



Contemporary diagnostics: moving towards integrated technology platforms

Dr. Giorgio Roscigno
FIND CEO

GLI Conference
Les Pensières
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Partnering for better diagnosis for all



World Health
Organization

REGIONAL OFFICE FOR

Africa

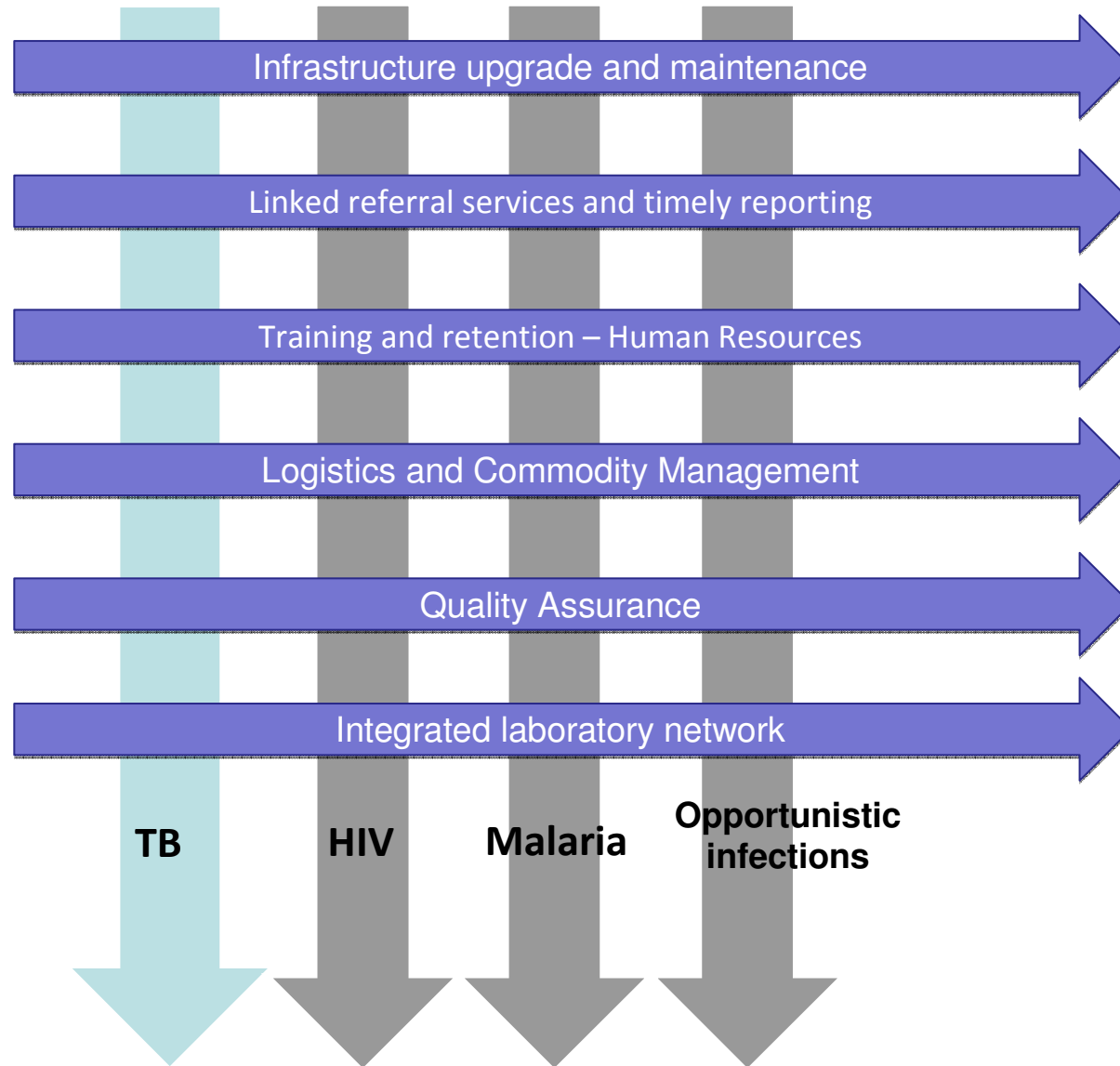
The Maputo Declaration



Maputo Declaration on Strengthening of Laboratory Systems

- Call on donors and implementing partners to ensure that in supporting laboratory strengthening that proper consideration is given to **fostering national ownership**.
- Call on donors and development partners to commit to **work collaboratively with each other and with coordination from the national governments to support strengthening of laboratory systems in order to create one unified, integrated national laboratory network**. These laboratory strengthening efforts should seek to build public private partnerships.

