On-demand diagnosis of TB and rifampin-resistance using the Xpert MTB/RIF assay: the present and the future.

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Conflict of interest

Dr. Alland is the Principal Investigator of two NIH grants which include Cepheid as collaborators.

Is a member of a group of investigators who receive royalty payments for molecular beacons licenses.

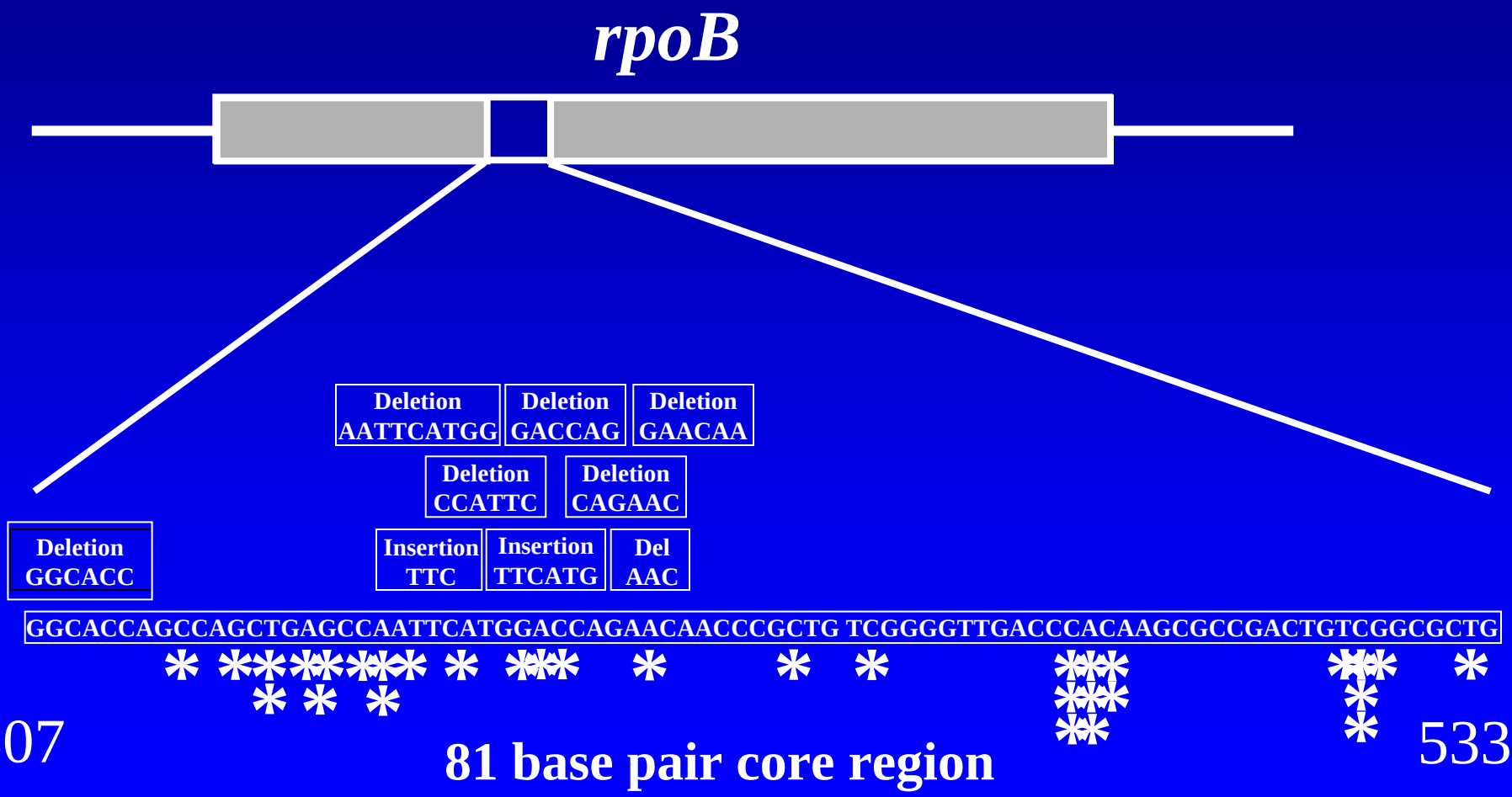
Cepheid has licensed molecular beacons for use in the Xpert MTB/RIF assay.

Xpert MTB/RIF assay development goals

- Sensitive and specific *M. tuberculosis* detection
- Detection of rifampin resistance
- Rapid, simple virtually hands-free operation.
- Reduction in biohazard

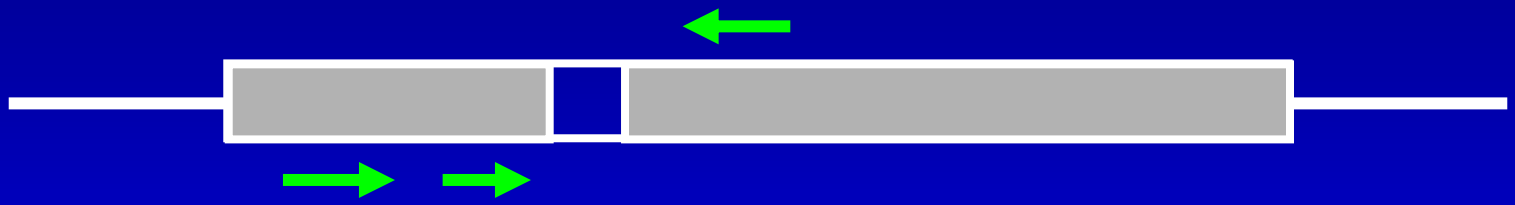
Rifampin resistance

- Mutations map to a single "core region" of the *rpoB* gene
- Accounts for ~ 95% of clinical rifampin-resistance.



