Xpert MTB/RIF

10th Jan 2013

Points to cover

- Introduction
- Molecular Diagnostics (Rapid Dx)
- Gene Xpert- Advent
- Present Status
  - Pulmonary TB
  - Extrapulmonary TB
  - Pediatric TB
  - PLHIV- TB
- Cost effective analysis
- Conclusions

Introduction

“Reach the three million
A TB test, treatment and cure for all”

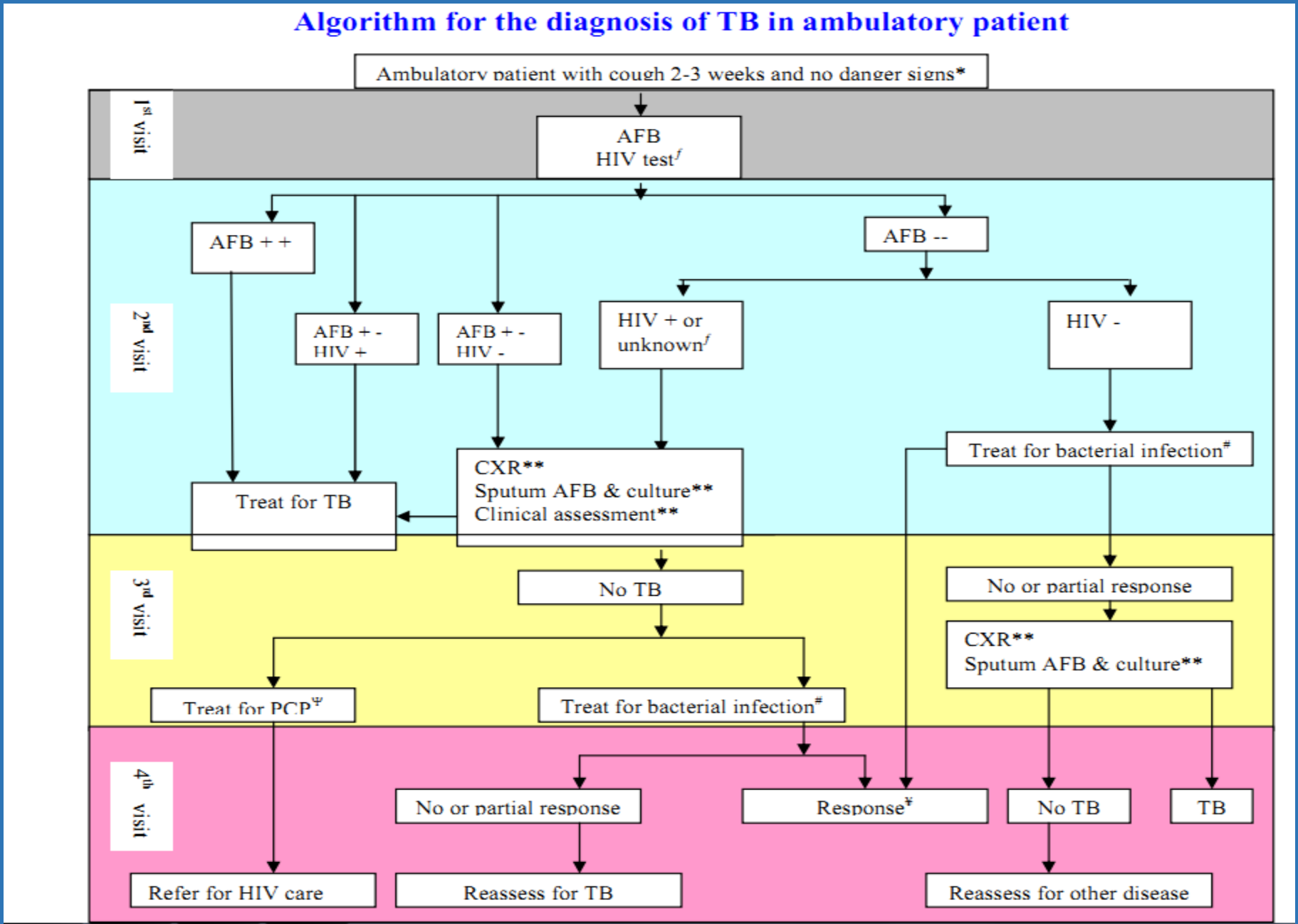
World TB day theme for 2014

TB Burden (GTR 2013)

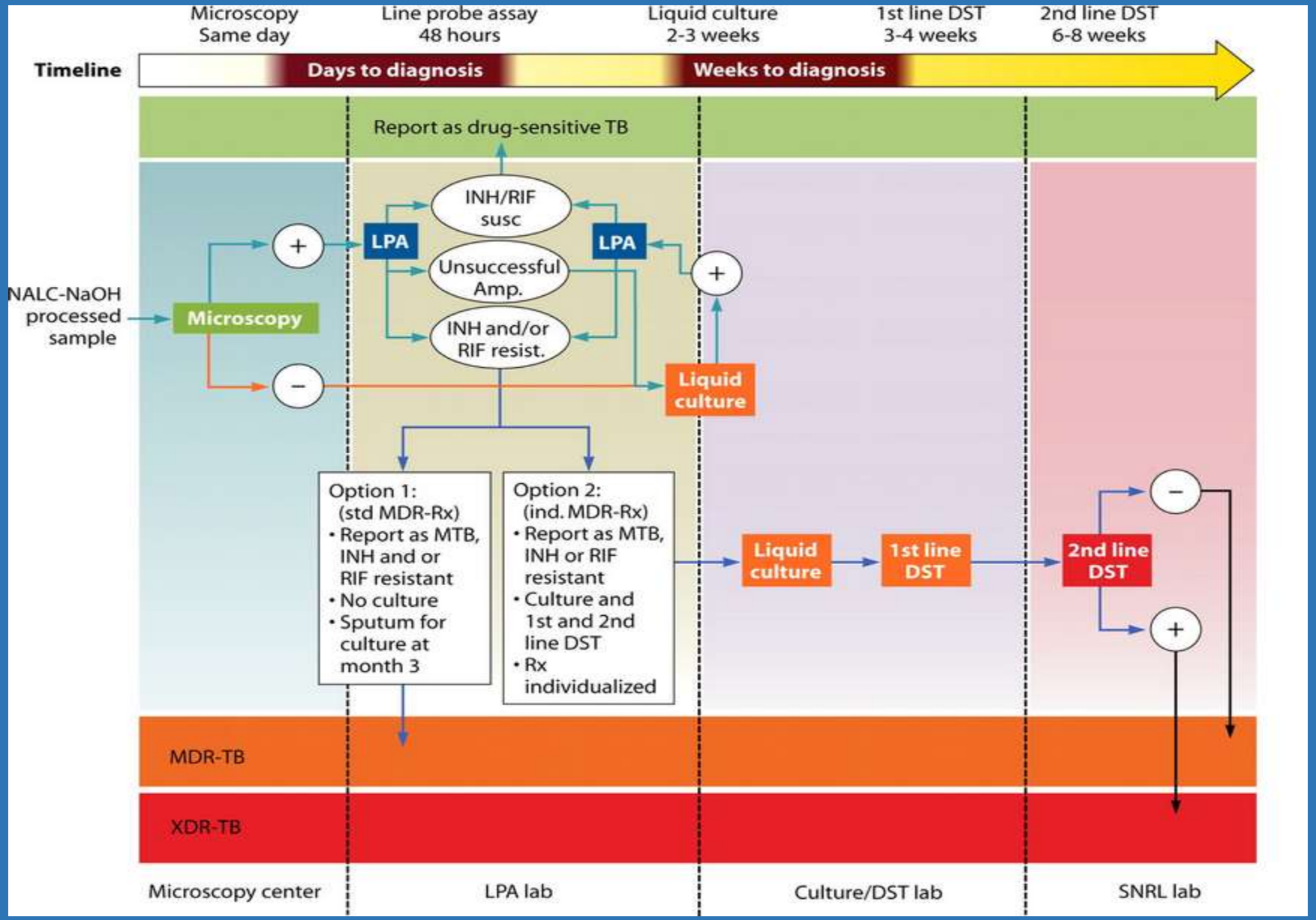
- 8.6 million patients
- 1.1 million TB patients are PLHIV
- 1.3 million deaths
- 4,50,000 MDR (3.6% new, 20% Prev. treated)
- 9.6 % of MDR are XDR TB
- 26% patients from INDIA

- ‘3 million cases missed notification in 2012’

Diagnosis of TB



Diagnosis of MDR TB



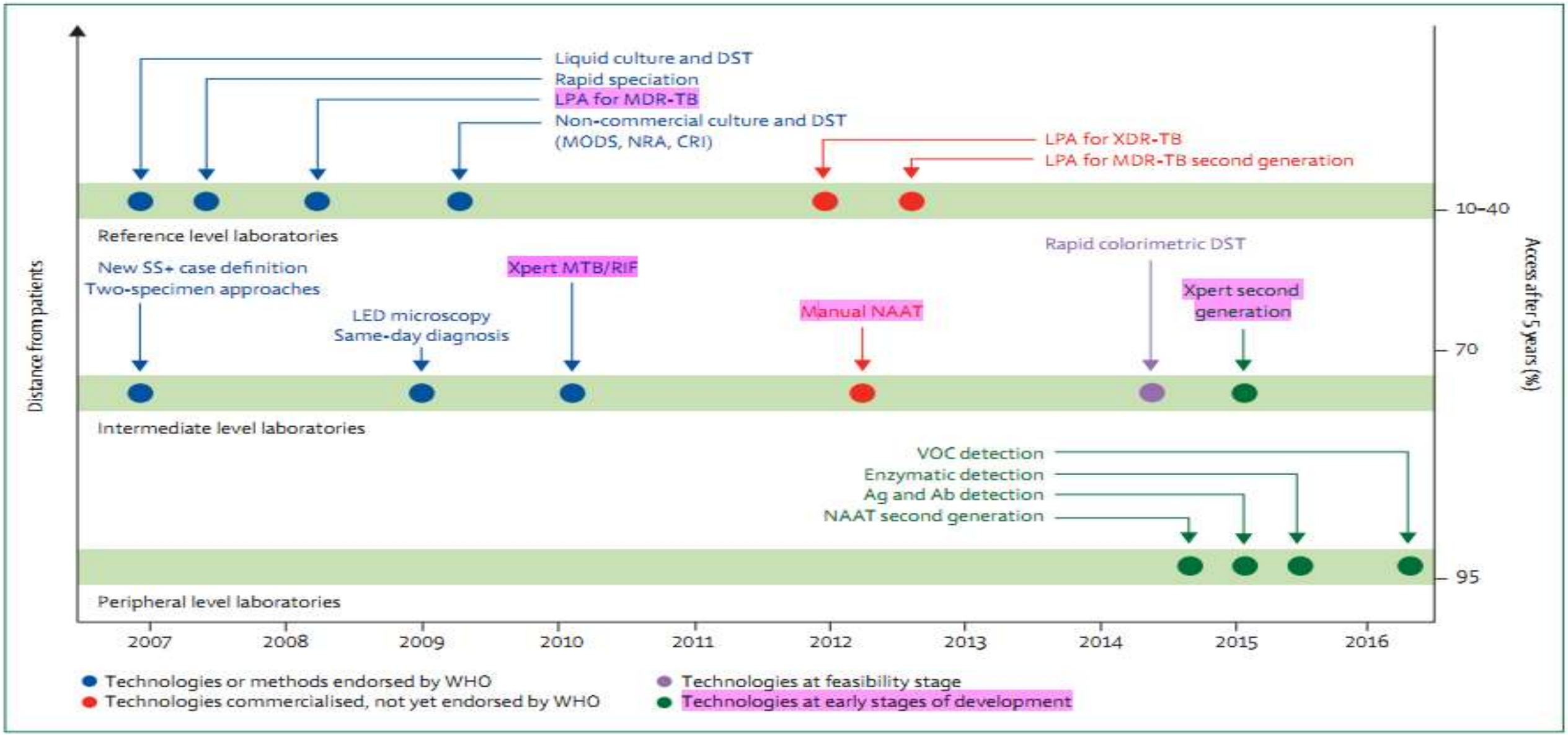


Figure: **Development pipeline for new tuberculosis diagnostics**

Reference level laboratories refer to national level facilities. Intermediate level laboratories refer to district and subdistrict level facilities. Peripheral level laboratories refer to community level facilities. Reproduced from WHO's global tuberculosis control report, 2012,[1] by permission of the World Health Organization. Ab=antibody. Ag=antigen. CRI=colorimetric redox indicator assay. DST=drug susceptibility test. LED=light emitting diode. LPA=line-probe assay. MDR-TB=multidrug-resistant tuberculosis. MODS=microscopic observation drug susceptibility. NAAT=nucleic acid amplification tests. NRA=nitrate reductase assay. SS+=sputum smear-positive. VOC=volatile organic compound. XDR-TB=extensively drug-resistant tuberculosis.

