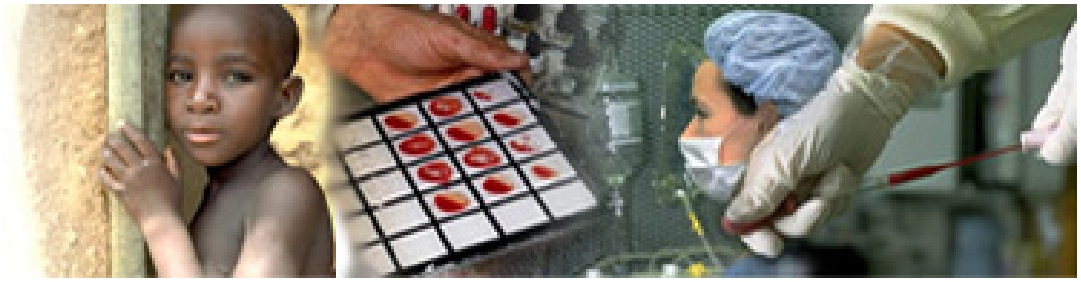
foundation
for innovative new diagnostics



Value chain in action: Xpert MTB/RIF

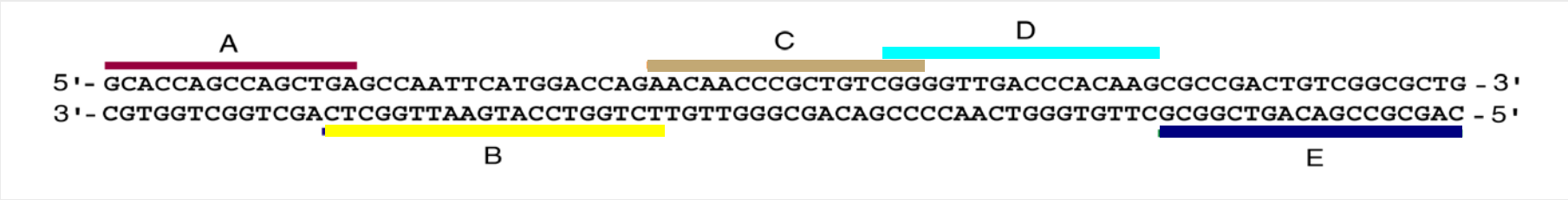
Advanced TB Diagnostic Research
July 5-8, 2011
Catharina Boehme

Partnering for better diagnosis for all

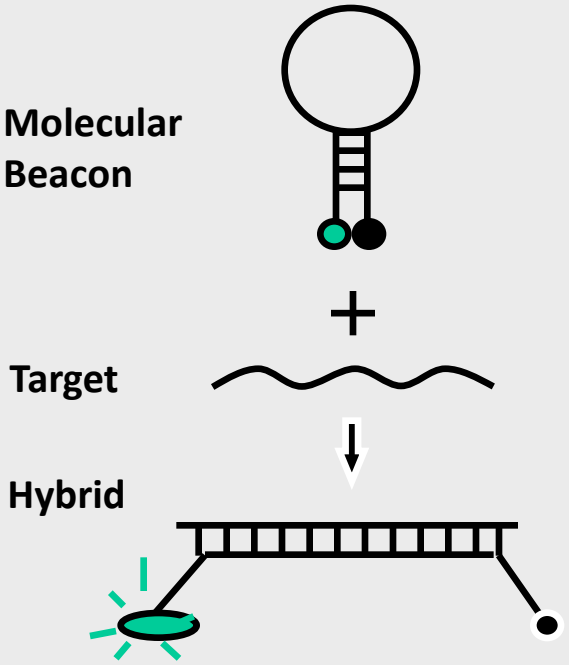
Conflict of interest

- FIND is a non-profit foundation devoted to developing and rolling out diagnostic tools for poverty-related diseases.
- In this role, FIND has development partnerships with industry.
- Xpert MTB/RIF has been developed through a partnership between Cepheid, FIND and UMDNJ with support from BMGF and NIH.
- FIND has no financial beneficial participation in any form.

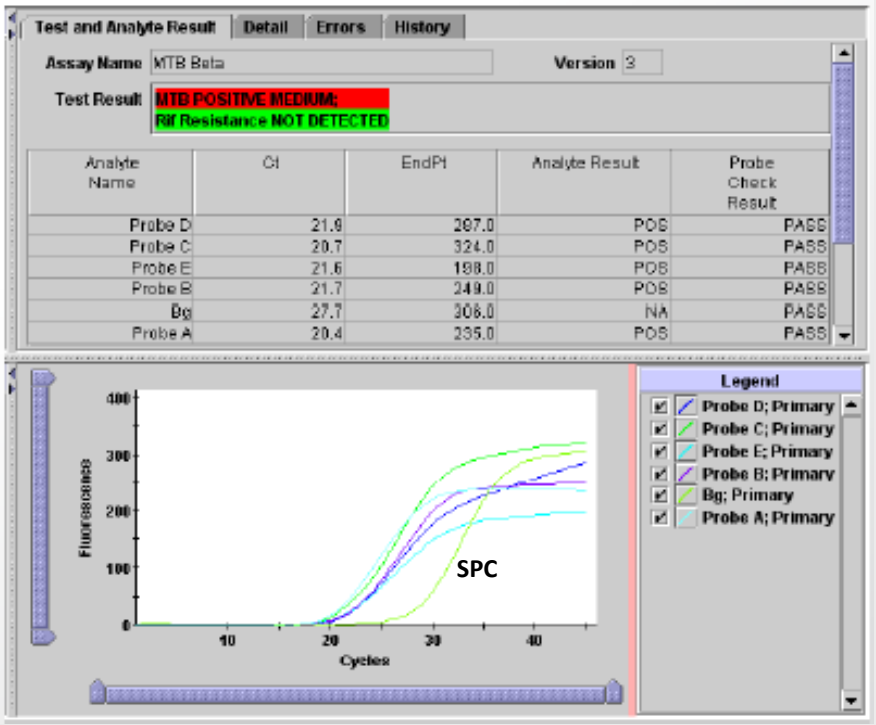
Xpert MTB/Rif molecular beacon assay



The PCR target is the 81 bp region of the rpoB gene: 5 probes bind to wildtype, but not mutant target

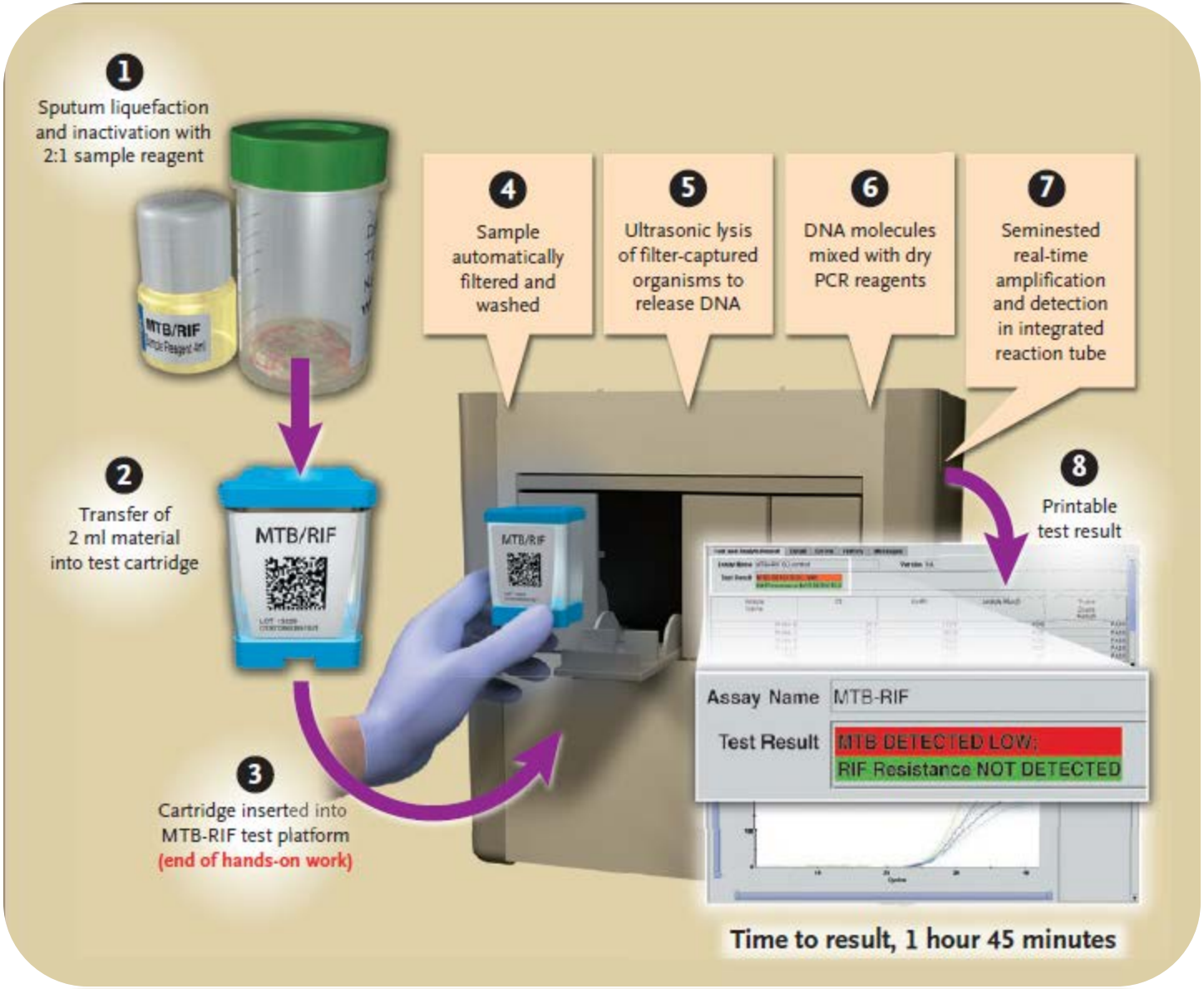


Each probe is labeled with a different fluorescent dye, permitting simultaneous detection