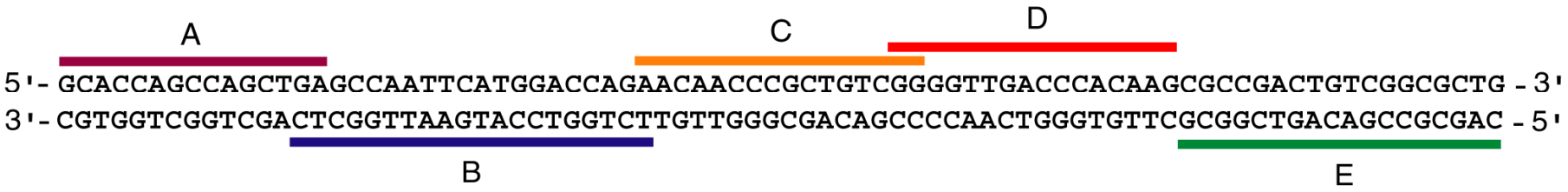
Adapted from: Musser. 1995. *Clin. Microbiol. Rev.* 8:496.

The *rpoB* core region is flanked by *M. tuberculosis* – specific DNA sequences.

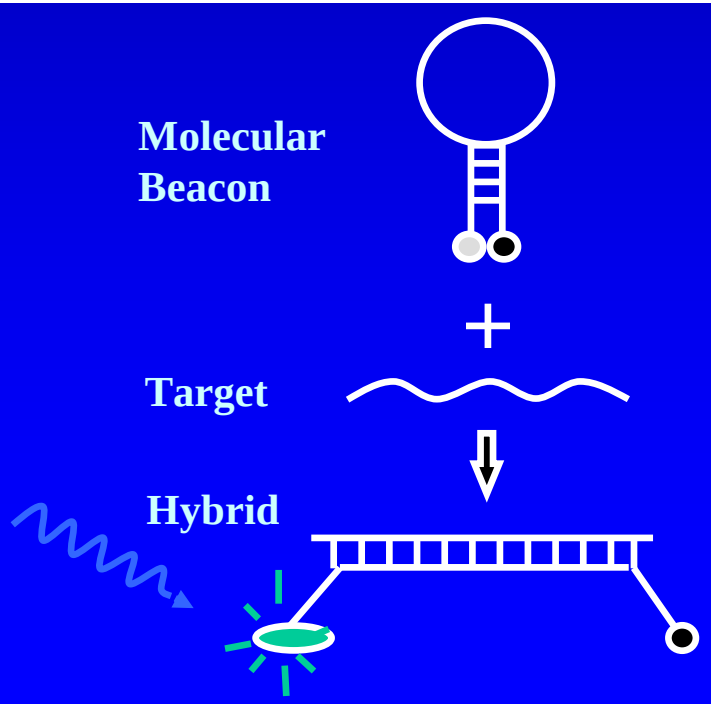


Thus, it is possible to test for *M. tuberculosis* and rifampin-resistance simultaneously, targeting a single amplicon

A molecular beacon assay to detect TB and mutations in the *rpoB* core region

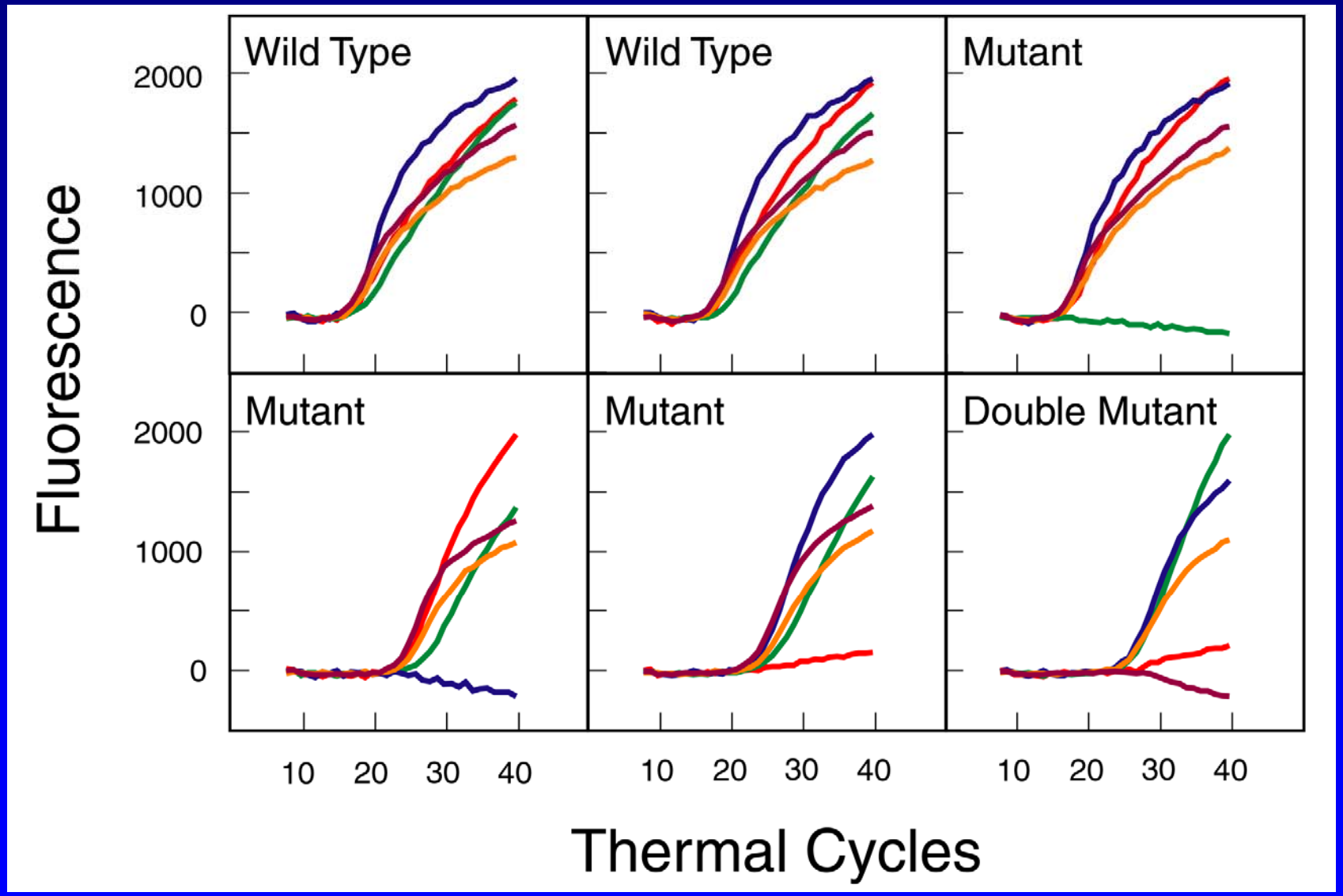


By labeling each molecular beacon with a different fluorophore, it is possible to perform the entire assay in a single well.



