

Global Implementation of Xpert MTB/Rif

David H Persing, MD, PhD

Chief Medical and Technology Officer
Cepheid, Sunnyvale, CA, USA

Consulting Professor of Pathology
Stanford University, Stanford, CA, USA

“The Machine that will help end TB”



In November 2011, Jabu Ngcobo, 25, felt a pain in her side and went to the KwaMsane clinic, which resembles a trailer park.

“I was all along thinking I had MDR TB because my two brothers and my sister had it,” says Ngcobo.

Cohen, J. MIT Technology
Review, Dec 2012

“The Machine that will help end TB”



Ngcobo’s speedy diagnosis and recovery were made possible by a machine called a GeneXpert, which sits atop a counter inside one of the trailers....

.....and resembles a high-end espresso maker.

Cohen, J. MIT Technology Review, Dec 2012

Not to be mistaken for a double shot decaf latte...



- Sputum processing was the greatest challenge to Xpert test development
 - Often bloody and full of inflammatory cells
 - Cell-free DNA content makes it viscous and gravity-defying
 - Small number of bacilli in many samples requires large sample volume
 - Requirement for inactivation

Xpert MTB/RIF - A result of successful partnerships

Genetic basis for Rifampin Resistance in *M. tuberculosis* has been known since the early 1990s

rpoB

RpoB mutations are a good surrogate marker of MDR TB in areas endemic for MDR TB

RpoB mutations are not as predictive of MDR in the US because of low prevalence, but carry Rx implications nonetheless



GGCACCAGCCAGCTGAGCCAATTCATGGACCAGAACAACCCGCTG TCGGGGTTGACCCACAAGCGCCGACTGTCGGGCGCTG



507

81 base pair core region

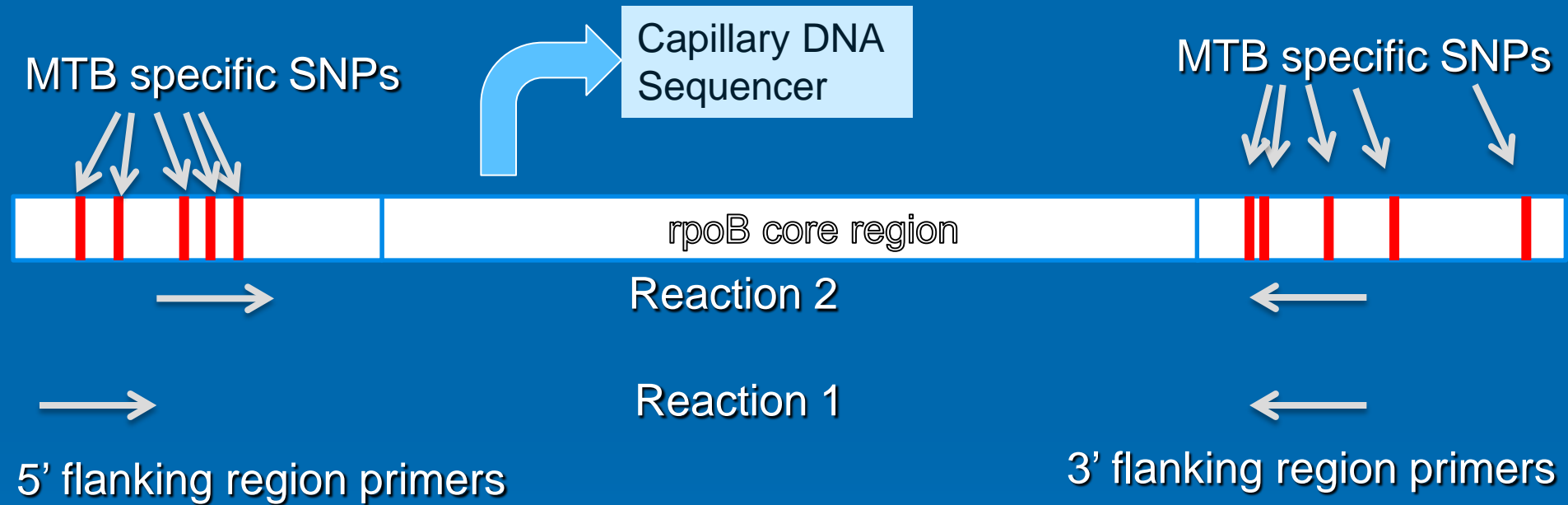
533

Sequence-specific Detection of MTB

Hunt et al, DMID 1994 18:219

	151				* * *	200
M. tuberculosis (S)					a g	a
M. tuberculosis (R)					a g	a
M. tuberculosis (S)					a g	a
M. tuberculosis (S)					a g	a
M. tuberculosis (R)					a g	a
rpoB					a g	a
M. tuberculosis (R)					a g	a
M. triviale						
M. tuberculosis (S)					a g	a
M. smegmatis	g	g				
M. phlei	g	g				
M. fortuitum	g	g		g tc		
Rhodococcus sp.				tc		
M. marinum	t					
Nocardia sp.				tc		
Streptomyces sp.		t	c			
M. avium-intracellulare	g				c	
M. kansasii			c		c	
Nocardia sp.		g		g	tc t	
Actinomyces sp.	a	g			tc	ca g
Corynebacterium sp.	t	t	g	c	tc	
Consensus sequence	GTCGCCGCGA	TCAAGGAGTT	CTTCGGCACC	AGCCAGCTGT	C-CAGTTCAT	

First generation MTB/rif: Mayo Clinic RpoB Assay Design



We used the test in cases like this:

- 50 year old diabetic man from Saudi royal family
- 20 year history of treatment for pulmonary TB
- Poorly compliant WRT TB and diabetes medication
- Not high-ranking enough to merit use of the family private jet....so....

