Xpert MTB/RIF

Automated molecular detection of TB and MDR screening in peripheral laboratories

Introduction to the technology

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Xpert MTB/RIF

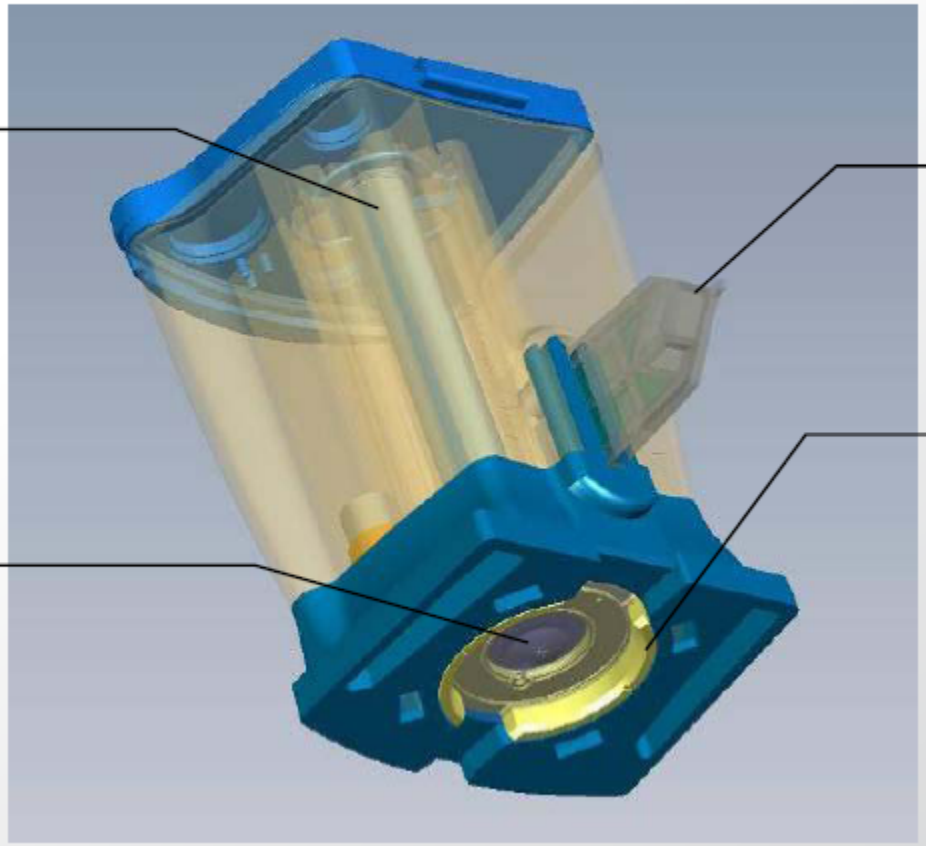
GeneXpert

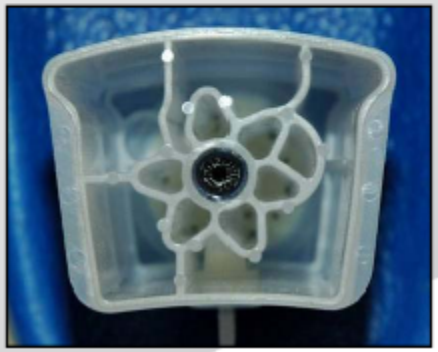
5	20	80	Samples per shift	500-1000

Multi-disease technology platform

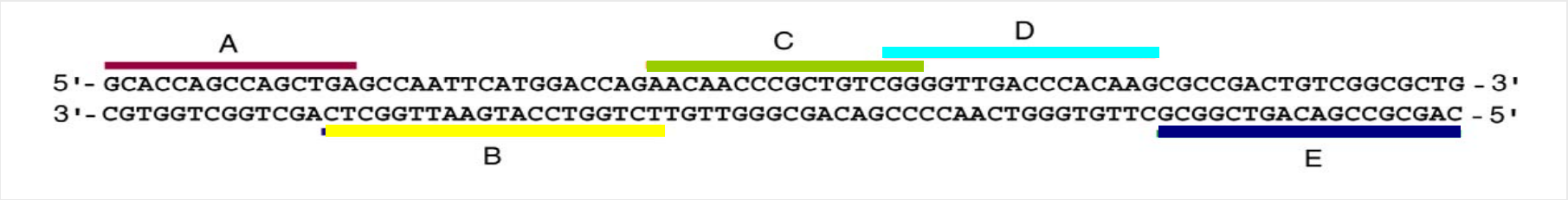
Table 34: Existing test cartridges on the GeneXpert platform	
Staphlococcus aureus colonization	*Bordetella pertussis*
Vancomycin resistance	*Bordetella parapertussis*
Clostridium difficile	HSV Type 1
MRSA from tissue or blood	HSV Type 2
Group B *Streptococcus*	RSV Type A
Enteroviral meningitis	RSV Type B
Coagulation disorders	Norovirus GI
Anthrax	Norovirus GII
	Flu A
	Flu B
	Leukemia (BCR-ABL)