Comparison of Various Diagnostic Tests for Diagnosis of TB

	Microscopy	LED Microscopy	GeneXpert MTB/RIF	LAMP	Solid Culture	Liquid Culture
Threshold (CFU/ml)	10,000	-	131 (106-176)	-	~100	10-50
Turnaround time	1-2 days	1day	90 min	-	4-8 week	Days - 2 week
Sensitivity	50-60 %	10% >than ZN staining	~90 %	88 %	Reference	Reference
Specificity			98%	94 %	Reference	Reference
Technical expertise	Required	Required	Minimal	Required	Required	Required
Biosafety			Better than Microscopy			
Other						Prone to contaminatio n

- Boehme CC *et al. Semin Respir Crit Care Med 2013;34:17 – 31.*
- Lawn SD *et al. Lancet Infect Dis 2013; 13: 349–61.*

Comparison of Various Diagnostic Tests for Detection of Drug resistance in TB

	GeneXpert MTB/RIF	LPA	MODS	CRI	Nitrate reductase A	Solid Culture	Liquid Culture
Turnaround time	90 min	1-2 days	7 days	5-10 days	7-14 days	4-8 week	Days - 2 week
Sensitivity	~90 %	97 % (R) 84% (H)	92%	89- 100%	>94% (R) >92% (H)	Reference	Reference
Specificity	98%	99%	96%			Reference	Reference
Technical expertise	Minimal	High	High	High	HIgh	High	High
Biosafety	Better than Microscopy	Atleast BSL II					Atleast BSL III
Additional Comments	Can be done in usual lab	Can not replace Culture	-	-	-	-	Prone to contaminati on

- Boehme CC *et al. Semin Respir Crit Care Med 2013;34:17 – 31.*
- Lawn SD *et al. Lancet Infect Dis 2013; 13: 349–61.*

History of GeneXpert- Platform

- Originally developed by Cepheid Inc. for the detection of anthrax
- Onboard sample preparation with fully-automated rt-PCR amplification and detection
- Cartridge-based system incorporates microfluidics technology and fully automated nucleic acid analysis
- Expanding range of different organisms, genes may be detected using pathogen-specific cartridges within the same platform
- Machines with 1, 4, 16 and 48 modules are available, permitting multiple assays to be run concurrently and independently

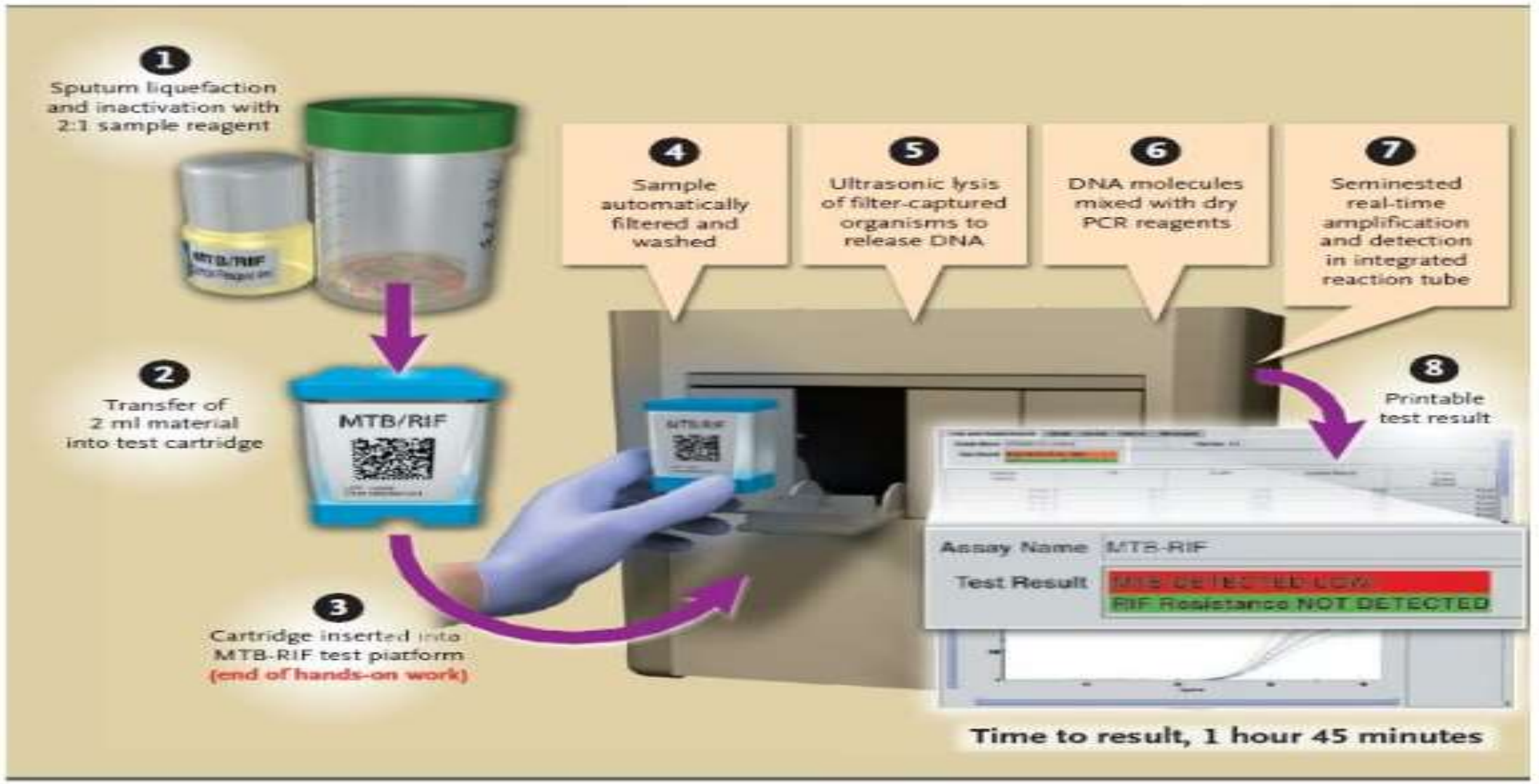
-Lawn SD *et al. Lancet Infect Dis 2013; 13: 349–61*

Target- Why Rifampicin?

- Amenable to rapid detection- 95% of all rifampicin-resistant mutations localized within the 81 bp core region of rpoB gene
- These mutations are highly predictive of rifampicin resistance
- Core region is flanked by M tuberculosis complex-specific DNA sequences
- M tuberculosis and rifampicin resistance can be tested simultaneously by targeting one amplicon
- Rifampicin resistance is strongly, although not invariably, indicative of MDR tuberculosis

-Lawn SD *et al. Lancet Infect Dis 2013; 13: 349–61*

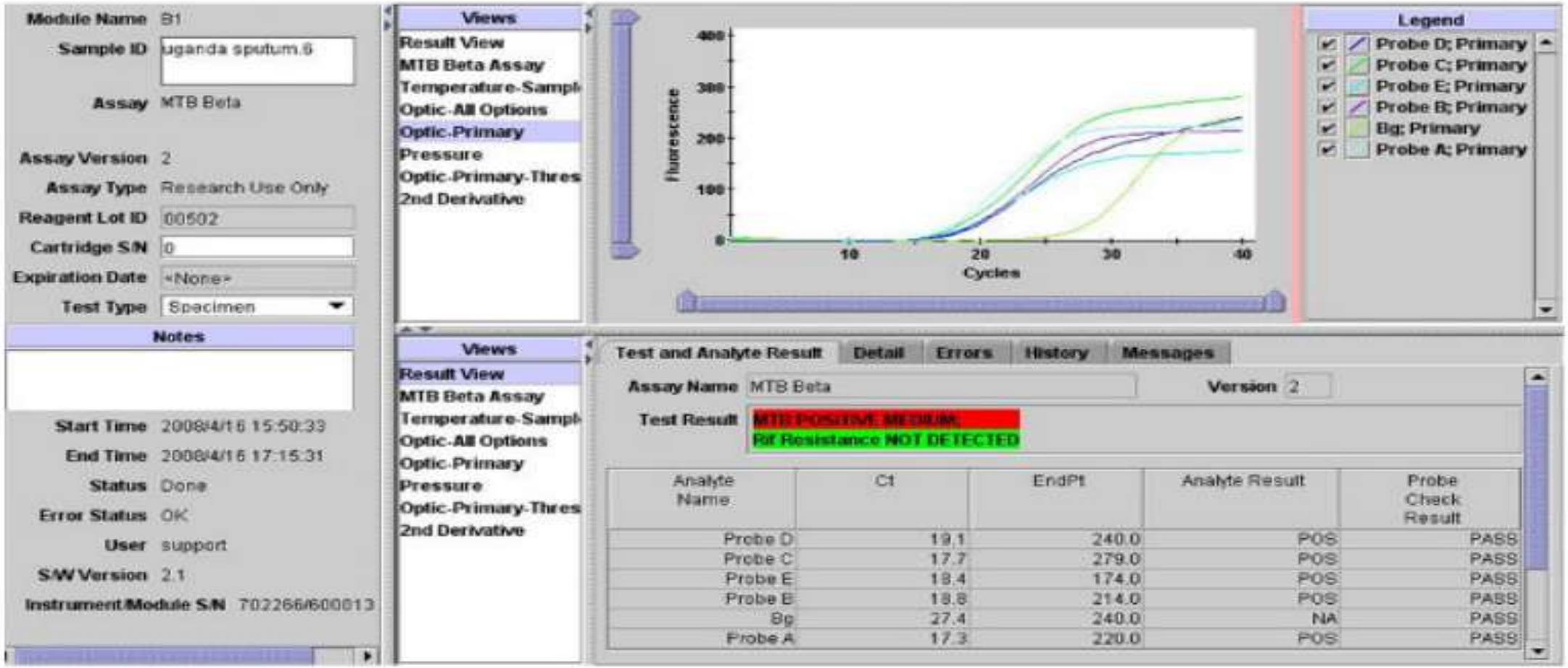
It's "GeneXpert MTB/RIF"



Assay procedure for the MTB/RIF test

- http://www.finddiagnostics.org/programs/tb/find_activities/automated_naat.html

Rifampin susceptible sample



Analyte Name	C1	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

- http://www.finddiagnostics.org/programs/tb/find_activities/automated_naat.html

TIMELINE – FROM CONCEPT TO IMPLEMENTATION

May 2006 – FIND and the University of Medicine and Dentistry of New Jersey partner with Cepheid to develop a novel TB test, with funding from the US NIH and the Bill & Melinda Gates Foundation (BMGF)

