

GeneXpert MTB-Rif

10 July 2018



TASK™

APPLIED SCIENCE

▶ CONTENT

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- **History & Use Today**
- **Principles of the Test**
- **Sensitivity & Specificity**
- **Samples / Specimens**
- **Advantages & Disadvantages**
- **Considerations of Implementation**
- **New Developments**

▶ GeneXpert MTB/Rif

- Said to have revolutionized TB diagnosis
- Xpert MTB/Rif used with Cepheid GeneXpert® Dx system
- Tests for:
 1. Detection of MTB DNA
 2. Detection of rifampicin resistance – *rpoB* gene



▶ GeneXpert MTB/Rif

1
Sputum liquefaction
and inactivation with
2:1 sample reagent



2
Transfer of
2 ml material
into test cartridge



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

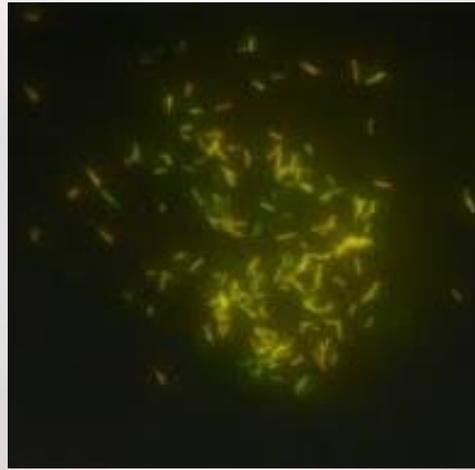
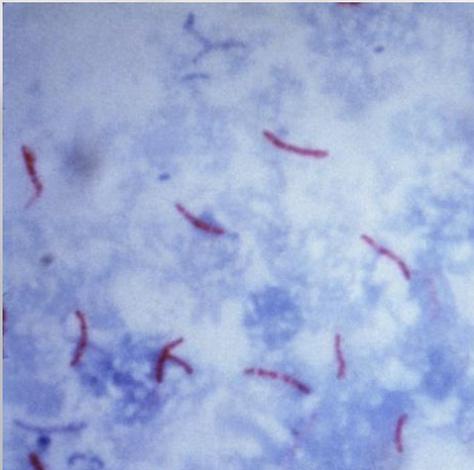
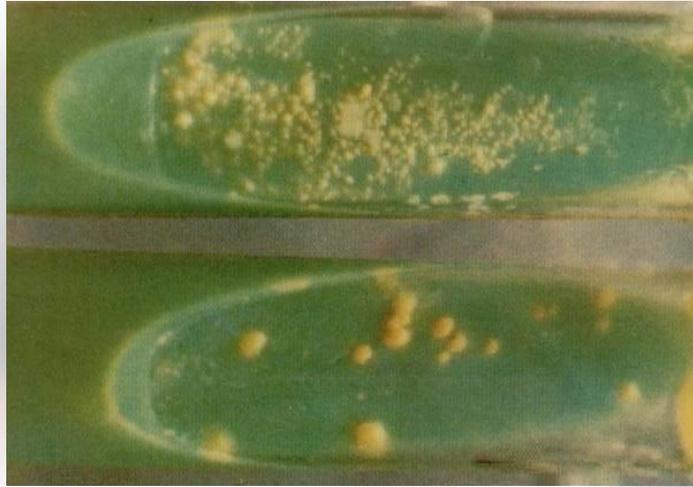
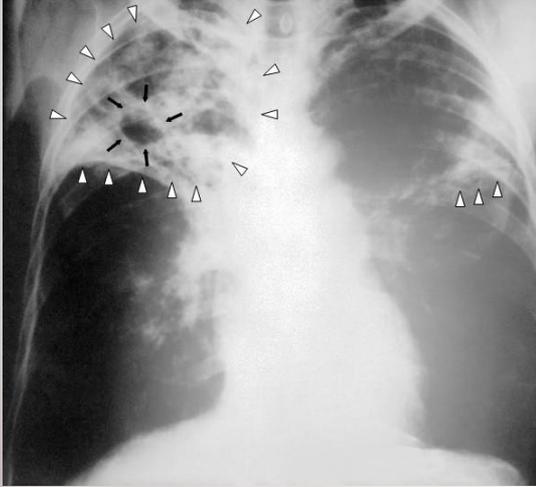
7
Seminested
real-time
amplification
and detection
in integrated
reaction tube