Cartridge Design and Operating Principle

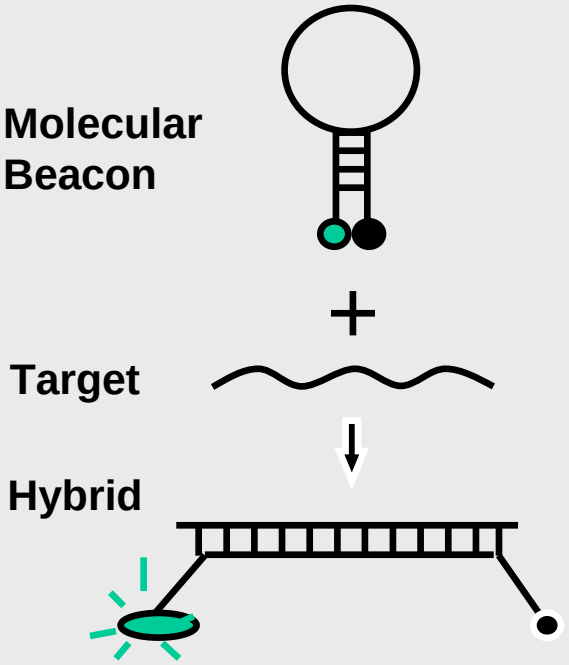




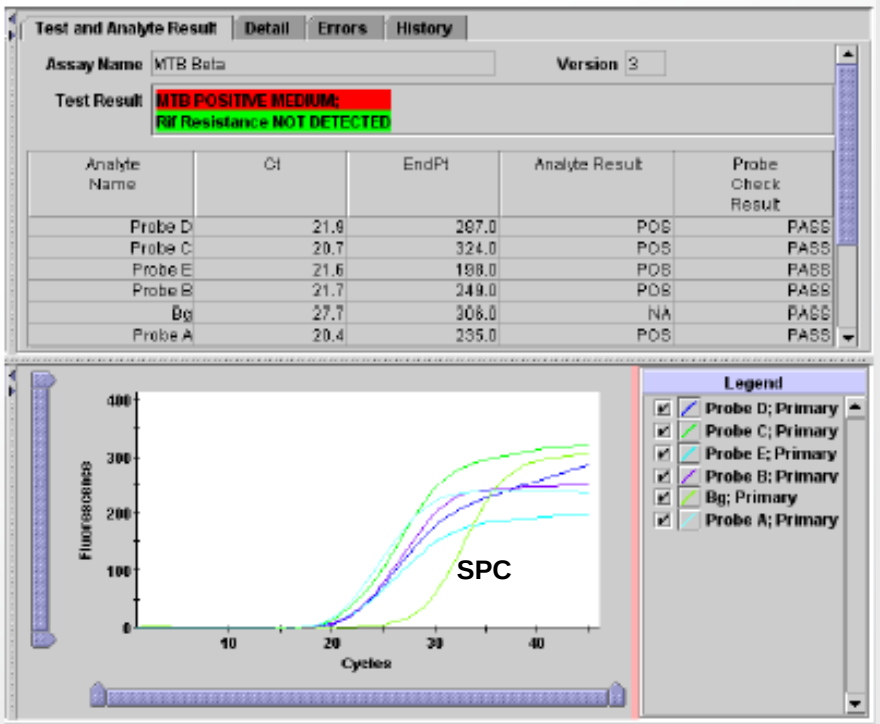
Xpert MTB/Rif molecular beacon assay



The PCR target is the 81 bp region of the rpoB gene: 5 probes bind to wildtype, but not mutant target