May 2009 – Demonstration studies underway

September 2010 – Expert Group issues strong recommendation to WHO based on scientific evidence; WHO's Strategic and Technical Advisory Group for TB further reviews evidence and makes policy recommendations

December 2010 – After organization of a Global Consultation, *WHO recommends Xpert MTB/RIF*

August 2012 – A public-private partnership between the US President's Emergency Plan for AIDS Relief (PEPFAR), the US Agency for International Development (USAID), UNITAID, and BMGF allows for a drop in price of the Xpert MTB/RIF test cartridge from 16.86 USD to 9.98 USD

May 2013 – Expert Group reviews updated evidence base on use of Xpert MTB/RIF for diagnosis of pulmonary, extrapulmonary and paediatric TB and rifampicin resistance, and issues updated recommendations to WHO

October 2013 –WHO updates recommendations on Xpert MTB/RIF, with an expanded scope of use

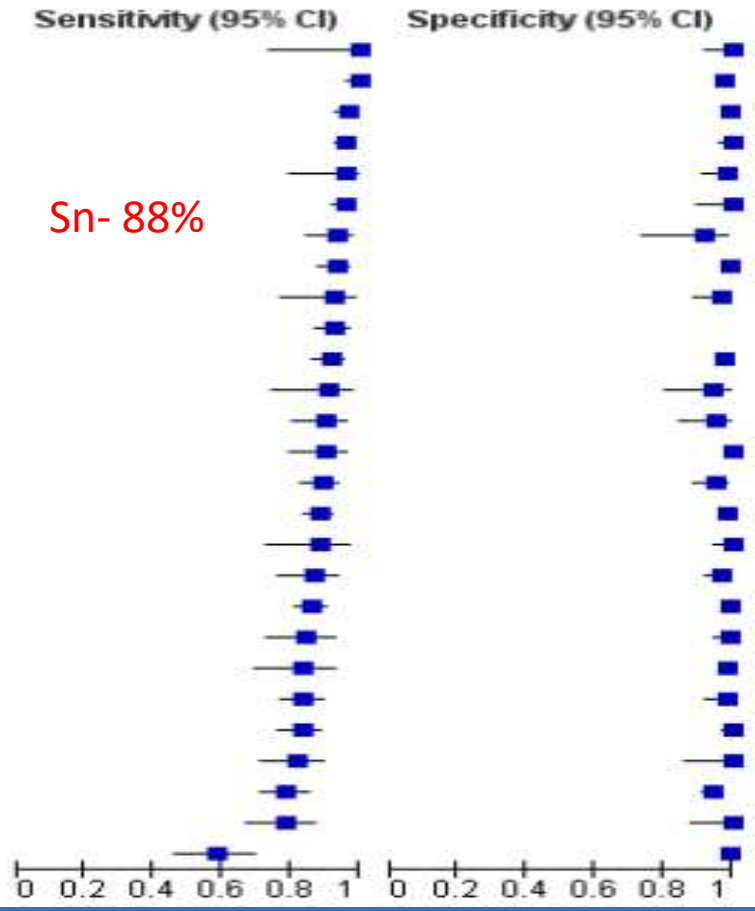
- WHO Xpert fact sheet Oct 2013

Performance of Xpert MTB/RIF in Clinical setting Pulmonary TB

Initial Test replacing microscopy

Figure 5. Forest plots of Xpert sensitivity and specificity for TB detection, Xpert used as an initial test replacing smear microscopy. The individual studies are ordered by decreasing sensitivity. TP = True Positive; FP = False Positive; FN = False Negative; TN = True Negative. Between brackets are the 95% CI of sensitivity and specificity. The figure shows the estimated sensitivity and specificity of the study (blue square) and its 95% CI (black horizontal line). Xpert specificity could not be estimated in one study.

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Malbruny 2011	12	0	0	46	1.00 [0.74, 1.00]	1.00 [0.92, 1.00]
Boehme 2011e	101	16	0	671	1.00 [0.96, 1.00]	0.98 [0.96, 0.99]
Boehme 2011b	171	3	6	825	0.97 [0.93, 0.99]	1.00 [0.99, 1.00]
Boehme 2010b	201	0	8	101	0.96 [0.93, 0.98]	1.00 [0.96, 1.00]
Ciftci 2011	24	1	1	59	0.96 [0.80, 1.00]	0.98 [0.91, 1.00]
Boehme 2010e	179	0	8	35	0.96 [0.92, 0.98]	1.00 [0.90, 1.00]
Bowles 2011	60	2	4	23	0.94 [0.85, 0.98]	0.92 [0.74, 0.99]
Boehme 2010c	136	1	10	185	0.93 [0.88, 0.97]	0.99 [0.97, 1.00]
Miller 2011	27	2	2	58	0.93 [0.77, 0.99]	0.97 [0.88, 1.00]
Friedrich 2011	117	0	9	0	0.93 [0.87, 0.97]	Not estimable
Boehme 2011f	136	5	12	234	0.92 [0.86, 0.96]	0.98 [0.95, 0.99]
Ioannidis 2011	29	2	3	32	0.91 [0.75, 0.98]	0.94 [0.80, 0.99]
Teo 2011	56	2	6	42	0.90 [0.80, 0.96]	0.95 [0.85, 0.99]
Hanif 2011	54	0	6	146	0.90 [0.79, 0.96]	1.00 [0.98, 1.00]
Marlowe 2011	116	4	14	82	0.89 [0.83, 0.94]	0.95 [0.89, 0.99]
Boehme 2011a	203	4	26	303	0.89 [0.84, 0.92]	0.99 [0.97, 1.00]
Zeka 2011	31	0	4	68	0.89 [0.73, 0.97]	1.00 [0.95, 1.00]
Scott 2011	58	3	9	104	0.87 [0.76, 0.94]	0.97 [0.92, 0.99]
Boehme 2011c	201	2	32	669	0.86 [0.81, 0.90]	1.00 [0.99, 1.00]
Rachow 2011	49	1	9	101	0.84 [0.73, 0.93]	0.99 [0.95, 1.00]
Boehme 2010d	36	3	7	215	0.84 [0.69, 0.93]	0.99 [0.96, 1.00]
Boehme 2010a	123	1	24	68	0.84 [0.77, 0.89]	0.99 [0.92, 1.00]
Boehme 2011d	121	0	24	144	0.83 [0.76, 0.89]	1.00 [0.97, 1.00]
Helb 2010	67	0	15	25	0.82 [0.72, 0.89]	1.00 [0.86, 1.00]
Theron 2011	111	19	30	320	0.79 [0.71, 0.85]	0.94 [0.91, 0.97]
Moure 2011	61	0	17	29	0.78 [0.67, 0.87]	1.00 [0.88, 1.00]
Lawn 2011	42	2	30	320	0.58 [0.46, 0.70]	0.99 [0.98, 1.00]

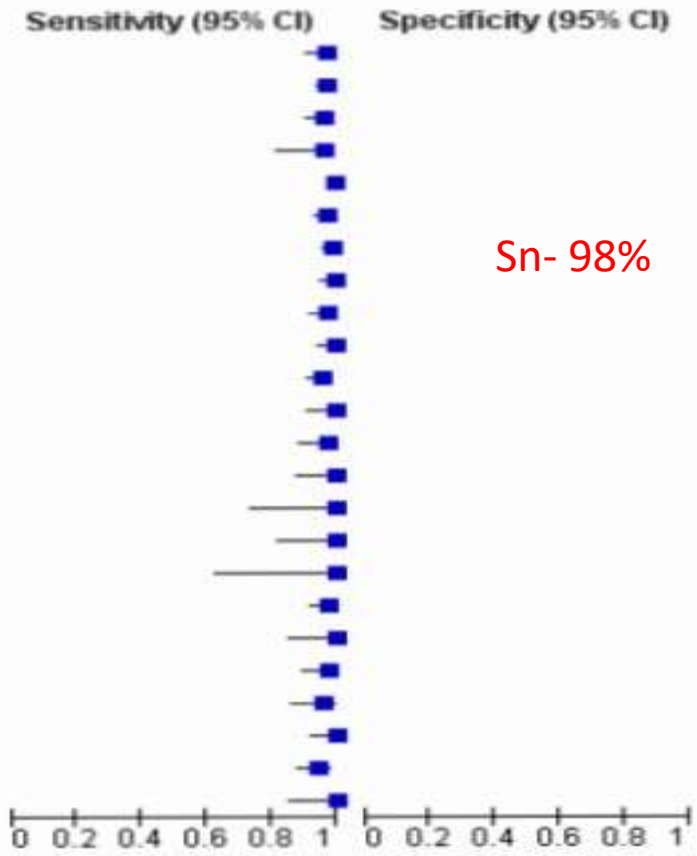


-Cochrane Review 2013

Follow-up to Negative smear

Figure 9. Forest plot of Xpert sensitivity for TB detection in smear-positive subgroup. The squares represent the sensitivity and specificity of one study, the black line its CI. TP = true positive; FP = false positive; FN = false negative; TN = true negative. Xpert specificity could not be estimated in these studies.

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Boehme 2010a	77	0	2	0	0.97 [0.91, 1.00]	Not estimable
Boehme 2010b	193	0	4	0	0.98 [0.95, 0.99]	Not estimable
Boehme 2010c	92	0	3	0	0.97 [0.91, 0.99]	Not estimable
Boehme 2010d	28	0	1	0	0.97 [0.82, 1.00]	Not estimable
Boehme 2010e	161	0	0	0	1.00 [0.98, 1.00]	Not estimable
Boehme 2011a	135	0	3	0	0.98 [0.94, 1.00]	Not estimable
Boehme 2011b	134	0	1	0	0.99 [0.96, 1.00]	Not estimable
Boehme 2011c	80	0	0	0	1.00 [0.95, 1.00]	Not estimable
Boehme 2011d	91	0	2	0	0.98 [0.92, 1.00]	Not estimable
Boehme 2011e	70	0	0	0	1.00 [0.95, 1.00]	Not estimable
Boehme 2011f	127	0	5	0	0.96 [0.91, 0.99]	Not estimable
Bowles 2011	40	0	0	0	1.00 [0.91, 1.00]	Not estimable
Hanif 2011	45	0	1	0	0.98 [0.88, 1.00]	Not estimable
Helb 2010	29	0	0	0	1.00 [0.88, 1.00]	Not estimable
Ioannidis 2011	12	0	0	0	1.00 [0.74, 1.00]	Not estimable
Lawn 2011	19	0	0	0	1.00 [0.82, 1.00]	Not estimable
Malbruny 2011	8	0	0	0	1.00 [0.63, 1.00]	Not estimable
Marlowe 2011	85	0	2	0	0.98 [0.92, 1.00]	Not estimable
Miller 2011	24	0	0	0	1.00 [0.86, 1.00]	Not estimable
Rachow 2011	50	0	1	0	0.98 [0.90, 1.00]	Not estimable
Scott 2011	47	0	2	0	0.96 [0.86, 1.00]	Not estimable
Teo 2011	43	0	0	0	1.00 [0.92, 1.00]	Not estimable
Theron 2011	89	0	5	0	0.95 [0.88, 0.98]	Not estimable
Zeka 2011	24	0	0	0	1.00 [0.86, 1.00]	Not estimable



