Molecular Detection of MDR-TB

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Mycobacterium tuberculosis

- **Nearly 2 million deaths/yr worldwide**
- **High Impact on HIV- associated mortality in Africa**
- **Drug resistance is emerging**
- **Rifampin resistance is found in virtually all MDR MTB strains**
- **Rifampin resistance proposed as a surrogate for MDR TB**

Characteristics of an Ideal TB test

Elaine Thomas/WHO, via The New York Times

Fewer resistance tests exist for the newer, second-line drugs needed to treat drug-resistant tuberculosis, and many of them are difficult to perform.

A lack of reliable tests slows fight against TB

By Lawrence K. Altman

In the escalating battle against extensively drug-resistant tuberculosis, conflicting findings from laboratory tests have hampered efforts to control the spread of the disease.

Some of the conflicts come from a lack of standardized testing methods and others from subtle but critical differences in the way the tests are performed.

The most celebrated example of such discordant findings involved Andrew Speaker, the Atlanta lawyer who caused an international health scare after traveling to Europe in May with what was believed to be extensively drug-resistant tuberculosis, known as XDR. This month, Speaker's doctors downgraded his type of tuberculosis to multidrug-resistant, or MDR, after repeating tests that initially gave a different result

tions for such procedures made by a similar agency panel in 2001.

The overwhelming majority of tuberculosis cases are caused by bacterial strains that yield to the standard, or first-line, anti-TB drugs. Newer, second-line drugs are used if a strain of tuberculosis is MDR or XDR, which are resistant to the first-line drugs. If tuberculosis strains are not tested for drug resistance as soon as they are found in a patient, the problem may be detected too late to permit a cure.

Tuberculosis resistance develops when drugs are misused or mismanaged. For example, patients may fail to complete their full course of treatment. Health care providers may prescribe the wrong treatment, the wrong dose or the wrong length of time for taking the drugs. Another problem occurs when drugs are not available, or when the drugs are of poor quality.

What alarms health officials is the

oratories use pure powders of an anti-tuberculosis drug's active ingredient. But even such powders can be affected by heat and other factors, leading to inconsistent findings.

Another reason is that the many steps involved in the laboratory process increase chances for human error.

In 2001, the WHO panel said the latest knowledge was "very incomplete regarding how to best perform" resistance tests of second-line drugs and the usefulness of the tests in treating such cases. Since then, experience

If TB strains are not tested for drug resistance as soon as they are found, it may be too late for a cure.

to isoniazid and streptomycin, according to published research by Barror Lerner of Columbia University, where she was treated.

As more tuberculosis strains have become resistant to more drugs in recent years, health officials have come up with the MDR and XDR designations. Such forms are much costlier to control than standard tuberculosis.

In 2006, the Centers for Disease Control and the WHO revised the definition of XDR in part because of a lack of standardization of the tests for certain antibiotics needed to treat it.

The WHO says that of the 424,000 MDR tuberculosis cases that occur in the world each year, 25,000 are XDR but the organization believes that fewer than 5 percent of resistant cases are detected. The agency bases its opinion in part on the high prevalence of multidrug resistance found in an expanding number of provinces surveyed in

- Direct-specimen detection of MTB
- Sensitivity of culture with goal of eliminating negative cultures
- Simultaneous detection of drug resistance
- On-demand availability (no batching requirement)
- Decentralized Platform technology to reduce or eliminate sample shipping
- Rapid (<2 hours)
- Portable
- Low skill requirements
- Highly reliable

(Oh, and impossibly cheap)

WHO Stop TB Effort

- 900 WHO TB Reference labs worldwide
  - Liquid or solid culture
  - Implementing molecular methods
  - Drug resistance testing
  - Specimens transported over long distances
  - Delayed results
  - Many patients LTF or deceased by the time results are back
- 19,000 microscopy centers
  - Specimen collection
  - Patient management
  - DOT therapy
  - The key: putting the right technology in the right place