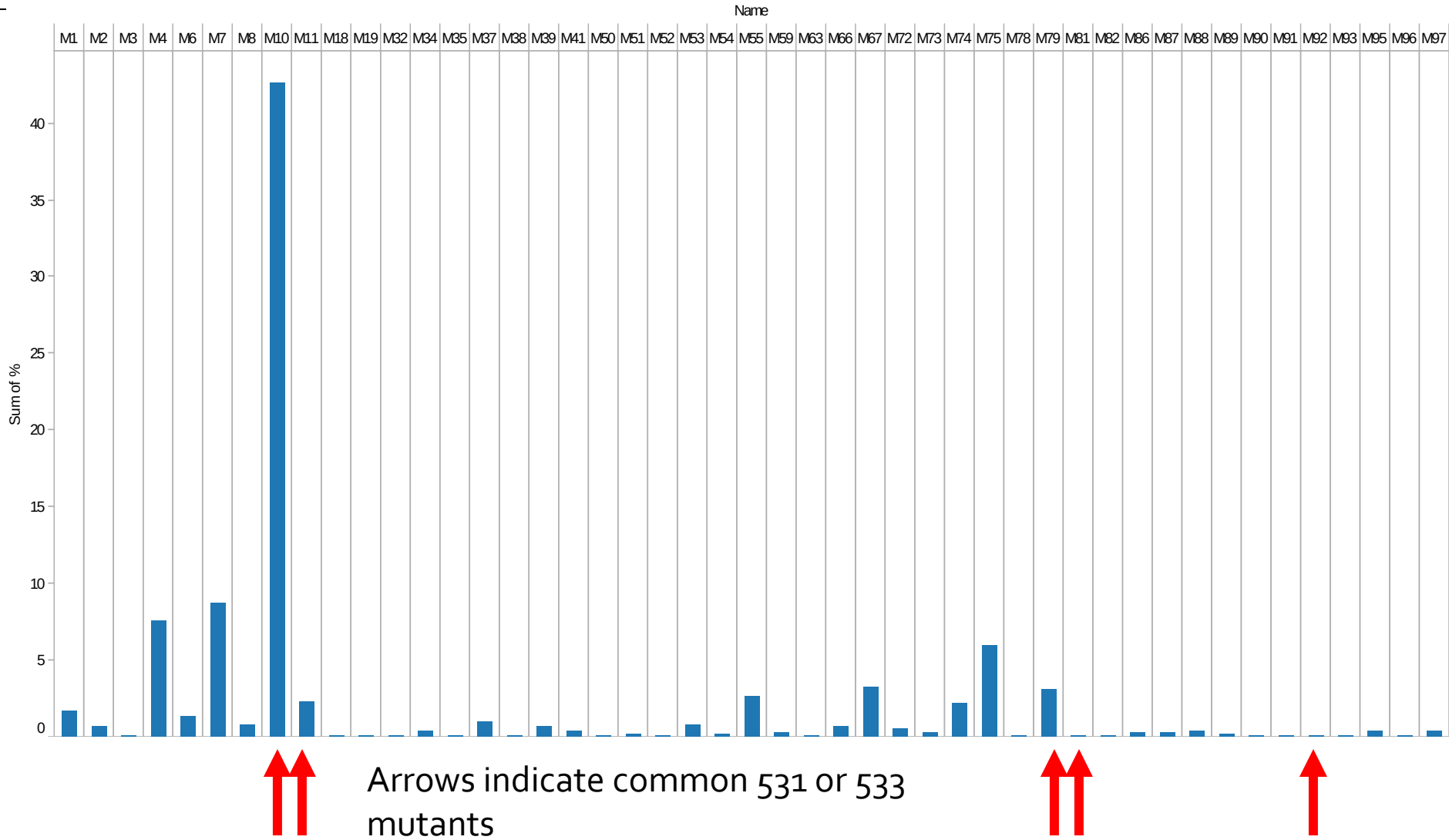


Each probe is labeled with a different fluorescent dye, permitting simultaneous detection



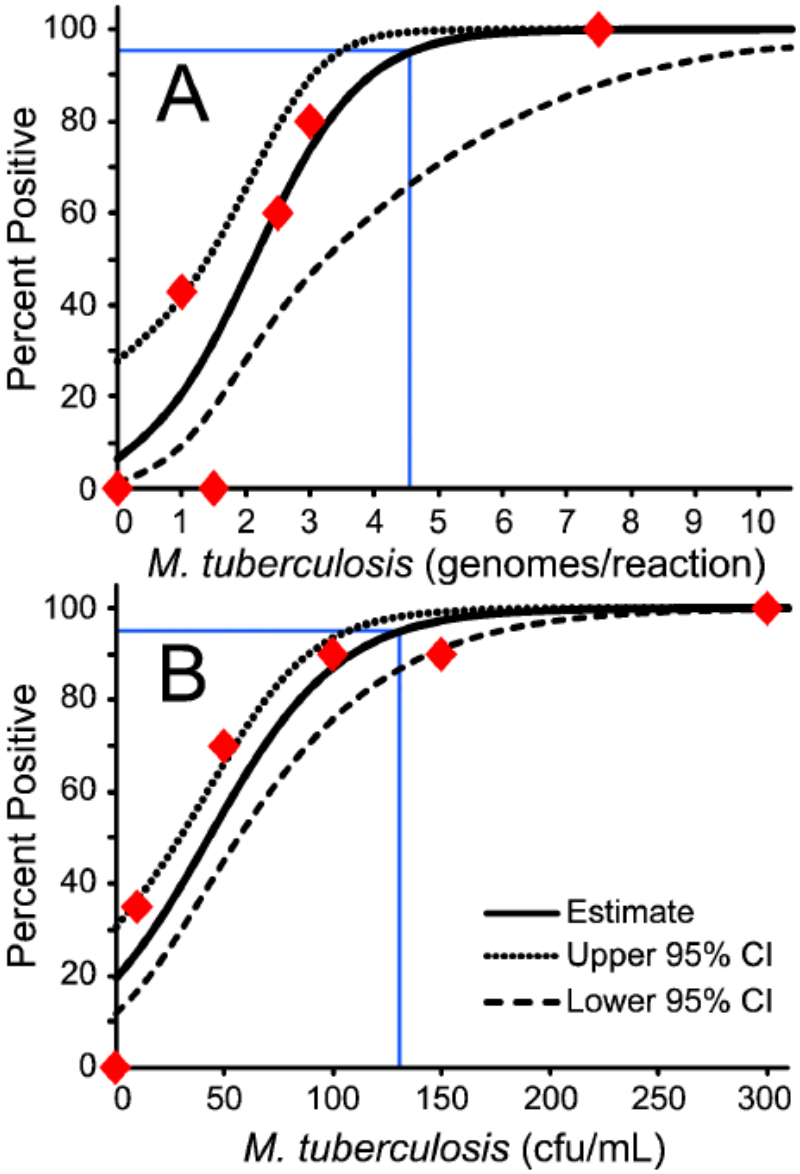
Example of Rif-Sensitive Profile – 5 probes & SPC show fluorescence