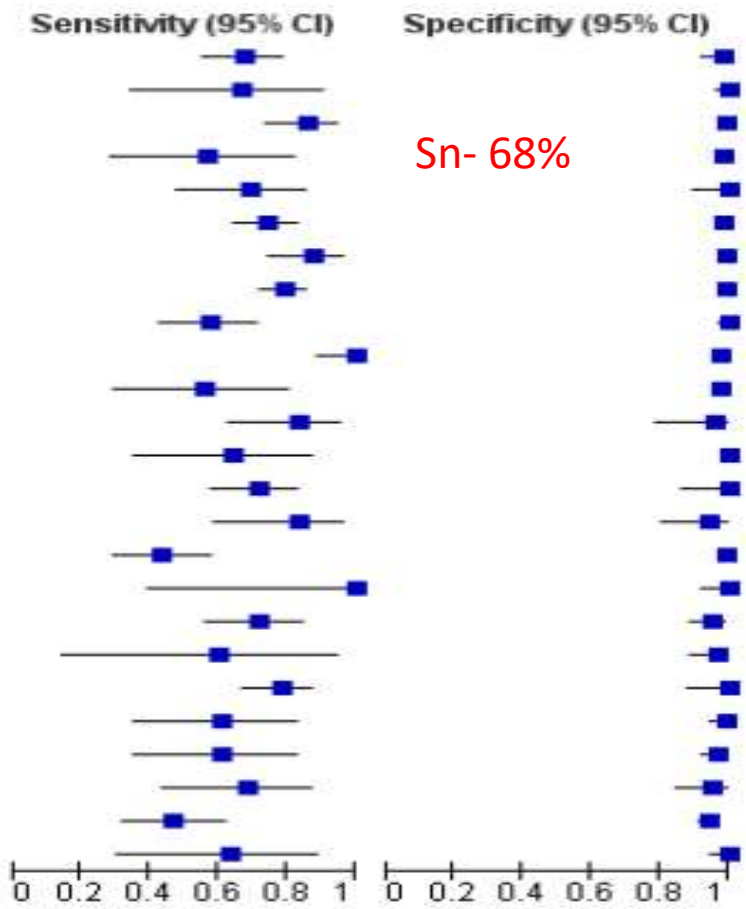
Sn- 98%

-Cochrane Review 2013

Follow-up to Negative smear

Figure 7. Forest plots of Xpert for TB detection, Xpert used as an add-on test following a negative smear microscopy result. TP = True Positive; FP = False Positive; FN = False Negative; TN = True Negative. Between brackets the 95% CI of sensitivity and specificity. The figure shows the estimated sensitivity and specificity of the study (blue square) and its 95% CI (black horizontal line).

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Boehme 2010a	46	1	22	68	0.68 [0.55, 0.78]	0.99 [0.92, 1.00]
Boehme 2010b	8	0	4	101	0.67 [0.35, 0.90]	1.00 [0.96, 1.00]
Boehme 2010c	44	1	7	185	0.86 [0.74, 0.94]	0.99 [0.97, 1.00]
Boehme 2010d	8	3	6	215	0.57 [0.29, 0.82]	0.99 [0.96, 1.00]
Boehme 2010e	18	0	8	35	0.69 [0.48, 0.86]	1.00 [0.90, 1.00]
Boehme 2011a	68	4	23	303	0.75 [0.65, 0.83]	0.99 [0.97, 1.00]
Boehme 2011b	37	3	5	825	0.88 [0.74, 0.96]	1.00 [0.99, 1.00]
Boehme 2011c	121	2	32	669	0.79 [0.72, 0.85]	1.00 [0.99, 1.00]
Boehme 2011d	30	0	22	144	0.58 [0.43, 0.71]	1.00 [0.97, 1.00]
Boehme 2011e	31	16	0	671	1.00 [0.89, 1.00]	0.98 [0.96, 0.99]
Boehme 2011f	9	5	7	234	0.56 [0.30, 0.80]	0.98 [0.95, 0.99]
Bowles 2011	20	1	4	23	0.83 [0.63, 0.95]	0.96 [0.79, 1.00]
Hanif 2011	9	0	5	146	0.64 [0.35, 0.87]	1.00 [0.98, 1.00]
Helb 2010	38	0	15	25	0.72 [0.58, 0.83]	1.00 [0.86, 1.00]
Ioannidis 2011	15	2	3	32	0.83 [0.59, 0.96]	0.94 [0.80, 0.99]
Lawn 2011	23	2	30	320	0.43 [0.30, 0.58]	0.99 [0.98, 1.00]
Malbruny 2011	4	0	0	45	1.00 [0.40, 1.00]	1.00 [0.92, 1.00]
Marlowe 2011	31	4	12	82	0.72 [0.56, 0.85]	0.95 [0.89, 0.99]
Miller 2011	3	2	2	58	0.60 [0.15, 0.95]	0.97 [0.88, 1.00]
Moure 2011	61	0	17	29	0.78 [0.67, 0.87]	1.00 [0.88, 1.00]
Rachow 2011	11	1	7	102	0.61 [0.36, 0.83]	0.99 [0.95, 1.00]
Scott 2011	11	3	7	104	0.61 [0.36, 0.83]	0.97 [0.92, 0.99]
Teo 2011	13	2	6	42	0.68 [0.43, 0.87]	0.95 [0.85, 0.99]
Theron 2011	22	19	25	320	0.47 [0.32, 0.62]	0.94 [0.91, 0.97]
Zeka 2011	7	0	4	68	0.64 [0.31, 0.89]	1.00 [0.95, 1.00]



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Xpert in Diagnosis of PTB

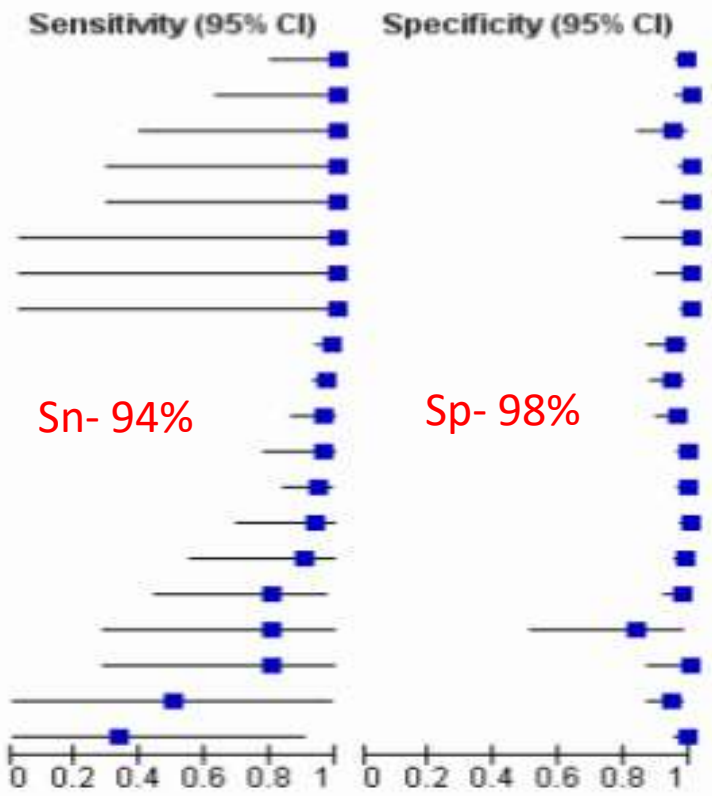
- Pooled sensitivity in HIV Negative- 89% (95% CI 81-94%)
- Pooled sensitivity in PL-HIV 80% (95% CI 67% - 88%)
  - PLHIV Smear +ve, Culture +ve – 100% (82-100%)
  - PLHIV Smear – ve , Culture +ve- 43% (30-58%)
- Fresh specimen > Frozen

-Cochrane Review 2013

Xpert MTB/RIF for Rif Detection in PTB

Figure 12. Forest plots of Xpert sensitivity and specificity for detection of rifampicin resistance, Xpert used as an initial test replacing conventional drug susceptibility testing as the initial test. The individual studies are ordered by decreasing sensitivity and decreasing number of true positives. The squares represent the sensitivity and specificity of one study, the black line its CI. TP = true positive; FP = false positive; FN = false negative; TN = true negative.

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Boehme 2010b	16	3	0	190	1.00 [0.79, 1.00]	0.98 [0.96, 1.00]
Bowles 2011	8	0	0	81	1.00 [0.63, 1.00]	1.00 [0.96, 1.00]
Lawn 2011	4	3	0	48	1.00 [0.40, 1.00]	0.94 [0.84, 0.99]
Friedrich 2011	3	0	0	90	1.00 [0.29, 1.00]	1.00 [0.96, 1.00]
Boehme 2010d	3	0	0	38	1.00 [0.29, 1.00]	1.00 [0.91, 1.00]
Malbruny 2011	1	0	0	16	1.00 [0.03, 1.00]	1.00 [0.79, 1.00]
Zeka 2011	1	0	0	34	1.00 [0.03, 1.00]	1.00 [0.90, 1.00]
Teo 2011	1	0	0	130	1.00 [0.03, 1.00]	1.00 [0.97, 1.00]
Boehme 2010e	119	3	2	61	0.98 [0.94, 1.00]	0.95 [0.87, 0.99]
Boehme 2011f	149	6	5	97	0.97 [0.93, 0.99]	0.94 [0.88, 0.98]
Boehme 2010a	47	4	2	90	0.96 [0.86, 1.00]	0.96 [0.89, 0.99]
Boehme 2011b	22	1	1	161	0.96 [0.78, 1.00]	0.99 [0.97, 1.00]
Boehme 2011a	47	1	3	160	0.94 [0.83, 0.99]	0.99 [0.97, 1.00]
Boehme 2010c	15	0	1	126	0.94 [0.70, 1.00]	1.00 [0.97, 1.00]
Boehme 2011c	9	3	1	175	0.90 [0.55, 1.00]	0.98 [0.95, 1.00]
Boehme 2011e	8	2	2	91	0.80 [0.44, 0.97]	0.98 [0.92, 1.00]
Scott 2011	4	2	1	10	0.80 [0.28, 0.99]	0.83 [0.52, 0.98]
Ioannidis 2011	4	0	1	27	0.80 [0.28, 0.99]	1.00 [0.87, 1.00]
Theron 2011	1	5	1	84	0.50 [0.01, 0.99]	0.94 [0.87, 0.98]
Boehme 2011d	1	1	2	112	0.33 [0.01, 0.91]	0.99 [0.95, 1.00]



-Cochrane Review 2013

Xpert in Diagnosis of PTB

Systematic review and Meta-analysis

- Studies published up to October 2011
- 18 studies, 10,224 patients
- 15 reported on Dx of PTB,
- Pooled sn- 90·4% (95% CI 89·2–91·4), sp- 98·4% (98·0–98·7).
  - Sm –ve- 75·0%
  - Sm +ve- 98·7%

Similar to Cochrane review, however current G4 version, launched in Dec 2011might differ in performance

- G4 version in Africa- Specificity of 99.5%

-Chang K, J Infect 2012; 64: 580–88

- Muhammad Osman *et al.* JCM, Nov 2013

Extrapulmonary TB

Study	Tissue	Lymph node	CSF	Gastric	Pleural	Urine	Cavitary fluid	Pericardial fluid	Stool	Pus	Other	Total sensitivity
Ligthelm et al[122]		28/29 (97%)										28/29 (97%)
Hillemann et al[96]	20/29 (69%)			7/8 (87.5%)		5 /5 (100%)			2/2 (100%)			35/44 (77.3%)
Teo et al[137]			2/3 (66%)	4/4 (100%)			1/1 (100%)				7/7 (100%)	14/15 (93.3%)
Vadwai et al[99]	54/70 (77%)		1/3 (33%)	Body fluids 16/21 (76%)						54/56 (96%)		125/150 (83%)
Miller et al[139]												7/8 (88%)
Zeka et al[138]					0/4 (0%)							21/31 (68%)
Causse et al[131]												39/41 (95%)
Friedrich et al[132]					5/20 (25%)							5/20 (25%)
Hanif et al[133]												12/12 (100%)
Armand et al[130]	3/5 (60%)	8/16 (50%)			3/7 (43%)	0/1 (0%)				3/3 (100%)		17/32 (53%)
Moure et al[136]	5/12 (42%)	24/34 (71%)	2/2 (100%)	2/3 (67%)	7/26 (27%)	2/3 (67%)		1/1 (100%)	2/2 (100%)	13/17 (76%)	5/8 (63%)	63/108 (58%)
Tortoli et al[121]			11/13 (85%)	45/58 (78%)	5/15 (33%)	11/13 (85%)	40/47 (85%)	5/10 (50%)		71/82 (87%)		188/238 (79%)

Source: Based on literature published by September 2012 and categorized by sample type.