8
Printable
test result



Time to result, 1 hour 45 minutes

▶ Established Methods of TB Diagnosis



► History

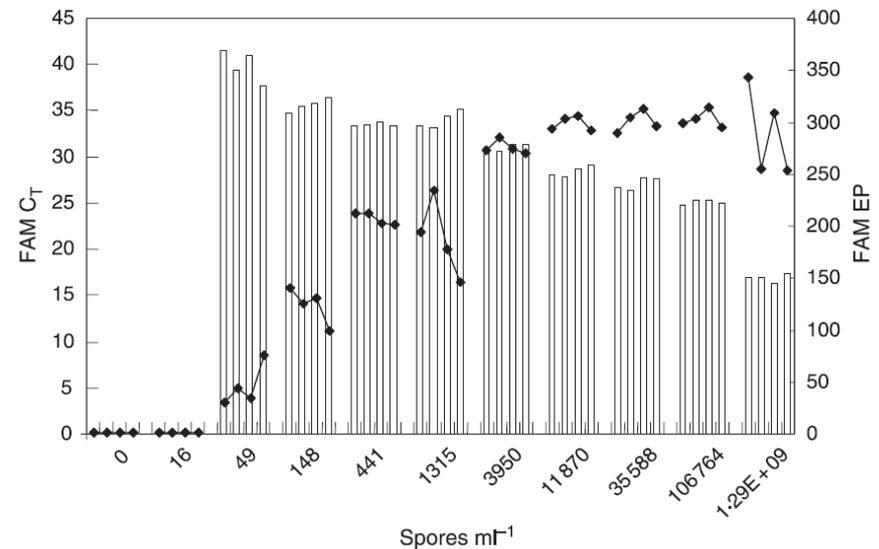
- Originally developed by Cepheid for detection of anthrax
- Deployed by United States Postal Service in mail sorting facilities (early 2000s)

Evaluation of the Cepheid GeneXpert® system for detecting *Bacillus anthracis*

M.P. Ulrich¹, D.R. Christensen¹, S.R. Coyne¹, P.D. Craw¹, E.A. Henschel¹, S.H. Sakai², D. Swenson², J. Tholath², J. Tsai², A.F. Weir² and D.A. Norwood¹

¹ Diagnostic Systems Division, United States Army Medical Research Institute of Infectious Diseases, Frederick, MD, USA

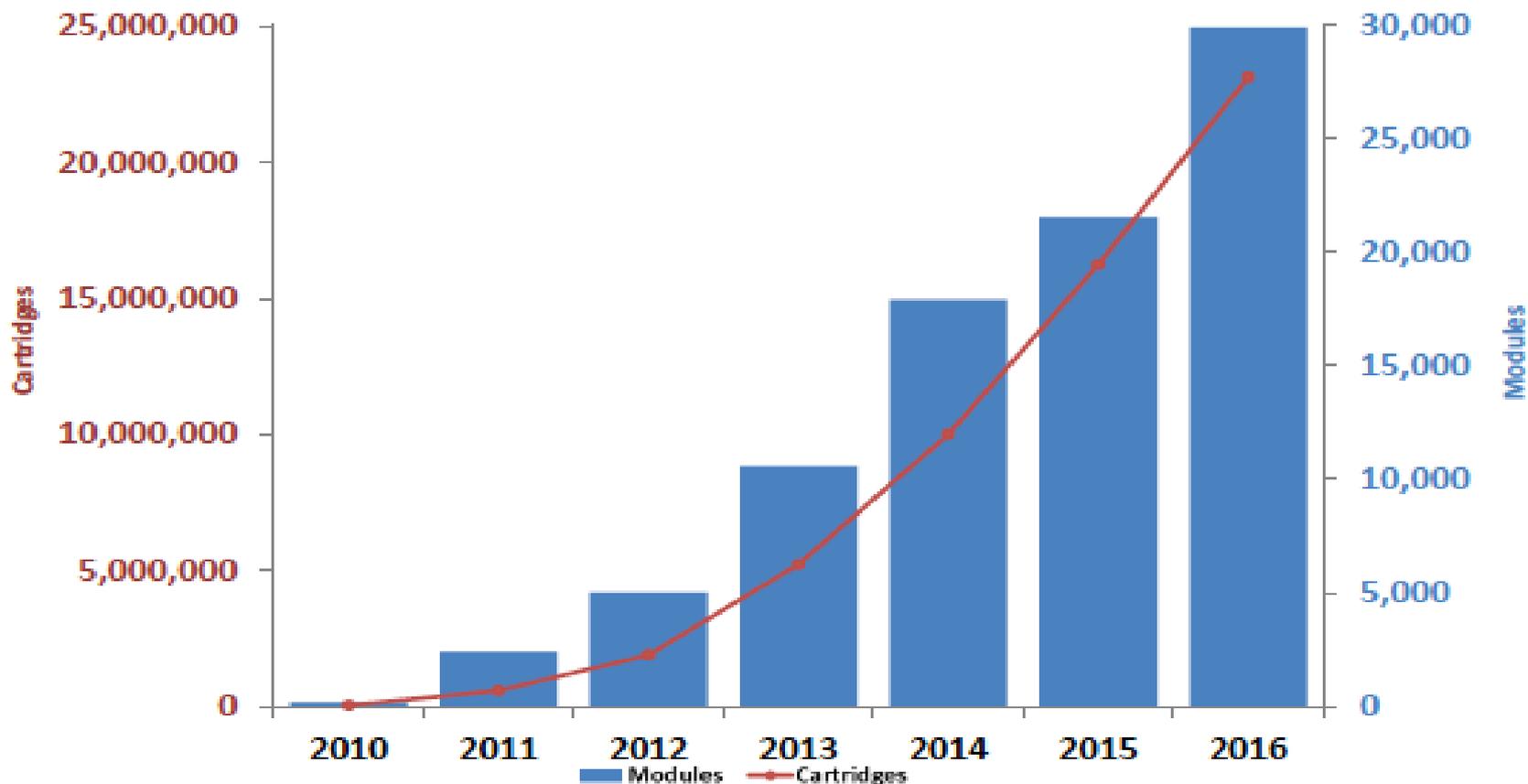
² Cepheid, 904 Caribbean Drive, Sunnyvale, CA, USA