Mutation Frequency



Frequency of mutation based on review of 4000+ strains

Limits of detection



Linearity of dynamic range

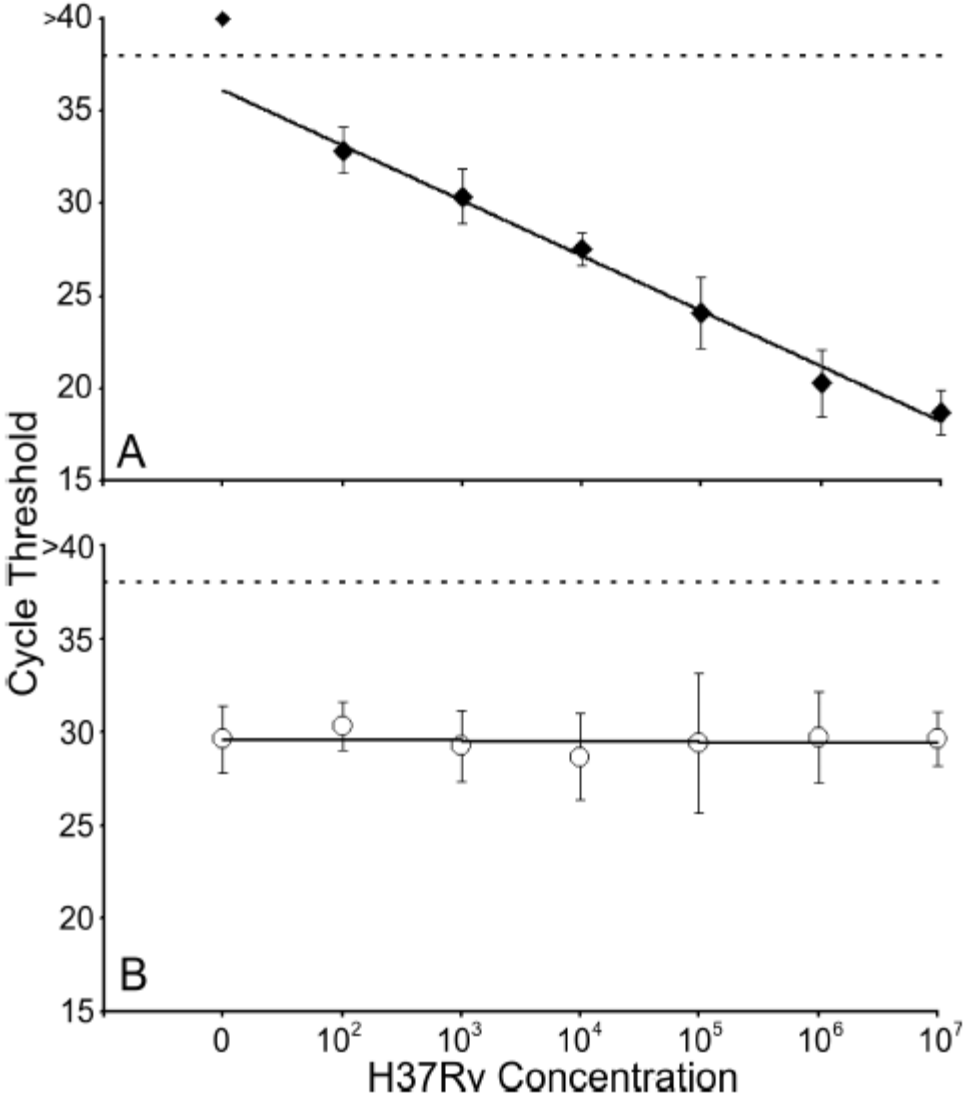


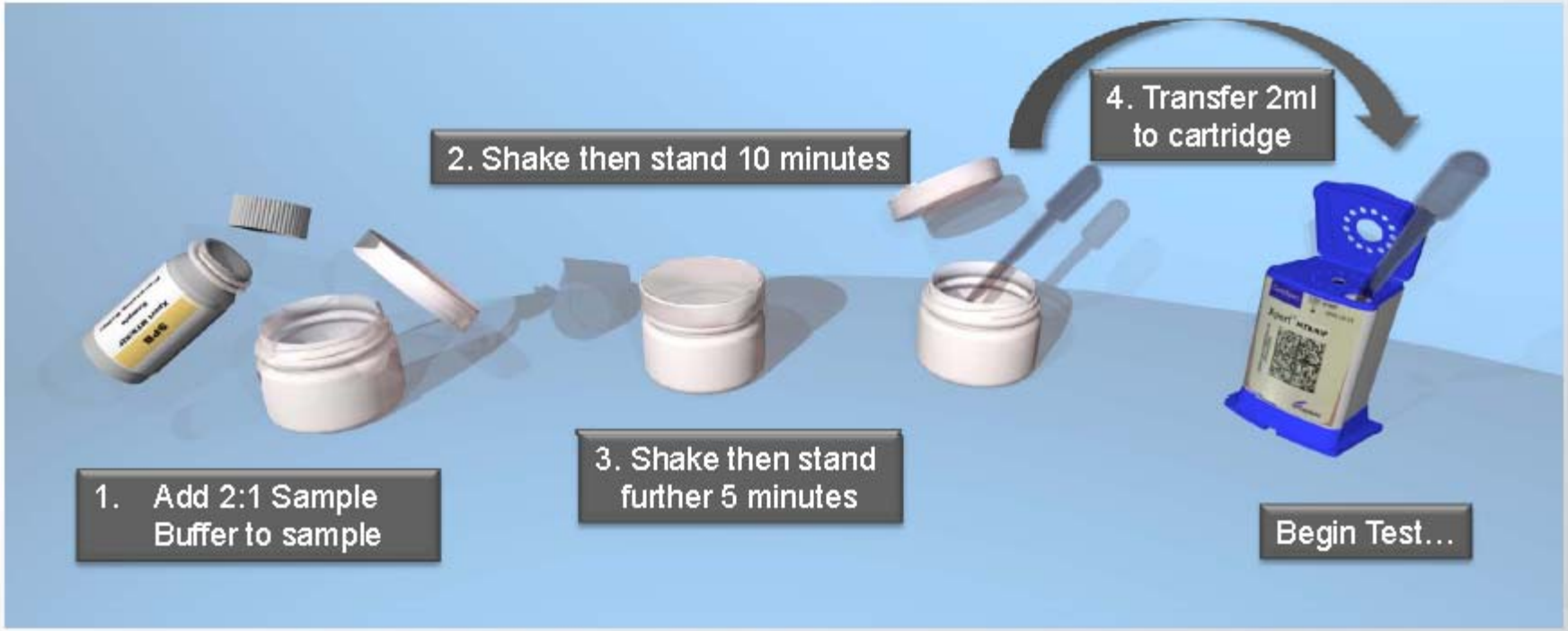
TABLE 1: Specificity Panel

BACTERIA[c]	
Acinetobacter baumanii	BEI NR-10146
Acinetobacter calcoaceticus	Clinical Strain
Actinomyces israellii	ATCC 12102
Actinomyces meyeri	Clinical Strain
Bacillus cereus	BEI NR-4198
Bacillus subtilis	Clinical Strain
Bordetella parapertussis	ATCC 151311D
Bordetella pertussis	Cepheid 500-0780
Campylobacter jejuni	BEI NR-3057
Chlamydia pneumonia	Cepheid VR 1360 CM-1
Citrobacter freundii	Clinical Strain
Corynebacterium diphtheriae	PI #581
Corynebacterium pseudodiphtheriticum	ATCC 10700
Corynebacterium xerosis	Clinical Strain
Enterobacter aerogenes	Clinical Strain
Enterobacter cloacae	Clinical Strain
Enterococcus avium	Clinical Strain
Enterococcus faecalis	Clinical Strain
Enterococcus faecium	Clinical Strain
Escherichia coli	Clinical Strain
Escherichia coli O157H7	ATCC 35150
Fusobacterium nucleatum	ATCC 25586D
Haemophilus influenzae	ATCC 49247
Haemophilus parahemolyticus	ATCC 10014
Haemophilus parainfluenzae	Clinical Strain
Klebsiella oxytoca	Clinical Strain
Klebsiella pneumoniae	Clinical Strain
Legionella pneumophila	ATCC 33152D