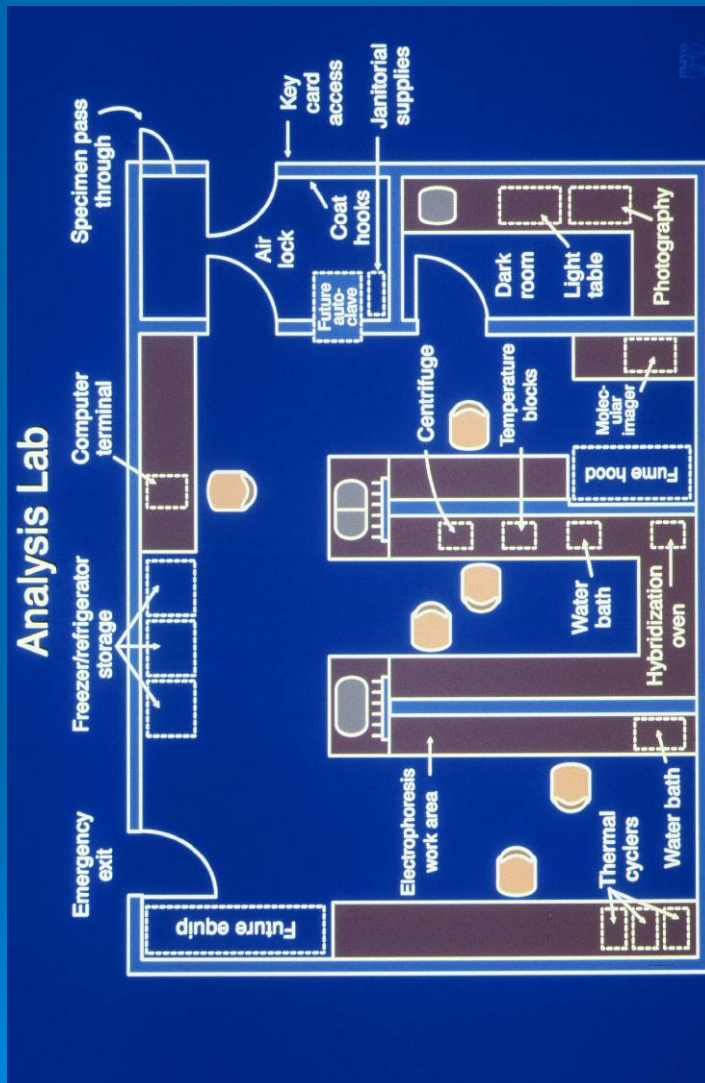
MDR-TB case, continued

- 8 hour flight aboard NWA (First class, London to Minneapolis)
- Productive cough, no protective mask
- Sat next to person who turned out to be HIV-positive
- Arrived at Mayo Clinic Rochester with few medical records
- Exposed multiple medical and admissions personnel before TB history was learned
- Follow-up study ordered by Mayo and MDH

Exposed to MDR-TB:

- 3 physicians
- 2 interpreters
- 8 registration and admissions clerks
- 2 X-ray technologists
- Unknown number of other patients in waiting areas
- Public exposure in local Rochester hotel and several restaurants
- An entire planeload of NWA passengers

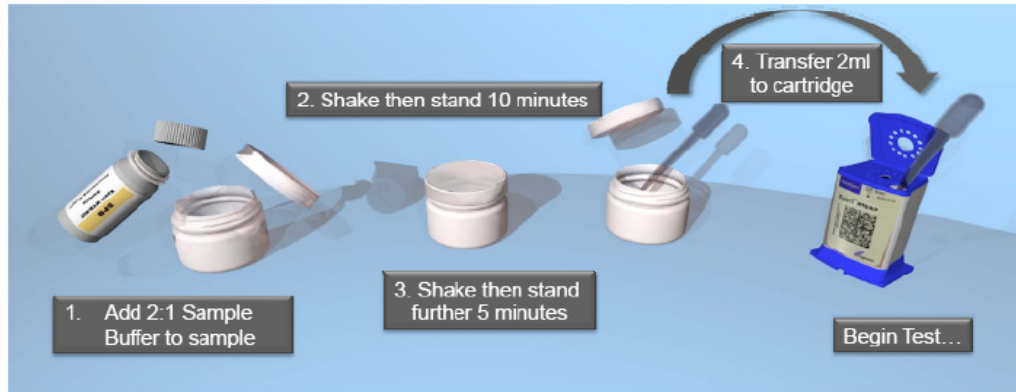
The “Dreaded” MTB/Rif Test at Mayo



- A herculean effort reserved only for emergencies
- Be careful of what you publish!
- 4000 Square Foot dedicated PCR lab
- 3 hermetically sealed rooms
- Specially trained medical technologists, 2 MD fellows, 1 PhD Director
- HEPA filtered air for each room (in and out)
- TAT about a week
- **Nested protocol was prone to PCR product contamination, requiring multiple negative controls per run**
- **Back-up “clean rooms” in the event of contamination**
- Cost: hundreds of thousands

Fast Forward
20 years

(Paradigm) Shift Happens: Xpert MTB/Rif



Automated sample preparation

Amplification and detection

< 2 h

Xpert™ MTB/Rif



Workflow

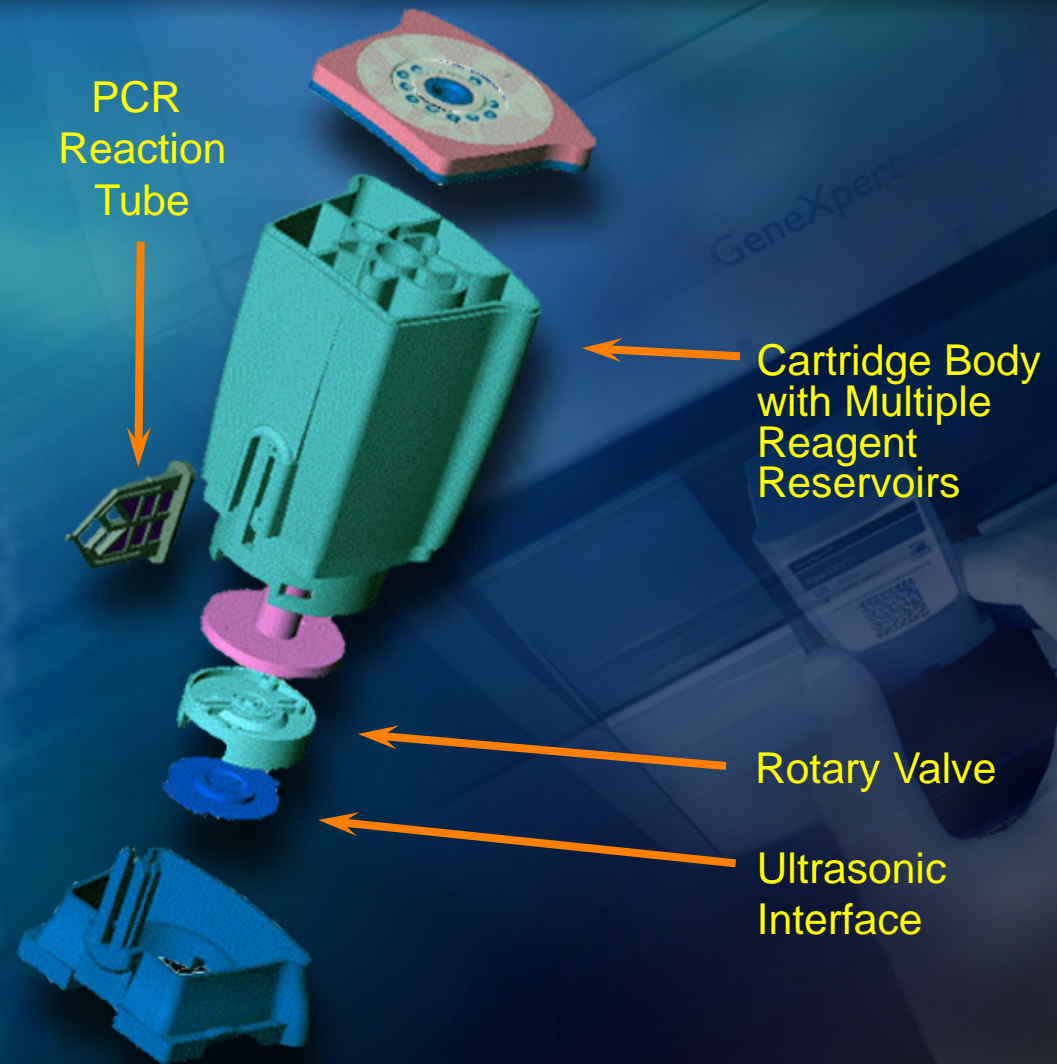
- fully automated with 1-step external sample prep.
- time-to-result < 2 h (walk away test)
- throughput: up to 1-48 tests / run
- no bio-safety cabinet
- closed system (no contamination risk)