FIND/Gates/NIAID Support for the GeneXpert TB Project

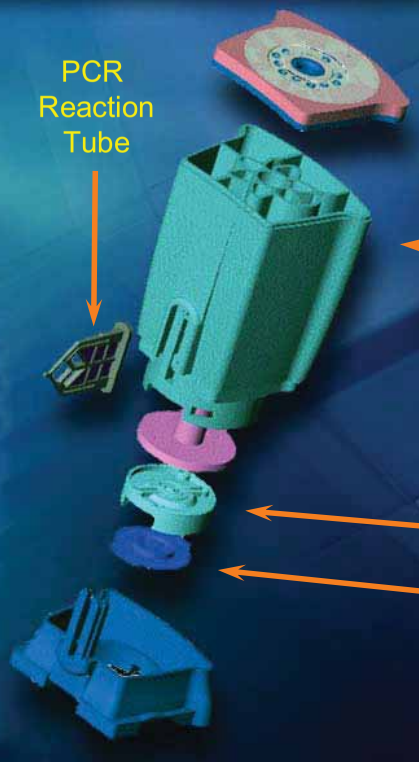
- Project Initiated in 2006
- FIND/Gates Support: $6.2 M
- NIAID support: $4.3M
- Collaborators:
  David Alland, UMDNJ
  Catherina Boehme
  Mark Perkins
  FIND

Sputum…..the final frontier

- It is usually highly viscous and thus incompatible with microfluidic devices
- It is often purulent
- It is often bloody
- Target organisms require concentration in order to be consistently detected
- Complicated off-line centrifugation and DNA extraction too slow and technically demanding for POC testing

GeneXpert Cartridge

- **Critical interface between *macrofluidic* requirements of sample processing with *microfluidics* of PCR**
- **Room-temp stability of reagents within lyophilized beads**
- **Contamination Control via enclosed, real-time PCR**
- **Nested PCR capability**
- **Universal sample prep**
  - Sputum, stool, blood, BAL, swabs
- **Built-in assay controls**



Patents - 6,374,684 & 6,391,541

GeneXpert Module

- Syringe motor drives fluid movement
- Valve motor directs access to chambers
- Independent thermal cycler allows for random-access design
- Adaptable to different Dx environments

Cepheid.