Example of Rif-Sensitive Profile – 5 probes & SPC show fluorescence

Assay procedure



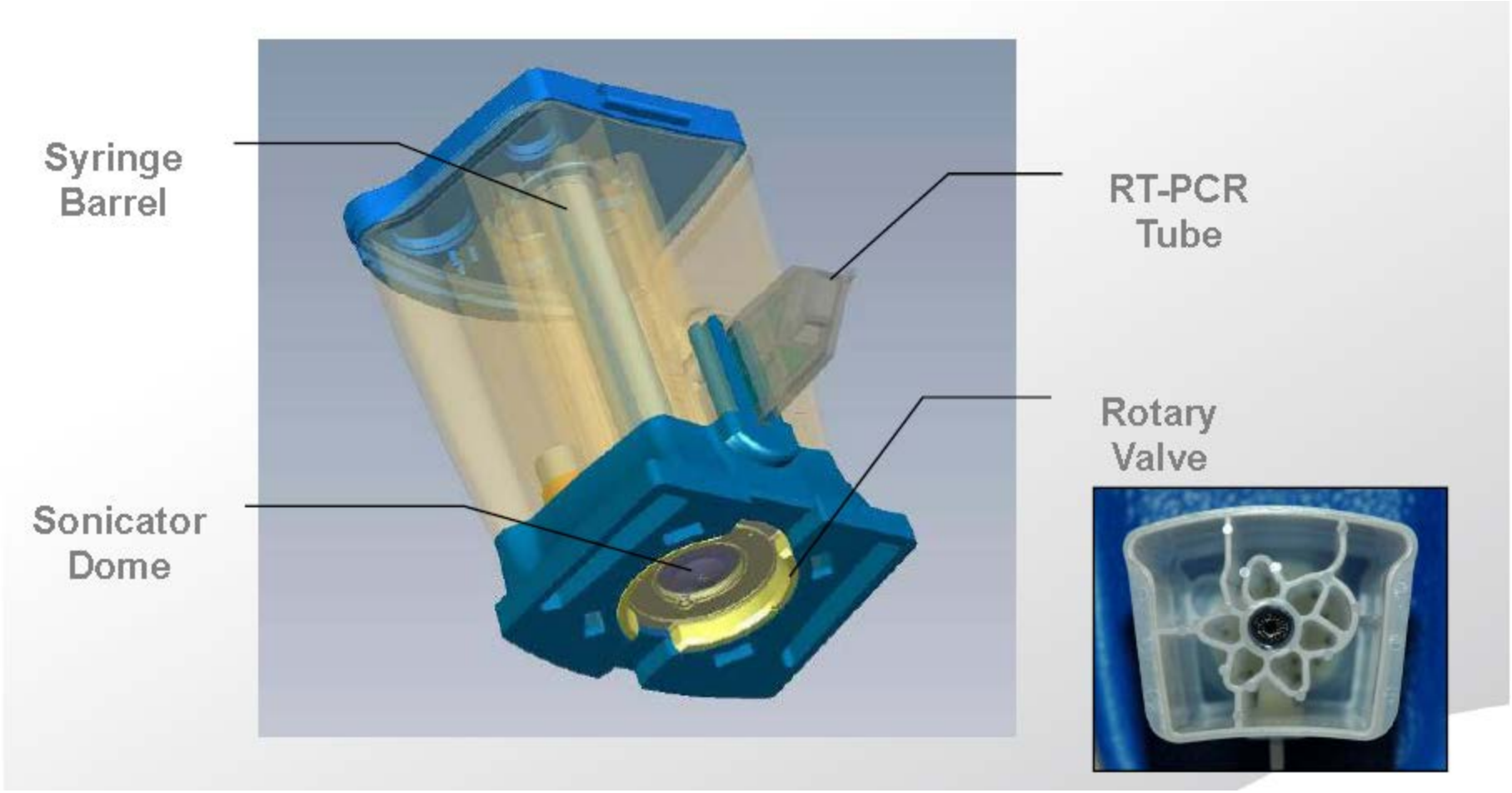
A multi-disease platform

GeneXpert

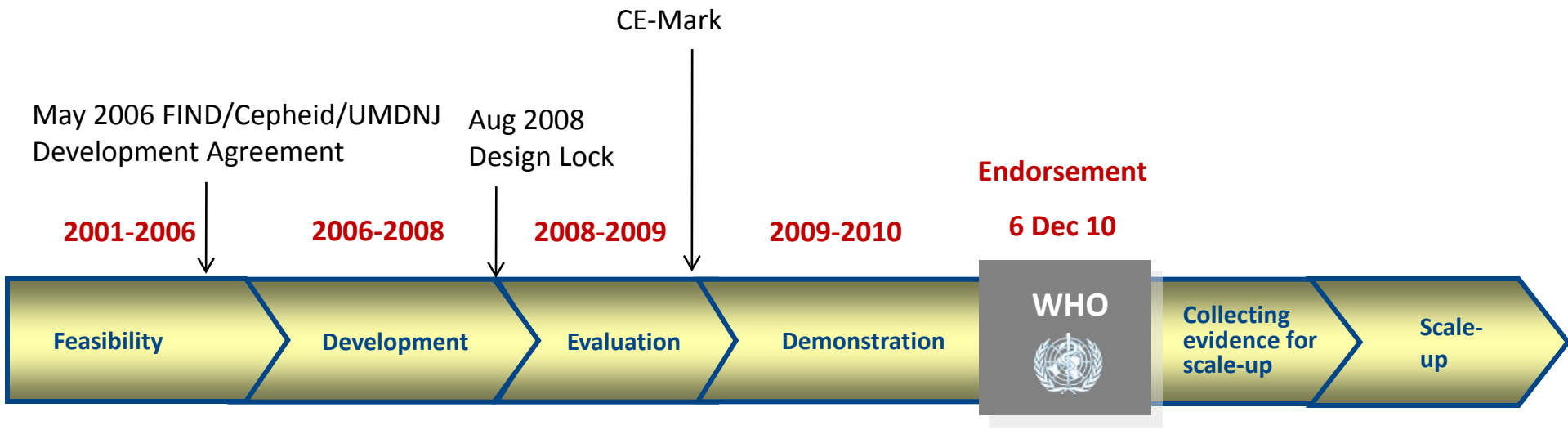
Xpert MTB/RIF

5	20	80	Samples per shift	500-1000

Glance inside the cartridge



Value Chain in Action



How it all began …

Anthrax Testing in US mail sorting centers

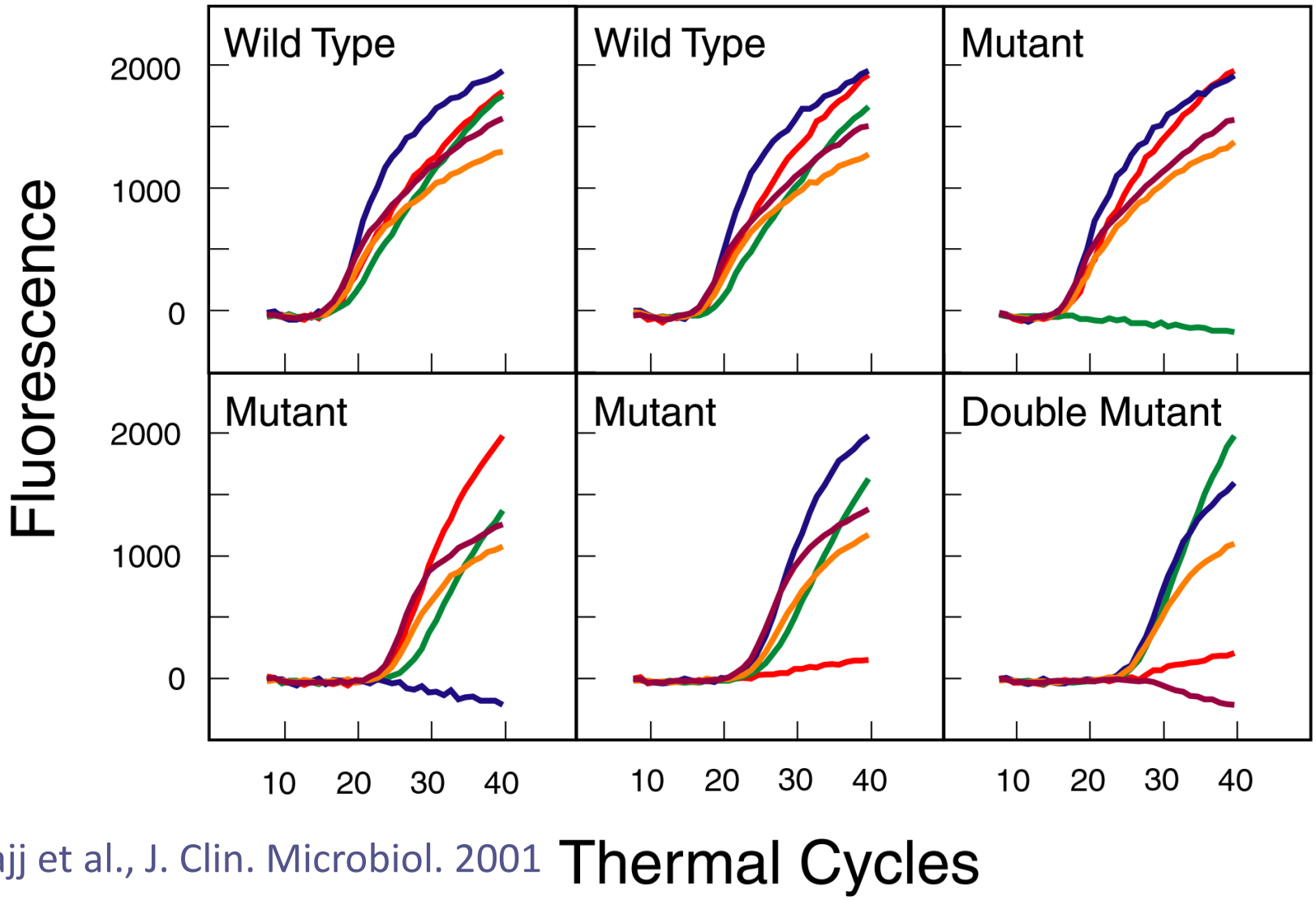


Sampling is continuous, PCR testing occurs hourly

- Installed in 275 sites across the country (USPS)
- System tests Hepa filtered air
- Est. 35 billion pieces of mail screened
- Over 3 million tests run
  - NO FALSE POSITIVES
- For the most specific of routine medical diagnostic tests, false positive rates are around 0.1%
  - This would translate to over 2,000 false positives for an equivalent number of medical Dx tests

