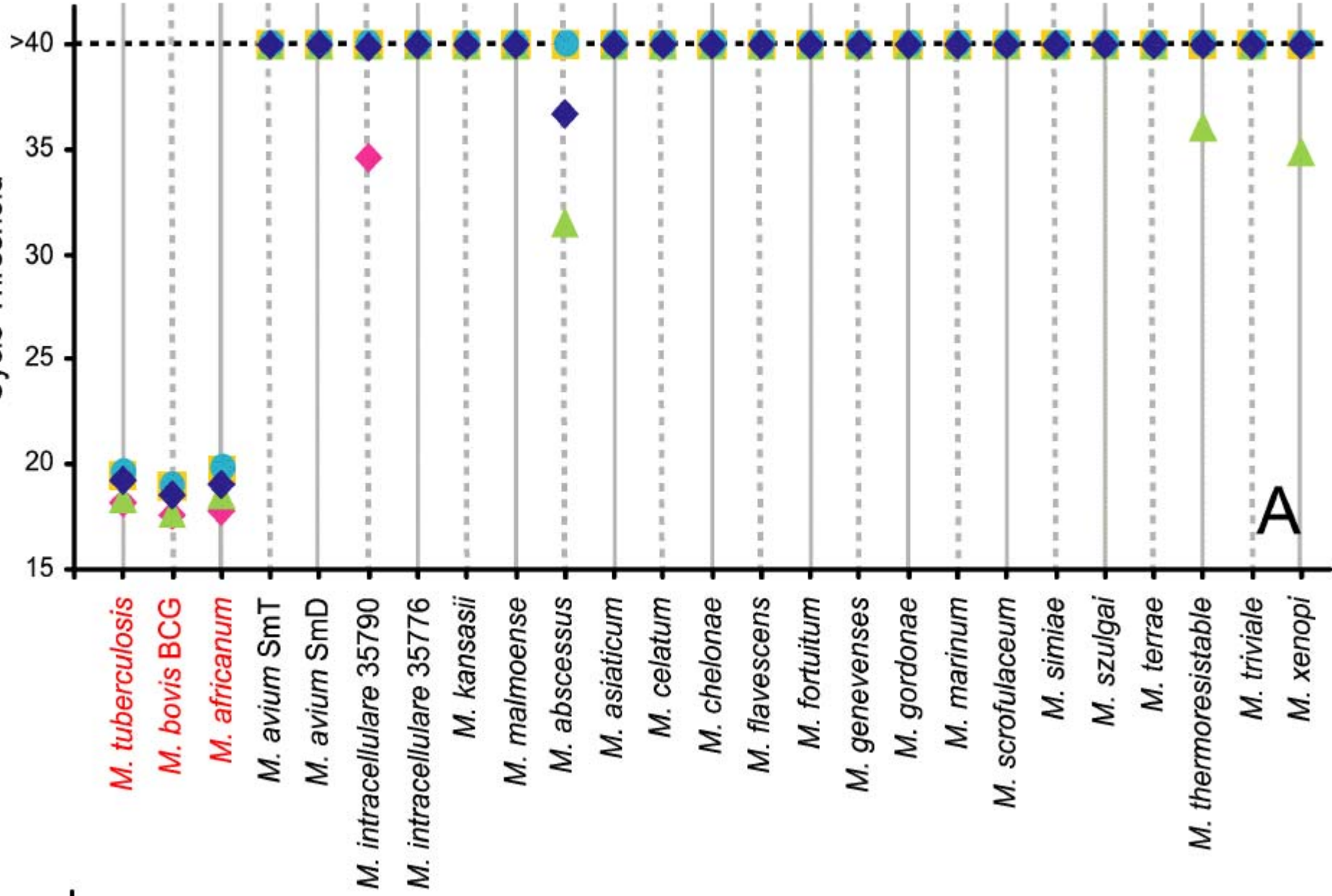


Figure 1: Typical results. No signal except for Bg internal control