▶ GXP Today(ish)

- Endorsed by WHO in 2010

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



As of 31 December 2016, a total of 6,659 GeneXpert instruments (comprising 29,865 modules) and 23,140,350 Xpert MTB/RIF cartridges had been procured in the public sector in 130 of the 145 countries eligible for concessional pricing.

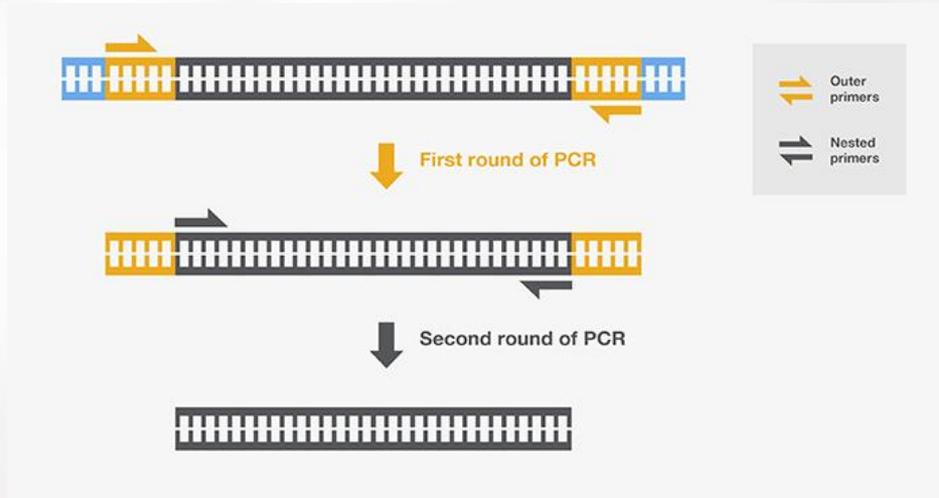
Data: Cepheid

▶ Principles of the Test

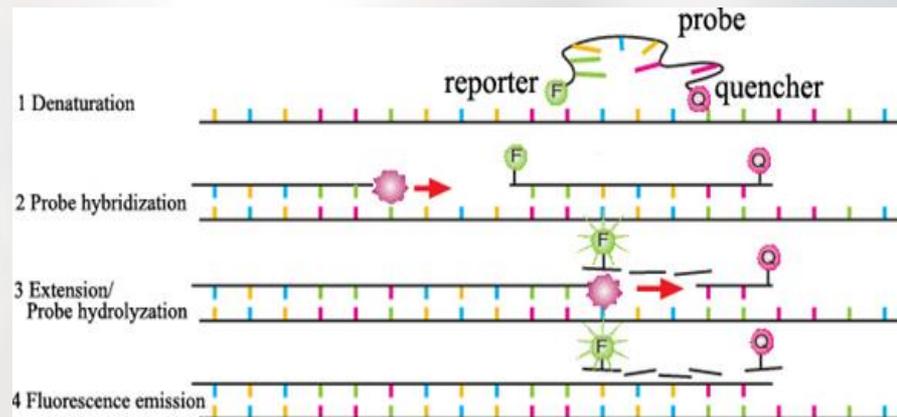
- **Nucleic acid amplification testing (NAAT)** searches genetic information in test sample to find MTB
 - Can analyze both DNA and RNA
- Able to analyze genetic mutations that cause drug resistance – i.e. Rifampicin resistance
- Fast testing method because genetic information is extracted and amplified, rather than waiting for bacteria to grow
- Limitations of NAAT:
 - Identifies genetic material – cannot differentiate if bacteria are alive or dead
 - Not a useful diagnostic tool if patient received TB treatment in previous 2 years