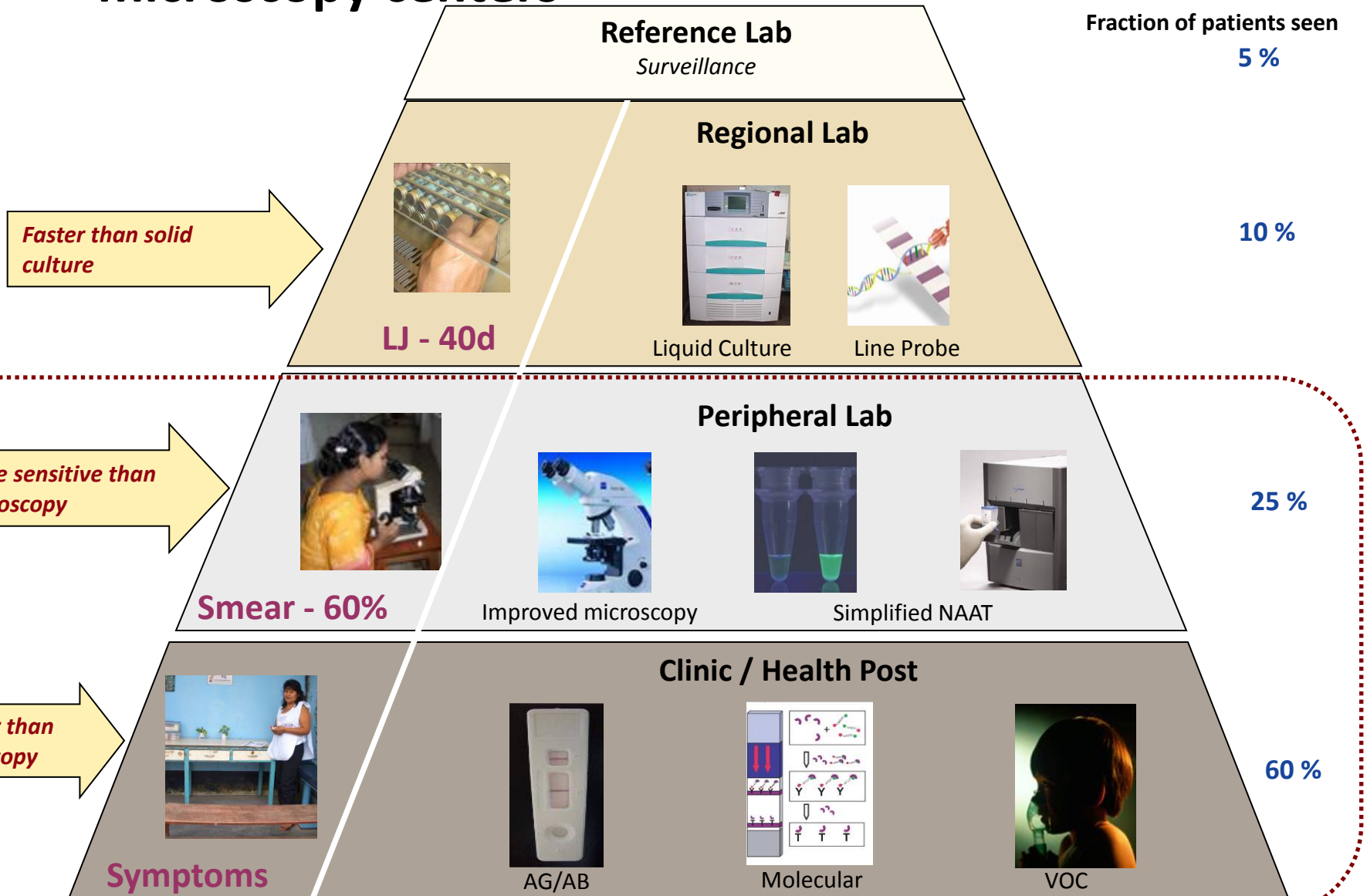
Rifampin-resistant TB contains 1-2 *rpoB* mutations

(95% sensitivity; Five-color PCR performed in a single well.)



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

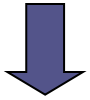
Simple & safe NAAT with culture-level accuracy at microscopy centers



Conventional NAAT

Extraction and purification DNA/RNA

Amplification



Detection

Target

Extraction and purification DNA/RNA

&

Amplification

&

Multiplex detection

(automated)

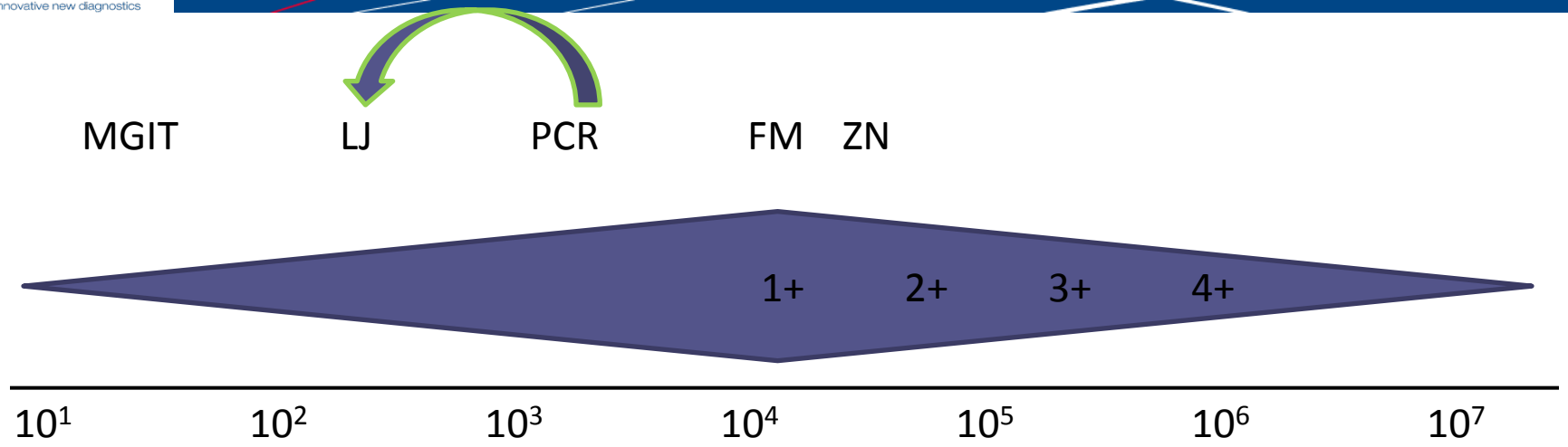
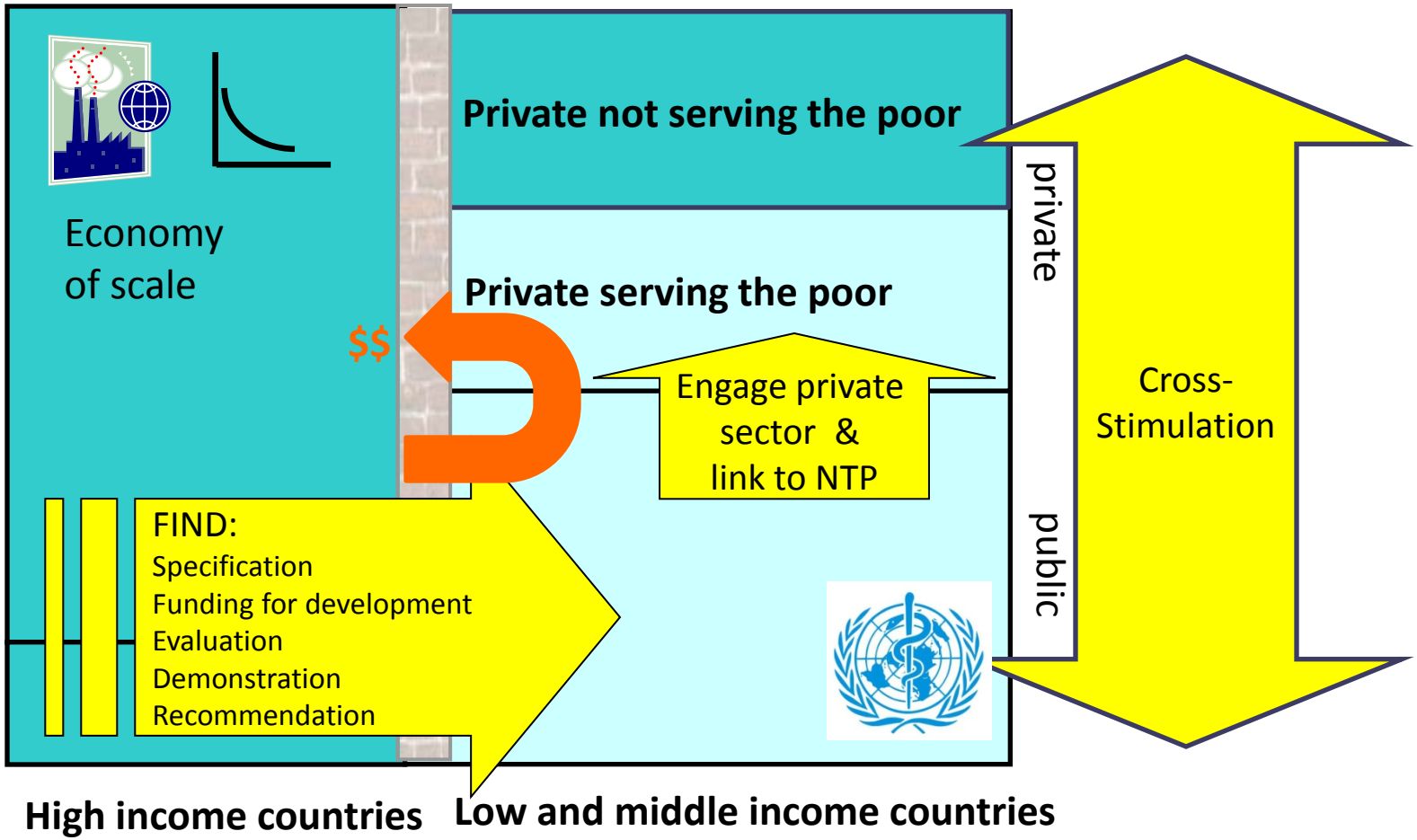