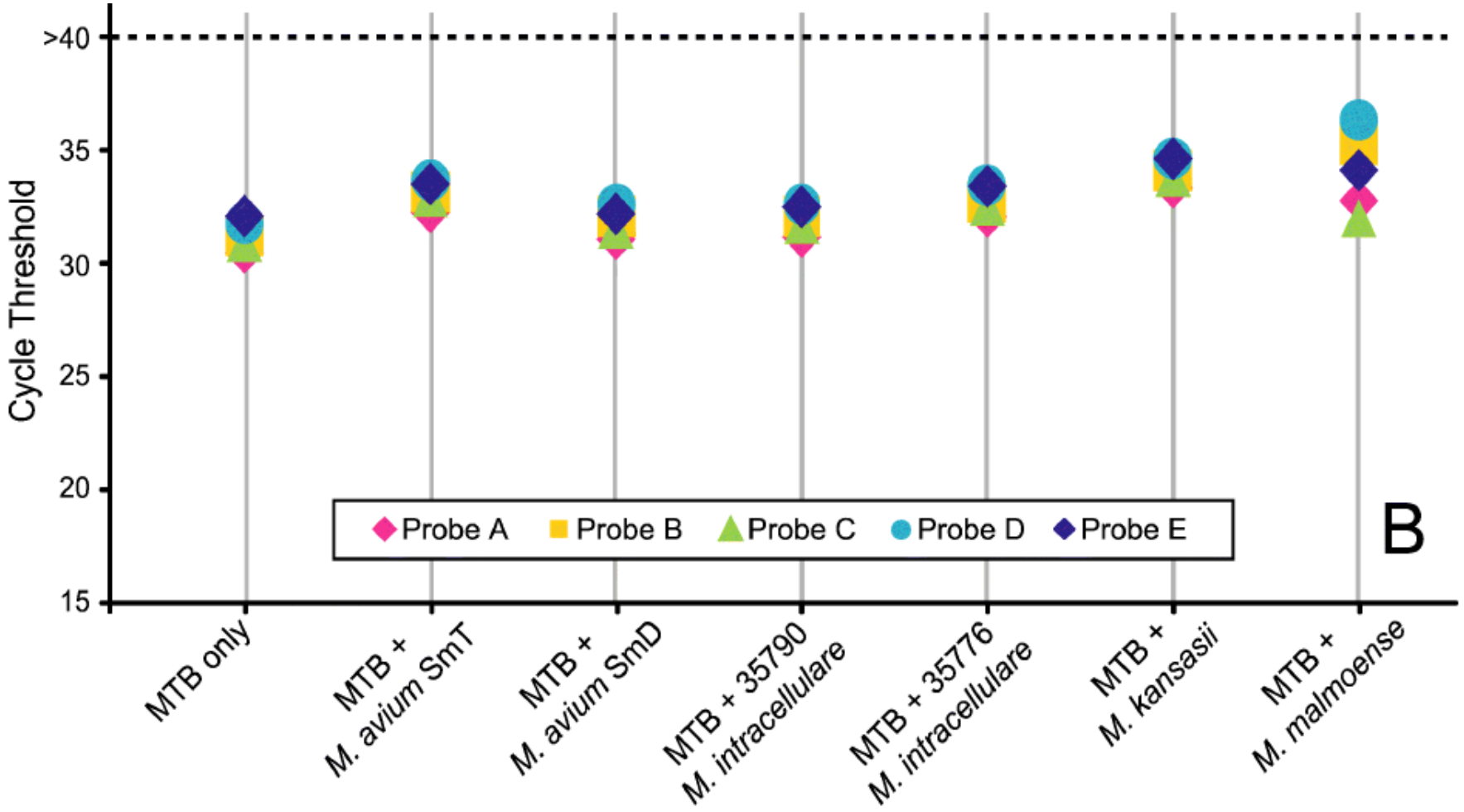
- Specificity is 100% after testing 65/97 organisms.