GeneXpert Systems

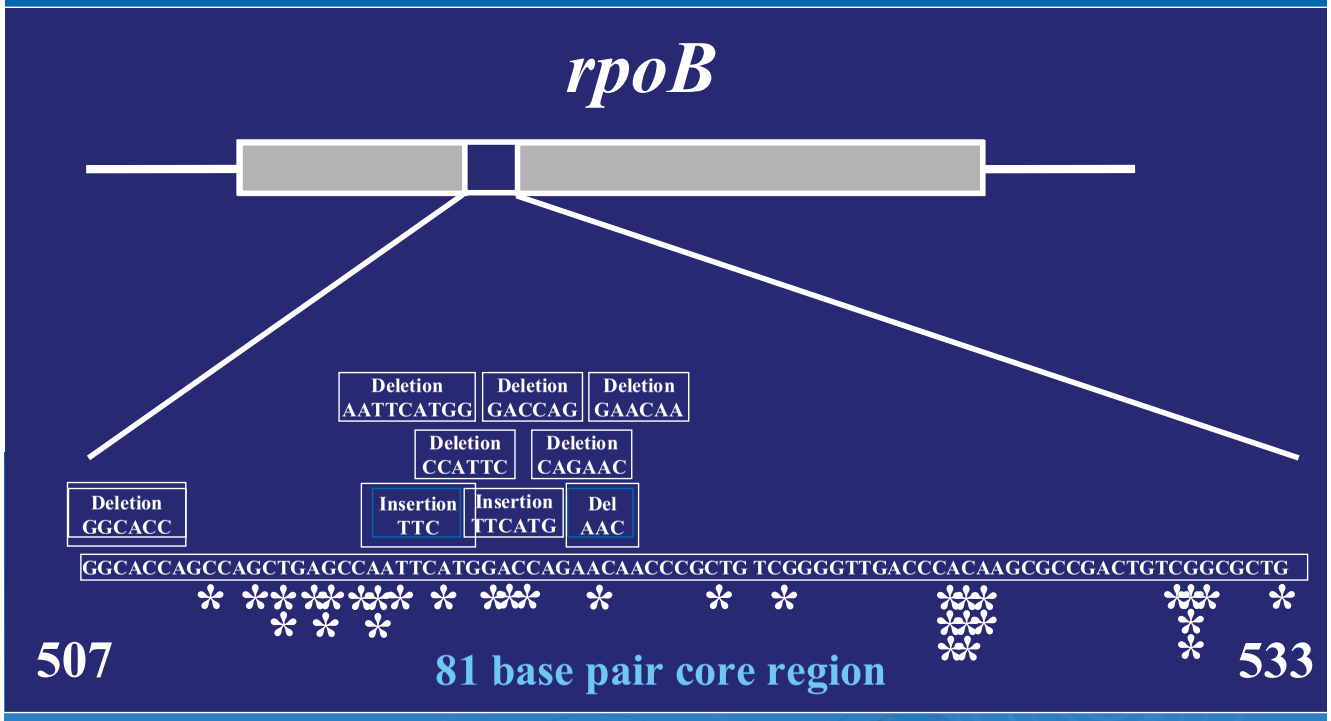
GeneXpert Module

GX-I | GX-IV | GX-XVI | GeneXpert Infinity-48 System

GeneXpert Menu: Now Thru 2013

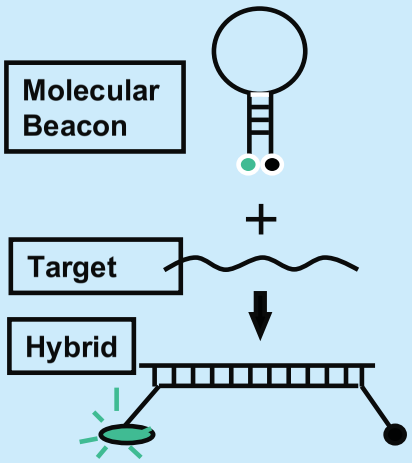
	Available now (CE-IVD)			2010	2011-2013
Women's Health	GBS			GC-CT	GC-CT *CLIA-waived* Vaginitis Panel GC-CT-Trich HPV HSV lesion
Healthcare Associated Infections	MRSA Nasal MRSA/SA/*mecA* BC MRSA/SA/*mecA* SSTI	*C. difficile* *vanA/vanB*	MRSA/SA/*mecA* Nasal MRSA Nasal *CLIA-Waived*	MRSA Next Gen	MRSA/SA SSTI *CLIA-waived* Noro/Rotavirus
Critical Infectious Disease	EV		MTB-RIF	Respiratory Virus Panel	Group A Strep TB Sepsis Fungal HSV-CSF
Immuno-Compromised					CMV EBV HIV HCV
Oncology			BCR-ABL		Bladder Cancer Monitor Bladder Cancer Diag Jak2 Kinase/BCL2/PML RARA
Genetics		HemosIL FII, FV			

Genetics of Rifampin Resistance in *M. tuberculosis*

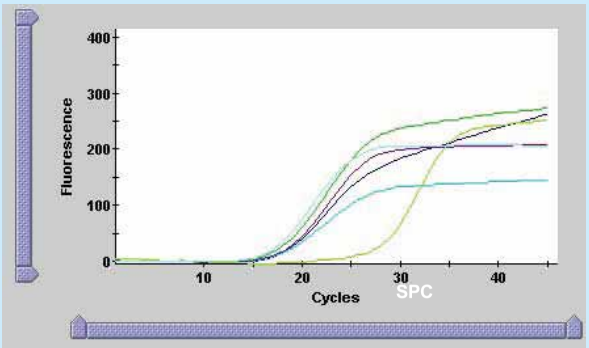


Assay design

The MTB assay target is the 81 bp region (RRDR) of the rpoB gene.