Integration of public health laboratory services

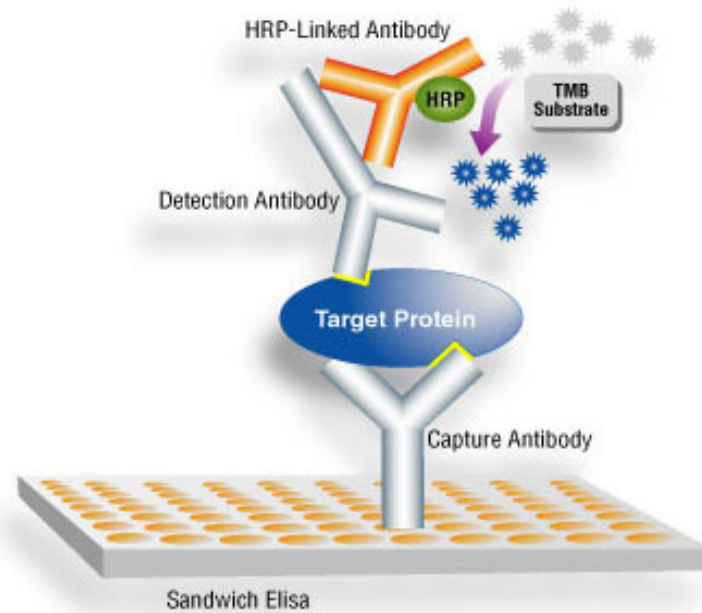
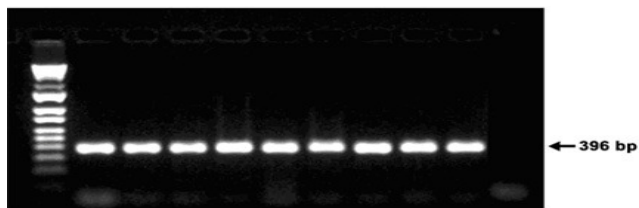


The nightmare scenario for service, maintenance and training...





FIND
foundation
for innovative new diagnostics







Fundamental
diagnostic: 1882

Evolution of TB diagnostics in the public sector



Fundamental
diagnostic: 2007



Fundamental
diagnostic: 1882

Evolution of TB diagnostics in the public sector



Fundamental
diagnostic: 2008



An extraordinary act of will





Complexity of conventional sputum decontamination in reference labs



1. Liquefaction

2. Sample decanted

3. Decontamination NaOH

4. Vortex

5. Phosphate Buffer



6. Centrifugation

7. Decant

8. Re-suspend

9. Inoculation

Simplicity of MDR-XDRTB COLOUR TEST for regional labs



Combined optimizations: single-step decontamination (Vasanthakuri et al 1987), microscopic observation of growth, direct susceptibility testing for MDRTB testing & XDRTB screening, selective culture media (Mitchison et al), colour indication of culture positivity

MDR-XDRTB Colour Test for Regional Labs

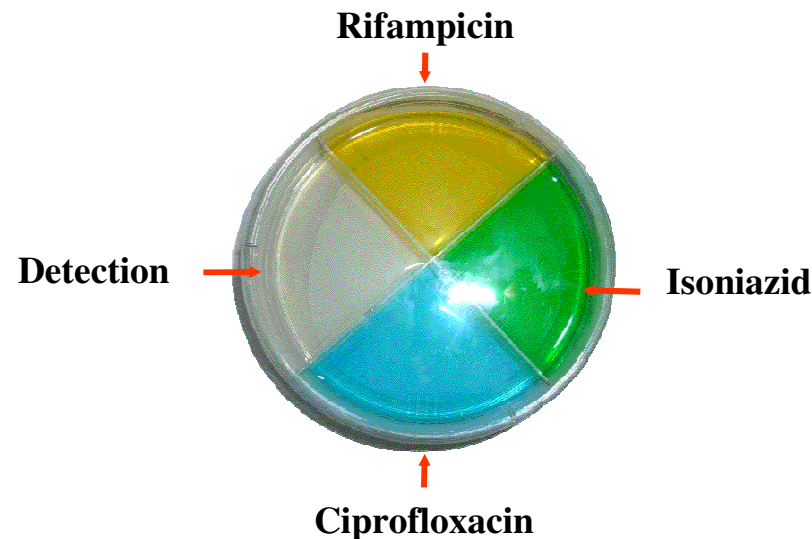
1

Liquefaction & decontamination in transport medium at room temperature



2

Direct application of 2 drops to selective thin layer agar for incubation in room air for MDRTB testing & XDRTB screening