Cycle Threshold



A

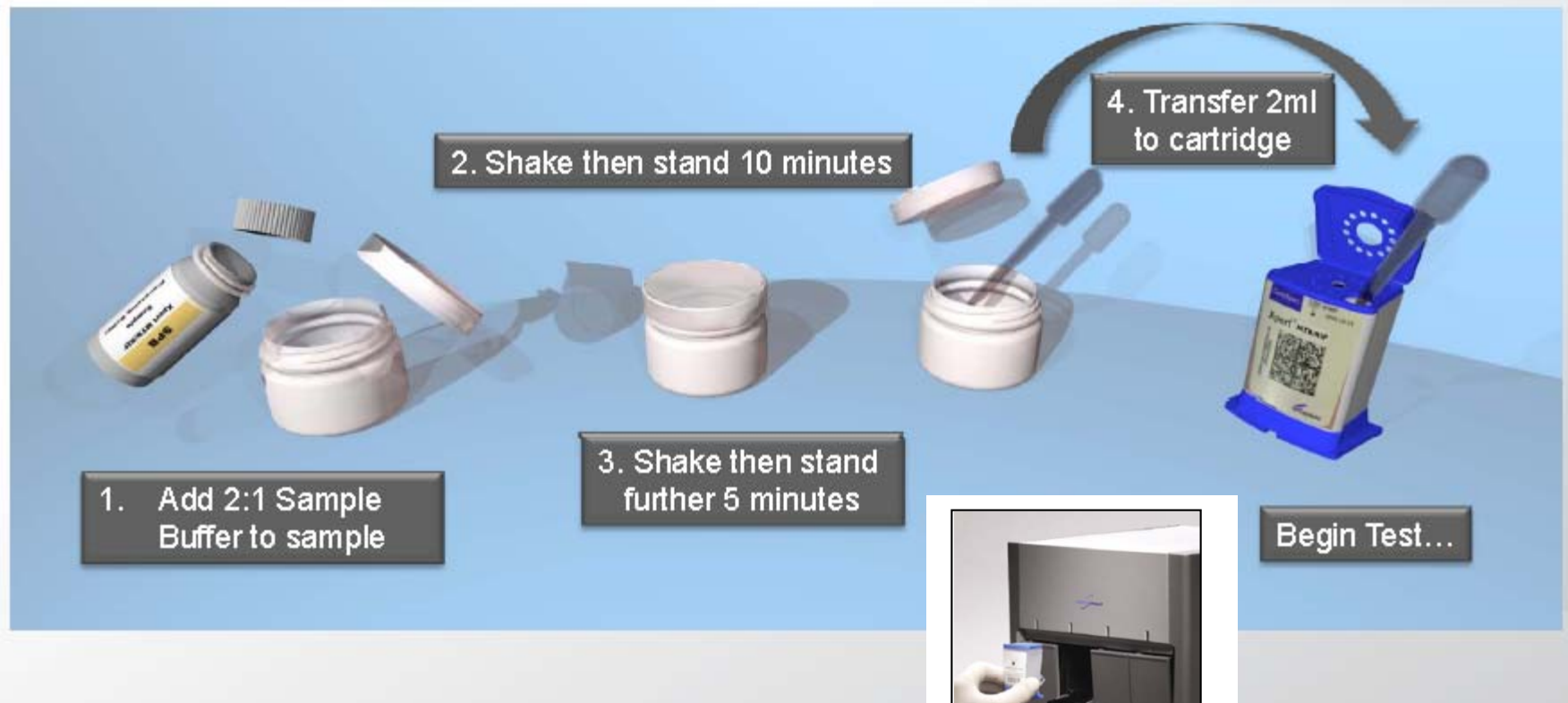
Interference: Sputum containing both 200 cfu/mL of *M. tuberculosis* and  $10^6$  cfu/mL of an NTM