A technology platform for

- TB & Rif Resistance
- TB Quinolone resistance
- Potential for HIV viral load

GeneXpert Cartridge

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



GeneXpert Module

- Syringe motor drives fluid movement
- Valve motor directs access to chambers
- Independent thermal cycler allows for random-access design
- Scalable from 1 to 80 modules in a system



GeneXpert Systems



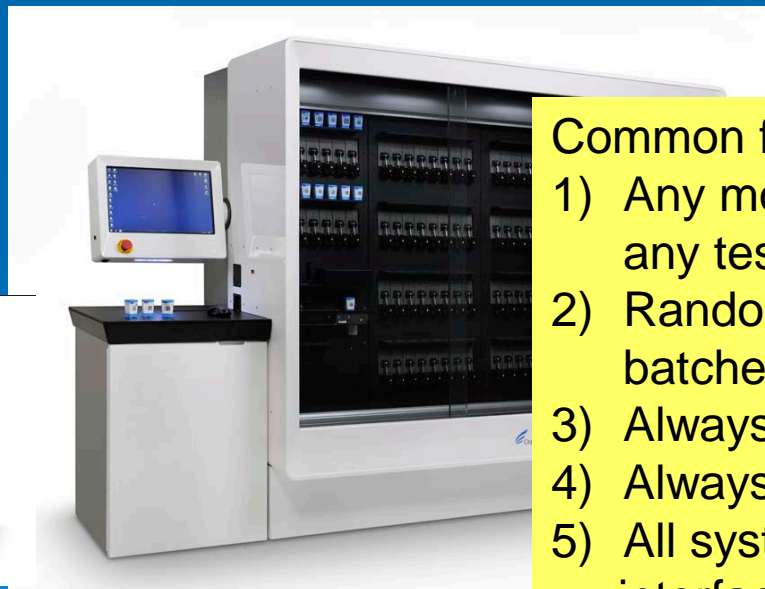
GeneXpert Module

GX-I GX-II

GX-IV

GX-XVI

GeneXpert Infinity-80 System



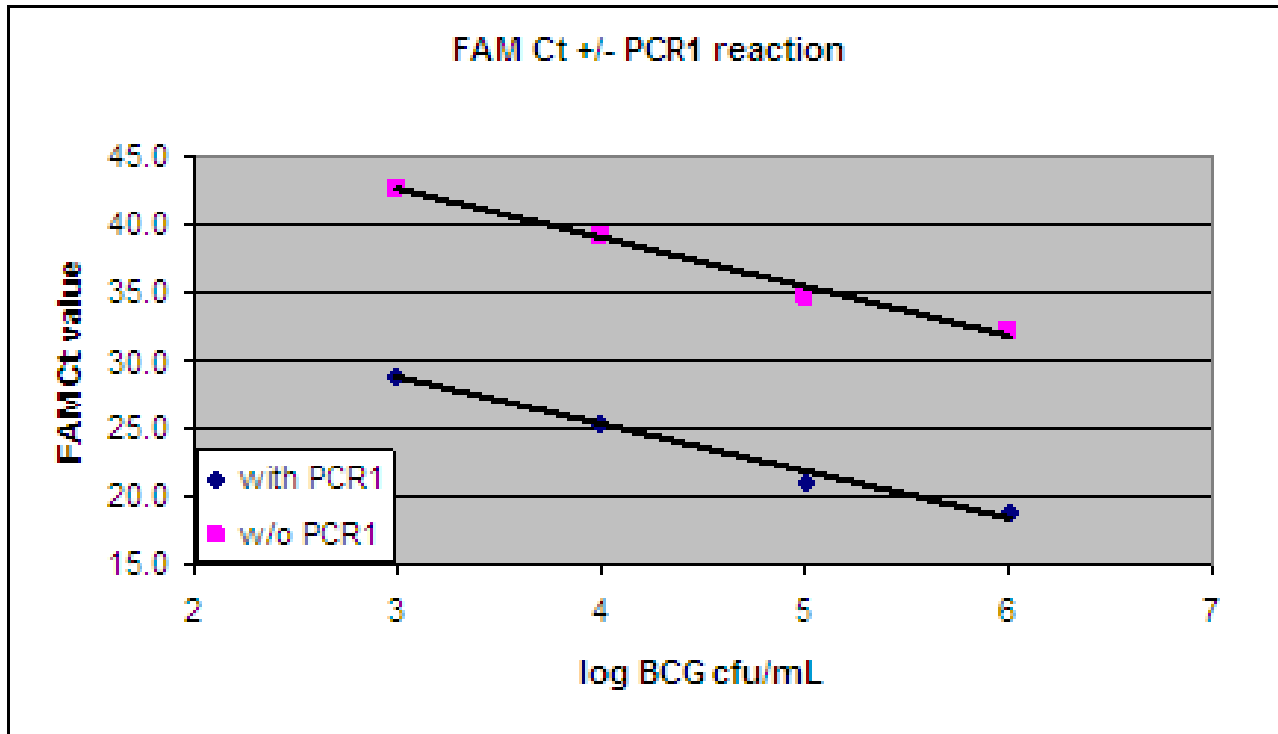
Common features:

- 1) Any module can run any test.
- 2) Random order or batches
- 3) Always On
- 4) Always Stat Capable
- 5) All systems are LIS interfaceable

How it Works: Fully Enclosed, Nested PCR Amplification in the GeneXpert Cartridge



Performance Improvement with Self-Contained Nested PCR



Xpert[®] MTB/RIF Performance

Rapid Molecular Detection of Tuberculosis and Rifampin Resistance'

Catharina Boehme, et al. New England Journal of Medicine, 1 Sept, 2010

The NEW ENGLAND
JOURNAL of MEDICINE

Rapid Molecular Detection of Tuberculosis and Rifampin Resistance

Catharina C. Boehme, M.D., Pamela Nabetta, M.D., Doris Hillemann, Ph.D., Mark P. Nicol, Ph.D., Shubhada Shenai, Ph.D., Fiorella Krapp, M.D., Jenny Allen, B.Tech., Rasim Tahiri, M.D., Robert Blakemore, B.S., Roxana Rustomjee, M.D., Ph.D., Ana Milovic, M.S., Martin Jones, Ph.D., Sean M. O'Brien, Ph.D., David H. Persing, M.D., Ph.D., Sabine Ruesch-Geddes, M.D., Eduardo Gotuzzo, M.D., Camilla Rodrigues, M.D., David Alland, M.D., and Mark D. Perkins, M.D.

ABSTRACT

BACKGROUND

Global control of tuberculosis is hampered by slow, insensitive diagnostic methods, particularly for the detection of drug-resistant forms and in patients with human immunodeficiency virus infection. Early detection is essential to reduce the death rate and interrupt transmission, but the complexity and infrastructure needs of sensitive methods limit their accessibility and effect.

METHODS

We assessed the performance of Xpert MTB/RIF, an automated molecular test for *Mycobacterium tuberculosis* (MTB) and resistance to rifampin (RIF), with fully integrated sample processing in 1730 patients with suspected drug-sensitive or multidrug-resistant pulmonary tuberculosis. Eligible patients in Peru, Azerbaijan, South Africa, and India provided three sputum specimens each. Two specimens were processed with N-acetyl-L-cysteine and sodium hydroxide before microscopy, solid and liquid culture, and the MTB/RIF test, and one specimen was used for direct testing with microscopy and the MTB/RIF test.