3

Colour growth detection & microscopy confirmation of morphology



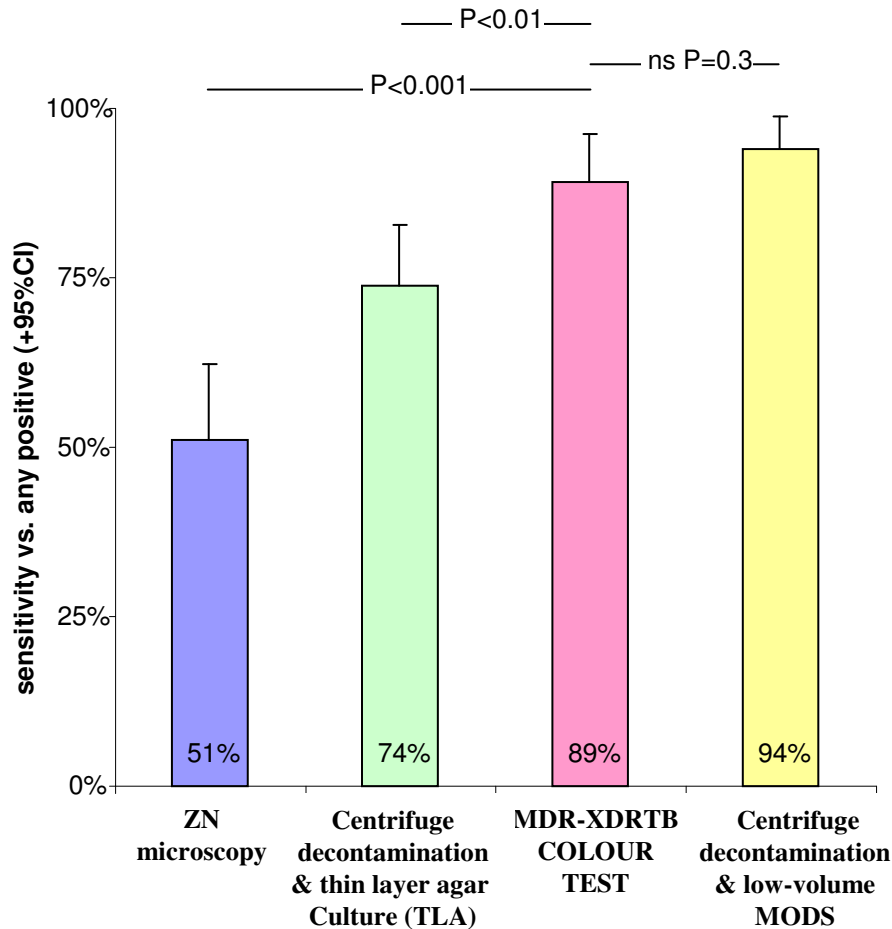
Biosafety similar to sputum microscopy because sputum is smeared directly onto the plate which is then permanently double-sealed until autoclaving



MDR-XDRTB Colour Test Performance (n=214)

Gold standard=culture positive in any test (n=84/214)

TB diagnostic sensitivity

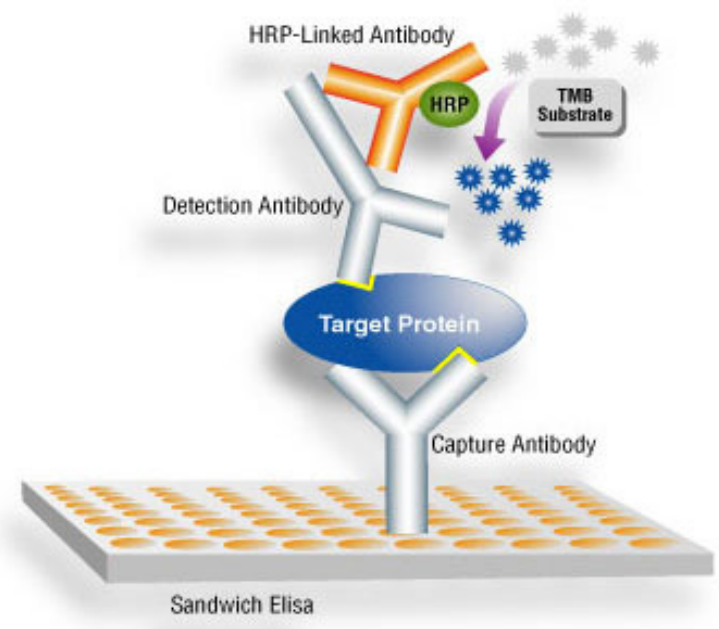


Concurrent Drug Susceptibility Testing

COLOUR TEST	Direct MODS		Total
	MDR	not-MDR	
MDR	9	3	12
not-MDR	1	68	69
Total	10	71	81

COLOUR TEST	Indirect TEMA		Total
	MDR	not-MDR	
MDR	8	4	12
not-MDR	1	51	52
Total	9	55	64

Colour test had 2% contamination (all fungal) & median time to positive result was 16 days



The urgent need for a POC test

WHY



- **4 M undiagnosed cases**

WHO Global TB Report 2008

- **Diagnostic delays fuel transmission & severity**

Liam, 1997, Int J Tub & Lung Dis

WHAT



- **Simple &**
- **Accurate &**
- **Robust &**
- **Rapid Test**
- **For qualitative TB case detection**
- **At the lowest level of health system: the health posts**