Table 1: Pooled values (95% CI) of sensitivity and specificity of five commercial NAATs for pulmonary TB in 60 published studies (Greco, Girardi et al. 2006)

Test	AFB+		AFB-	
	Sensitivity	Specificity	Sensitivity	Specificity
Amplicor (PCR)	96 (94-97)	83 (80-86)	61 (57-65)	97 (96.8-97.4)
Cobas Amplicor (PCR)	96 (95-97)	74 (68-8)	64 (59-69)	99 (99.2-99.4)
BDP (SDA)	98 (96-99)	89 (84-93)	71 (66-76)	97 (96.4-97.4)
E-MTD (TMA)	97 (95-98)	96 (93-97)	76 (70-80)	97 (96.6-97.4)
LCx (LCR)	96 (94-98)	71 (64-78)	57 (50-64)	98 (97.8-98.5)

PCR: polymerase chain reaction; SDA: strand displacement amplification; TM: transcription mediated amplification; LCR: ligase chain reaction.

High tech development for low and middle income countries



What did it take?

- Development of the assay (integrated specimen processing; internal process control; molecular beacon technology, nested PCR)
- From 4 color to 6 color instrument (hardware and software)
- Development of new dyes (to avoid royalties)
- Reagents on board
- Reagents on Board Automated Line (Robal)

- R&D and PM team (8 FT; 6 PT)
- Instrumentation team (5 FT)
- Clinical Team (2 FT; plus study teams at sites (10 FT/site))
- 20 Mio
- 2500 banked samples & 1000 fresh samples during development; 9000 patients enrolled after design lock

WHO Review & Policy Development for Xpert: Setting a precedent

- Process
- Speed
- Level of involvement

The evidence base for WHO review

1. Multi-centre clinical evaluation studies	➢ 1,730 subjects in five evaluation sites (four countries)
2. Multi-centre demonstration studies	➢ 6,648 subjects in nine evaluation sites (six countries)
3. Single-centre evaluation studies	➢ 4,575 subjects in 12 studies (nine countries)

Literature overview - pTB

	Citation	Population	Sample type	Sensitivity	Sensitivity in S-C+	Specificity	RIF Sensitivity	RIF Specificity	Comments
Pulmonary samples	Helb et al , JCM, Jan 2010	Vietnam, Uganda	Direct, Frozen	89% (130/146)	72% (38/53)	100% (45/45)	100% (9/9)	98% (54/55) w sequencing 100%	NA
	Boehme et al, NEJM, Sep 2010	SA, Peru, Azerbaijan, India	Direct & Pellet, Fresh	92% (675/732)	72% (124/171)	99% (604/609)	98% (200/205)	98% (505/515)	NA
	Armand et al, JCM, Mar 2011	France	Pellet, Frozen	NA	57%	100%	75% (3/4)	100%	Some EPTB IS6110
	Moure et al, JCM, Mar 2011	Spain	Pellet, Frozen	NA	75% (64/85)	100% (40/40)	100% (6/6)	100% (58/58)	
	Malbruny et al, IUTLD, Mar 2011	France	Pellet, Fresh	100% (17/17)	NA	100% (74/74)	NA	NA	Some EpTB
	Marlowe et al, JCM, Apr 2011	US	Pellet, Partially frozen	89% (116/130)	72% (31/43)	100% (82/82)	NA	97% (113/116)* no sequencing	MOTT
	Theron et al, AJRCCM, Apr 2011	South Africa (SA)	Pellet, Frozen	87% (101/116)	55% (12/22)	94% (320/339)	NA	99% (151/152)	Most Xp + / cul - = clin TB
	Boehme et al, Lancet, Apr 2011	SA, Uganda, Peru, Azerbaijan, India, Philipp	Direct, Fresh	90% (933/1033)	77% (296/385)	99% (2846/2876)	94% (236/250)	98% (796/810)	NA
	Rachow et al, PLOS One, June 2011	Tanzania	Direct, Frozen	88.4 (61/69)	61.1 (11/18)	99.0 (81/82)	NA	100% (69/69)	HIV pos MOTT

Multi-center evaluation study

- Boehme CC et al. The New England Journal of Medicine. 2010 Sep 9;363(11):1005-15.
- July 2008 – March 2009
- 5 reference laboratories with high quality gold standard

	UPCH
HIV	2%
TB (C+)	61%
MDR TB	7%

	STI
HIV	5%
TB (C+)	42%
MDR TB	31%

	Hinduja
HIV	5%
TB (C+)	60%
MDR TB	67%

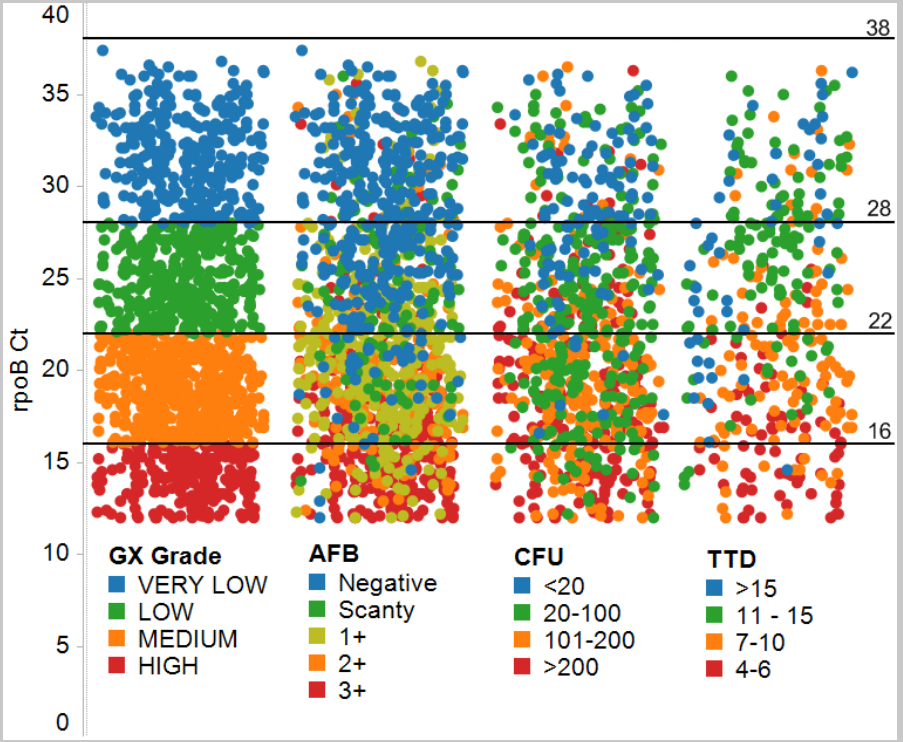
	UCT	SAMRC
HIV	77%	72%
TB (C+)	39%	13%
MDR TB	10%	9%

Evaluation Study Results

- **1730** TB/MDR suspected patients enrolled in Azerbaijan, India, Peru, South Africa
- A single, direct Xpert detected **92.2% of all C+ patients.**
- **Sensitivity in S-C+ patients was 72.5%** and **increased to 90.2%** when three samples were tested. **Specificity was 99%.**
- A single, direct Xpert identified a greater proportion of culture-positive patients than did a single LJ culture.
- Xpert MTB/RIF detected rifampicin resistance with 99.1% sensitivity and excluded resistance with 100% specificity.

Other take-away messages from evaluation study

- Performance from NaOH-treated pellet – equivalent to raw sputum
- Moderate sensitivity gain in S-C+ with 2nd (+13%) & 3rd test (+5%)
- Low indeterminate rate



Correlation of semi-quantitative results

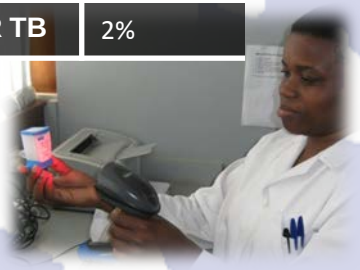
Multi-center implementation studies

- Boehme CC et al. **Lancet**. 2011 Apr 30;377(9776):1495-505.
- **9** settings of intended use in **6 countries**
  - (Sub)-District (3), microscopy centers (3), MDR screening / ER (3)
- **Diverse** laboratory conditions (temp, space, staff background)
- **6648** TB or MDR-TB suspected patients screened from diverse populations

Lima	Peru
HIV	3%
TB (C+)	17%
MDR TB	8%

Kampala	Uganda
HIV	80%
TB (C+)	42%
MDR TB	2%

Baku	Azerbaijan
HIV	6%
TB (C+)	47%
MDR TB	22%



Vellore	India
HIV	<1%
TB (C+)	10%
MDR TB	7%

Cape Town	South Africa
HIV	77% (K), 30% (P)
TB (C+)	26%
MDR TB	4%

Manila	Philippines
HIV	<1%
TB (C+)	20%
MDR TB	54%

Single, direct Xpert in routine settings: Performance similar to solid culture

	Sensitivity All C+	Sensitivity S+C+	Sensitivity S-C+	Specificity Non-TB
Lima, Peru	96.6%	99.3%	88.1%	99.6%
Baku, Azerbaijan	88.6%	97.8%	74.7%	98.7%
Cape Town, SA	86.3%	100.0%	79.1%	99.7%
Kampala, Uganda	83.4%	97.8%	57.7%	100.0%
Vellore, India	100.0%	100.0%	100.0%	97.7%
Manila, Philippines	91.9%	96.2%	56.3%	97.9%
TOTAL	**90.3% (933/1033)**	**98.3% (637/648)**	**76.9% (296/385)**	**99.0% (2846/2876)**

- Routine smear microscopy (2-3 smears per patient) had a sensitivity of 61%.
- Xpert identified at least as many patients as a single LJ culture (89.8%; CI 87- 92%).
- High positive and negative predictive values in all settings
- 2.5% indeterminate rate; 0.3% after repetition. Culture indet. rate 4.7%.