## Xpert MTB Cartridge Controls

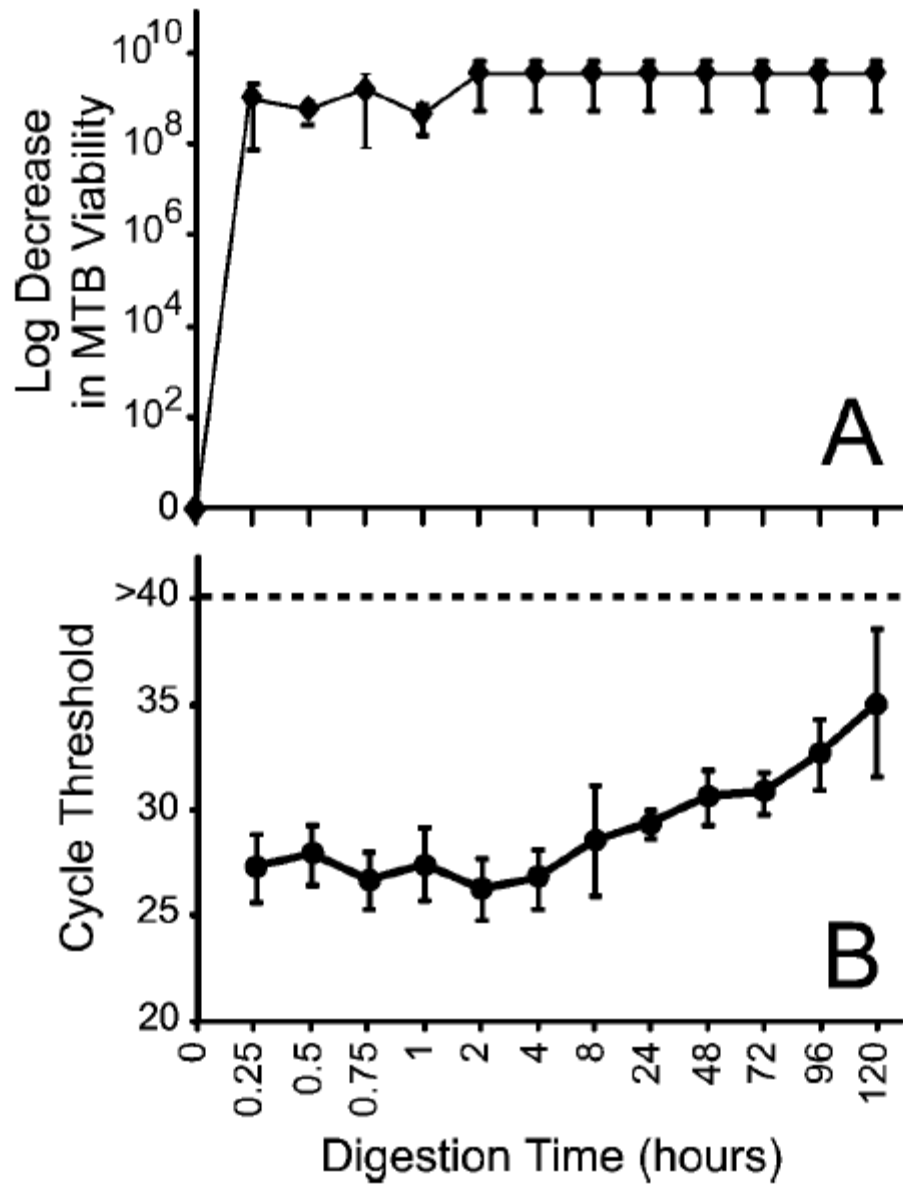
- Probe Check Control
  - Is performed prior to PCR.
  - Is performed to ensure:
    - Rehydration of beads
    - Flow in cartridge works
    - That probe and dye are stable
- Sample processing control/extraction control
  - Ensure that the sample was correctly lysed and extracted.
  - Runs through the complete process
  - Is freeze-dried beads with *Bacillus globigii* spores . The spores are difficult to break open and to process.
  - Detects PCR inhibitors associated with the sample