Rifampin-resistant TB contains 1-2 *rpoB* mutations (95% sensitivity).

(Five-color PCR performed in a single well.)



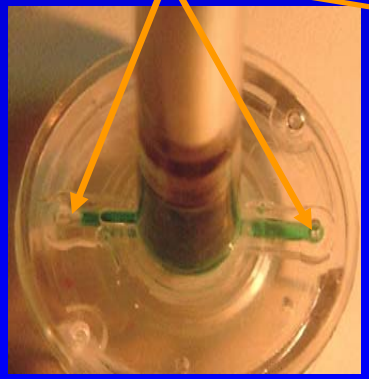
El Hajj et al., J. Clin. Microbiol. 2001

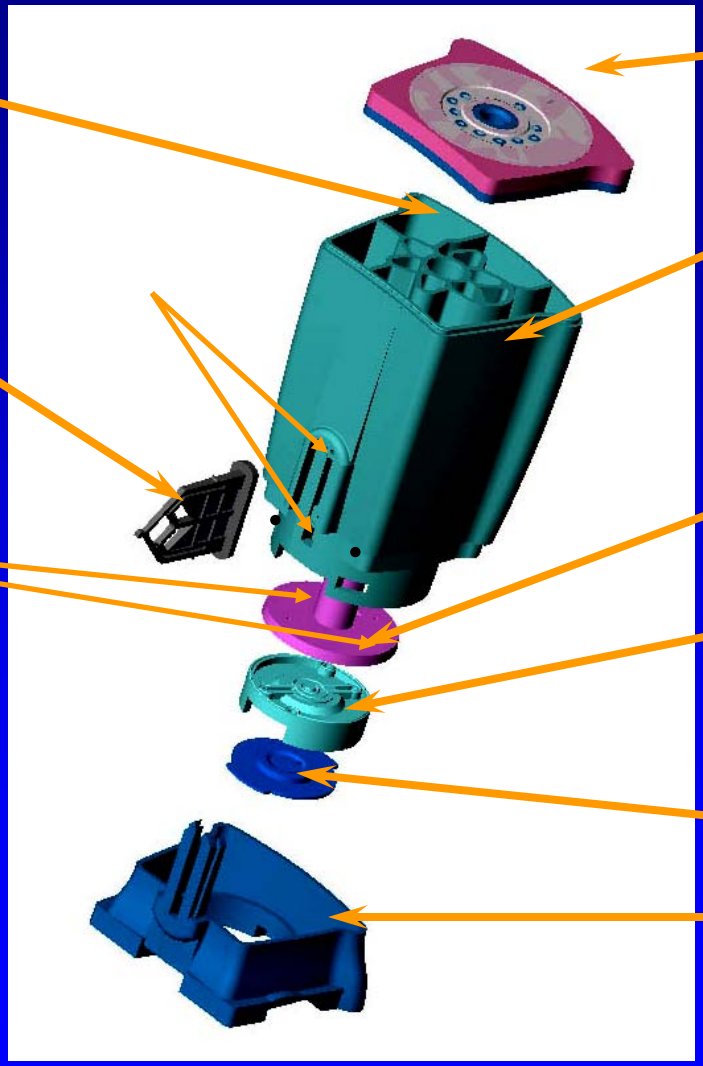
Cephied GenXpert Cartridge Exploded View

Molded Prefilter
bottom of chamber 3

PCR
Tube

Valve body Ports





Lid

Cartridge Body
(11 Fluid Chambers and
overmolded gasket)