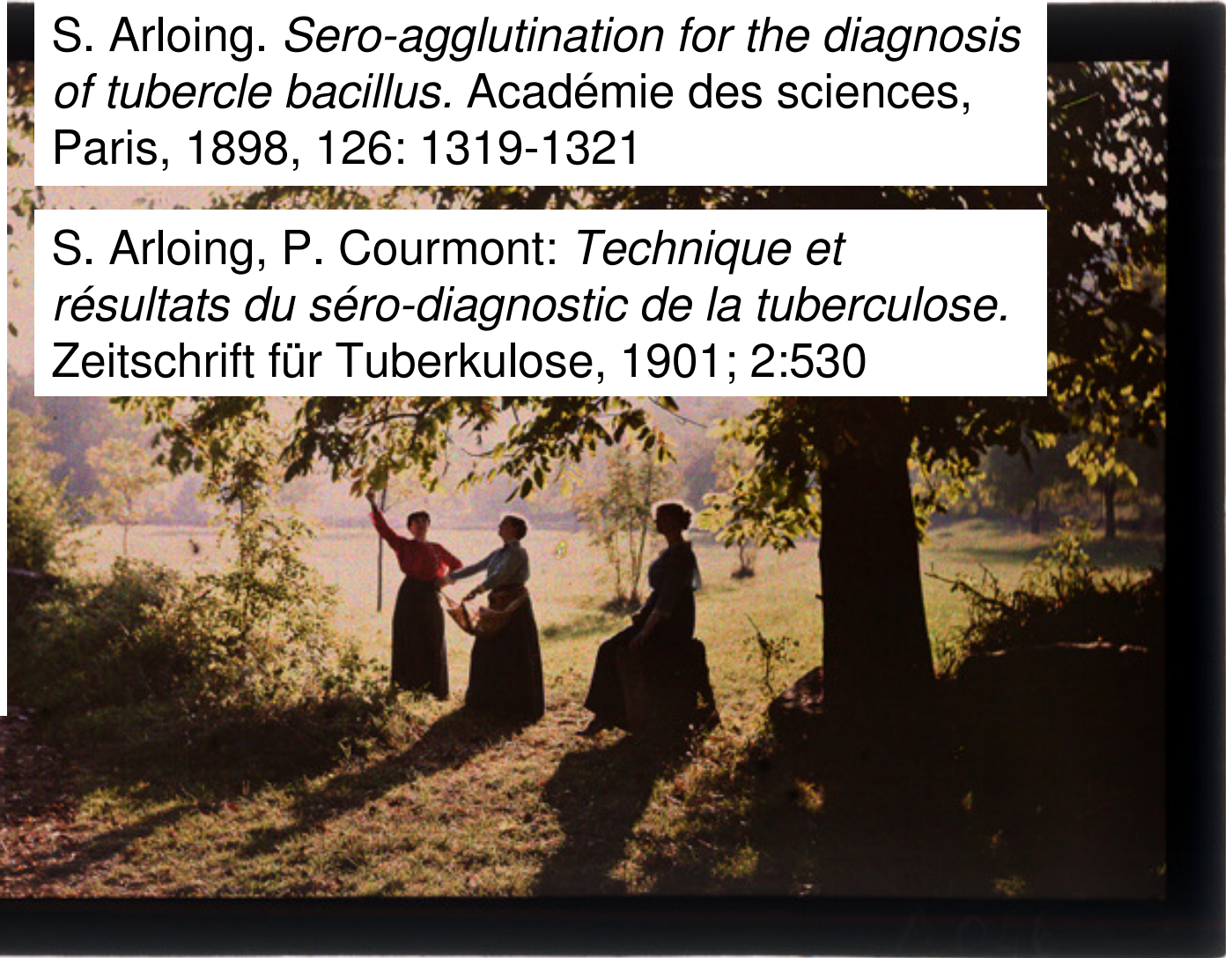
Serodiagnosis of TB





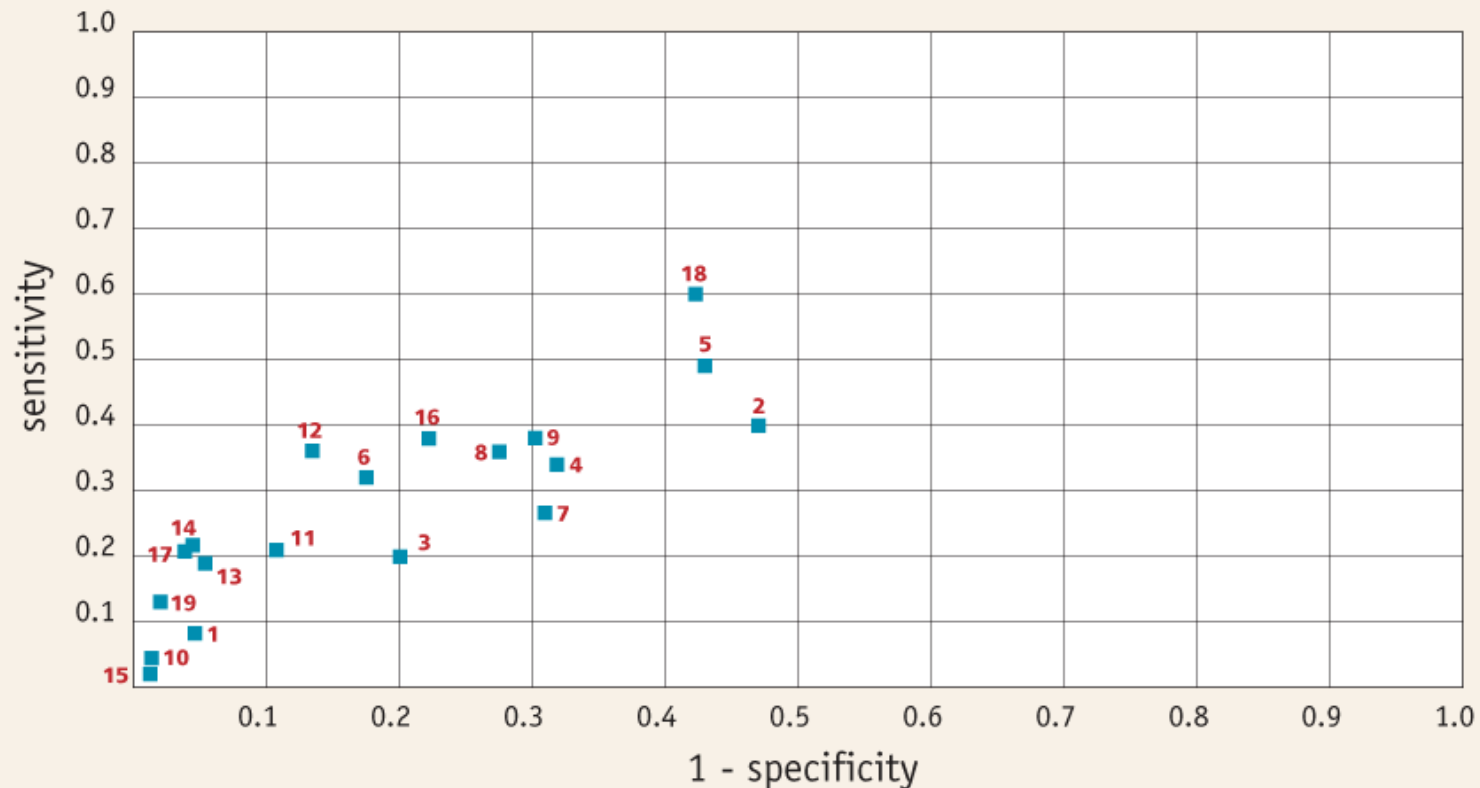
S. Arloing. *Sero-agglutination for the diagnosis of tubercle bacillus*. Académie des sciences, Paris, 1898, 126: 1319-1321

S. Arloing, P. Courmont: *Technique et résultats du séro-diagnostic de la tuberculose*. Zeitschrift für Tuberkulose, 1901; 2:530



Fernand Arloing, Femmes cueillant des fruits

Figure 4. ROC curve of commercial rapid tests for the diagnosis of pulmonary tuberculosis (all patients, n=355)



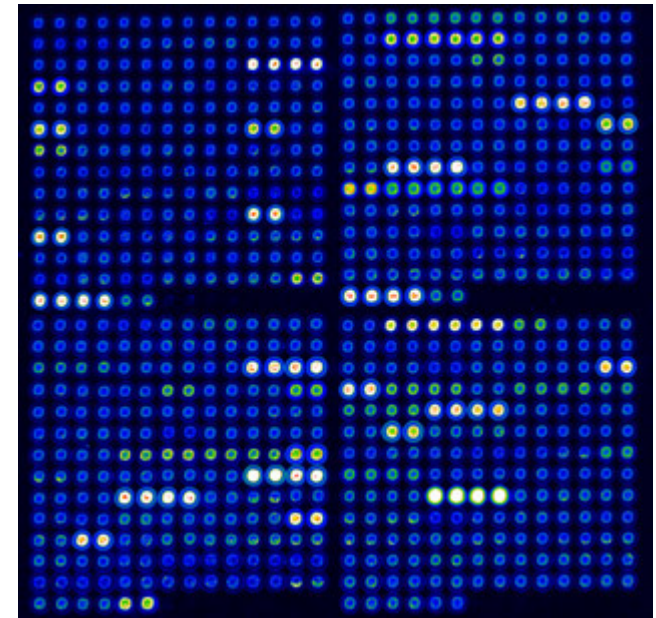