RESULTS

Among culture-positive patients, a single, direct MTB/RIF test identified 551 of 561 patients with smear-positive tuberculosis (98.2%) and 124 of 171 with smear-negative tuberculosis (72.5%). The test was specific in 604 of 609 patients without tuberculosis (99.2%). Among patients with smear-negative, culture-positive tuberculosis, the addition of a second MTB/RIF test increased sensitivity by 12.6 percentage points and a third by 5.1 percentage points, to a total of 90.2%. As compared with phenotypic drug-susceptibility testing, MTB/RIF testing correctly identified 200 of 205 patients (97.6%) with rifampin-resistant bacteria and 504 of 514 (98.1%) with rifampin-sensitive bacteria. Sequencing resolved all but two cases in favor of the MTB/RIF assay.

CONCLUSIONS

The MTB/RIF test provided sensitive detection of tuberculosis and rifampin resistance directly from untreated sputum in less than 2 hours with minimal hands-on time. (Funded by the Foundation for Innovative New Diagnostics.)

10.1056/NEJM0907847 NEJM.010

The New England Journal of Medicine
Downloaded from www.nejm.org on September 2, 2010. For personal use only. No other uses without permission.
From the NEJM Archive Copyright © 2010 Massachusetts Medical Society.

From the Foundation for Innovative New Diagnostics, Geneva (C.C.B., P.N., M.D.P.); Forschungszentrum Borstel, Borstel, Germany (D.H., S.R.-G.); the Department of Clinical Laboratory Sciences, University of Cape Town, and National Health Laboratory Service, Cape Town (M.P.N., A.M.), and the Unit for Clinical and Biomedical TB Research, South African Medical Research Council, Durban (J.A., R.K.)—all in South Africa; P.D., Hinduja National Hospital and Medical Research Centre (Hinduja), Mumbai, India (S.S., C.K.); Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru (F.K., E.G.); Special Treatment Institution, Baku, Azerbaijan (R.T.); the Division of Infectious Diseases, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, Newark (R.B., D.A.); Cepheid, Sunnyvale, CA (M.J., D.H.P.); and the Department of Biostatistics and Biinformatics, Duke University Medical Center, Durham, NC (S.M.O.). Address reprint requests to Dr. Boehme at the Foundation for Innovative New Diagnostics, Ave de Bude 16, 1202 Geneva, Switzerland, or at catharina.boehme@finddiagnostics.org.

N Engl J Med 2010.
Copyright © 2010 Massachusetts Medical Society.

- Studied >1,700 Patients
 - Peru, Azerbaijan, South Africa and India
- Smear Positive Patients
 - 98.2% Sensitivity, 99.2% Specificity
- Smear Negative, Culture Positive Patients
 - 90.2% Sensitivity with Three Samples
 - 72.5% Sensitivity with One Sample
- Patients with Rifampin Resistance
 - 97.6% Sensitivity, 98.1% Specificity

EDITORIALS



Tuberculosis Diagnosis — Time for a Game Change

Peter M. Small, M.D., and Madhukar Pai, M.D., Ph.D.

- Shorter time to effective Rx for TB patients may result in better outcomes, lower overall costs
- Shorter time to effective Rx may reduce TB transmission pressure at family, community levels
- Transmission dynamics of TB and MDR-TB may be affected: an essential step toward global control of TB

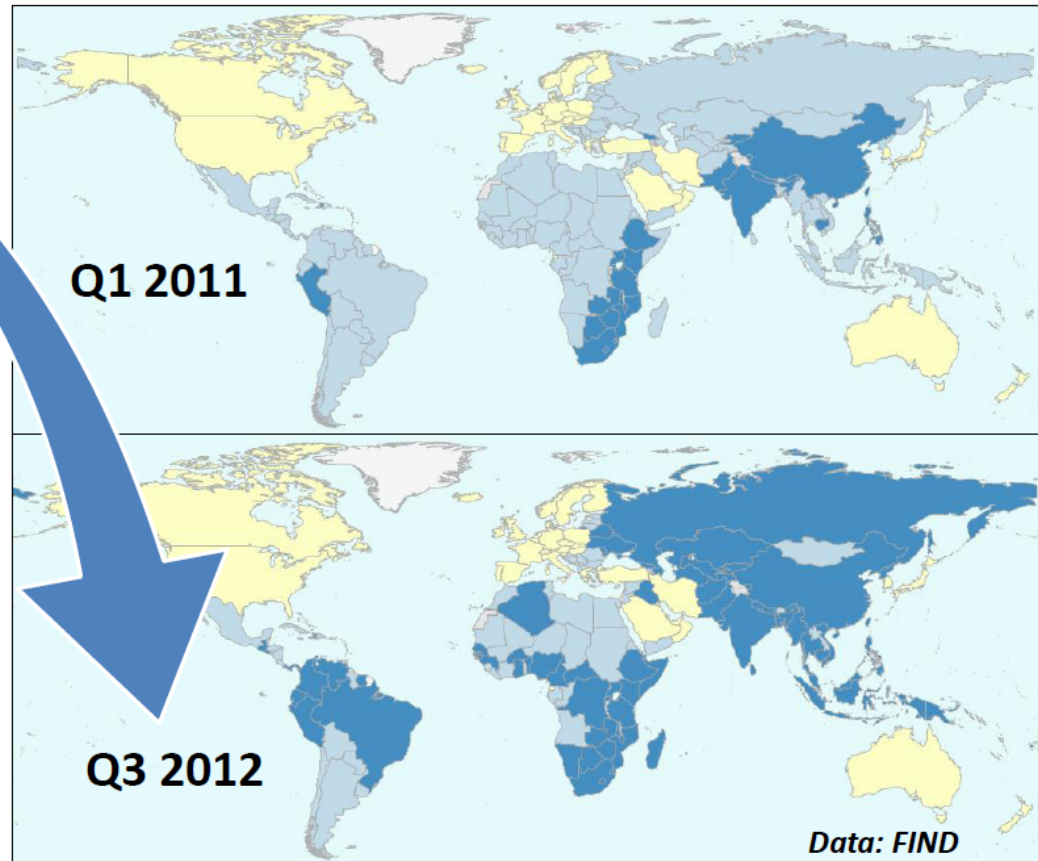
WHO Endorsment Leads to Rapid Uptake

Policy impact (1)



99 GeneXperts
(524 modules)
in the public sector
in **23** countries

898 GeneXperts
(4,660 modules)
in the public sector
in **73** countries



High Burden Developing Country Program

- **Concessionary pricing program supported by Unitaid, Pefpar and BMGF**
- **Since Q1 2011:**
 - **1,123 GeneXpert® Systems Placed in 83 of 145 eligible countries**
 - **Nearly 3 Million Xpert MTB/RIF Tests Run in Support of HBDC Programs**
- **South Africa, India, China, and Brazil, Among Others, have Xpert-Based Programs at Various Stages**