- Boehme CC *et al. Semin Respir Crit Care Med 2013;34:17 – 31*

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Journals.ASM.org

Evaluation of GeneXpert MTB/RIF for Diagnosis of Tuberculous Meningitis

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Tuberculous meningitis (TBM) is the most severe form of tuberculosis. Microbiological confirmation is rare, and treatment is often delayed, increasing mortality and morbidity. The GeneXpert MTB/RIF test was evaluated in a large cohort of patients with suspected tuberculous meningitis. Three hundred seventy-nine patients presenting with suspected tuberculous meningitis to the Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam, between 17 April 2011 and 31 December 2012 were included in the study. Cerebrospinal fluid samples were tested by Ziehl-Neelsen smear, mycobacterial growth indicator tube (MGIT) culture, and Xpert MTB/RIF. Rifampin (RIF) resistance results by Xpert were confirmed by an MTBDR-Plus line probe assay and all positive cultures were tested by phenotypic MGIT drug susceptibility testing. Overall, 182/379 included patients (48.0%) were diagnosed with tuberculous meningitis. Sensitivities of Xpert, smear, and MGIT culture among patients diagnosed with TBM were 59.3% (108/182 [95% confidence interval {CI}, 51.8 to 66.5%]), 78.6% (143/182 [95% CI, 71.9 to 84.3%]) and 66.5% (121/182 [95% CI, 59.1 to 73.3%]), respectively. There was one false-positive Xpert MTB/RIF test (99.5% specificity). Four cases of RIF resistance (4/109; 3.7%) were identified by Xpert, of which 3 were confirmed to be multidrug-resistant (MDR) TBM and one was culture negative. Xpert MTB/RIF is a rapid and specific test for the diagnosis of tuberculous meningitis. The addition of a vortexing step to sample processing increased sensitivity for confirmed TBM by 20% ($P = 0.04$). Meticulous examination of a smear from a large volume of cerebrospinal fluid (CSF) remains the most sensitive technique but is not practical in most laboratories. The Xpert MTB/RIF represents a significant advance in the early diagnosis of this devastating condition.

Pediatric TB

	Country	Summary of findings
Nicol et al, 2011[42]	South Africa	Prospective study of inpatients (n=452) with median age 19 months (maximum 15 years) and suspected TB: from two induced sputum samples, the Xpert MTB/RIF assay diagnosed 75·9% (44 of 58) of culture-positive cases (specificity 98·8%) compared with 37·9% using smear microscopy; in smear-negative cases, the incremental yield of the second Xpert MTB/RIF test was 27·8%
Rachow et al, 2012[43]	Tanzania	Prospective study of 164 children aged <14 years (median 5·8 years): of 28 microbiologically confirmed cases, the Xpert MTB/RIF assay diagnosed 100% (7 of 7) smear-positive cases and 66·6% (14 of 21) smear-negative cases with 100% specificity; the incremental yields of testing second and third samples were 20% and 16%, respectively
Zar et al 2012[44]	South Africa	Prospective study of inpatients (n=535) with median age 19 months (maximum 15 years) and suspected TB: the yield of two Xpert MTB/RIF assay tests on nasopharyngeal aspirates from culture-confirmed cases was 65% (41 of 63) compared with 33% (21 of 63) by smear microscopy
Bates et al, 2013[45]	Zambia	Prospective study of inpatients (n=930) with median age 24 months (maximum 15 years) and suspected TB: in culture-positive cases (n=58), the Xpert MTB/RIF assay was more sensitive than smear microscopy when testing sputum samples (90·0% vs 30·0%) or gastric lavage aspirates (68·8% vs 25·0%) and specificity was 99·3%
Tortoli et al, 2012[39]	Italy	Study of the diagnosis of extrapulmonary TB in adults and children with a wide range of different sample types (tissue biopsies, pleural fluid, gastric aspirates, pus, CSF, and urine) that used a composite reference standard of culture, radiology, histology, and treatment response: the sensitivity in samples from children (86·9%) tended to be higher than that in samples from adults (77·6%), possibly as a result of the types of clinical samples in each group

TB=tuberculosis. MTB=*Mycobacterium tuberculosis*. RIF=rifampicin.

Table 2: **Studies of the Xpert MTB/RIF assay for diagnosis of tuberculosis in children**

-Lawn SD *et al. Lancet Infect Dis 2013; 13: 349–61*

TB in PLHIV

	Country	Clinical population	Patient selection	Sensitivity of smear microscopy, % (95% CI)	Sensitivity of single Xpert MTB/RIF assay test, % (95%CI)
Studies of outpatients					
Boehme et al, 2011[28]	South Africa, Uganda, India, Peru, Azerbaijan, Philippines	Outpatients (HIV+ and HIV−)	Presentation with suspected TB with cough ≥2 weeks	HIV+: 44·6% (37·7–51·6); HIV−: 68·6% (63·5–73·3); p<0·001	HIV+: 82·4% (76·7–86·9); HIV−: 90·7% (87·2–93·4); p=0·08
Theron et al, 2011[49]	South Africa	Outpatients (HIV+ and HIV−)	Presentation with suspected TB	HIV+: 50·0% (36·1–63·9); HIV−: 73·2% (62·7–81·6); p=0·01	HIV+: 69·6% (55·2–80·1); HIV−: 82·9% (73·4–89·6); p=0·09
Scott et al, 2011[50]	South Africa	Outpatients (mostly HIV+) with suspected TB with cough for ≥2 weeks	Presentation with suspected TB with cough ≥2 weeks	HIV+: 54% (38–69)	HIV+: 84% (69–93)
Lawn et al, 2011[51]	South Africa	Outpatients (HIV+) enrolling in an antiretroviral treatment clinic	Unselected patients screened for TB irrespective of symptoms before antiretroviral therapy	HIV+: 22·2% (13·3–33·6)	HIV+: 58·3% (46·1–69·8)
Studies of hospital inpatients					
O'Grady et al, 2012[52]	Zambia	Hospital medical inpatient admissions (HIV+ and HIV−)	All who could produce sputum samples	HIV+: 52·8% (45·1–60·4); HIV−: 48·6% (33·0–64·4); p=0·71	HIV+: 88·2% (81·9–92·6); HIV−: 74·3% (56·4–87·0); p=0·033
Balcells et al, 2012[53]	Chile	Hospital medical inpatients (HIV+)	Admission with suspected TB and symptoms >10 days	HIV+: 66·7% (39·1–86·2)	HIV+: 91·7% (64·6–98·5)
Carriquiry et al, 2012[54]	Peru	Hospital medical inpatients (HIV+)	Admission with suspected TB and cough >10 days plus abnormal chest radiograph plus additional symptoms	HIV+: 68·9% (54·3–80·6)	HIV+: 86·3% (74·3–93·2)

GeneXpert MTB/RIF for EBA/Response to ATT

The early bactericidal activity of antituberculosis agents is usually determined by measuring the reduction of the sputum mycobacterial load over time on solid agar medium or in liquid culture. This study investigated the value of a quantitative PCR assay for early bactericidal activity determination. Groups of 15 patients were treated with 6 different antituberculosis agents or regimens. Patients collected sputum for 16 h overnight at baseline and at days 7 and 14 after treatment initiation. We determined the sputum bacterial load by CFU counting (log CFU/ml sputum, reported as mean ± standard deviation [SD]), time to culture positivity (TTP, in hours [mean ± SD]) in liquid culture, and Xpert MTB/RIF cycle thresholds (C_T, n [mean ± SD]). The ability to discriminate treatment effects between groups was analyzed with one-way analysis of variance (ANOVA). All measurements showed a decrease in bacterial load from mean baseline (log CFU, 5.72 ± 1.00; TTP, 116.0 ± 47.6; C_T, 19.3 ± 3.88) to day 7 (log CFU, −0.26 ± 1.23, $P = 0.2112$; TTP, 35.5 ± 59.3, $P = 0.0002$; C_T, 0.55 ± 3.07, $P = 0.6030$) and day 14 (log CFU, −0.55 ± 1.24, $P = 0.0006$; TTP, 54.8 ± 86.8, $P < 0.0001$; C_T, 2.06 ± 4.37, $P = 0.0020$). The best discrimination between group effects was found with TTP at day 7 and day 14 ($F = 9.012$, $P < 0.0001$, and $F = 11.580$, $P < 0.0001$), followed by log CFU ($F = 4.135$, $P = 0.0024$, and $F = 7.277$, $P < 0.0001$). C_T was not significantly discriminative ($F = 1.995$, $P = 0.091$, and $F = 1.203$, $P = 0.316$, respectively). Culture-based methods are superior to PCR for the quantification of early antituberculosis treatment effects in sputum.

Kayigire XA *et al. J. Clin. Microbiol. 2013, 51(6):1894.*

We report an innovative approach to selectively amplify DNA derived from viable *Mycobacterium tuberculosis* in clinical specimens, which is useful for monitoring mycobacterial load in pulmonary TB patients during anti-TB treatment.

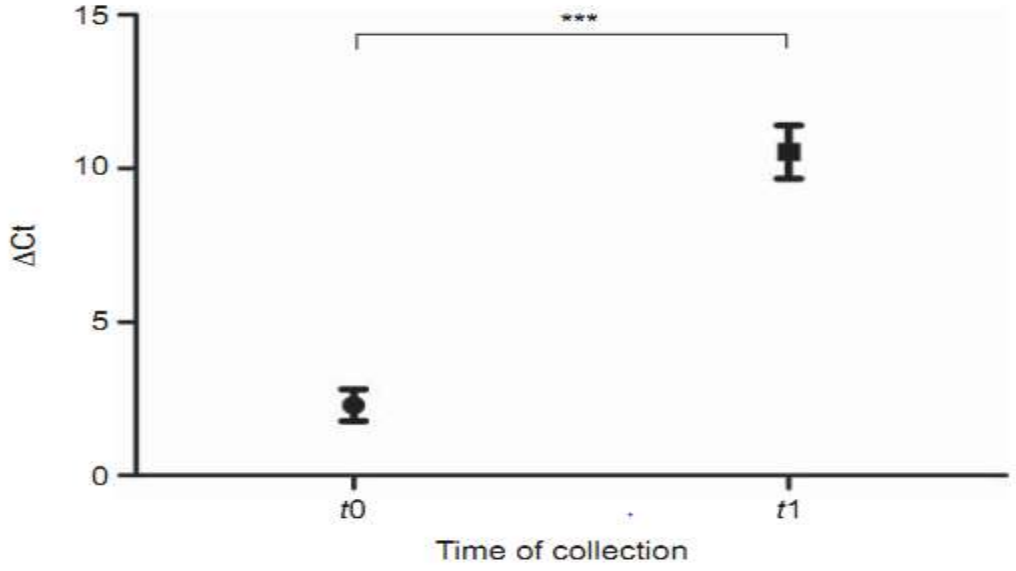


FIGURE 1. Comparison between the mean difference in threshold cycle (ΔCt) (propidium monoazide (PMA) treated minus PMA untreated) obtained from sputum samples collected before starting treatment (*t*0) and 10–20 days after the beginning of anti-tuberculosis therapy (*t*1). Errors bars represent ± SEM values. ***: $p<0.001$.