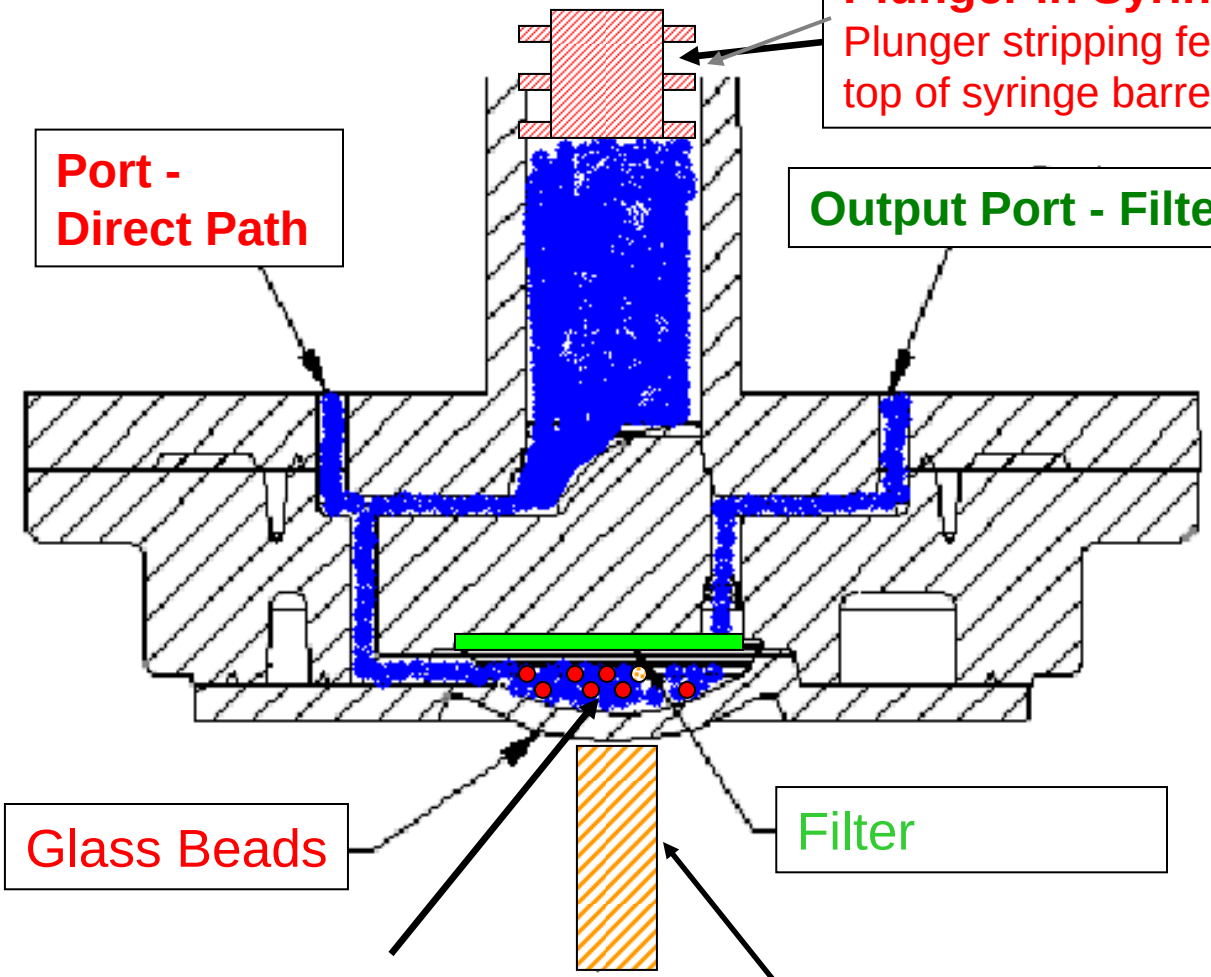
Syringe Barrel

Rotary Valve/Filter and
Ultrasonic Lysis or
SPBRegion

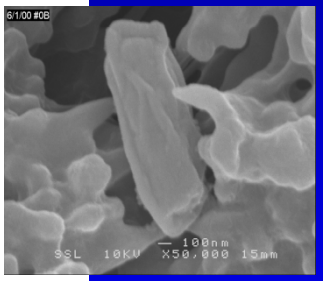
Cap/Ultrasonic Interface

Cartridge Foot

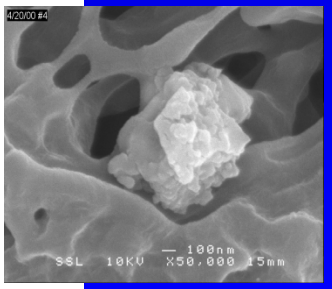
Cartridge A – Valve Body

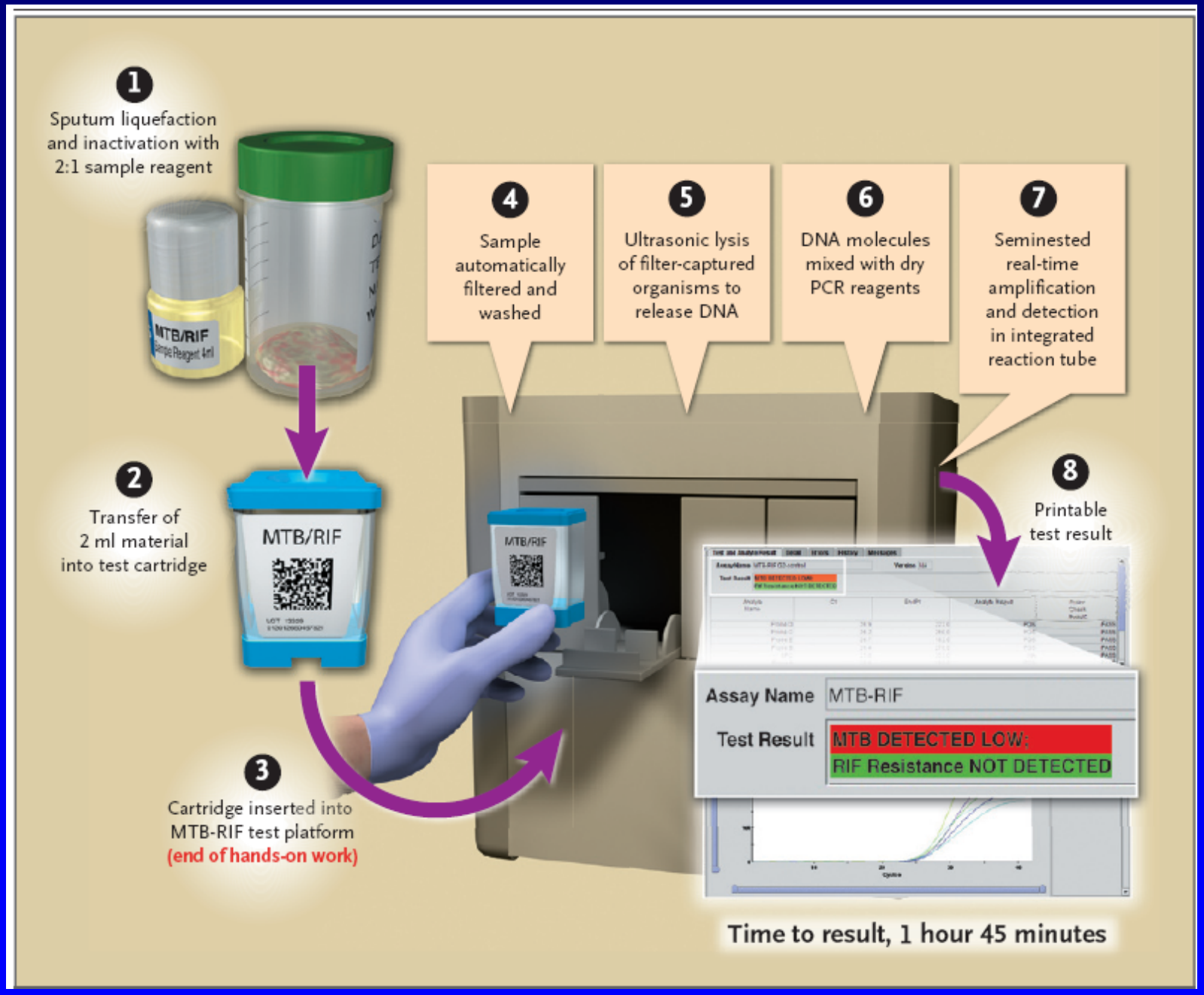


Before

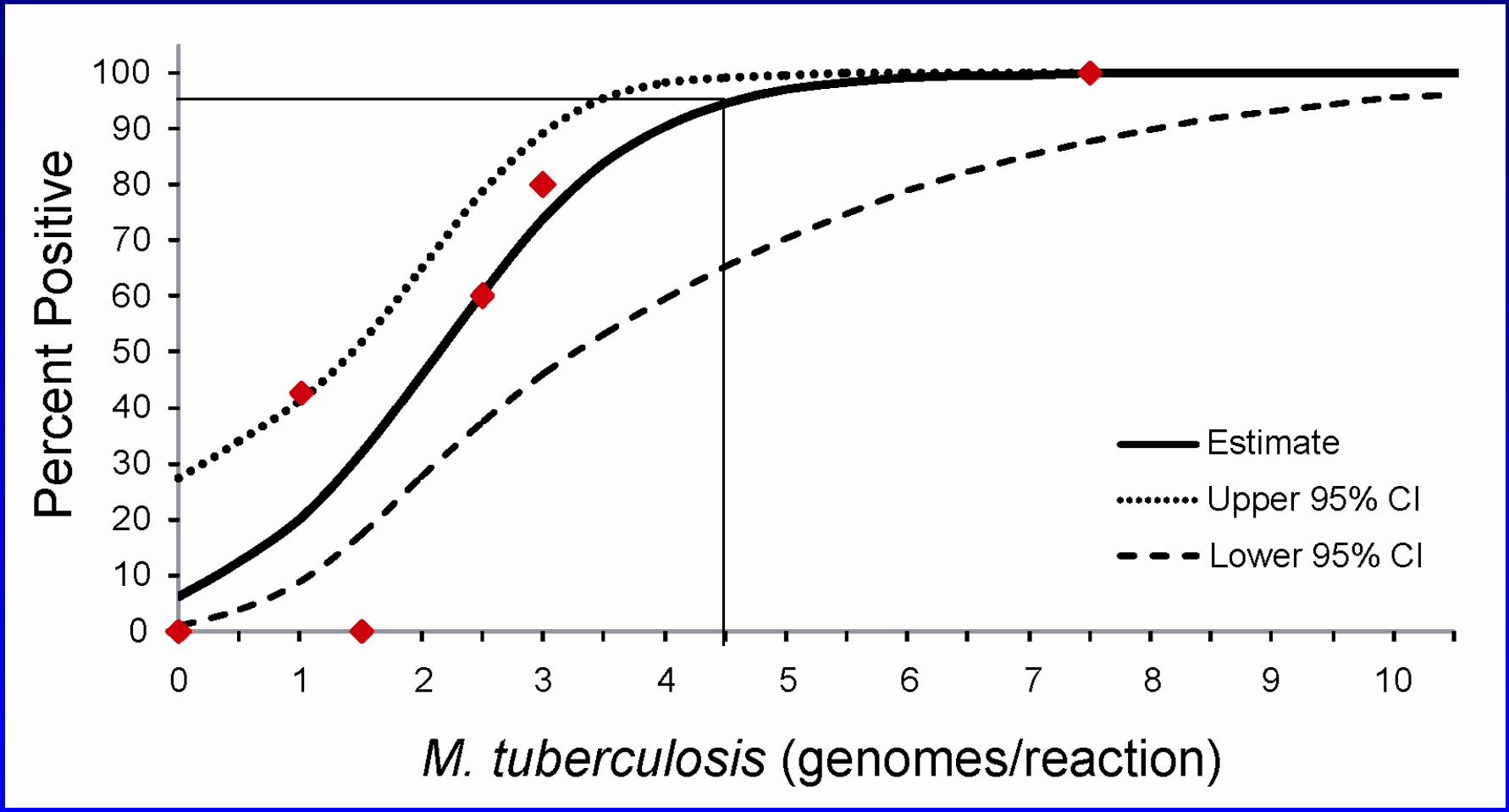


After



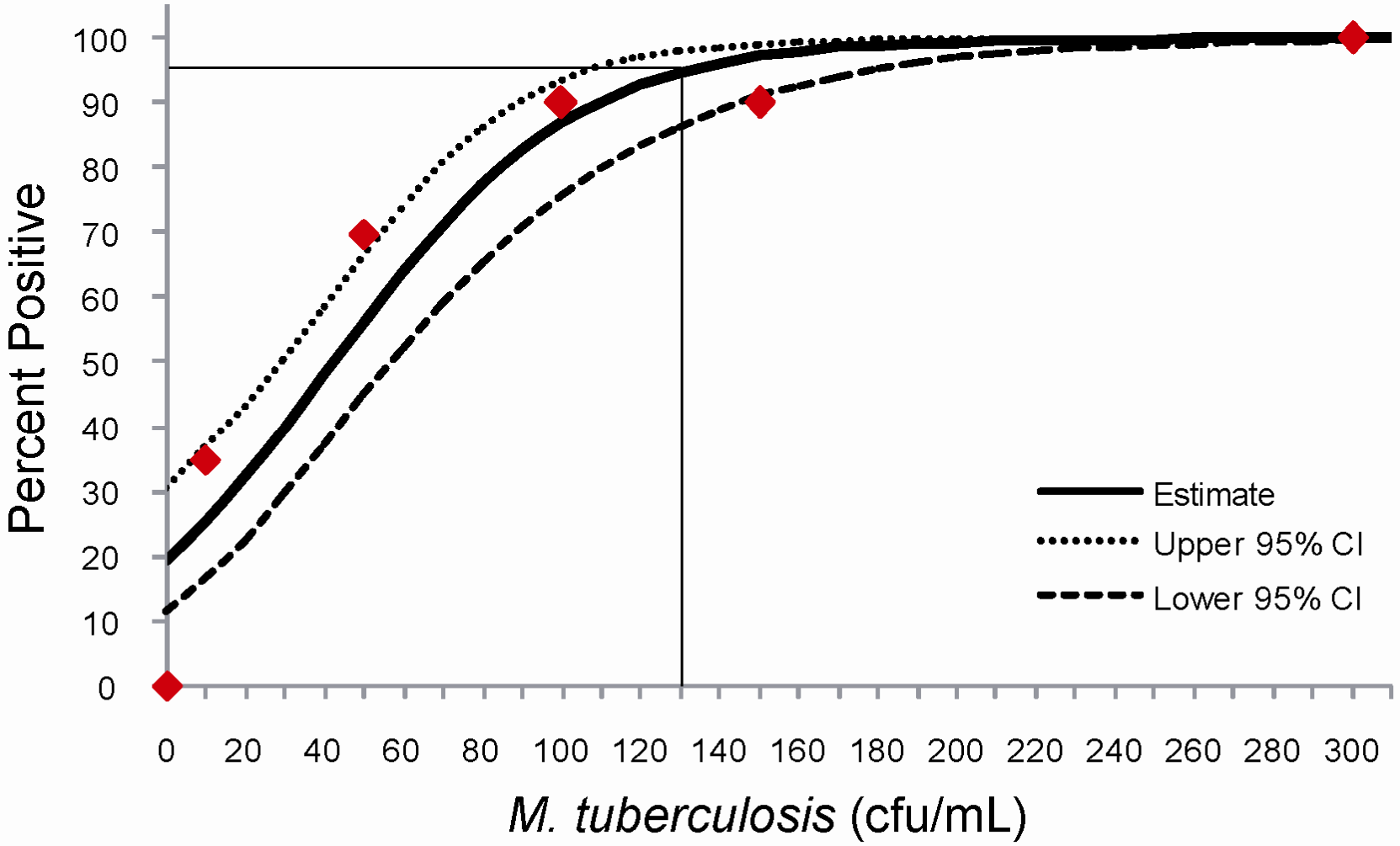

1
Sputum liquefaction and inactivation with 2:1 sample reagent
MTB/RIF
2
Transfer of 2 ml material into test cartridge
MTB/RIF
3
Cartridge inserted into MTB-RIF test platform
(end of hands-on work)
4
Sample automatically filtered and washed
5
Ultrasonic lysis of filter-captured organisms to release DNA
6
DNA molecules mixed with dry PCR reagents
7
Seminested real-time amplification and detection in integrated reaction tube
8
Printable test result
Assay Name
MTB-RIF
Test Result
MTB DETECTED LOW;
RIF Resistance NOT DETECTED
Time to result, 1 hour 45 minutes

Limit of detection (LOD) of *M. tuberculosis* DNA



Based on 20 replicates per concentration tested

Limit of detection (LOD) of *M. tuberculosis* cells spiked into sputum