Sensitivity & specificity of Xpert and smear stratified by HIV status

	HIV pos	HIV neg	HIV neg / unknown	P-Value
Sensitivity in C+				
Smear microscopy	**44.6%** (86/193) [37.7% - 51.6%]	**68.6%** (234/341) [63.5% - 73.3%]	**72.3%** (613/848) [69.2% - 75.2%]	<.0001
Xpert MTB/RIF	**82.4%** (173/210) [76.7% - 86.9%]	**90.7%** (304/335) [87.2% - 93.4%]	**92.3%** (760/823) [90.3% - 94.0%]	0.0849
Sensitivity in S+C+ Xpert MTB/RIF	**97.7%** (84/86) [91.9% - 99.4%]	**99.0%** (204/206) [96.5% - 99.7%]	**98.4%** (553/562) [97.0% - 99.2%]	0.2167
Sensitivity in S-C+ Xpert MTB/RIF	**71.8%** (89/124) [63.3% - 78.9%]	**77.5%** (100/129) [69.6% - 83.9%]	**79.3%** (207/261) [74.0% - 83.8%]	0.8976
Specificity in Non-TB				
Smear microscopy	**100.0%** (660/660)	**99.4%** (1054/1060)	**99.4%** (3040/3058)	0.2545
Xpert MTB/RIF	**99.2%** (389/392)	**99.3%** (748/753)	**98.9%** (2457/2484)	0.2246

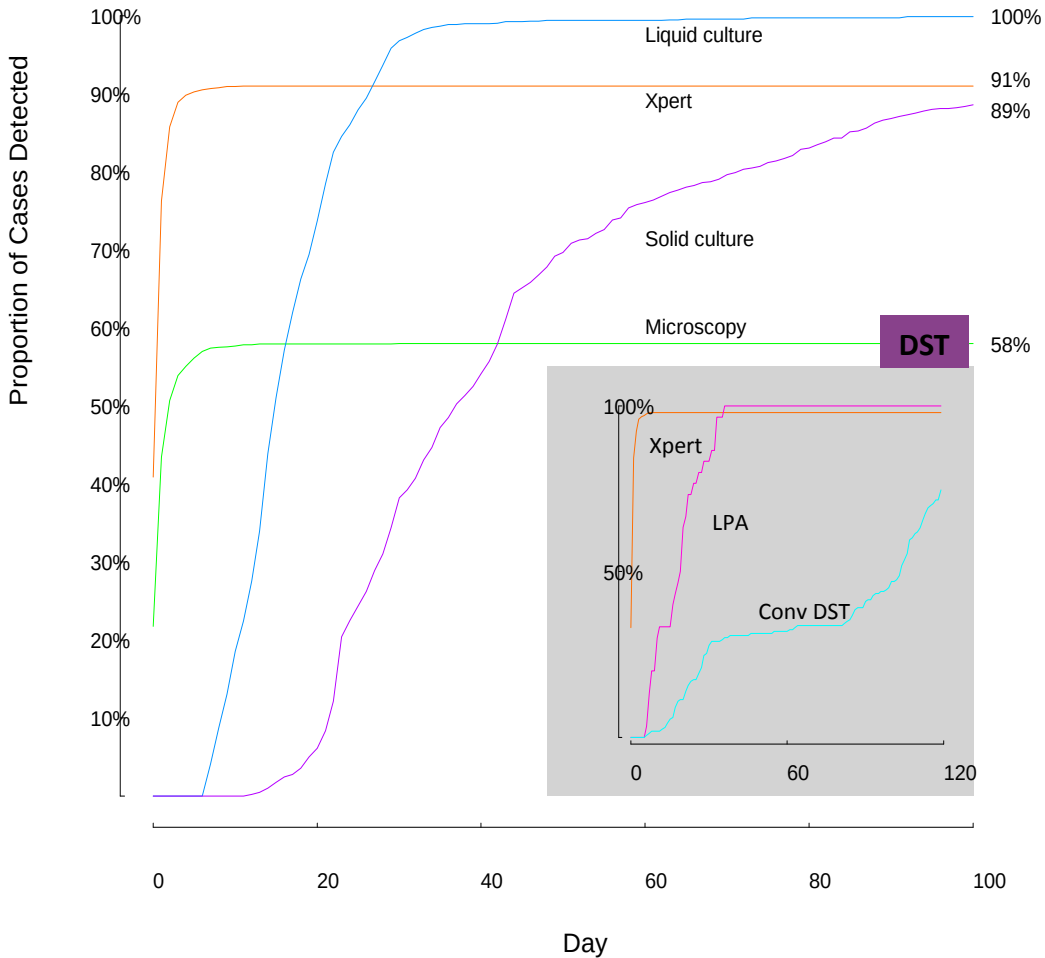
Sensitivity and specificity of Rif resistance detection

	Sensitivity in RIF-resistant	Specificity in RIF-sensitive
Total		
%	**94.4%**	**98.3%**
(Correct / total)	**(236/250)**	**(796/810)**
[CI]	**[90.8% - 96.6%]**	**[97.1% - 99.0%]**

- RIF resistance was a good marker for MDR at all sites.
- Positive predictive value suboptimal in low MDR-prevalence settings
- Confirmation of resistance by culture at present recommended for low MDR prevalence settings
- Optimization ongoing

Case detection rate increased by >30%
0.7 d mean time to detection from sputum collection

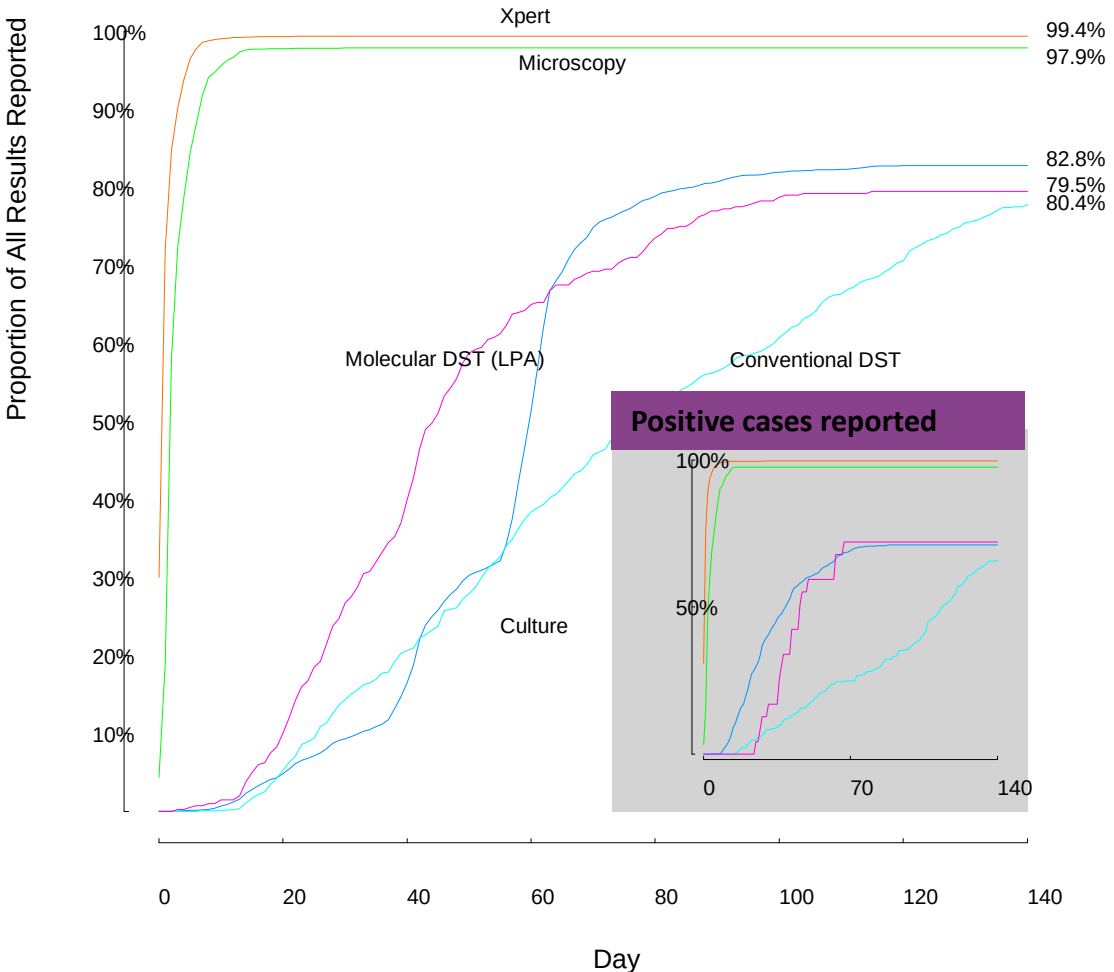
Proportion of cases detected over time, by test method;
Maximum proportion detected