Paolo miotto. ERJ

Impact Of GeneXpert MTB/RIF

Impact Of GeneXpert MTB/RIF

Author, Year	Setting	Comparison	Sample size	Impact	Comments
Boehme CC, 2011	Multicentric, 9 Centres	Baseline vs Implementation group	6648	Early Dx 0/1/16/30 days Median time tp Rx 5 vs 56 days	Outcome variables not evaluated
Yoon C, 2012	Inpatients, Uganda	Baseline vs implementation group	477	Improved % microbiological Dx	No improvement in 2 month mortality
Hanrahan CF, 2013	OPD, South Africa, POC Xpert	Xpert +ve vs -ve	641 69% PLHIV	Early Dx 0/14/14/144 days	No improvement in outcomes at 6 months
Menzies NA, 2012	Multicentric, 5 African countries	Status quo vs introduction of Xpert	Calibrated dynamic mathematical model	Avert 13200 TB Cases Avert 182000 TB Deaths Prevalence will decrease by 28%, 2022 Cost effective over 20 yrs	
Dowdy DW, 2013	Africa	Same day Microscopy vs Xpert vs Both	Compartmental Model for Africa	Micro- 11% inci, 11.8% Death Red^{n} Xpert- 9.3% incid, 23.8 % Death Red^{n} Combined- 18.7 %, 33.1%	

Impact of GeneXpert MTB/RIF

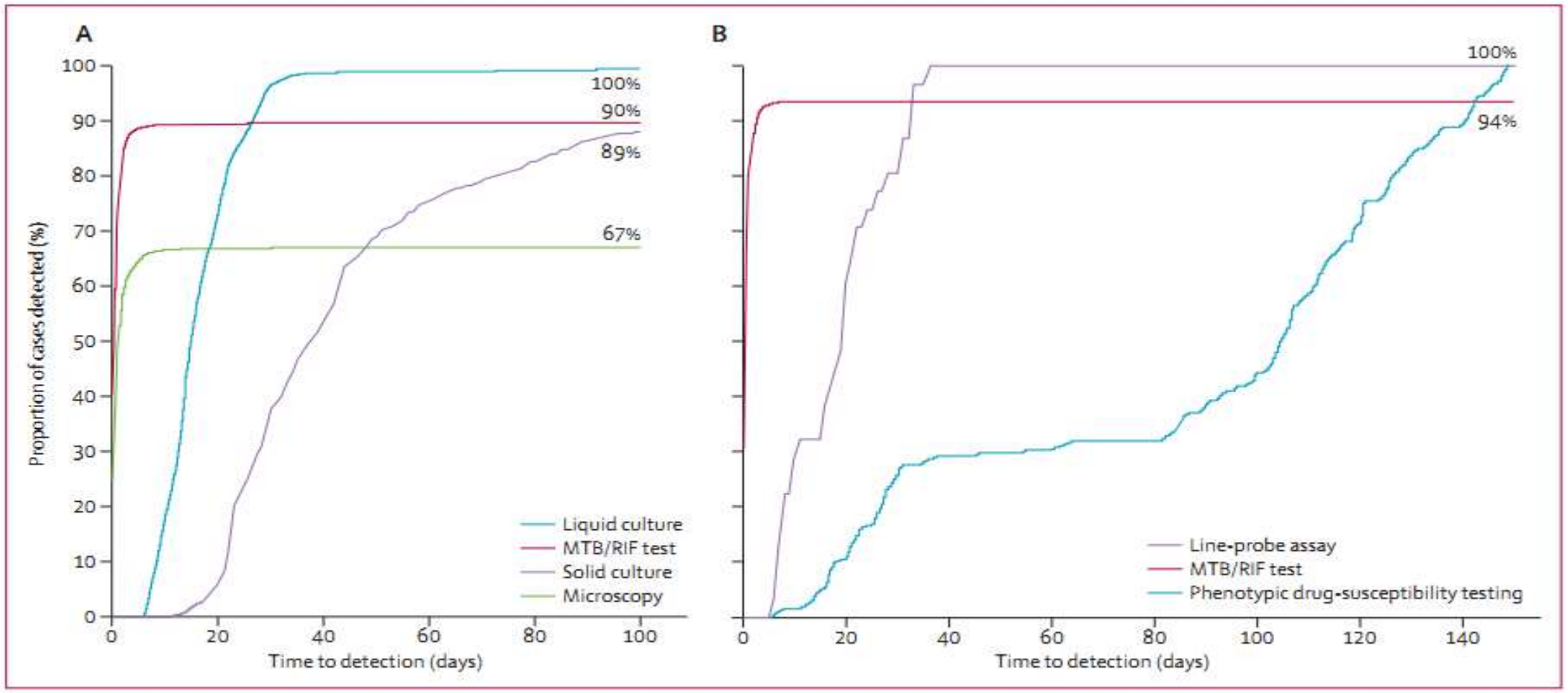
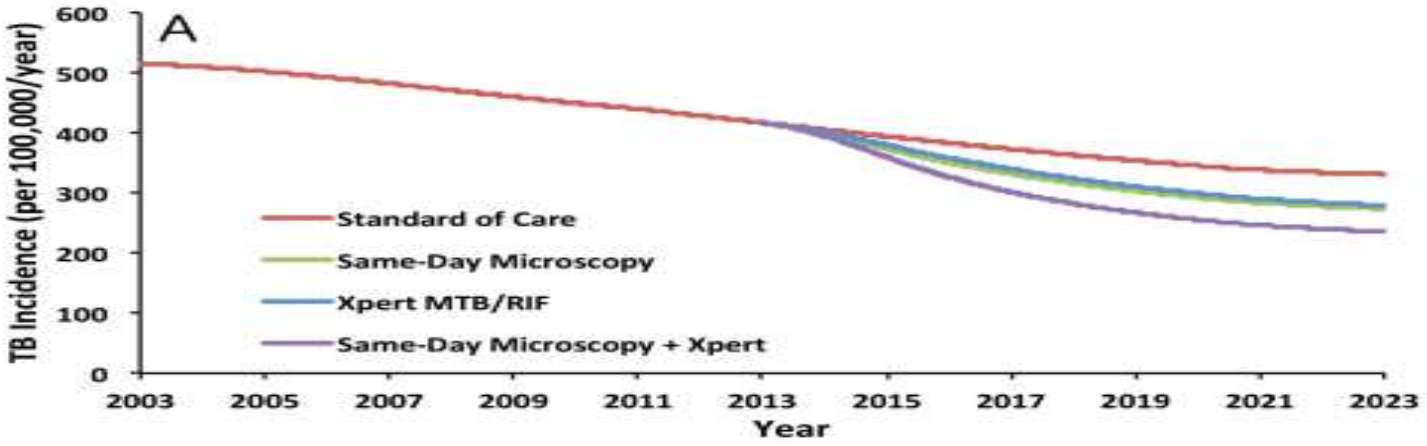


***Figure 2*: Proportion of tuberculosis cases detected by each method in culture-positive patients**
Percentages are the maximum proportion of cases detected by every method. (A) Tuberculosis case detection. (B) Detection of rifampicin resistance. Time to detection was defined as time between date of sputum sample collection and date of positive result. MTB=*Mycobacterium tuberculosis*. RIF=rifampcicin.

- Boehme CC *et al. Lancet 2011; 377: 1495–1505*

Impact of GeneXpert MTB/RIF



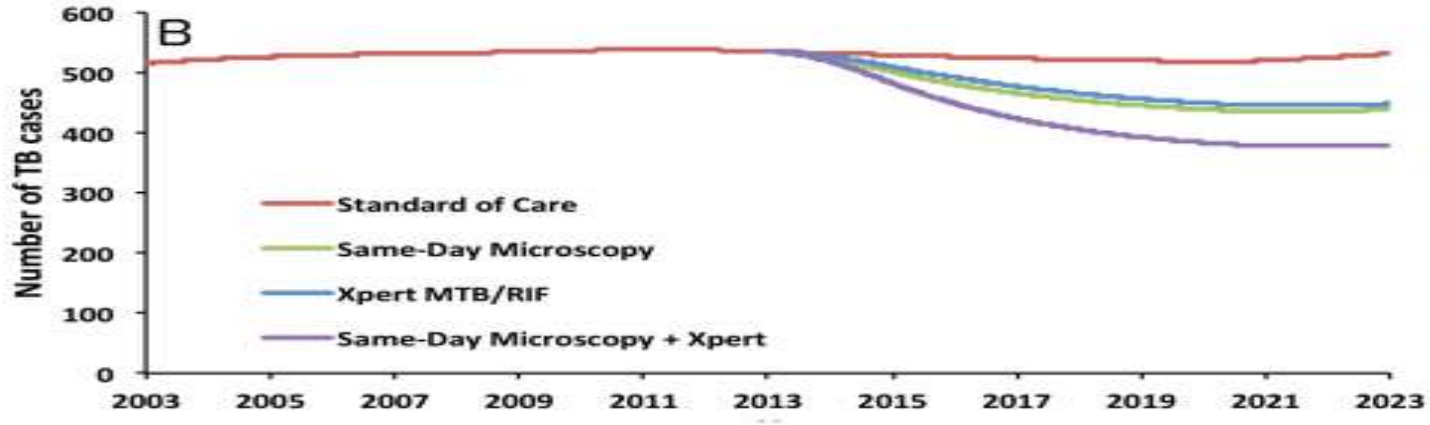


Figure 2. Projected Trajectory of TB Incidence in Africa, 2013–2022. Panel A shows the TB incidence rate (per 100,000 population per year), while Panel B shows the projected number of TB cases per year in an area with an adult population of 10 million in 2002, assuming constant 2.25% population growth.
doi:10.1371/journal.pone.0070485.g002

Impact of Xpert on PTB Rx and Outcome

	Smear microscopy (N=758)	Xpert MTB/RIF (N=744)	p value
All patients with a positive result (by any means) *			
By day 1	99/758 (13%)	178/744 (24%)	<0·0001
By day 2	107/758 (14%)	183/744 (25%)	<0·0001
By day 3	109/758 (14%)	185/744 (25%)	<0·0001
By day 14	165/758 (22%)	196/744 (86%)	0·0380
By day 28	199/758 (26%)	212/744 (29%)	0·33
By day 56	204/758 (27%)	215/744 (29%)	0·39

Feasibility, accuracy, and clinical effect of point-of-care Xpert MTB/RIF testing for tuberculosis in primary-care settings in Africa: a multicentre, randomised, controlled trial

Article in Press: Corrected Proof

Grant Theron, Lynn Zijenah, Duncan Chanda, Petra Clowes, Andrea Rachow, Maia Lesosky, Wilbert Bara, Stanley Mungofa, Madhukar Pai, Michael Hoelscher, David Dowdy, Alex Pym, Peter Mwaba, Peter Mason, Jonny Peter, and Keertan Dheda

Lancet, The

Anticipated benefits

- Increase case detection, esp. smear-ve
- Reduction in time to diagnosis and treatment
- Reduced patient default during investigation
- Reduced morbidity, mortality, and tuberculosis transmission
- Increased detection and Rx of MDR TB
- Reduced need of culture
- Reduced biohazard

Limitations

- Lack of a battery-operated system
- Annual calibration
- Limited range of operating temperatures
- Detects Dead bacilli, Not suitable for monitoring Rx response

- Rif resistance >15% PPV- >90%
- Rif Resistance <5% PPV <70%
- Does not detect INH resistance

- Boehme CC *et al. Semin Respir Crit Care Med 2013;34:17 – 31*
- Lawn SD *et al. Lancet Infect Dis 2013; 13: 349–61*