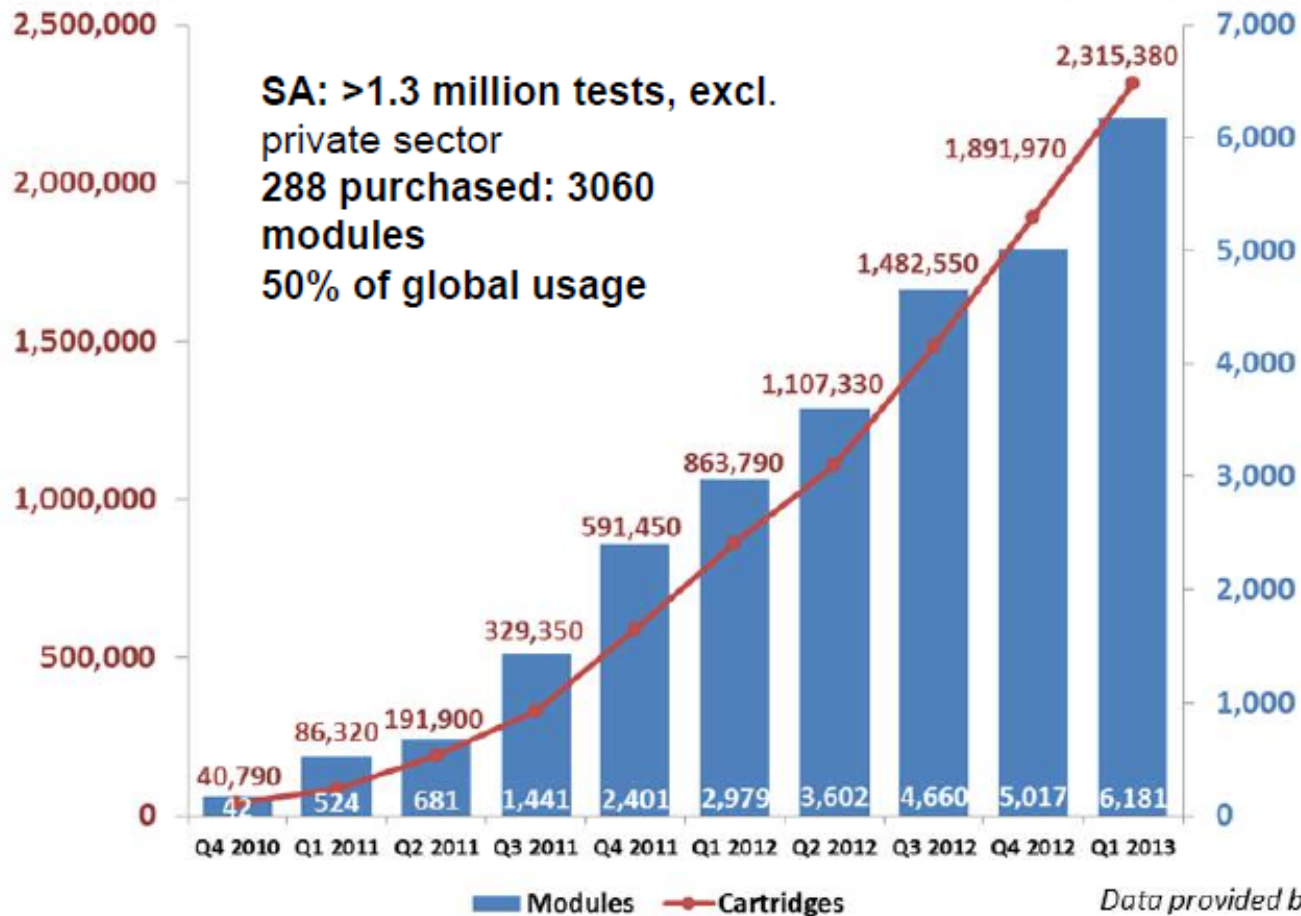
“.... it has the potential to revolutionize TB care, and WHO will treat it as a top priority.”

Dr. Mario Raviglione, World Health Organization



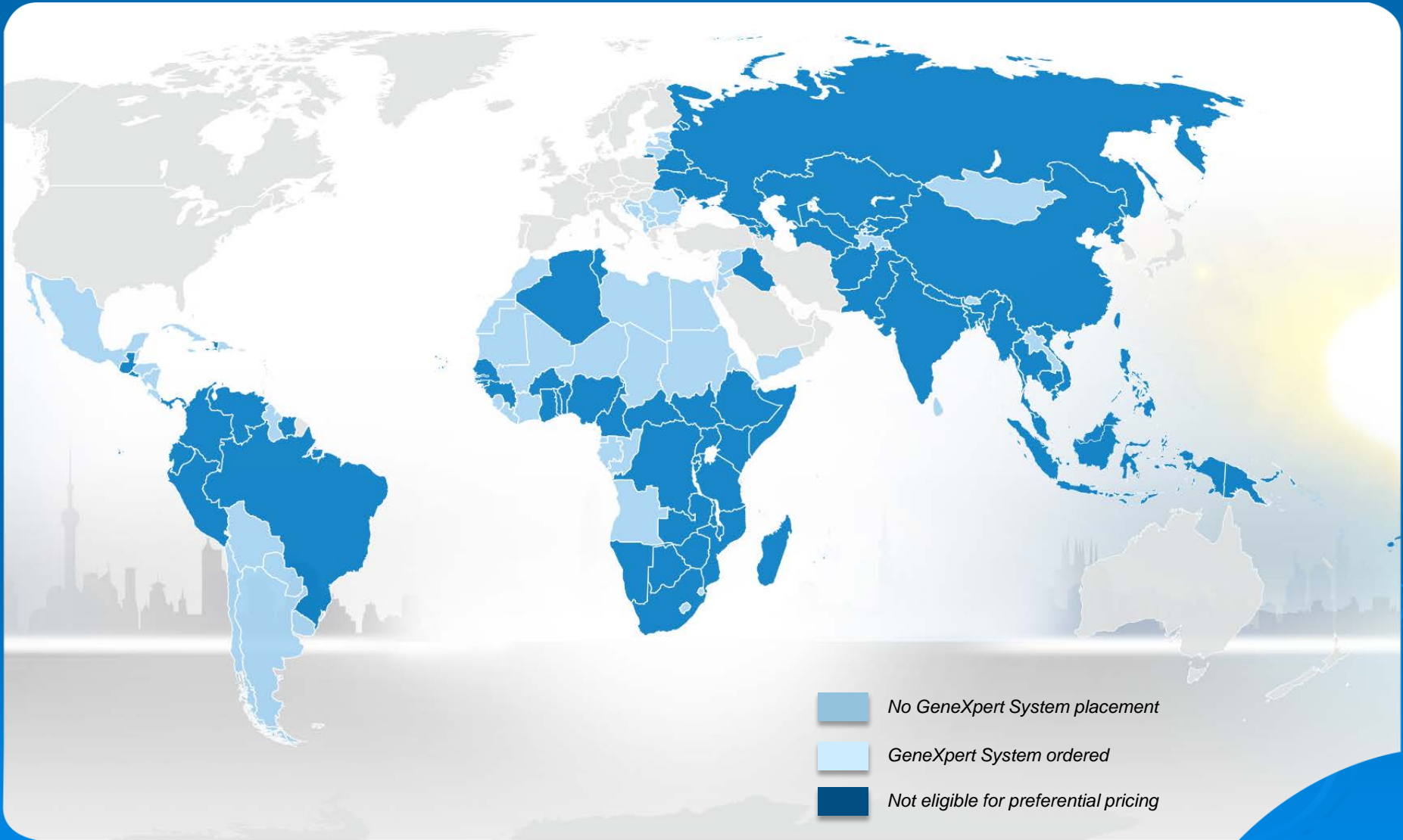
WHO data: Xpert MTB/Rif

Cumulative number of GeneXpert instrument modules and Xpert MTB/RIF cartridges procured under concessional pricing