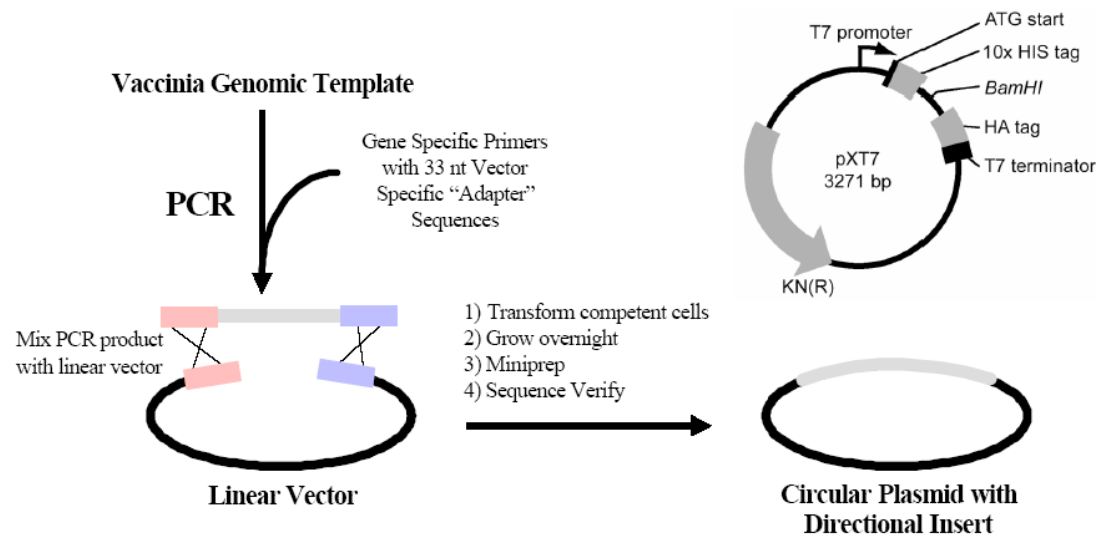
- 1.** ABP Diagnostics **2.** Advanced Diagnostics **3.** American Bionostica **4.** Ameritek USA **5.** Bio-Medical Products **6.** Chembio Diagnostic Systems **7.** CTK Biotech **8.** Hema Diagnostic Systems **9.** Laboratorios Silanes **10.** Millennium Biotechnology **11.** Minerva BiOTECH **12.** Mossman Associates **13.** Pacific Biotech **14.** Premier Medical Products **15.** Princeton Biomeditech **16.** Span Diagnostics **17.** Standard Diagnostics **18.** Unimed International **19.** VEDA.LAB