Challenges

- Increase in budget
- Use of the assay in centralized laboratories might blunt the potential effect
- Increased diagnostic capacity should be matched by increases in treatment capacity
- Diagnostic algorithms need to be redefined
- Need for robust supply chains and storage facilities for bulky cartridges with short shelf-life

Cost Effective analysis- Global

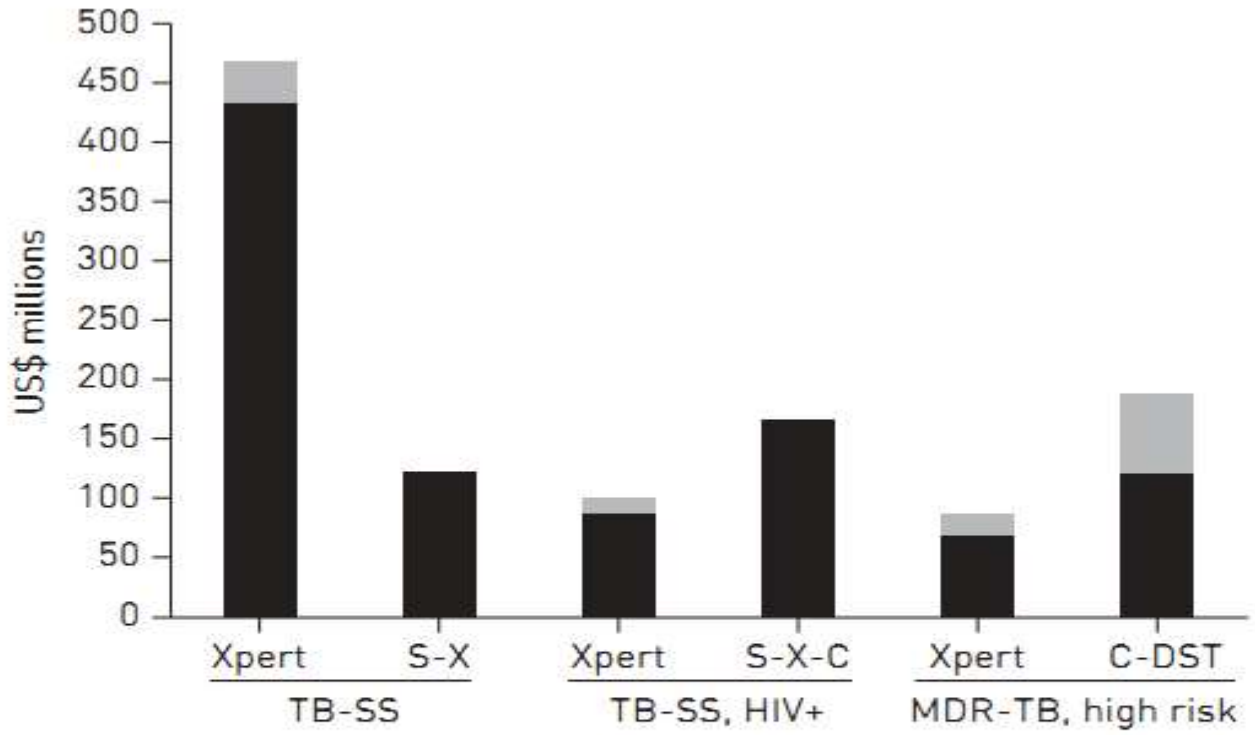
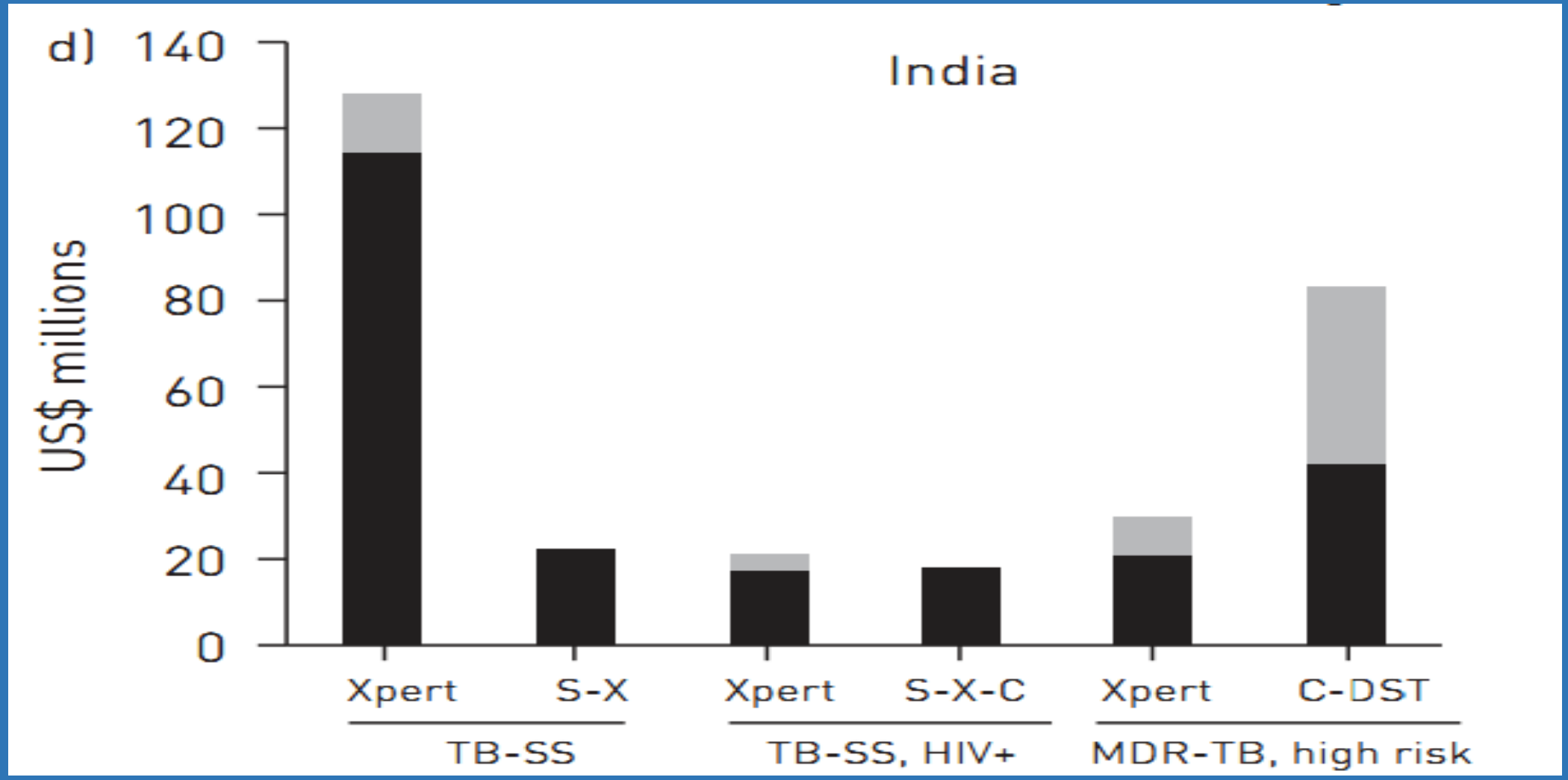


FIGURE 1 Global estimates of the annual cost of tuberculosis (TB) and multidrug-resistant (MDR)-TB diagnosis using Xpert MTB/RIF, compared with the costs of conventional diagnostics following World Health Organization-recommended algorithms in US$ millions using 2011 prices. Estimates include costs for solid and/or liquid media for culture and drug susceptibility testing (C-DST). The light grey section of the bar depicts the additional cost for liquid media of culture and/or DST. S: smear microscopy; X: radiograph; c: culture; TB-SS: people with signs and symptoms of tuberculosis; HIV+: HIV-positive.

Pantoja A *et al. Eur Respir J 2013; 42: 708–720*

Cost Effective analysis- India



Pantoja A *et al. Eur Respir J 2013; 42: 708–720*

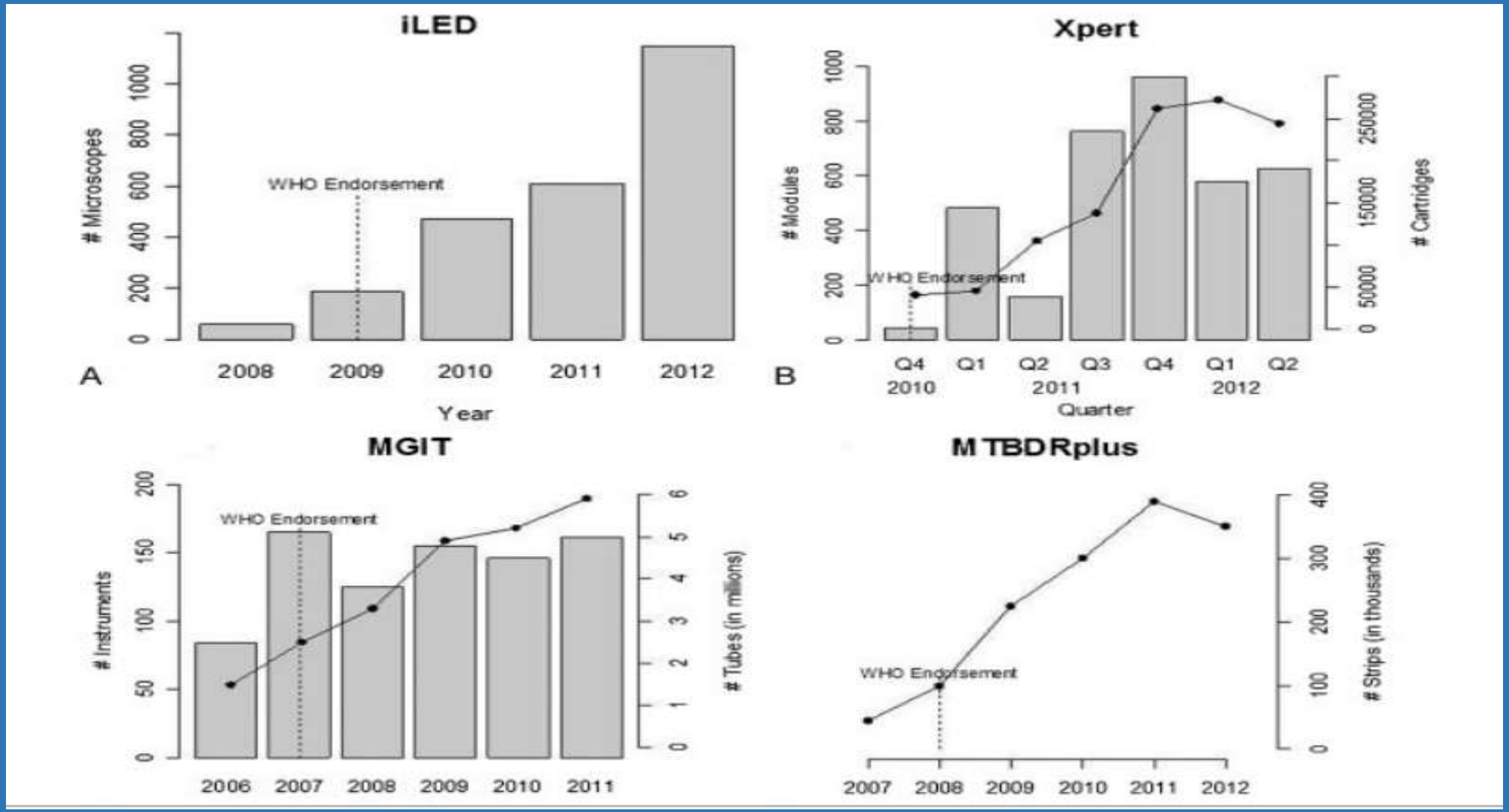
Cost Effective analysis

Methods and Findings: We estimate the impact of the introduction of Xpert on the costs and cost-effectiveness of TB care using decision analytic modelling, comparing the introduction of Xpert to a base case of smear microscopy and clinical diagnosis in India, South Africa, and Uganda. The introduction of Xpert increases TB case finding in all three settings; from 72%–85% to 95%–99% of the cohort of individuals with suspected TB, compared to the base case. Diagnostic costs (including the costs of testing all individuals with suspected TB) also increase: from US$28–US$49 to US$133–US$146 and US$137–US$151 per TB case detected when Xpert is used "in addition to" and "as a replacement of" smear microscopy, respectively. The incremental cost effectiveness ratios (ICERs) for using Xpert "in addition to" smear microscopy, compared to the base case, range from US$41–$110 per disability adjusted life year (DALY) averted. Likewise the ICERS for using Xpert "as a replacement of" smear microscopy range from US$52–$138 per DALY averted. These ICERs are below the World Health Organization (WHO) willingness to pay threshold.