► Principles of the Test

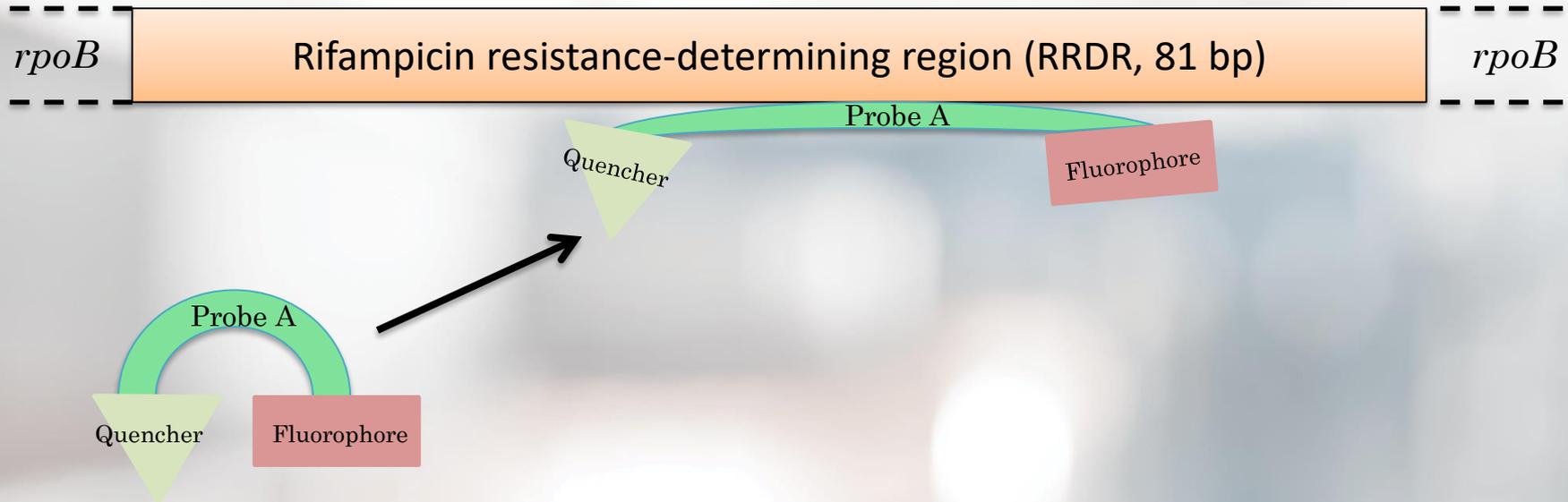
- Nested PCR



- Real-time PCR



► Principles of Detection



- A normal PCR is conducted to produce multiple copies of the RRDR
- 5 probes (A to E) or molecular beacons are designed to bind at the amplified RRDR causing an extended distance between fluorophore and quencher
- Definitions of binding:
 - 5 probes: MTB positive, no rifampicin resistance
 - 4, 3, 2 probes: MTB positive, rifampicin resistance
 - 1 and 0 probes: MTB not detected