- Xpert → Median 0d; IQR 0-1
- Smear → Median 1d; IQR 0-1
- LC→ Median 16d; IQR 13-21
- SC → Median 30d; IQR 23-43
- LPA → Median 20d; IQR 10-26
- DST → Median 106d; IQR 30-124

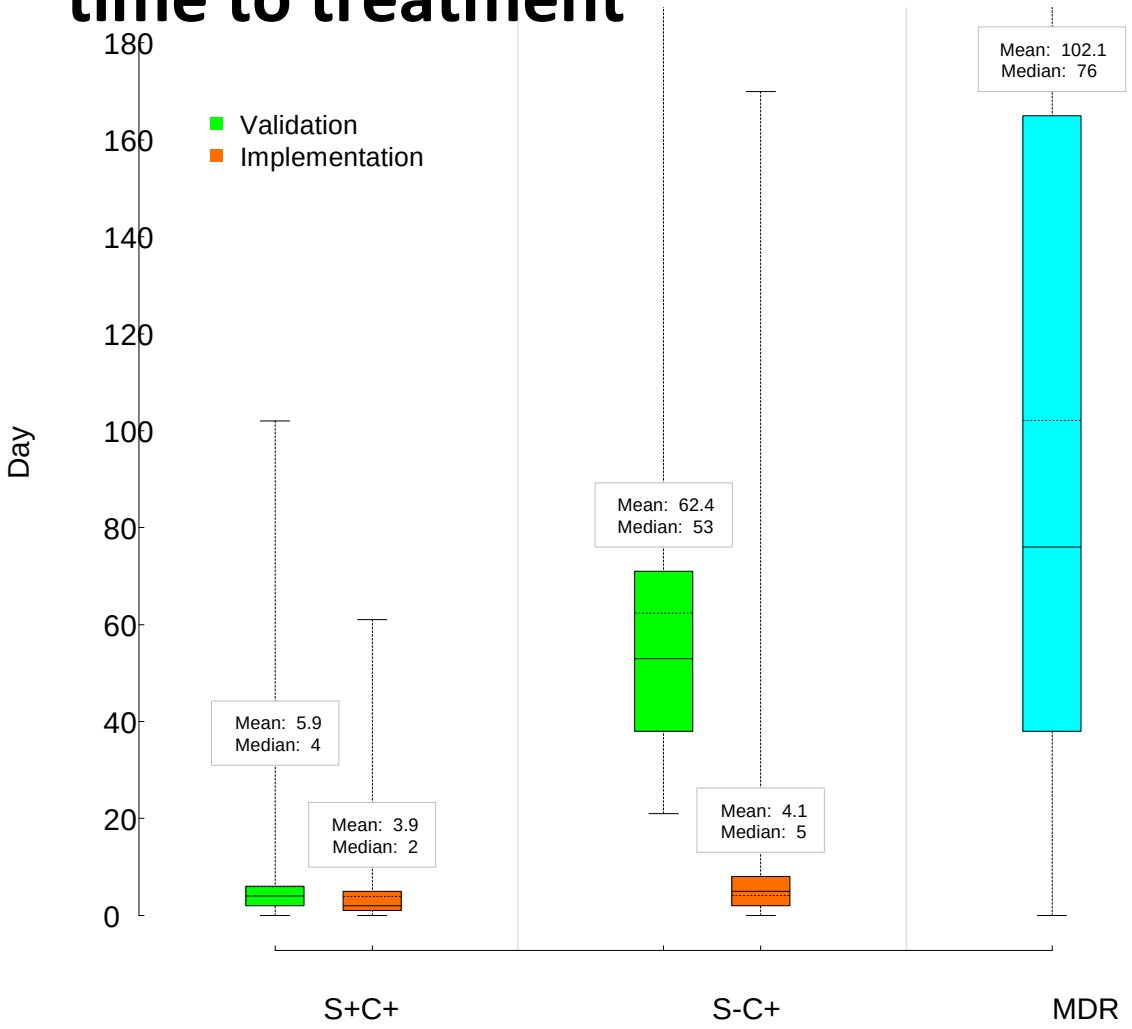
Almost all Xpert and microscopy results reported; 20% NOT reported for other methods,

Proportion of results reported over time, by test method;
Maximum proportion reported



Definition:
30 d after result (Xp, smear, culture);
150 days after collection (DST)

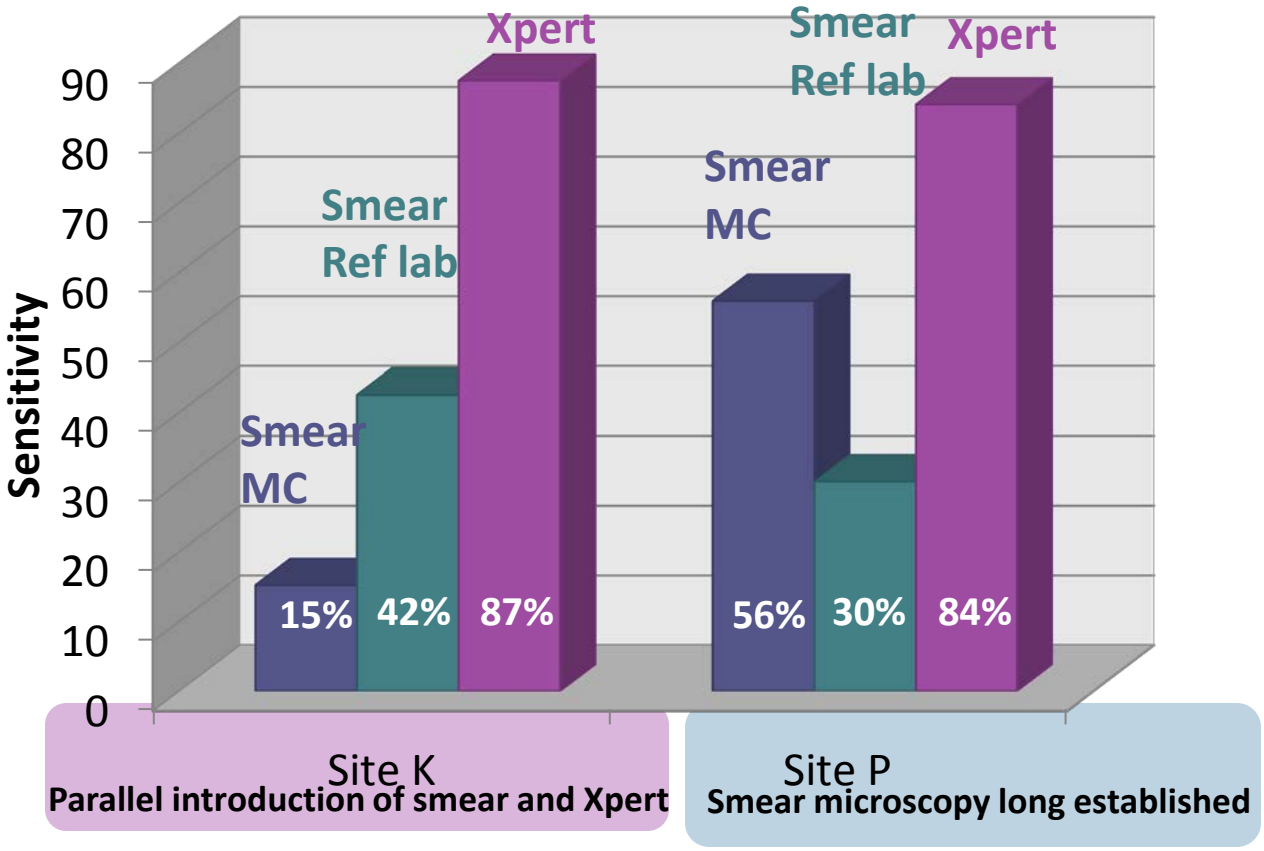
Xpert implementation translates into shortened time to treatment



- Validation → Tx based on routine tests.
- Implementation → Tx based on Xpert.
- MDR → conventional DST (or LPA).

- Drop out rate: 39.3% before to 14.7% after implementation
- Almost 10% of patients did not come back to provide a 2nd or 3rd specimen.

Xpert performance seems less dependent on user skills, motivation or daily workload than smear



- Example: South Africa, Xp training duration 2 d
- Xpert performance identical at both sites

Operational performance and robustness

Variable	Performance / outcome
Indeterminate rate	2.5% and 0.3% after repetition. Culture indeterminate rate 4.7%.
DNA contamination events	None observed (swabs, neg controls)
Training needs	2 days for non-experienced lab techs.
User appraisal	Less difficult than microscopy; user friendly; user-independent read-out.




Considerations for implementation

Variable	Performance / outcome
Preventive maintenance	Annual calibration (logistics and costs)
Storage and operating temperature	2-30°C; cartridges require substantial storage space
Electrical supply and back-up power	power outage reported; uninterruptable power supply with UPS (400 VA) for 20 min. Serial car batteries tested.
Biosafety requirements	Same as smear microscopy*.
Waste management	As for sputum containers; additional waste volume compared to smear microscopy.