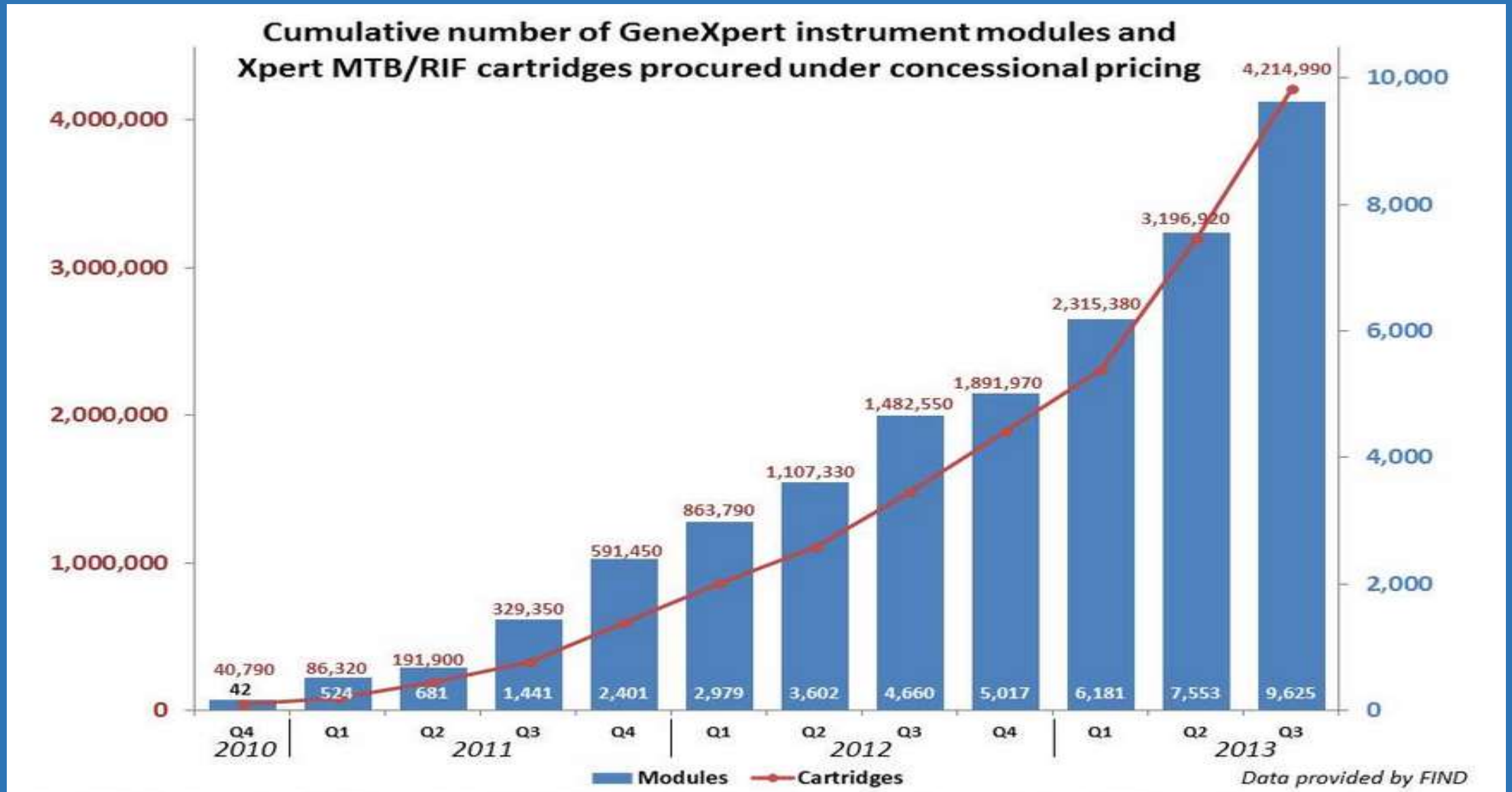
Conclusions: Our results suggest that Xpert is a cost-effective method of TB diagnosis, compared to a base case of smear microscopy and clinical diagnosis of smear-negative TB in low- and middle-income settings where, with its ability to substantially increase case finding, it has important potential for improving TB diagnosis and control. The extent of cost-effectiveness gain to TB programmes from deploying Xpert is primarily dependent on current TB diagnostic practices. Further work is required during scale-up to validate these findings.

Vassal A et al. PLoS Med 8 (11): e100 1120

Scale up so far



- Boehme CC *et al. Semin Respir Crit Care Med 2013;34:17 – 31*



As of 30 September 2013, a total of 1,843 GeneXpert instruments (comprising 9,625 modules) and 4,214,990 Xpert MTB/RIF cartridges had been procured in the public sector in 95 of the 145 countries eligible for concessional pricing.

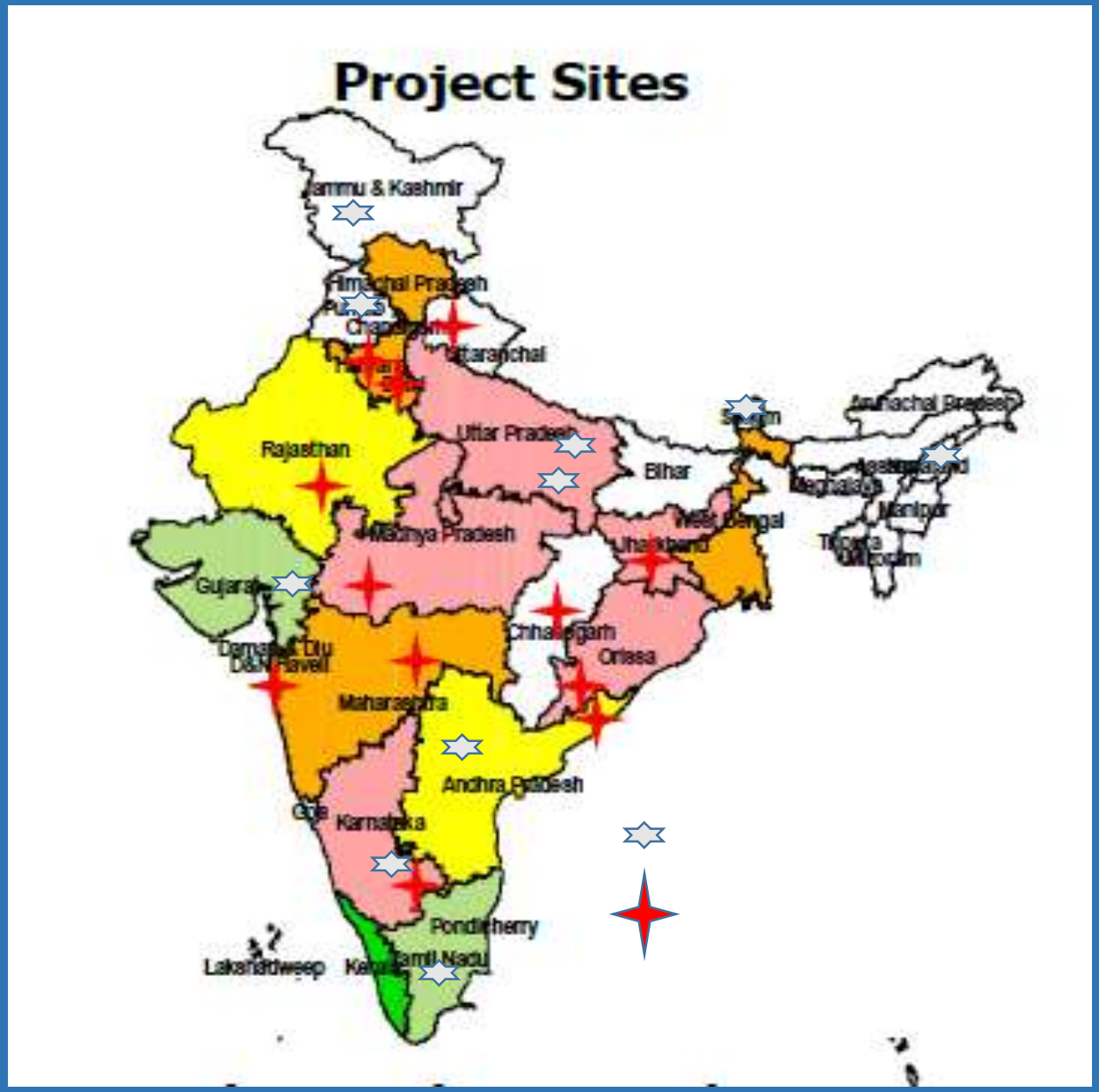
http://who.int/tb/laboratory/GeneXpert_rollout_large.jpg

Pilot of Xpert-MTB-Rif (CB-NAAT) in India

- 10 CB-NAAT sites under EXPANDx TB project to supplement the routine DST capacity

- RNTCP-WHO-FIND CB NAAT assessing feasibility of introducing CBNAAT for TB suspects in RNTCP across 18 TU sites in 12 states from March 2012. Interim Results expected shortly and will be placed before National Technical Committee

- 950 CBNAAT machines planned for every district and Medical College in India by 2017



Choice of Diagnostic Technology

MDR Diagnostic Technology	**Choice**
Molecular DST (e.g. CBNAAT or LPA DST)	First
Liquid culture isolation and LPA DST	Second
Solid culture isolation and LPA DST	Third
Liquid culture isolation and Liquid DST	Fourth
Solid culture isolation and DST	Fifth

UPDATED WHO RECOMMENDATIONS
AS OF OCTOBER 2013

For diagnosis of pulmonary TB and rifampicin resistance:

Strong recommendation:

- Xpert MTB/RIF should be used as the initial diagnostic test in adults and children presumed to have MDR-TB or HIV-associated TB

Conditional recommendations (recognising major resource implications):

- Xpert MTB/RIF may be used as the initial diagnostic test in adults and children presumed to have TB
- Xpert MTB/RIF may be used as a follow-on test to microscopy in adults presumed to have TB but not at risk of MDR-TB or HIV-associated TB, especially in further testing of smear-negative specimens

For diagnosis of extrapulmonary TB and rifampicin resistance:

Strong recommendation:

- Xpert MTB/RIF should be used as the initial diagnostic test in testing cerebrospinal fluid specimens from patients presumed to have TB meningitis

Conditional recommendation:

- Xpert MTB/RIF may be used as a replacement test for usual practice (including conventional microscopy, culture, and/or histopathology) for testing of specific non-respiratory specimens (lymph nodes and other tissues) from patients presumed to have extrapulmonary TB

For all WHO TB diagnostics policy documents:
http://www.who.int/tb/laboratory/policy_statements/