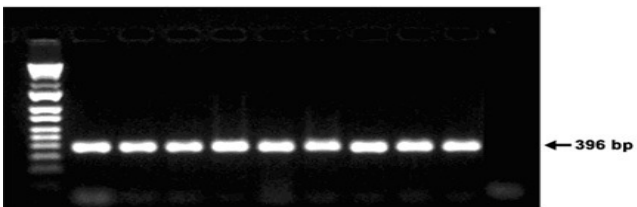


Sensitivity of selected antigens at >95% specificity level compared to healthy controls

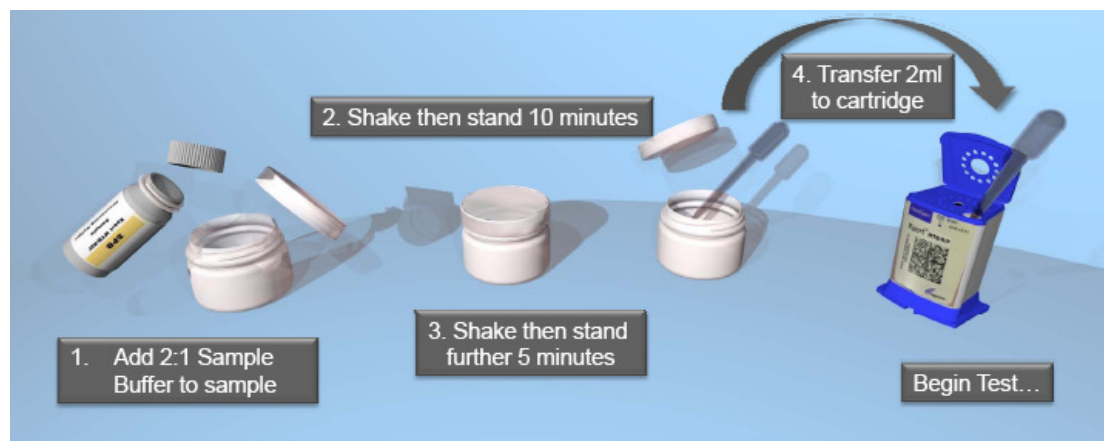
Antigen	Europe, HIV– (n=71)	Africa, HIV– (n=79)	Africa, HIV+ (n=77)
TB9.7	35%	79 %	91%
CFP10:ESAT6*	25%	64%	49%
TB10.2	21%	45%	48%
TB15.3	41%	75%	65%
TB16.3	55%	81%	88 %
TB 51	31%	76%	48%
TB51.7	57%	83%	78%
aCry:MPT83	26%	83%	58%
38 kDa	19%	29%	15%

Whole proteome screening of *M. tuberculosis* for diagnostic antigens





Integrated NAAT for TB/Rif: An update



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

Xpert MTB/Rif: FIND Evaluation studies



Rigorous performance evaluation at 5 sites (>1500 TB suspects)
 Included 2 sites with high HIV prevalence (80%) & 2 with high MDR TB prevalence (>30%)

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



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



Peru
UPCH



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

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



South Africa
UCT
SAMRC

Xpert MTB/Rif: FIND evaluation studies

		AFB-		AFB+			
		Culture Positive	Culture Negative	Culture Positive			
Combined	Xpert MTB/RIF	MTB Detected	70	3	275	PPV	99.1%
		MTB Not Detected	7	171	0	NPV	96.1%
		Sensitivity		Specificity			
		98.0%		98.3%			