*Banada PP., et al. Containment of bioaerosol infection risk by the Xpert MTB/RIF assay and its applicability to point-of-care settings. J Clin Microbiol 2010; 48 (10): 3551-7




Aerosol Viability During Manual Steps

Mean cfu/m^3 air detected over 3 experiments

5 X 10^8 cfu BCG spiked into sputum.	Anderson impactor	BioSampler
SR added and sample immediately pipetted in and out of three Xpert TB cartridge over 15 min time period (equivalent to loading >30 cartridges)	6	67
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

GRADE summary

Xpert MTB/RIF	Absolute difference per 1000 persons				Quality of evidence
Pre-test prevalence 10%	TP	TN	FP	FN	
TB detection	92	891	9	8	
R detection	95	891	9	5	
Overall quality of evidence					Moderate
Desirable vs undesirable effects					Highly favourable
Patient values and preferences					No data
Cost and requirements					Moderate cost
Added value to conventional methods					Significant

Recommendations

1. Xpert MTB/RIF should be used as the initial diagnostic test in individuals suspected of having MDR-TB or HIV-associated TB (strong recommendation)

2. Xpert MTB/RIF may be used as a follow-on test to microscopy where MDR and/or HIV is of lesser concern, especially in smear-negative specimens (conditional recommendation, recognising major resource implications)

Remarks

- **Recommendations also apply to children**, based on generalisation of data from adults and acknowledging the limitations of microbiological diagnosis of TB (including MDR-TB) in children;

- **Access to conventional microscopy, culture and DST is still needed** for monitoring of therapy, for recovering isolates for drug susceptibility testing other than rifampicin (including second-line anti-TB drugs); and for prevalence surveys and/ or surveillance;

- **Recommendations apply to Xpert MTB/RIF use in sputum specimens** (including pellets from decontaminated specimens), as data on the utility of Xpert MTB/RIF in extra-pulmonary specimens are still limited;

- **Recommendations support the use of one sputum specimen** for diagnostic testing, acknowledging that multiple specimens increase the sensitivity of Xpert MTB/RIF but have major resource implications.

Changing TB control dynamics

- Changes in diagnostic and screening algorithms
- Increased capacity needed to treat TB and MDR-TB
- Need to re-define TB case and outcome definitions
- Monitoring of impact on case detection and cure
- Resource awareness by donors/funders
- Use in non-traditional TB settings (HIV, private sector)
- Innovative new partnerships needed

Global Consultation: 30 Nov - 2 Dec 2010

The Roadmap: Collecting evidence for scale up

Roadmap for rolling out Xpert MTB/RIF
for rapid diagnosis of TB and MDR-TB

6 December 2010

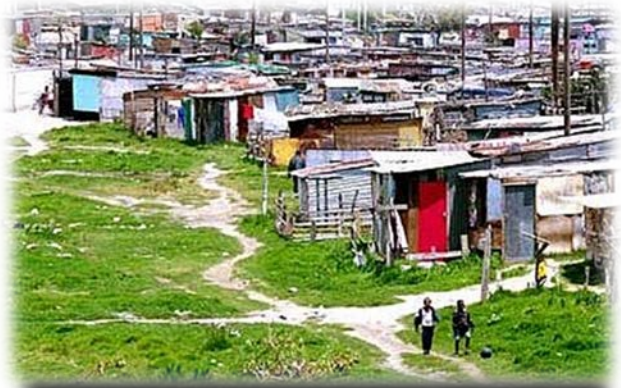
WHO THE STOP TB DEPARTMENT

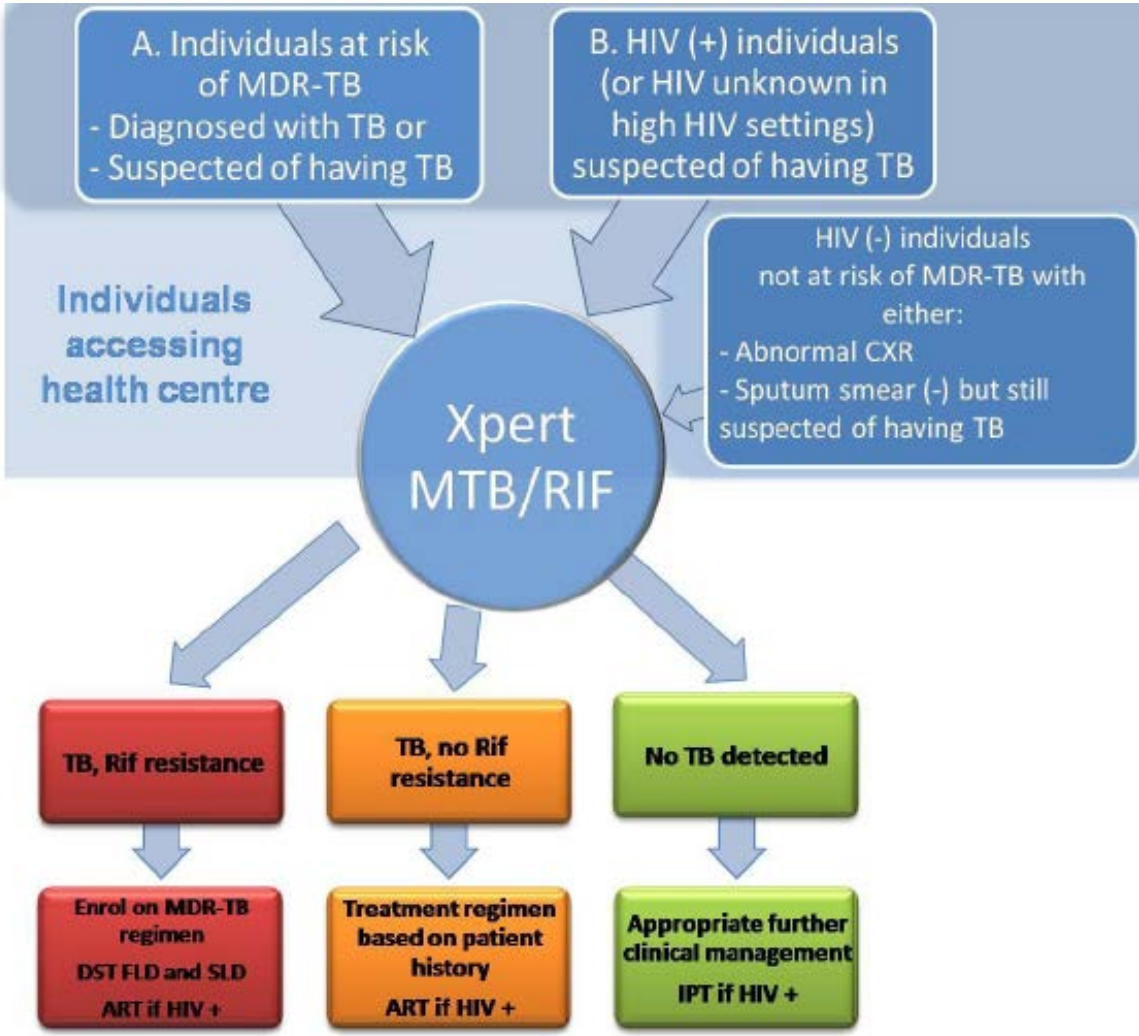
World Health Organization

Operational research agenda:

1. Cost and cost-effectiveness of the algorithms in different epidemiological and risk settings
2. Additional yield, sensitivity, specificity, and predictive values
3. Impact on treatment and patient management
4. Impact on access to care by different socio-economic groups
5. Performance of Xpert MTB/RIF in remote and peripheral settings
6. Performance of Xpert MTB/TB in extra-pulmonary and paediatric TB
7. Models to engage the private sector and strengthen linkages with national TB programmes

WHO recommendation on use of Xpert MTB/RIF





A phased roll out

World Health Organization

Rapid Implementation of the Xpert MTB/RIF diagnostic test

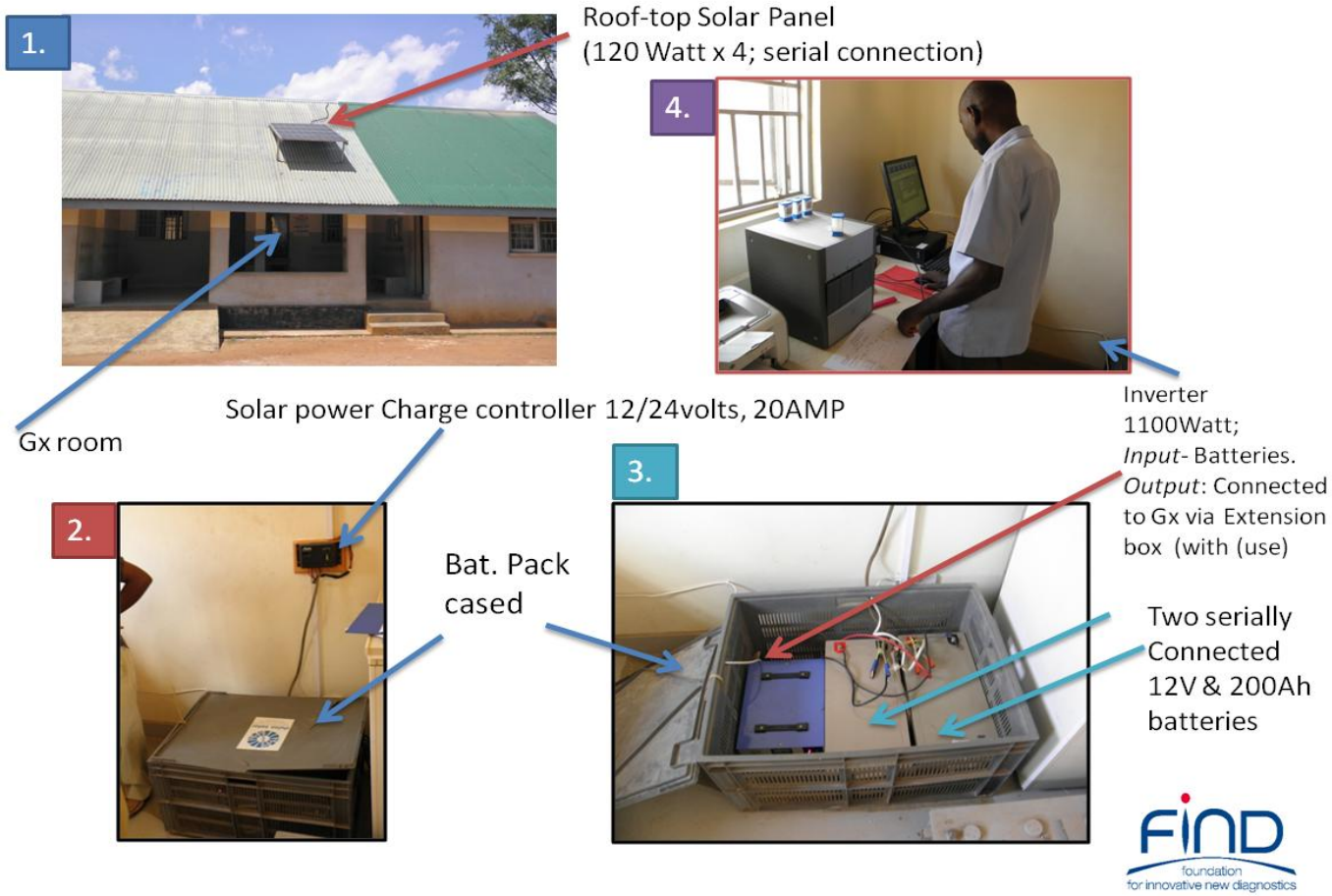
Technical and operational 'How-to' Practical considerations