# Simple Sample Processing – Direct Sputum

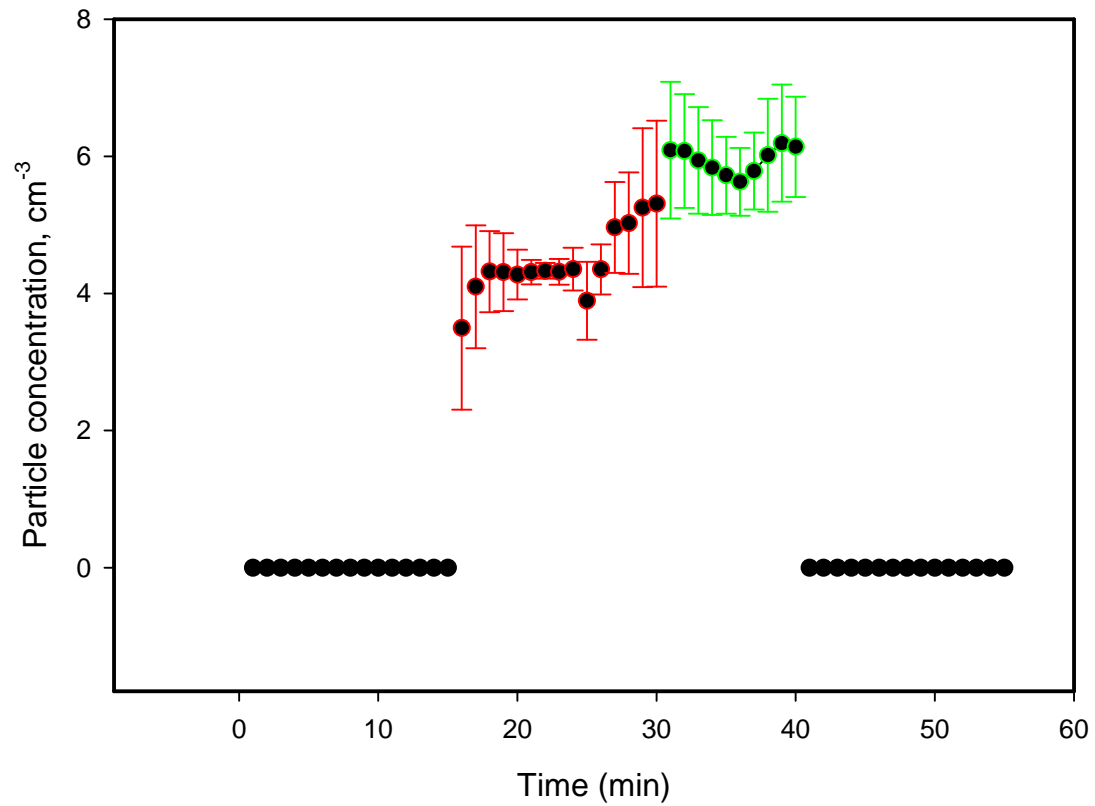


# Inactivation procedure

	Starting cfu/mL	Diluent	Average cfu/ plate for each replicate	Average cfu/plate	Average cfu/mL	Average log Reduction	Percent Reduction
Study 1	$3.5 \times 10^7$ BCG	7H9 media			<10	$>3.5 \times 10^6$	>99.9
Study 2	$3.5 \times 10^7$ BCG	sputum			1.5	$2.3 \times 10^7$	>99.9
Study 3	$3.3 \times 10^7$ H37Rv	sputum	12, 13, 21	15.3	153	$2.15 \times 10^5$	99.9
Study 4	$3.63 \times 10^8$ H37Rv	sputum	4, 6, 2	4	40	$9.1 \times 10^6$	>99.9
Study 5	$4.0 \times 10^8$ H37Rv	sputum	2, 2, 3	2.33	23.3	$1.7 \times 10^7$	>99.9
Average log kill						$1.06 \times 10^7$	