Leuconostoc mesenteroides	Clinical Strain
Listeria grayi	ATCC 25401
Listeria monocytogenes	BEI NR-4211
Moraxella catarrhalis	ATCC 8176
Morganella morganii	Clinical Strain
Mycoplasma pneumoniae	ATCC 15531D
Neisseria gonorrhoeae	ATCC 49226
Neisseria lactamica	ATCC 23971
Neisseria meningitidis	Clinical Strain
Neisseria mucosa	ATCC 69695
Nocardia asteroides	ATCC 19247
Nocardia cyriageorgica	ATCC BAA-1516
Nocardia farcinica	ATCC 3318
Pasteurella multocida	ATCC 3160
Peptostreptococcus anaerobius	ATCC 49031D
Porphyromonas gingivalis	ATCC 33277D-5
Prevotella melaninogenica	ATCC 25845D-5
Propionibacterium acnes	Clinical Strain
Proteus mirabilis	Clinical Strain
Proteus vulgaris	BEI DD-460
Providencia alcalifaciens	PI #368
Pseudomonas aeruginosa	ATCC 27853
Rhodococcus equi	ATCC 14187
Salmonella enterica	BEI NR-515
Salmonella typhi	Clinical Strain
Serratia marcescens	Clinical Strain
Shigella boydii	Clinical Strain
Shigella flexneri	Clinical Strain
Staphylococcus aureus	ATCC 25953
Staphylococcus capitis	Clinical Strain
Staphylococcus epidermidis	ATCC 12228
Staphylococcus haemolyticus	Clinical Strain
Staphylococcus hominis	Clinical Strain
Staphylococcus lugdunensis	Clinical Strain
Stenotrophomonas maltophilia	Clinical Strain
Streptococcus equi	Clinical Strain
Streptococcus pyogenes	ATCC 19615