As of 31 March 2013, a total of 1,123 GeneXpert instruments (comprising 6,181 modules) and 2,315,380 Xpert MTB/RIF cartridges had been procured in the public sector in 83 of the 145 countries eligible for concessional pricing. http://who.int/tb/laboratory/GeneXpert_rollout_large.jpg

HBDC Has Extended GX Footprint Globally



Same-day turnaround time and reporting make a difference

Test and Treat: A New Standard for Smear-Positive Tuberculosis

J. Lucian Davis, MD, MAS*†

David W. Dowdy, MD, PhD, ScM‡

Saskia den Boon, MSc, PhD†

Nicholas D. Walter, MD†§

Achilles Katamba, MBChB, PhD†||

Adithya Cattamanchi, MD, MAS*†

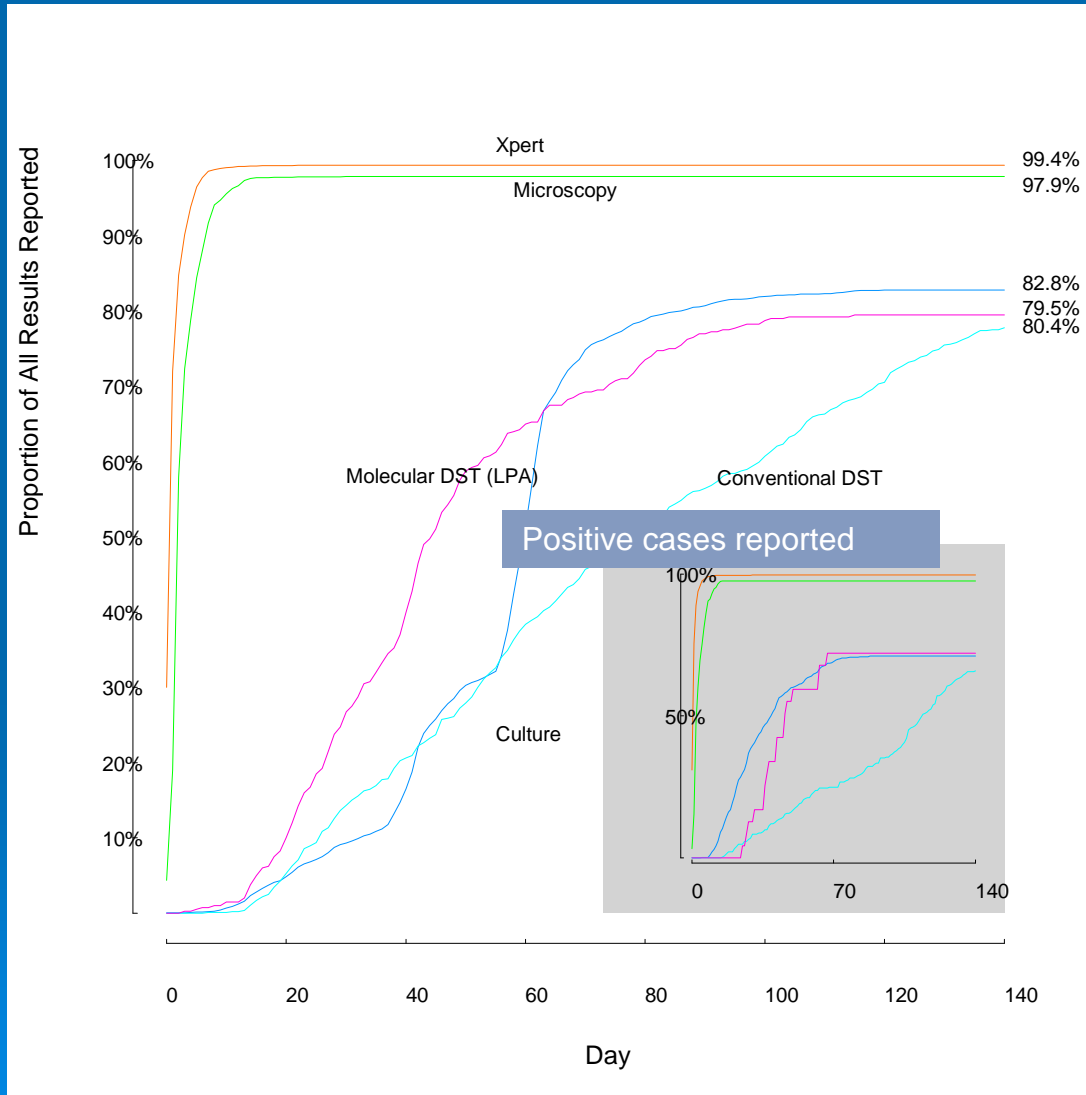
“same-day reporting of results is more likely to result in successful treatment initiation than either same-day or 2-day collection with delayed reporting”

JAIDS 2012

Xpert MTB/Rif:

Potential for increasing the number of same-day reports for both sensitive and resistant TB

Demonstration Studies: Impact of Rapid Testing



Decentralized Xpert MTB/Rif testing in South Africa

INT J TUBERC LUNG DIS 16(5):701-710
© 2012 The Union

Correspondence

Location of Xpert® MTB/RIF in centralised laboratories in South Africa undermines potential impact

“Ultimately, the diagnosis-treatment gap will only be closed by rapid point-of-care diagnostic assays that can be used during the patient’s first clinic visit to permit immediate treatment decisions...”
Lawn S et al.

Implementation of Xpert MTB/RIF for routine point-of-care diagnosis of tuberculosis at the primary care level

Kate Clouse, Liesl Page-Shipp, Heather Dansey, Bridgette Moatlhodi, Lesley Scott, Jean Bassett,
Wendy Stevens, Ian Sanne, Annelies Van Rie

“POC use of Xpert is feasible at the primary healthcare level but must be accompanied by financial, operational and logistical support.” Clouse K et al. SAMJ 2012