Sensitivity for in S+/C+ = 100%, in S-/C+ = 91%

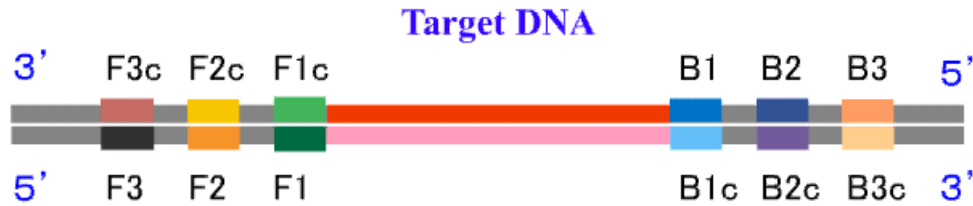
		DST RIF Resistant	DST Sensitivity				
		Combined	Xpert MTB/RIF			RIF Resistance Detected	58
	RIF Resistance Not Detected		2	280	NPV	99.3%	
		Sensitivity		Specificity			
		96.7%		98.6%			

High accuracy for Rif detection

Sequencing data for discrepant results suggest Xpert correct

Simple, manual NAAT

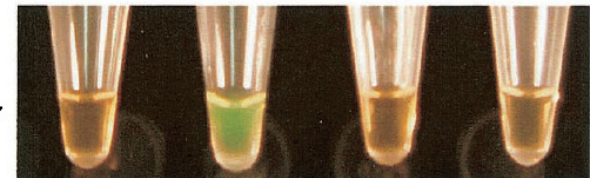
Loop-mediated Isothermal Amplification (LAMP)