Based on 20 replicates per concentration tested

Exclusivity Panel- NTM

M. avium SmT
M. avium SmD
M. intracellulare 35790
M. intracellulare 35776
M. kansasii
M. malmoense
M. abscessus
M. asiaticum
M. celatum
M. chelonae
M. flavescens
M. fortuitum
M. genevenses
M. gordonae
M. marinum
M. scrofulaceum
M. simiae
M. szulgai
M. thermoresistable
M; triviale
M. xenope

Exclusivity Panel

Acinetobacter baumanii	Legionella pneumophila	Staphylococcus aureus
Acinetobacter calcoaceticus	Leuconostoc mesenteroides	Staphylococcus capitis
Actinomyces israellii	Listeria grayi	Staphylococcus epidermidis
Actinomyces meyeri	Listeria monocytogenes	Staphylococcus haemolyticus
Bacillus cereus	Moraxella catarrhalis	Staphylococcus hominis
Bacillus subtilis	Morganella morganii	Staphylococcus lugdunensis
Bordetella parapertussis	Mycoplasma pneumoniae	Stenotrophomonas maltophilia
Bordetella pertussis	Neisseria gonorrhoeae	Streptococcus equi
Campylobacter jejuni	Neisseria lactamica	Streptococcus pyogenes
Candida albicans	Neisseria meningitidis	Streptococcus agalactiae
Chlamydia pneumonia	Neisseria mucosa	Streptococcus constellatus
Citrobacter freundii	Nocardia asteroides	Streptococcus mitis