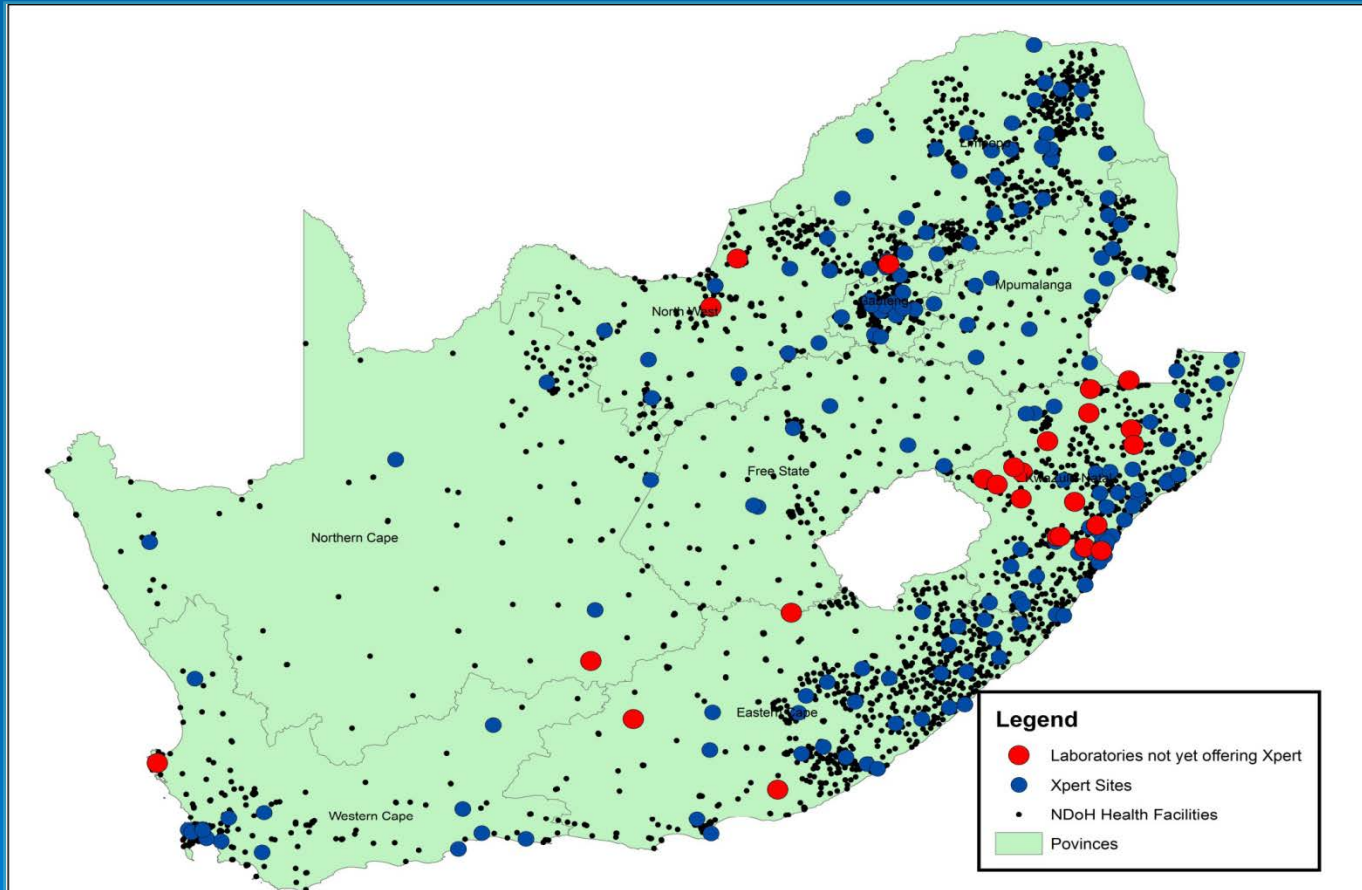
NHLS Implementation Plan

- **Three phases of implementation in microscopy centers**
 - **Phase I: Pilot in high burden districts in 9 provinces**
 - **Phase II: Completion of high burden districts**
 - **Phase III: XTEND Study (Aurum: BMGF funded, RCT)**
 - a. **Intervention arm**
 - b. **Control arm**
 - c/d. **Completion of all sites**
- **Full financial commitment attained for the National Program originally described, treasury support for test until 2016**
- **Program to be expanded for mines and correctional services with aspects of both surveillance, screening and case finding and Xpert to be used as front-line**

NHLS Experience to Date

- **Phases 1-3b completed; Phases 3c 85%; Phase 3d 53%**
- **175 testing centres established (~76% coverage)**
- **242 instruments: GX4 : Majority are 16 module instruments**
- **Placement of 7 infinities (Steve Biko, CHB, Leratong, Tshepong, Pelonomi, Greenpoint, PE)**
- **Train-the-Trainer courses conducted**
- **100 centers actively reporting on the Remote Connectivity website (TB test result geo-positioning in real time)**
- **Remote calibration active from November 2012**

South Africa: Increasing the Hubs and Spokes



GeneXpert Summary 01 March 2011 – 31 May 2013

- 1,451,361 assays to date nationally (4500-5000/day)
- 13.94% MTB positive cumulative (started out at 17%)
- Results demonstrating a downward trend
- Geographical variation

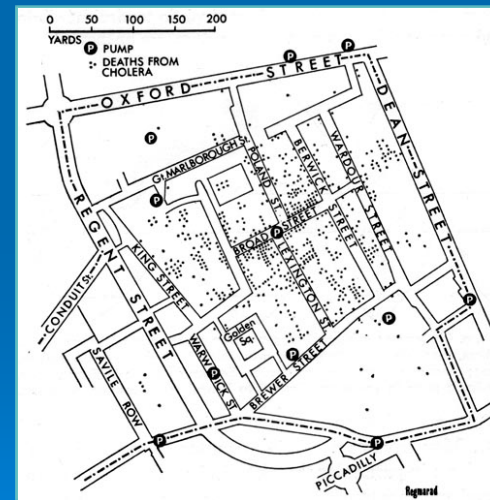
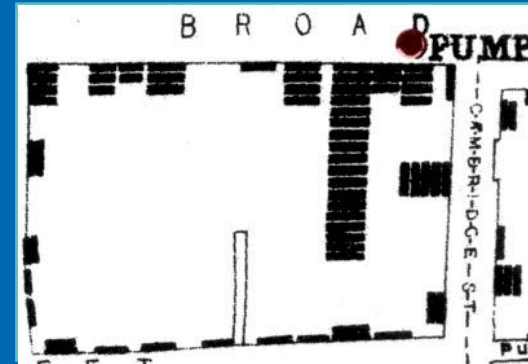
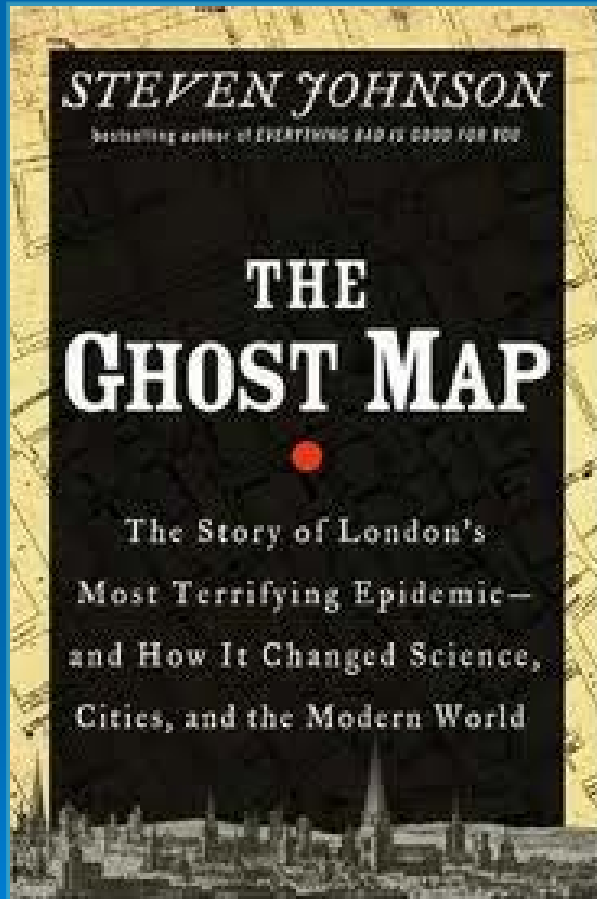
Province	MTB Detected	ITB Not Detecte	Test Unsuccessful	Total	% MTB Detected
Eastern Cape	35 083	200 611	6 811	242 505	14.47
Free State	20 192	139 846	710	160 748	12.56
Gauteng	23 354	156 940	5 560	185 854	12.57
Kwa-Zulu Natal	53 709	288 757	13 823	356 289	15.07
Limpopo	11 220	103 165	3 239	117 624	9.54
Mpumalanga	9 220	49 017	2 888	61 125	15.08
North West	12 865	74 021	4 275	91 161	14.11
Northern Cape	10 500	59 372	3 002	72 874	14.41
Western Cape	26 212	135 079	1 890	163 181	16.06
TOTAL	202 355	1 206 808	42 198	1 451 361	13.94

Decline in TB cases:

- improved diagnosis and less empirical treatment
- ARV coverage?,
- Adequate reporting from clinical side?
- Too early to tell?