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

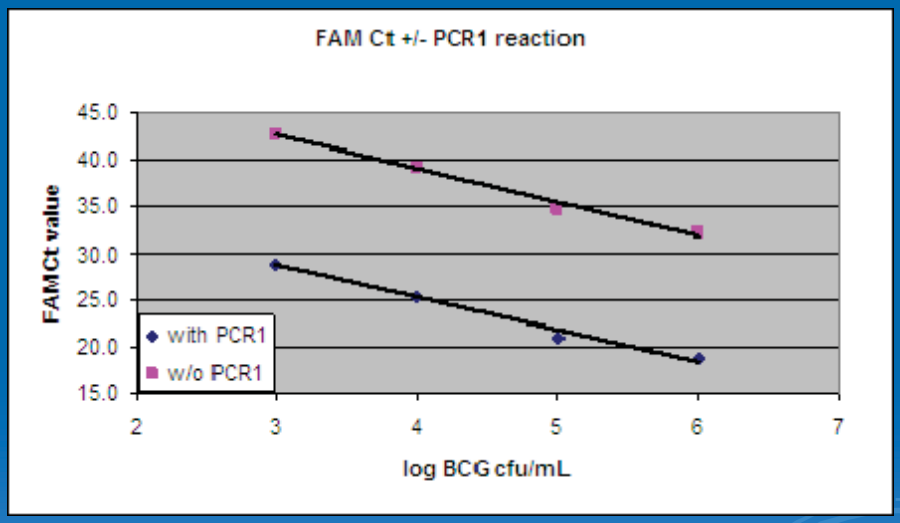


Example of Rif-Sensitive Profile – 5 probes are positive

Fully enclosed, nested PCR amplification in the GeneXpert

Cepheid®

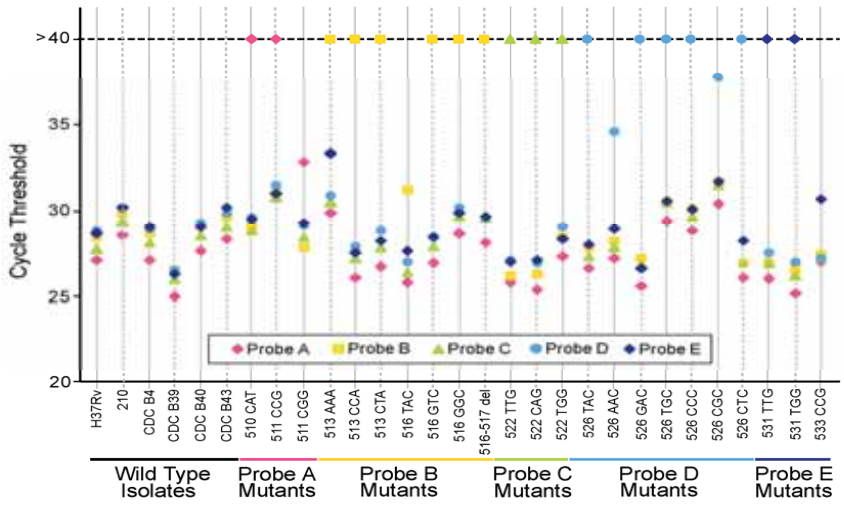
Performance Improvement with Self-Contained Nested PCR