MOH, SA
World TB Day 2011

Overcoming hurdles to rollout:

Power supply, Operating and storage temperature, Annual calibration

GeneXpert with Solar power, Luwero HC IV, Uganda



Xpert MTB/RIF Research Mapping Project

- Creation of an online tool mapping ongoing research activities related to Xpert MTB/RIF
- Basic information on the research project:
  - Research organization and collaborators
  - Objectives
  - Target population
- The tool will act as comprehensive platform for researchers, policy makers and implementers to link, communicate, collaborate

Rifampicin Resistance

ADF 1
- **Evaluation studies** (99% RIF sensitivity & 100% specificity)
- **Manufacturing scale up** (drop to 96% RIF specificity)

ADF 2
- **May 2010**
- **Demonstration studies** (95% RIF sensitivity & 98% specificity)

EGM / STAG

ADF 3
- **Oct 2010**
- **RIF specificity increase**

ADF 4 Cartridge
- **Q2 –Q3 2011**
- **RIF specificity & sensitivity increase**

ND
- Further refinements as part of post-marketing surveillance

Literature overview - ExpTB

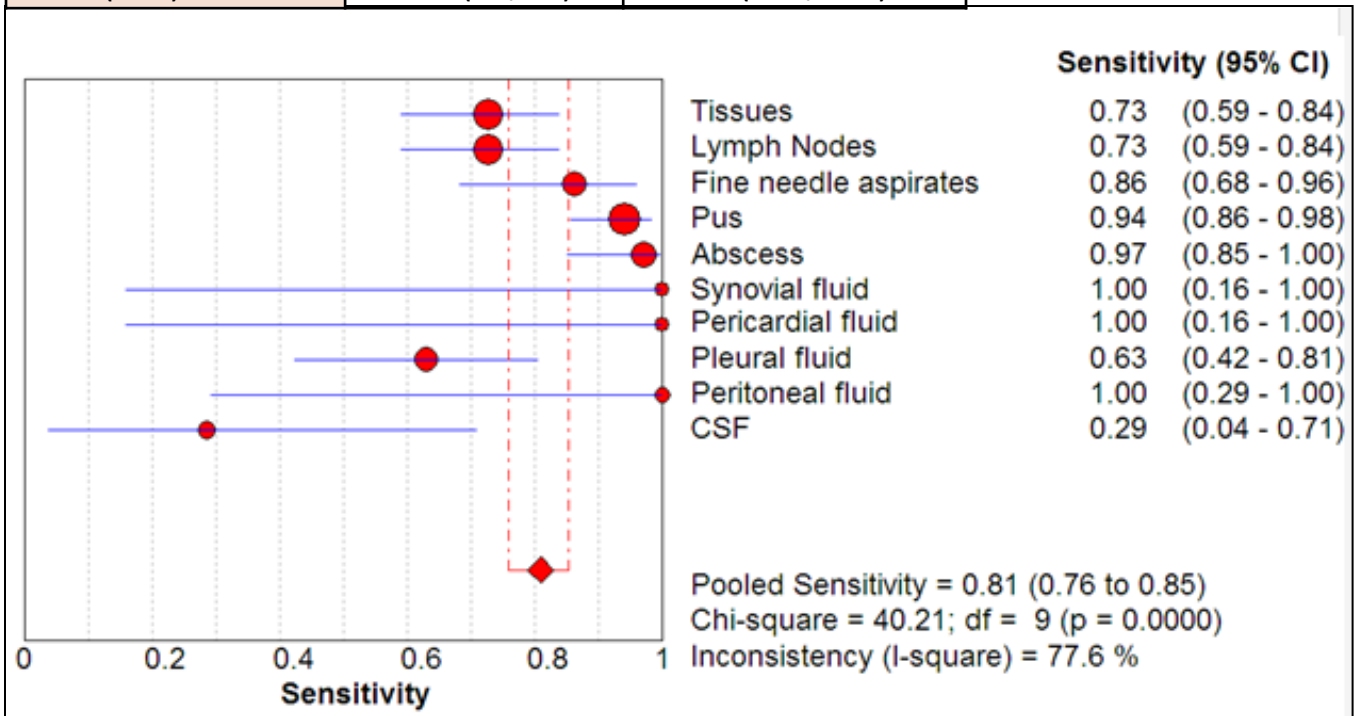
	Citation	Population	Sample type	Sensitivity	Sensitivity in S-C+	Specificity	RIF Sensitivity	RIF Specificity	Comments
EPTB	Hillemann et al, JCM, Jan 2011	Germany	Direct, Fresh	77.3% (34/44)	NA	98.2% (411/418)	NA	NA	NA
	Vadwai et al, JCM, May 2011	India	Direct, Fresh	80% (202/253)	NA	100% (231/232)	NA	NA	NA
	Causse et al, JCM, Jun 2011	Spain	Pellet, fresh (CSF and PL fluid direct)	95% (39/41)	95% (36/38)	100% (299/299)	NA	NA	Roche Cobas

Using Xpert for extra-pulmonary TB samples

(Hinduja Hospital, India & NRL, Germany)

Specimen type	Xp sensitivity	Xp specificity
Tissue (245)	69.0% (20/29)	98.4% (189/192)
CSF (19)	NA	100.0% (19/19)
Gastric fluid (30)	87.5% (7/8)	100.0% (19/19)
Pleural fluid (113)	NA	98.1% (103/105)
Stool (23)	100% (2/2)	91.7% (11/12)
Urine (91)	100% (5/5)	98.6% (70/71)
Total (521)	77.3% (34/44)	98.2% (411/418)

- 521 specimens
- Culture as a reference standard
- Hillemann, JCM, Jan 2011



- 453 specimens
- Composite reference standard: Sm, LJ, histology/cytology, ADA for CSF & fluids, CT/MRT for CSF, FU at 3 months

Screening for drug resistance to other drugs?

Drug	Gene Locus	Gene function	Percent of Resistance
Fluorochinolones	*gyr*A	DNA-Gyrase A	app.80-90%
Amikacin, Capreomycin, Kanamycin	*rrs* *tlyA*	16S rRNA Methylase	app. 80 %
Ethambutol	*embB*	Arabinosyl-tranferase	50 – 60%

The history of NAAT for TB

- Obvious advantages of NAAT recognized for slow-growing pathogen
- Low market penetration

1990s – the boom years.
Multiple commercial TB prototypes developed

QβR
Cycling probe
TMA
NASBA
LCR
PCR
SDA
bDNA

2000s – the lean years.
Very low uptake of TB NAAT outside Japan

Field narrowed:
SDA (BD)
TMA (GenProbe)
PCR (Roche)

2010s – the second coming.
New simpler testing platforms DEC biz opportunity seen

PCR-LPA*
rtPCR-beacon*
HDA
CPA
LAMP
RPA
READ PCR
LATE PCR

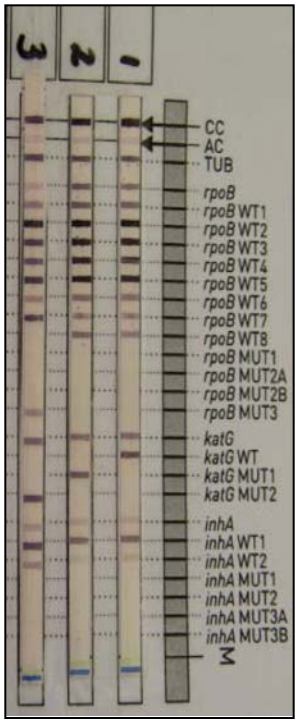
*WHO endorsed

Decentralization of molecular diagnostics

Platform expansion; Maximizing uptake (operational; costs)

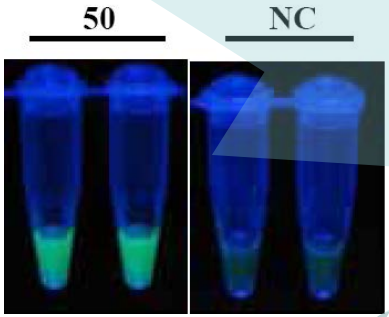
Less complexity, more robustness

1st generation MDR

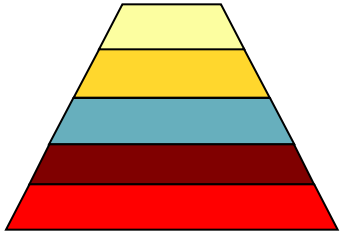


2nd generation automated MDR

1st generation manual detection

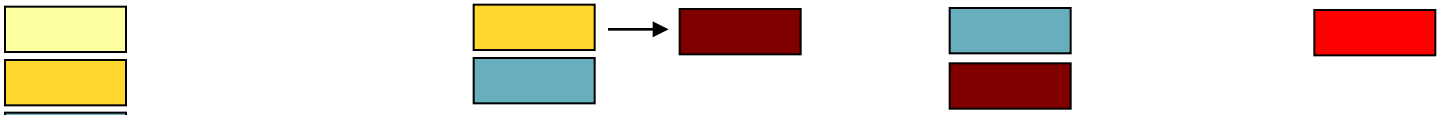


2nd generation manual detection



LPA | Xpert | LAMP | POC test

2008 | 2010 | 2011 | 2015



Thank you to all partners and patients who were part of these efforts!