Corynebacterium diptheriae	Nocardia asteroides	Streptococcus mutans
Corynebacterium pseudodiptheriticum	Nocardia cyriageorgica	Streptococcus pneumoniae
Corynebacterium xerosis	Nocardia farcinica	Streptococcus uberis
Cryptococcus neoformans	Pasteurella multocida	Veillonella parvula
Enterobacter aerogenes	Peptostreptococcus anaerobius	Stenotrophomonas maltophilia
Enterobacter cloacae	Porphyromonas gingivalis	Yersinia pestis
Enterococcus avium	Prevotella melaninogenica	**VIRUS**
Enterococcus faecalis	Propionibacterium acnes	Adenovirus
Enterococcus faecium	Proteus mirabilis	Herpes simplex virus 1
Escherichia coli	Proteus vulgaris	Herpes simplex virus 2
Escherichia coli O157H7	Providencia alcalifaciens	Influenzavirus A
Fusobacterium nucleatum	Pseudomonas aeruginosa	Influenzavirus B
Haemophilus influenzae	Rhodococcus equi	ParaInfluenza 2
Haemophilus parahemolyticus	Salmonella enterica	ParaInfluenza 3
Haemophilus parainfluenzae	Salmonella typhi	Respiratory Syncytial Virus A
Histoplasma capsulatum	Serratia marcescens	Respiratory Syncytial Virus B
Kingella kingae	Shigella boydii	Rhinovirus 6
Klebsiella pneumoniae	Shigella flexneri	Rhinovirus 16