Fluorescence

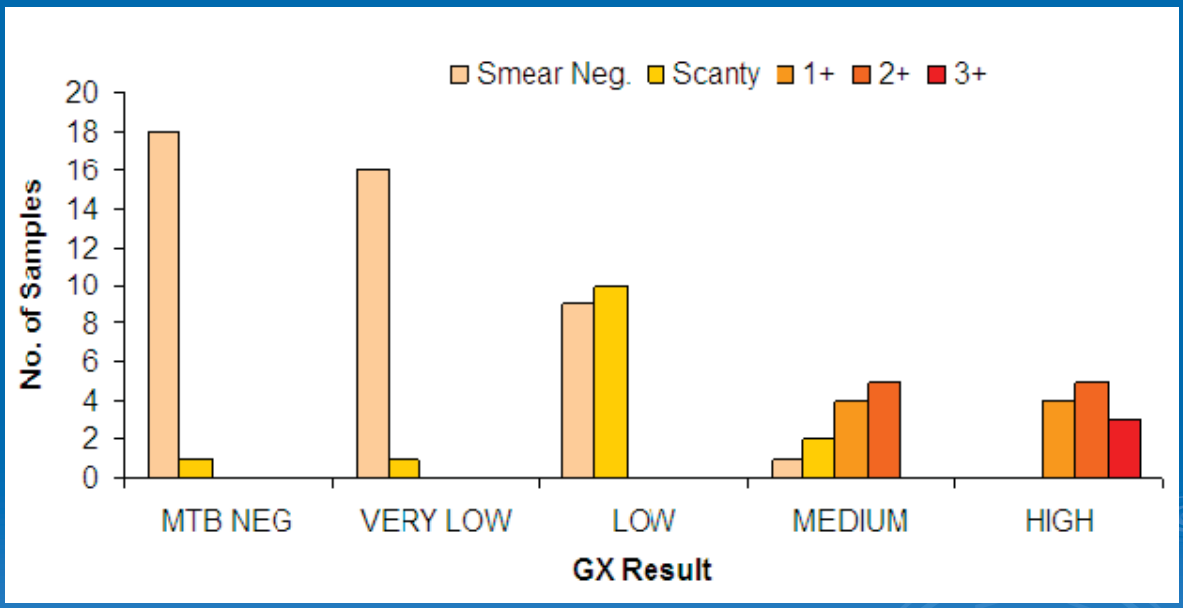
PCR Cycles

All common *rpoB* mutations detected by a Ct delay* in at least one probe



*For Rifampicin-resistant mutants, delta Ct between the earliest & latest Ct values is greater than 3.5 Ct.