► Principles of Detection

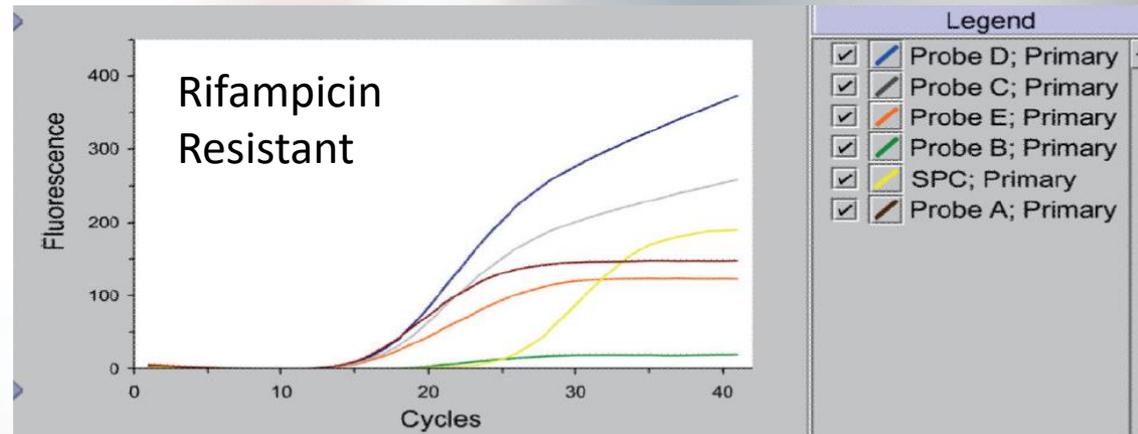
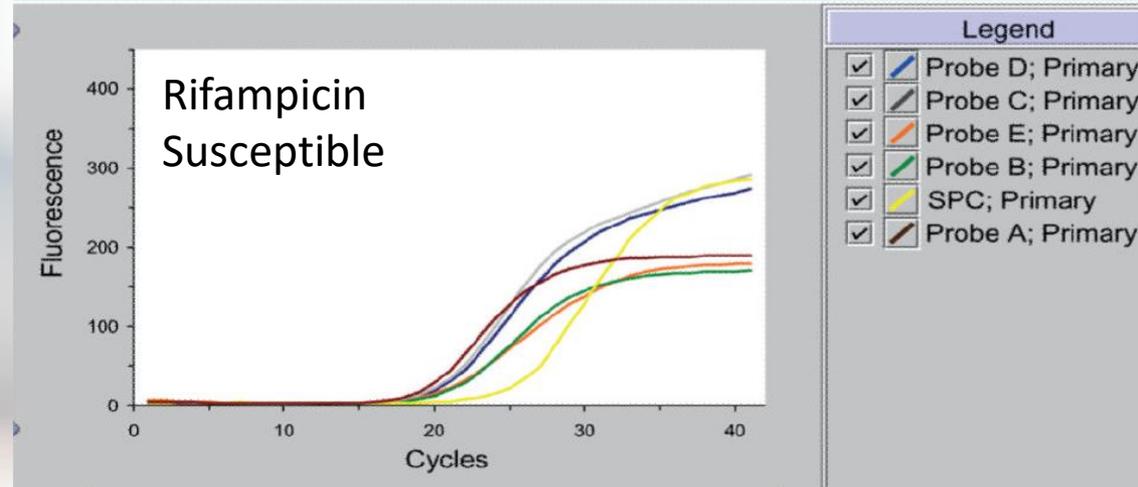
6 probes:

- 5 probes spanning *rpoB* gene
- >2 probes positive (Ct <38) confirm MTB

Estimates bacterial burden by measuring threshold-cycle (Ct)

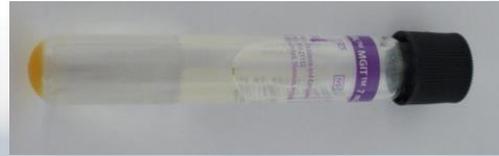
Semi-quantitative cut-offs:

- High → Ct <16
- Medium → Ct 16-22
- Low → Ct 22-28
- Very low → Ct 28-38



Blakemore R, Nabeta P, Davidow AL, Vadwai V, Tahirli R, Munsamy V, Nicol M, Jones M, Persing DH, Hillemann D, Ruesch-Gerdes S. A multisite assessment of the quantitative capabilities of the Xpert MTB/RIF assay. American journal of respiratory and critical care medicine. 2011 Nov 1;184(9):1076-84.

► Sensitivity & Specificity



Test result	“Gold standard” positive	“Gold standard” negative
Positive	True positive	False positive
Negative	False negative	True negative
	$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive} + \text{False negative}}$	$\text{Specificity} = \frac{\text{True negative}}{\text{True negative} + \text{False positive}}$

- A good medical screening test has a high sensitivity to identify all sick subjects
- False positive (healthy) subjects can be ruled out by a high specificity (second test)

► Sensitivity & Specificity

- Sensitivity:
 - 83 – 88 %, smear positive + negative
 - 46 – 63%, if smear negative
 - Can improve sensitivity by testing 3 samples
- Specificity:
 - 96 – 96%



► Correlations

Correlations between:

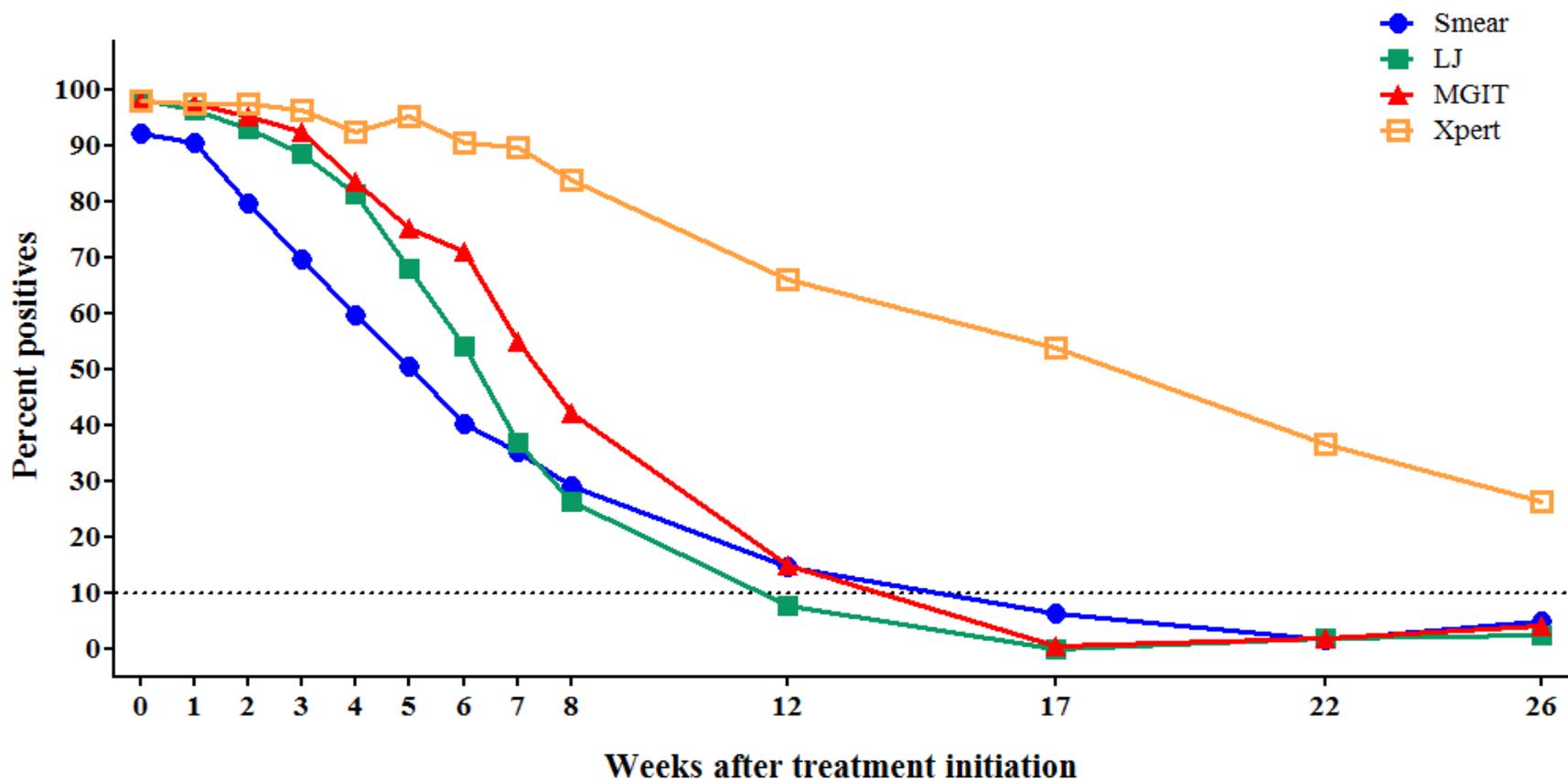
- Microscopy and Ct ($r = -0.77$)
- TTP and Ct ($r = 0.68$)
- CFU and Ct ($r = -0.56$)



▶ Disadvantages

Evaluation of the Xpert MTB/RIF assay for rapid identification of *Mycobacterium tuberculosis* DNA as a sputum biomarker of response to tuberculosis treatment: a prospective cohort study

Sven Friedrich, Andrea Rachow, Elmar Saathoff, Kasha Singh, Chacha Mangu, Rodney Dawson, Patrick Phillips, Amour Venter, Anna Bateson, Catharina Boehme, Norbert Heinrich, Robert Hunt, Alimuddin Zumla, Timothy McHugh, Stephen Gillespie, Andreas Diacon, and Michael Hoelscher