Inclusivity / Exclusivity results

Organism*	Xpert MTB/RIF Result		
	Mtb Positive		
	Resistance Detected	Resistance Not Detected	*Mtb* Negative
Mtb Rif R	37	0	0
Mtb Rif S	0	42	0
NTM	0	0	21
Bacteria	0	0	73
Fungi	0	0	4
Virus	0	0	8

**Mtb* tested at 4X limit of detection. All others tested at 10^6 genomes per reaction.

Phenotypic rifampin resistance versus Xpert MTB/RIF resistance. What causes discrepant results?

Affecting sensitivity

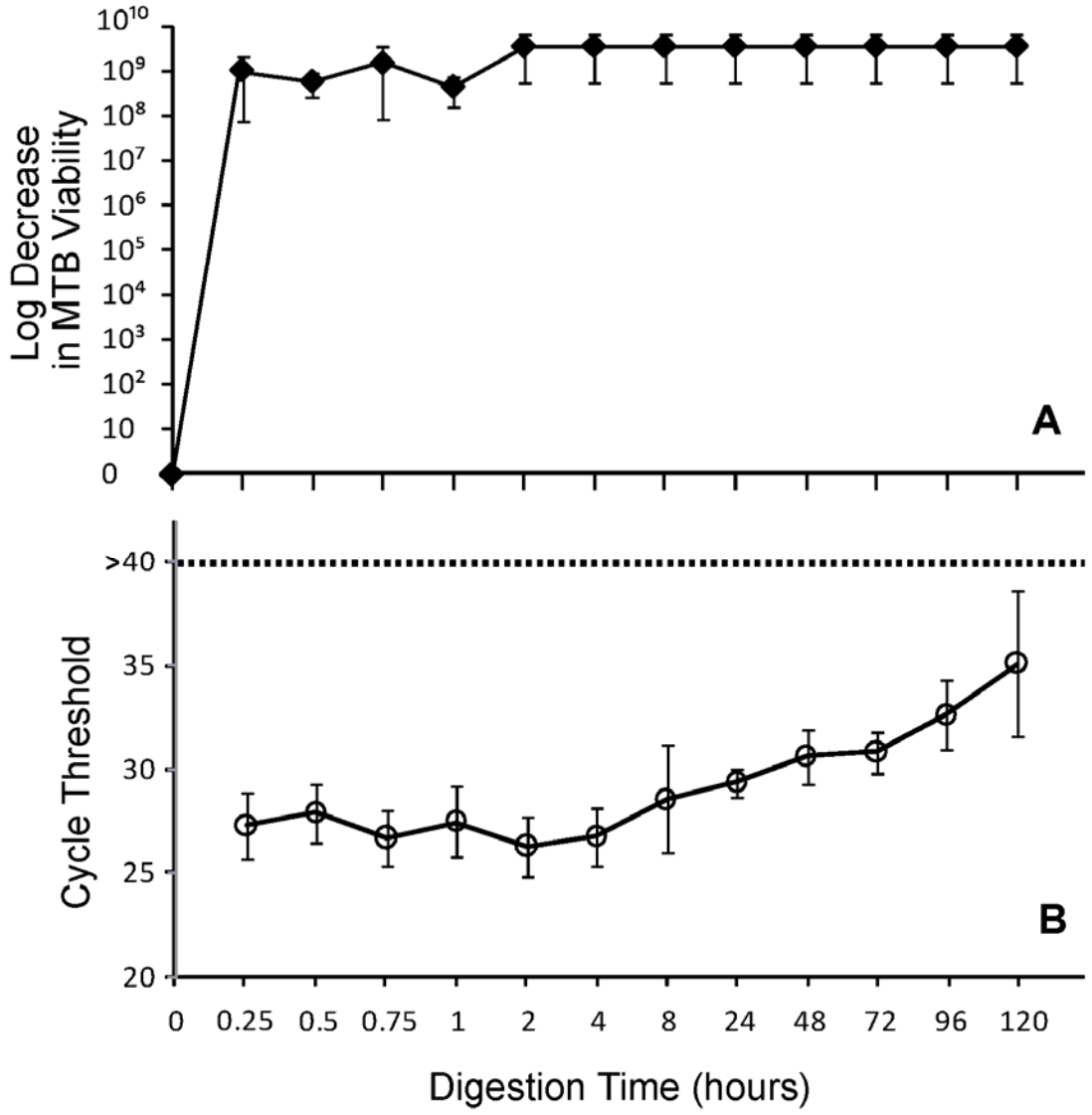
- Rifampin-resistant mutations occurring outside of the *rpoB* core region.
- Patients infected with both susceptible and resistant strains.

Affecting specificity

- Phenotypic drug-susceptibility test method.
- Liquid testing methods (MGIT) do not appear to be as accurate as LJ or agar proportions or molecular methods (Hain, Xpert or DNA sequencing).
- Xpert-specific issues to be discussed by Mark Perkins.