Streptococcus agalactiae	ATCC 12386
Streptococcus constellatus	Clinical Strain
Streptococcus mitis	Clinical Strain
Streptococcus mutans	Clinical Strain
Streptococcus pneumoniae	Clinical Strain
Streptococcus uberis	Clinical Strain
Veillonella parvula	ATCC 10790D-5
Stenotrophomonas maltophilia	Clinical Strain
Yersinia pestis	BEI NR-2644
FUNGUS	
Candida albicans	ATCC 14053D
Cryptococcus neoformans	Casadevall Lab
Histoplasma capsulatum	Nosanchuk Lab
Kingella kingae	ATCC 23332D
VIRUS	
Adenovirus[b]	ZM NATADV1-ST
Herpes simplex virus 1	Cepheid 500-1268
Herpes simplex virus 2	Cepheid 500-1269
Influenzavirus A[a]	Cepheid 500-0763
Influenzavirus B[a]	500-0764
Parainfluenza 2[b]	ZM NATPARA2-ST
Parainfluenza 3[b]	ZM NATPARA3-ST
Respiratory Syncytial Virus A[a]	Cepheid 500-0765
Respiratory Syncytial Virus B[a]	Cepheid 500-0765
Rhinovirus 6[a]	Cepheid
Rhinovirus 16[a]	Cepheid

foundation for innovative new diagnostics

- 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.

Simple Sample Processing – Direct Sputum



Rifampin Susceptible Sample

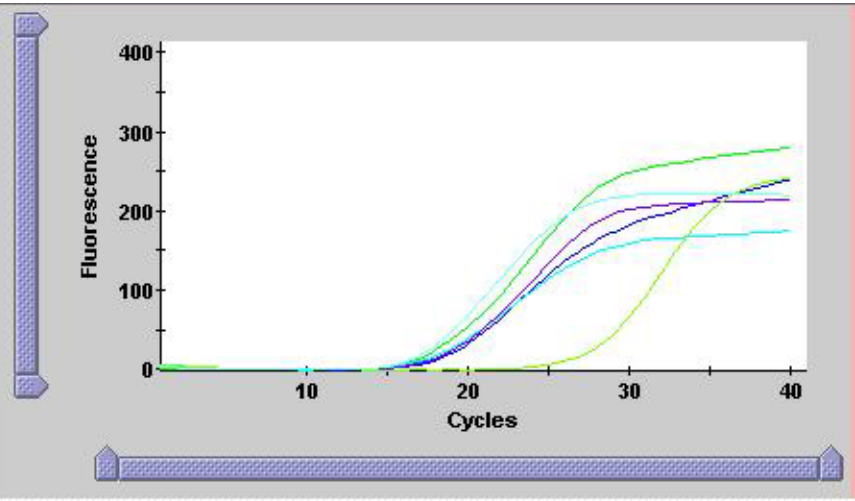
Module Name	B1
Sample ID	uganda sputum.6
Assay	MTB Beta
Assay Version	2
Assay Type	Research Use Only
Reagent Lot ID	00502
Cartridge S/N	0
Expiration Date	<None>
Test Type	Specimen