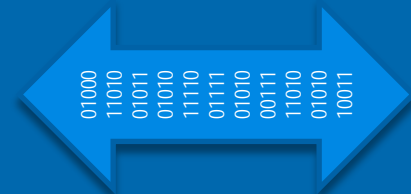
Sebaka Molapo, Leigh Berrie, and the NHLS GeneXpert Team
International TB Course, Montreal 2013

Incident case geopositioning: London cholera outbreaks in the 1850s

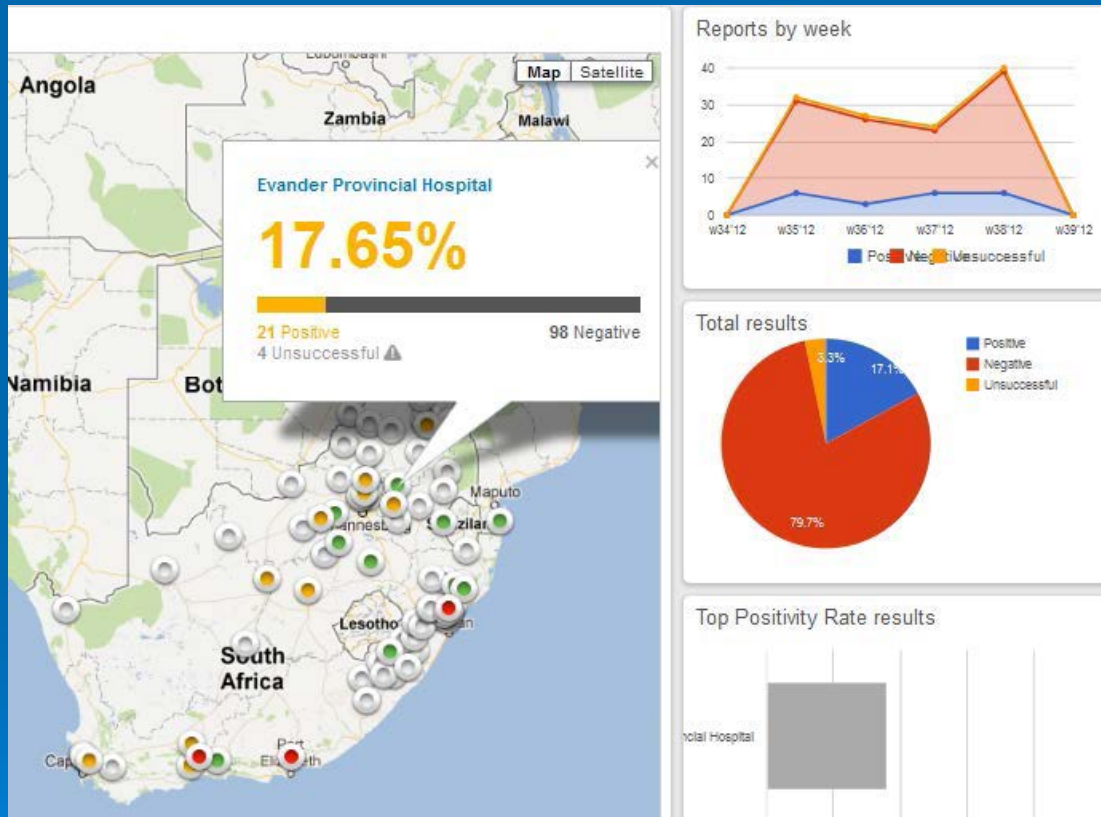


Remote Calibration and Connectivity

- Secure connection to the GeneXpert System
- Wired and wireless cloud-based networking
- NHLS: Remote calibration saves time and money
- Location specific data for fixed and mobile labs
- Data transfer via cell phone connection
- Define outbreak and drug resistance “hot spots”
- Generate hourly/daily “snapshots” of outbreak activity...a 21st century version of the Ghost Map



Real-time test data aggregation in South Africa



- De-identified test data reported directly from GeneXpert into Amazon cloud:
 - TB rates
 - TB resistance
 - Instrument/user problems
- Opt-in option for user
- Secure access to compiled information
- Pilot programs also in India, Burma
- Pilot program in US for Influenza (BARDA grant to JHU, Cepheid)

What about the US?

- **FDA Marketing Authorization of Xpert MTB/RIF on July 25, 2013**
- “[Xpert MTB/RIF] and the GeneXpert systems are intended to aid in the diagnosis of pulmonary tuberculosis and the selection of an initial treatment regimen when used in conjunction with clinical findings and other laboratory results. These devices do not provide confirmation of antibiotic susceptibility since other mechanisms of resistance may exist that may be associated with a lack of clinical response to treatment other than those detected by the device.”
- “The Xpert MTB/RIF Assay must be used in conjunction with mycobacterial culture to address the risk of false negative results and to recover the organisms for further characterization and drug susceptibility testing”

Current Climate for rapid MTB testing in the US

- 2 negative AFB smears used in most centers to release TB suspects from respiratory isolation, even though three are still recommended by CDC
- TAT on AFB smears is increasing due to centralization
- Significant need in the US for a rapid and accurate “rule-out TB” test in patients with nonspecific pulmonary findings
 - Endemic pockets in US
 - Prisons
 - Immigrant population from Middle east, Eastern Europe, Asia, India, Africa, Central America with active disease or at risk for reactivation
- 1 NAAT test in current CDC recommendations
- HOWEVER.....

It will take time for us figure out how to use this new tool.....

- No current indication for use of Xpert MTB/Rif as a smear replacement technology for infection control
- Smears still required to identify NTM
- Culture required to confirm resistance results
- Current PH infrastructure in place for sputum pellets and culture, but not for raw sputum

Conclusions

- Direct detection of rpoB in MTB was demonstrated over 20 years ago, but its impact was limited
- Next-Gen platform technology (MTB/Rif in particular) maximizes medical impact by bringing it closer to the patient, operating on-demand, and shortening TAT
- Similar impact will be seen for other infections of PH concern: Influenza, CT/NG, HPV, GI infections
- Next-Gen System connectivity enhances value of decentralized testing by employing advanced data reporting capabilities
- Radical democratization of molecular testing capabilities, for both ID and Oncology, is already underway and is having a worldwide impact



THANK YOU

**Fred Tenover and Ellen
Jo Baron both say hello**