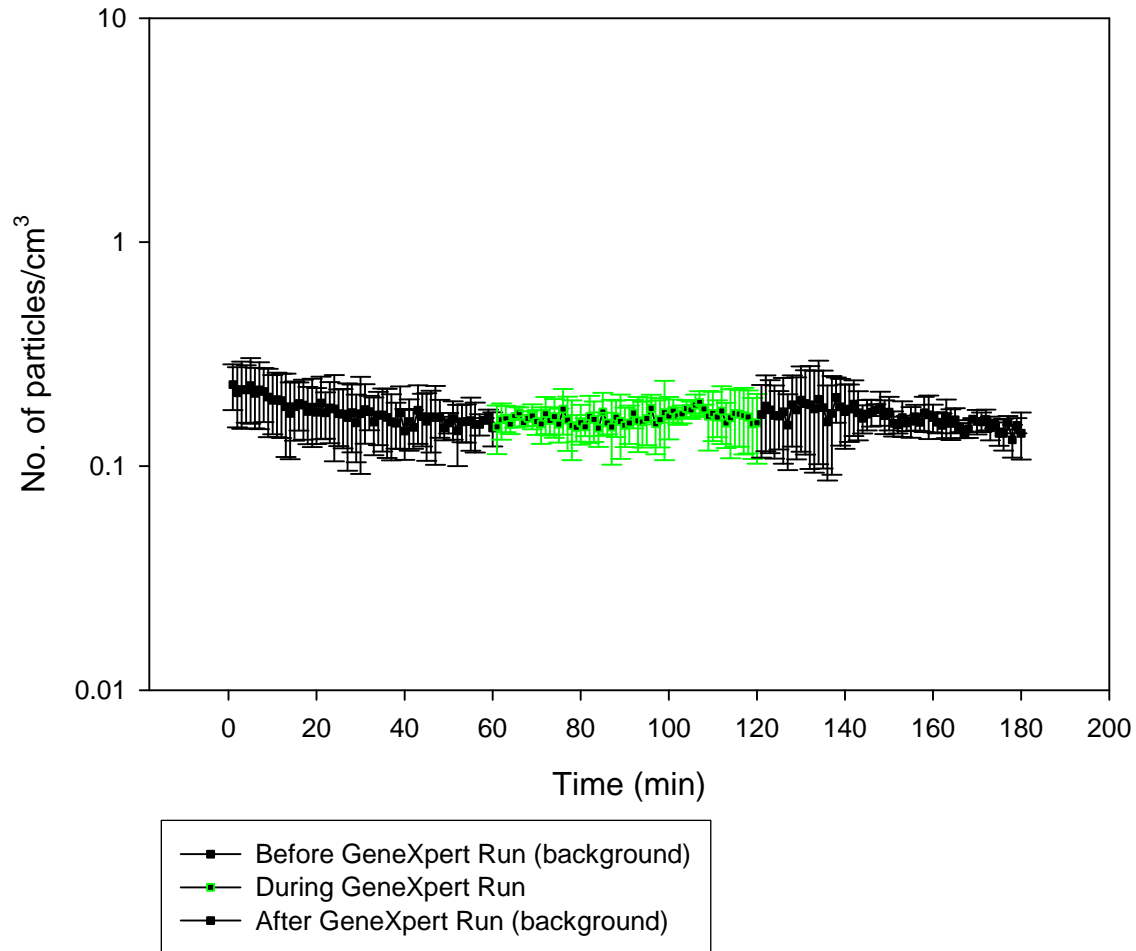


### During conventional slide preparation



- BSC air flow on
- BSC air flow off, background measurement
- BSC air flow off, during slide preparation
- BSC airflow on

### Concentration of airborne particles when GeneXpert is in operation (Sample - *Mycobacterium bovis* BCG + Sputum+SR)

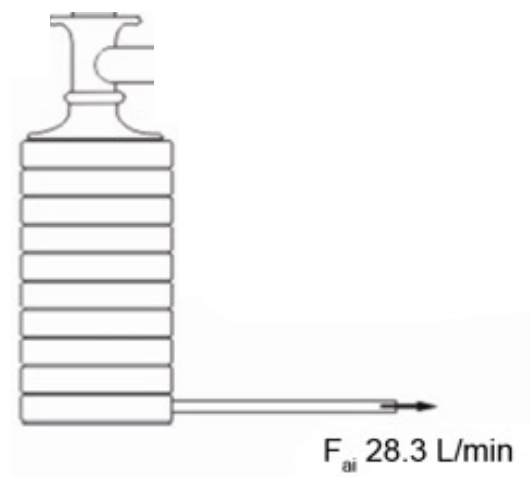


Error bars indicates SD of three repeats

Biosampler  
Liquid media



Anderson Impactor  
Solid media



# Aerosol Sampling: verifying the system



0.8 mL BCG in 7H9 media aerosolized over 10 min in a closed chamber

Mean cfu/m<sup>3</sup> air detected for 3 experiments

<u>Concentration BCG/mL.</u>	<u>Anderson impactor</u>	<u>BioSampler</u>
10 <sup>4</sup>	482	10,416
10 <sup>3</sup>	169	2,976
10 <sup>2</sup>	91	578
10	28	0