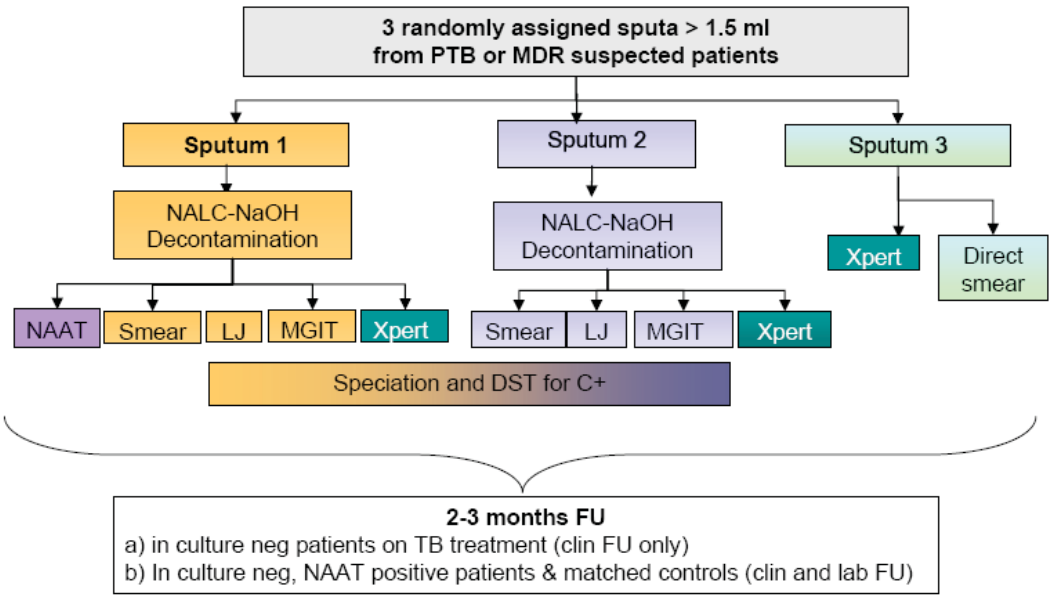
Semi-Quantitative results



Evaluation partner sites

Patient and specimen flow

- Enrolment of 1730 patients



Site 1 (Peru) TB detection results

			Smear Negative (AFB–)		Smear Positive (AFB+)		
			Culture Positive	Culture Negative	Culture Positive		
Site 1	Xpert MTB/RIF	MTB Detected	10	2	198	PPV	99.0%
		MTB Not Detected	2	107	0	NPV	98.2%
			Sensitivity	Specificity			
			99.0%	98.2%			

Site 2 (Azerbaijan) TB detection results

			AFB–		AFB+		
			Culture Positive	Culture Negative	Culture Positive		
Site 2	Xpert MTB/RIF	MTB Detected	57	16	76	PPV	89.3%
		MTB Not Detected	4	101	0	NPV	96.2%
			Sensitivity	Specificity*			
			97.1%	86.3%			

*For Site 2, 13 out of the 16 GX positive, smear and culture negative (false positive GX) results were from patients with a prior history of TB.

Site 1 and 2 combined TB detection results

			AFB–	AFB+		
			Culture Positive	Culture Negative	Culture Positive	
Combined	Xpert MTB/RIF	MTB Detected	67	18	274	PPV 95.0%
		MTB Not Detected	6	208	0	NPV 97.2%
			Sensitivity 98.3%	Specificity* 92.0%		

Rif Resistance Detection Results

			DST RIF Resistant	DST Sensitivity		
Combined	Xpert MTB/RIF	RIF Resistance Detected	57	5	PPV	91.9%
		RIF Resistance Not Detected	1	275	NPV	99.6%
			Sensitivity 98.3%	Specificity 98.2%		

Biosafety requirements for Xpert MTB/Rif

FIND target:

Equivalent biosafety infrastructure needs as for direct smear.

- 7 log killing in spiked sputum (15 min, 2:1 SR)
- >90% MGIT negativity for clinical 3+ sputa (15 min, 2:1 SR)
- Aerosol studies

Aerosol viability during manual steps

	Mean cfu/m³ air detected*	
5 X 10^8 cfu BCG spiked into sputum.	Anderson impactor	BioSample
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