► Sensitivity & Specificity in Xpert MTB/Rif Ultra Assay

Xpert MTB/RIF Ultra for detection of *Mycobacterium tuberculosis* and rifampicin resistance: a prospective multicentre diagnostic accuracy study

Susan E Dorman, Samuel G Schumacher, David Alland et al. 2017
The Lancet Infectious Diseases

	Xpert MTB/Rif	Xpert MTB/Rif Ultra	Difference
Sensitivity: all culture positive	83%	88%	5.4%
Sensitivity: smear-negative	46%	63%	17%
Sensitivity: HIV-negative	90%	91%	1.3%
Sensitivity: HIV-positive	77%	90%	13%
Specificity	98%	96%	-2.7%

- Xpert Ultra higher sensitivity in smear-negative PTB
- May improve early diagnosis in:
 - HIV+
 - Children
- Loss in specificity
- If used as single diagnostic method in medium-prevalence settings, 2.7% difference in specificity may lead to considerable over-treatment

► Samples

- Pulmonary TB:
 - Sputum
 - Tracheal aspirate
 - Gastric washings – paediatrics

- Extra-pulmonary TB:
 - Tissue
 - Cerebrospinal fluid (CSF)
 - Pleural fluid
 - Stool
 - Urine

TABLE 1. Comparison of Xpert assay with culture method results for detection of MTBC

Specimen type ^a	No. (%) of specimens						
	Xpert assay			Culture			
	Indeterminate	Negative	Positive	Negative	Positive for <i>M. tuberculosis</i>	Positive for NTM	Contaminated
Tissue (245)	6 (2.4)	216	23	3	1	2	3
CSF (19)	0	19	0	189	9	15	
Gastric fluid (30)	0	22	8	3	20	0	
Pleural fluid (113)	7 (6.2)	103	3	19	1		2
Stool (23)	3 (13.0)	15	5	6	7		1
Urine (91)	4 (4.4)	81	6	103	2		1
				2	11		4
				1	2		2
				4			
				70			11
				1	5		

^a A total of 521 specimens were tested. The numbers in parentheses are the number of each specimen type.

► Advantages & Disadvantages

Advantages

One step process – automated

Quick (results < 2 hours)

Requires fewer biosafety measures than culture/LPA, so can be used in lower-level laboratories

High sensitivity

High specificity

Can detect rifampicin resistance

Same machine can also be used for HIV, hepatitis C diagnoses / viral load monitoring

Can work on many extrapulmonary TB samples

Disadvantages

Reliant on electricity

Expensive

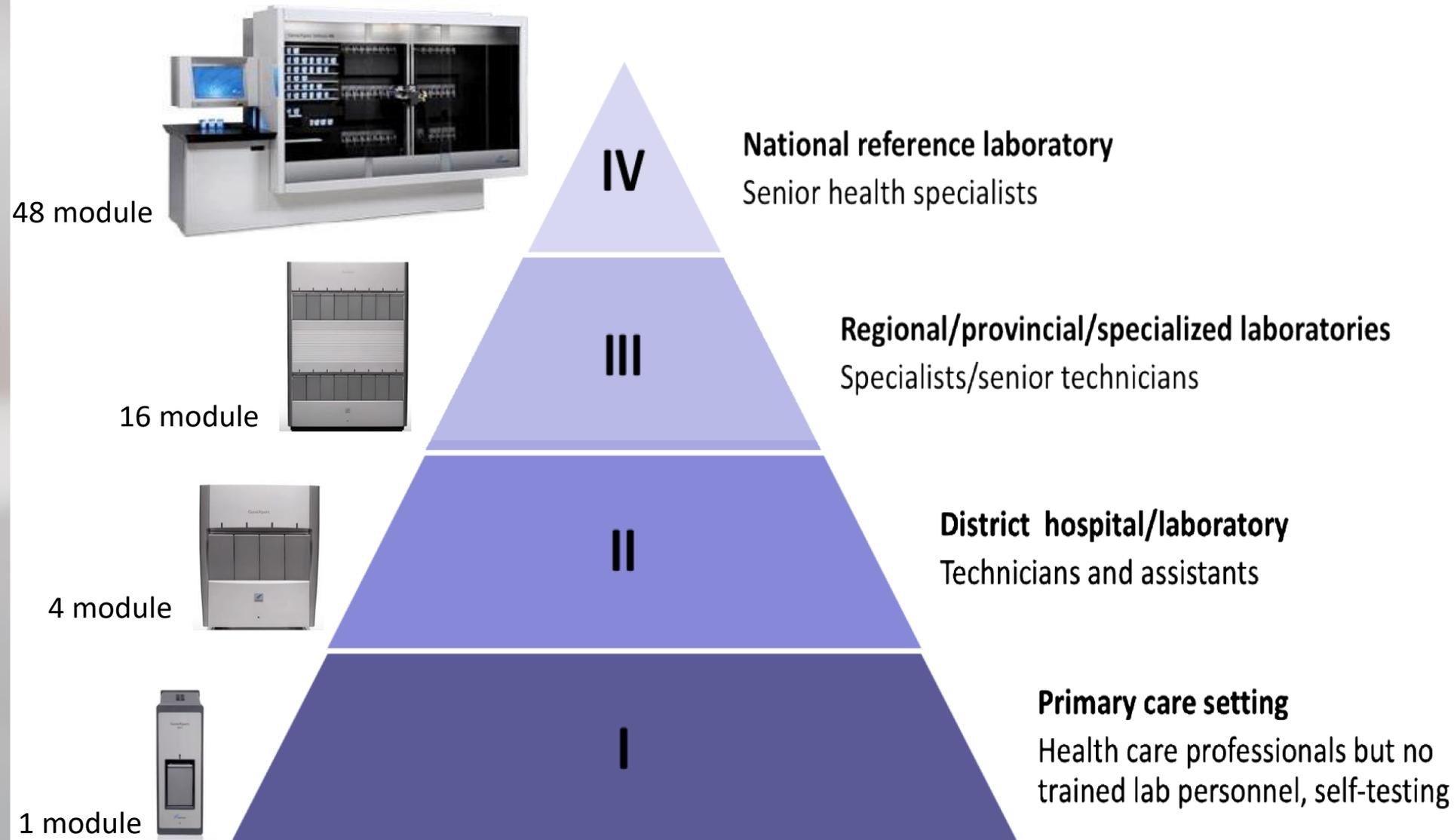
Cannot be used to track treatment progress