# Aerosol Viability During Manual Steps

Mean cfu/m<sup>3</sup> air detected over 3 experiments

5 X 10<sup>8</sup> cfu BCG spiked into sputum.

Anderson impactor

BioSampler

SR added and sample **immediately** pipetted in and out of three Xpert TB cartridge over 15 min time period (equivalent to loading >30 cartridges)

6

67

SR added **15 min wait** then sample pipetted in and out of three Xpert TB cartridge over 15 min time period (equivalent to loading >30 cartridges)

0

0

Sputum smeared/layered on 10 microscope slides over 10 min period.

16

324

# Aerosol Viability During GX Assay

Total cfu detected over all three runs

Sample type placed into Xpert TB cartridge (3 runs with 3 cartridge per condition)

Anderson impactor

BioSampler

5 X 10<sup>8</sup> cfu BCG spiked into water

0

0

5 X 10<sup>8</sup> cfu BCG spiked into sputum then treated with SR in standard protocol

0

0

5 X 10<sup>8</sup> cfu M. smegmatis spiked into sputum then treated with SR in standard protocol

0

0

## MTB / Rif-resistance test

*Essential features of future test*

### Workflow

- sputum
- simple 1-step external sample prep. procedure
- time-to-result < 2 h
- throughput:  $\geq 16$  tests / day / module
- no need for biosafety cabinet
- integrated controls
- true random access

### Performance

- specific for MTB
- sensitivity better than smear, similar to culture
- detection of rif-resistance via rpoB gene

### Product and system design

- test cartridges for GeneXpert System
- several GeneXpert modules can be combined in 1 workstation
- swap replacement of detection unit
- ~1 day technician training for non-mycobacteriologists

*GeneXpert System  
module*



*cartridge*



**Automated  
Sample Prep,  
Amplification  
and Detection**

**90 minutes**

# Xpert MTB/Rif: FIND Evaluation studies



Rigorous performance evaluation at 5 sites (>1500 TB suspects)  
 Included 2 sites with high HIV prevalence (80%) & 2 with high MDR TB prevalence (>30%)



	<b>UPCH</b>
<b>HIV</b>	2%
<b>TB (C+)</b>	61%
<b>MDR TB</b>	7%

	<b>STI</b>
<b>HIV</b>	5%
<b>TB (C+)</b>	42%
<b>MDR TB</b>	31%

**Peru**  
**UPCH**

**South Africa**  
**UCT**  
**SAMRC**

	<b>Hinduja</b>
<b>HIV</b>	5%
<b>TB (C+)</b>	60%
<b>MDR TB</b>	67%

	<b>UCT</b>	<b>SAMRC</b>
<b>HIV</b>	77%	72%
<b>TB (C+)</b>	39%	13%
<b>MDR TB</b>	10%	9%

		AFB-		AFB+			
		Culture Positive	Culture Negative	Culture Positive			
Combined	Xpert MTB/RIF	MTB Detected	70	3	275	PPV	99.1%
		MTB Not Detected	7	171	0	NPV	96.1%
		Sensitivity		Specificity			
		98.0%		98.3%			

**Sensitivity for in S+/C+ = 100%, in S-/C+ = 91%**

		DST RIF Resistant	DST Sensitivity				
		Combined	Xpert MTB/RIF			RIF Resistance Detected	58
	RIF Resistance Not Detected		2	280	NPV	99.3%	
		Sensitivity		Specificity			
		96.7%		98.6%			

**High accuracy for Rif detection**

**Sequencing data for discrepant results suggest Xpert correct**

# Acknowledgements



## UMDNJ

- David Alland
- Danica Helb

## Cepheid

- David Persing
- Martin Jones

## FIND

- Catharina Boehme
- Ranald Sutherland