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

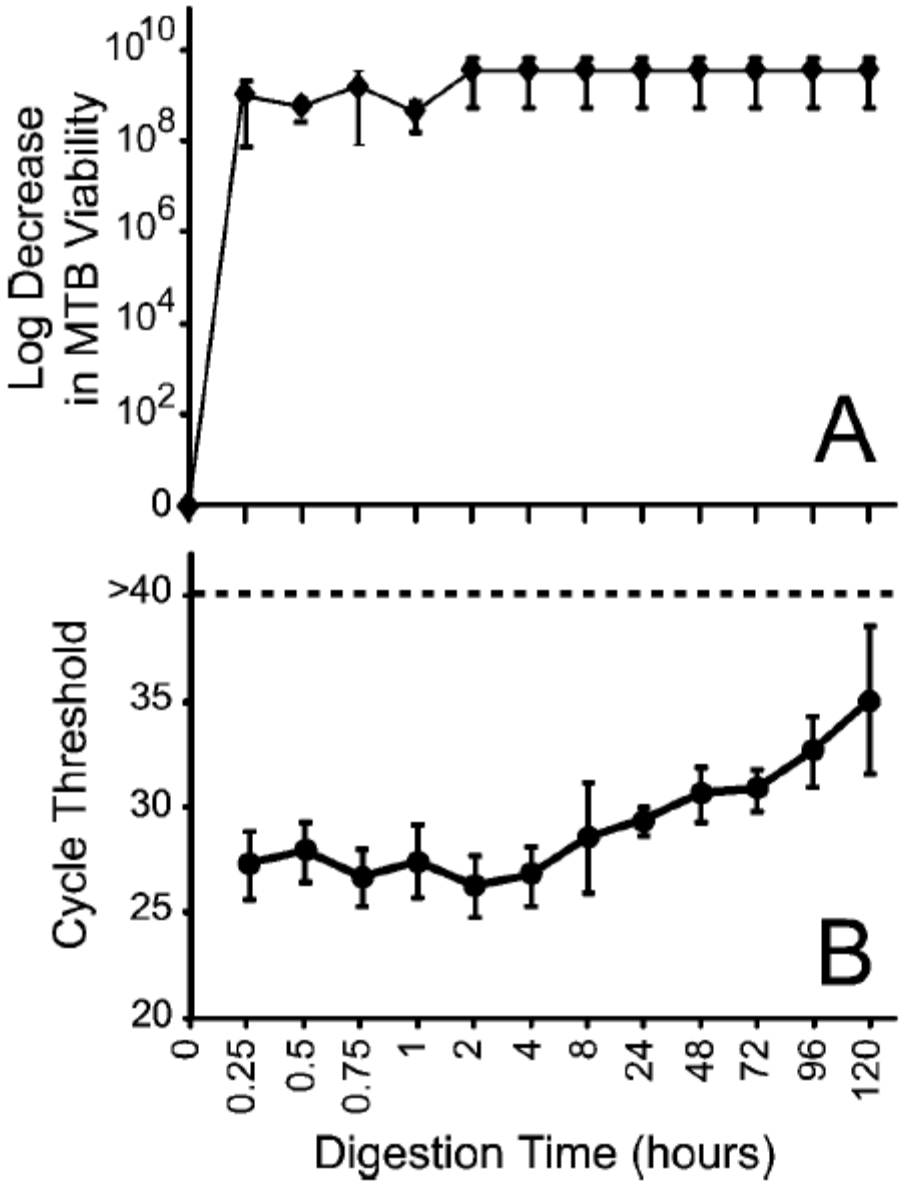
Assay Name: MTB Beta Version: 2

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

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	3.5×10^7 BCG	7H9 media			<10	$>3.5 \times 10^6$	>99.9
Study 2	3.5×10^7 BCG	sputum			1.5	2.3×10^7	>99.9
Study 3	3.3×10^7 H37Rv	sputum	12, 13, 21	15.3	153	2.15×10^5	99.9
Study 4	3.63×10^8 H37Rv	sputum	4, 6, 2	4	40	9.1×10^6	>99.9
Study 5	4.0×10^8 H37Rv	sputum	2, 2, 3	2.33	23.3	1.7×10^7	>99.9
				Average log kill		1.06×10^7	