Requires annual calibration

Cartridge shelf-life only 18 months



► For Different Test Volumes



▶ Samples per Shift



5

20

80

500-1000

► Costs

Item	Unit Cost (USD)	Shipping Cost (USD)
GeneXpert 2 Module with: Laptop / Desktop	12,280 / 11,780	600
GeneXpert 4 Module with: Laptop / Desktop	17,500 / 17,000	600
GeneXpert 16 Module with: Laptop / Desktop	71,500 / 71,100	700
Annual Calibration kit (XPRTCHECK-CE-5)	450	
Test Cartridge	9.98 *	1.28

*9.98 USD is concessional cost. Market price is 50 -150 USD.

▶ Xpert Omni

- Same test platform as Xpert MTB/Rif
- Single-module
- Battery powered
- Robust, for use in remote settings
- Cost of unit = \$5315
- Surcharge of \$1.50 / cartridge = \$11.50



► Xpert XDR / Extended Platform

An Xpert assay for detection of resistance to INH, FQ, SLIDs

Susan Dorman, MD

on behalf of the

TB Clinical Diagnostics Research Consortium

XXI Jornadas Internacionales sobre Tuberculosis

21 November 2017

- Tests for resistance to:
 - *rpoB* → Rifampicin
 - *katG* → High level isoniazid
 - *inhA* → Low level isoniazid and ethionamide
 - *gyrA* & *gyrB* → Fluoroquinolones
 - *rrs* → Kanamycin, amikacin and capreomycin
 - *eis* → Kanamycin and amikacin

Gene target	DNA Invest. Seq. assay	Mutation	No mutation	Sensitivity % (95% CI)	Specificity % (95% CI)
katG¹	Mutation	120	0	97.5 (92.4-99.4)	100 (97.4-100)
	No Mutation	3 (2)	181		
inhA promoter	Mutation	33	0	97.1 (82.9-99.8)	100 (98.3-100)
	No mutation	1	271		
gyrA²	Mutation	89	0	95.7 (88.7-95.7)	100 (97.7-100)
	No mutation	4 (4)	211		
gyrB³	Mutation	5	2 (1)	71.4 (34.0-92.6)	99.3 (98.4-99.8)
	No mutation	2 (2)	290		
rrs⁴	Mutation	30	0	96.7 (81.5-99.8)	100 (98.2-100)
	No mutation	1	268		
eis promoter⁵	Mutation	9	1	81.8 (47.7-96.8)	99.7 (97.9-99.9)
	No mutation	2 (1)	289		

THANK YOU



GeneXpert rolled out as first-line diagnostic for TB in South Africa - 31 March 2011

"If a minister can do it, it can't be that hard," said South African Health Minister Aaron Moatsoledi, demonstrating GeneXpert test for TB.