*over 3 experiments

- Aerosol generation < than during preparation of direct smear

Aerosol viability during Gx run

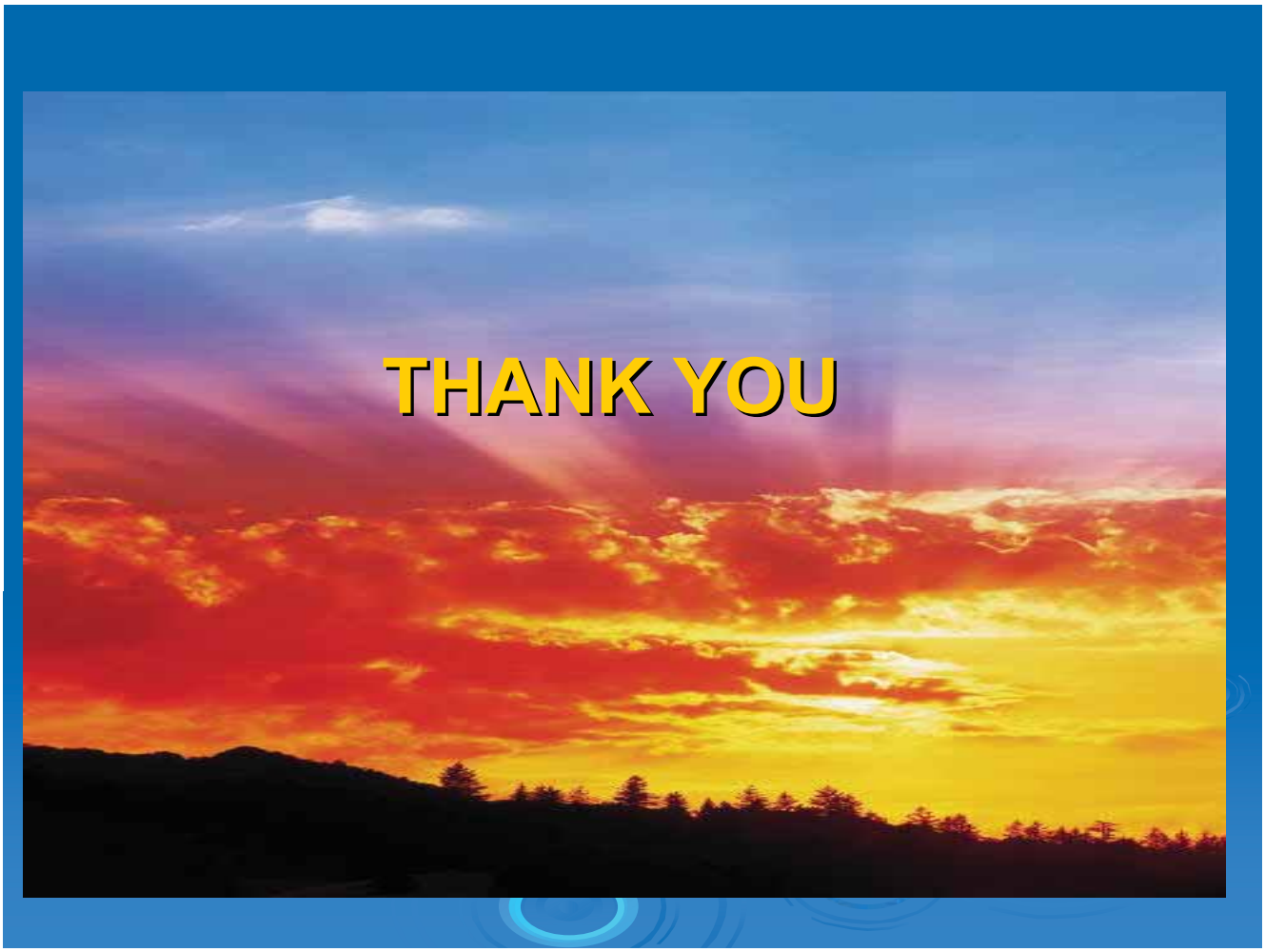
Sample type placed into Xpert TB cartridge (3 runs with 3 cartridge per condition)	Total cfu detected: Anderson impactor	Total cfu detected: BioSampler
5 X 10^8 cfu BCG spiked into water	0	0
5 X 10^8 cfu BCG spiked into sputum then treated with SR in standard protocol	0	0
5 X 10^8 cfu M. smegmatis spiked into sputum then treated with SR in standard protocol	0	0

*over 3 experiments

- GeneXpert = closed system

Conclusions – Xpert MTB/RIF Project

- The GX MDR TB assay generates TB ID results in less than 2 hours directly from sputum
- It simultaneously detects rifampin resistance
- Sputum processing is simple and does not require a centrifuge
- It can be performed by personnel with minimal training
- It does not require dedicated lab space
- It can be run on-demand (not batched) to generate real-time results
- It is the most sensitive molecular assay available
- It was released Ex-US on April 21, 2009 (CE mark)


THANK YOU