Log Decrease in MTB Viability
10^{10}
10^{8}
10^{6}
10^{4}
10^{2}
0
A
Cycle Threshold
>40
35
30
25
20
B
0
0.25
0.5
0.75
1
2
4
8
24
48
72
96
120
Digestion Time (hours)

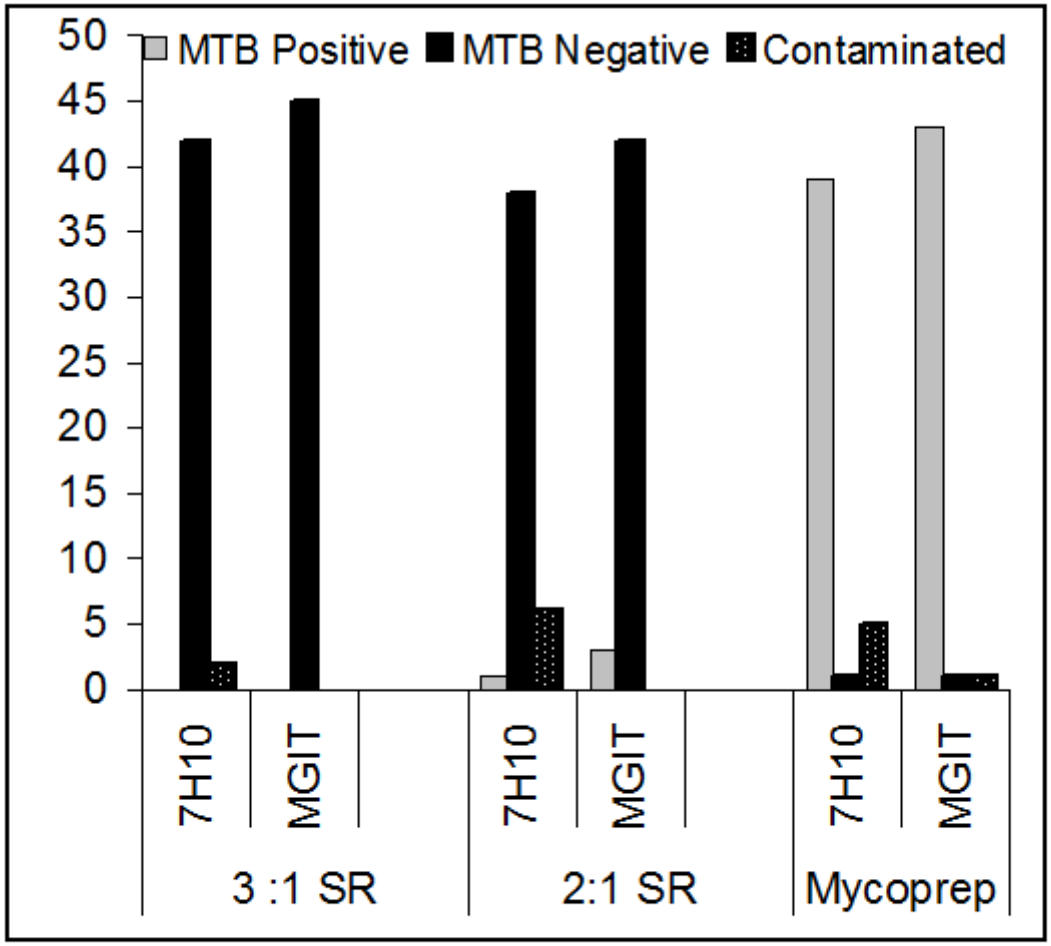


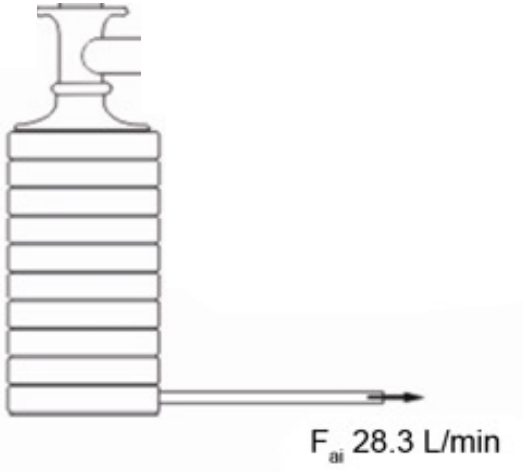
Figure 12: Inactivation effectiveness of SR on smear-positive sputum

Biosampler

Liquid media

Anderson Impactor

Solid media



Aerosol Viability During Manual Steps

	Mean cfu/m^3 air detected over 3 experiments	
5 X 10^8 cfu BCG spiked into sputum.	Anderson impactor	BioSampler
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