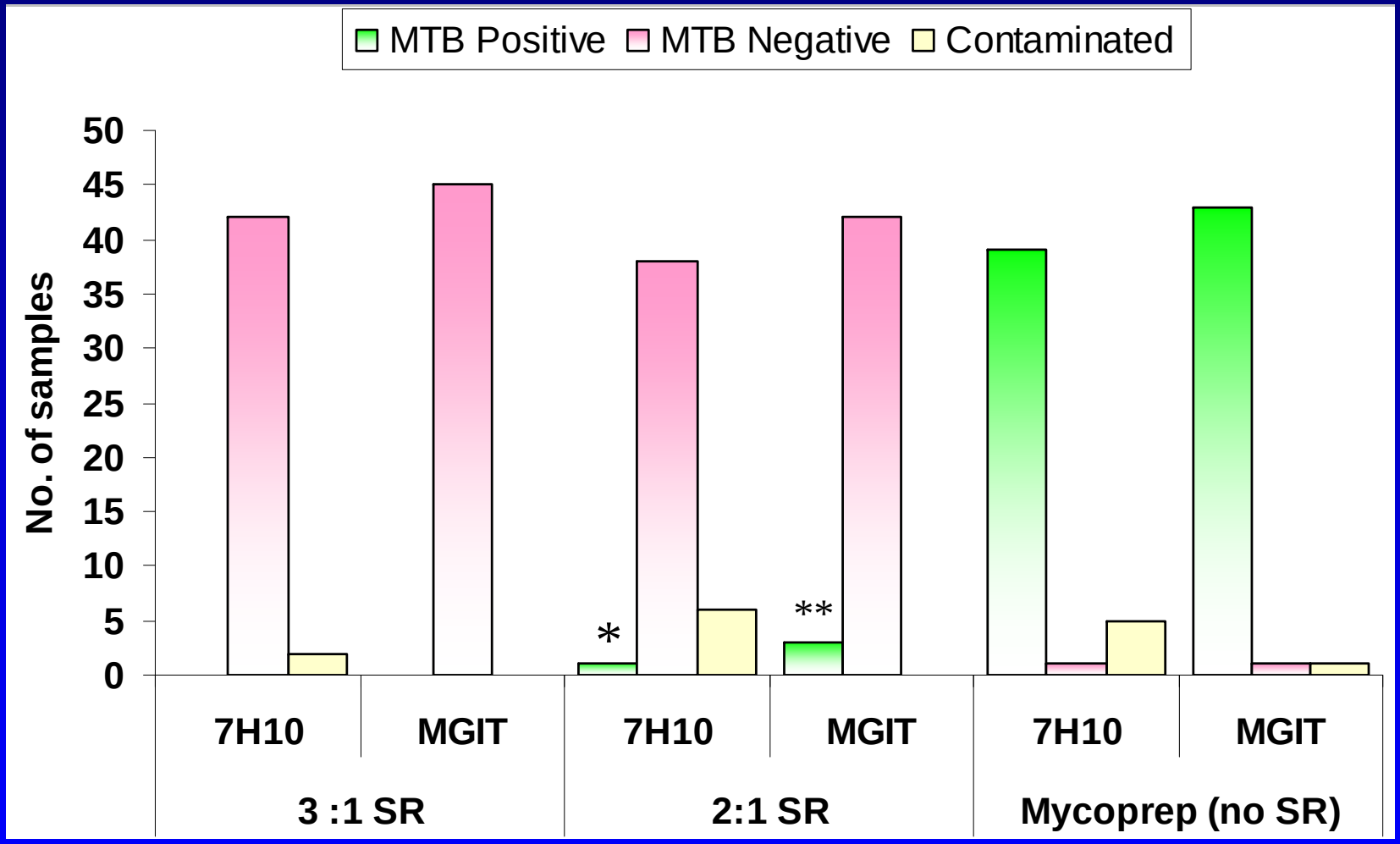
Biosafety?

Effect of Sample Reagent on Mtb viability (A) and assay sensitivity (B).



Based on 150 cfu in 1 ml sputum

Sample reagent (SR) tuberculocidal activity: 15 min treatment on strongly smear-positive sputum samples from TB patients



* Only 9 colonies present in the single solid media culture-positive sample

** Time to positive culture on three liquid culture-positive samples delayed an average of 13.1 days with SR treatment.

Six stage Andersen Impactor

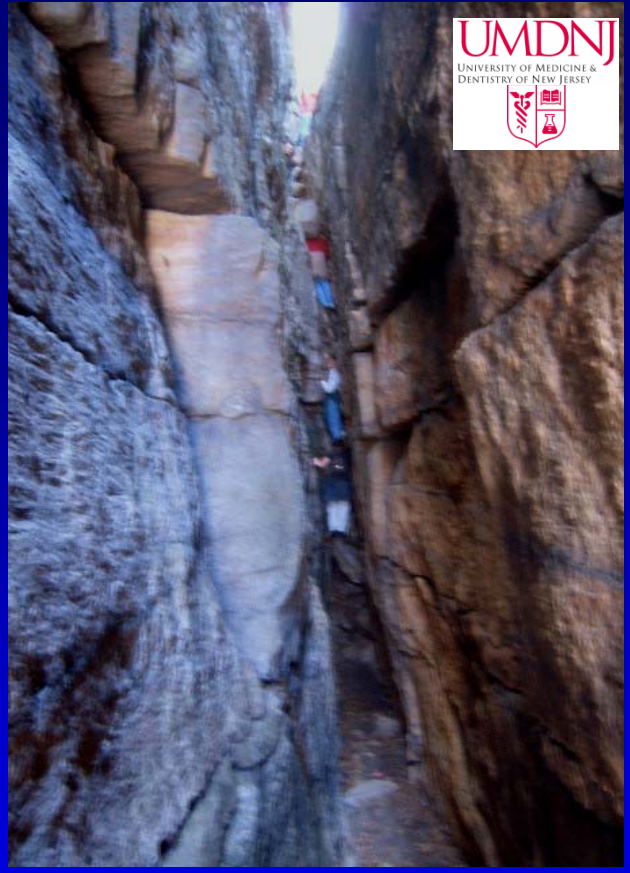
Solid culture

Biosampler

Liquid culture

Aerosol Viability During Manual Steps

5×10^8 cfu BCG spiked into sputum.	Mean cfu/m^3 air detected over 3 experiments	
	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



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