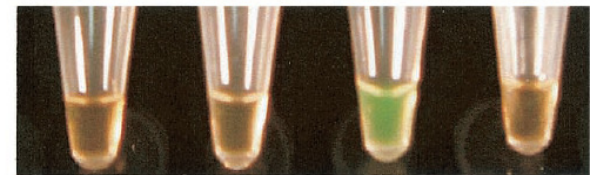
LAMP
w / MTB



LAMP
w / MAV



LAMP
w / MIN

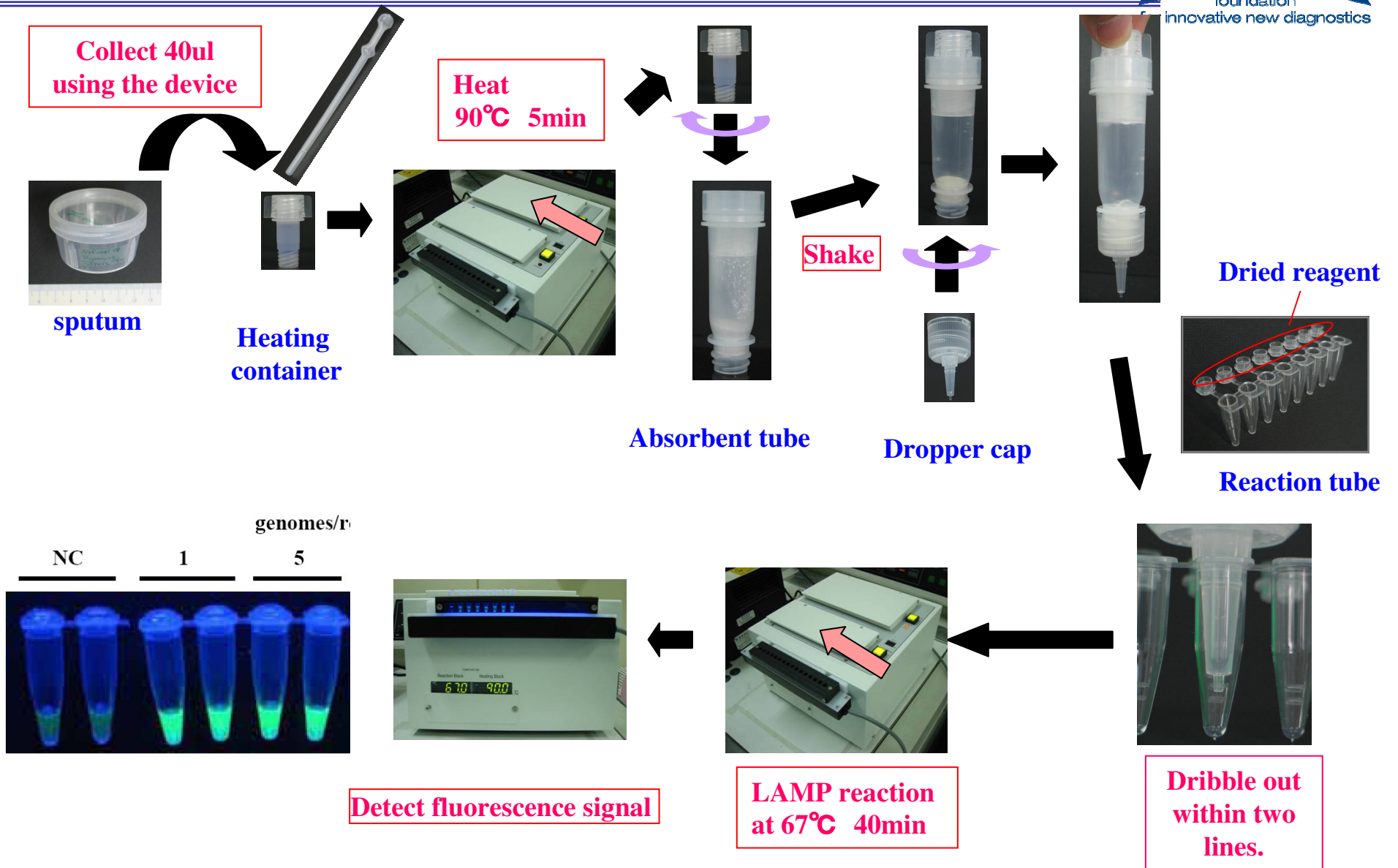


Ver.6.4

- Closed system
- Isothermal
- Rapid
- Multiprimer
- Visible readout



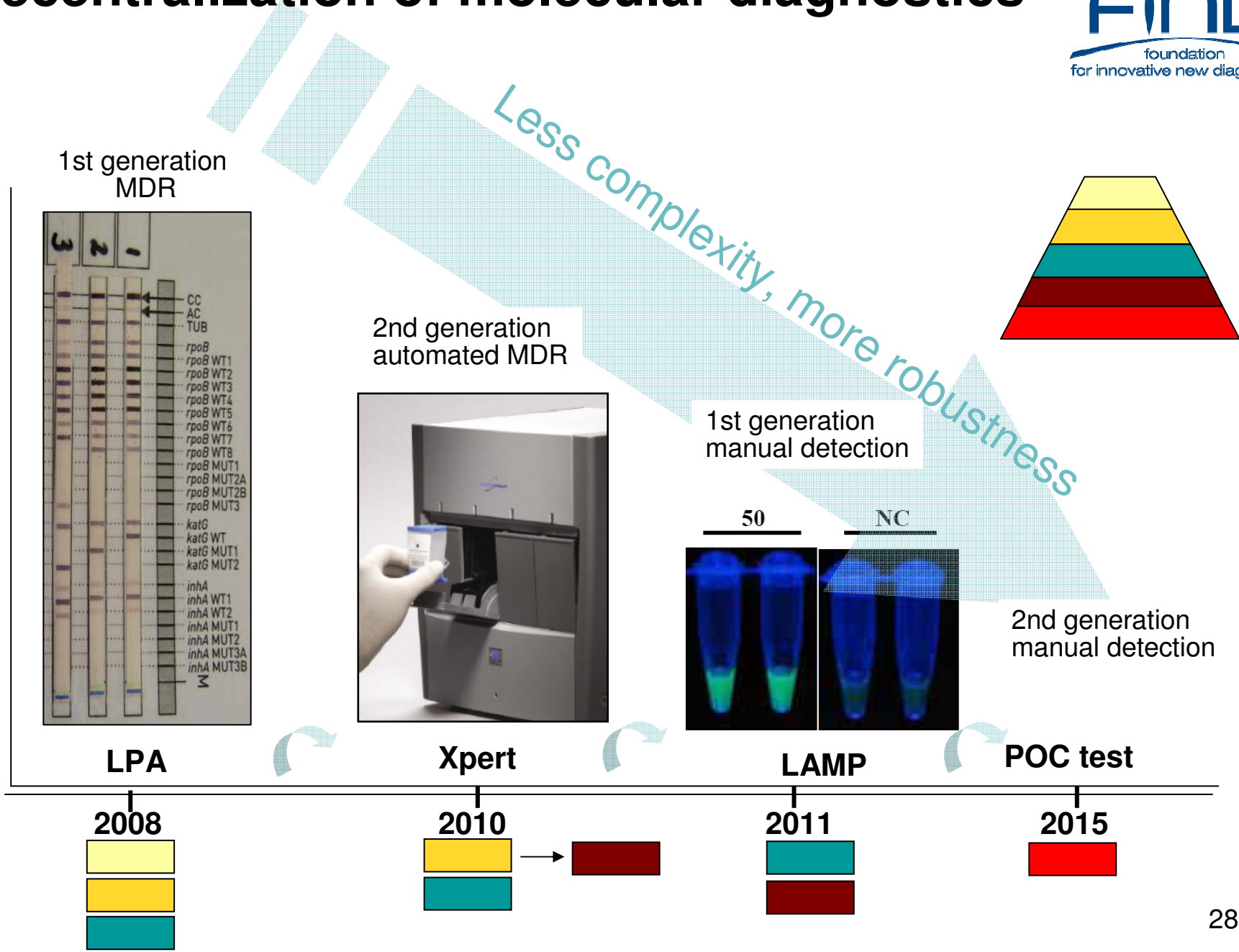
LAMP TB steps



Evaluation of PURE device performance

Experiment	Sample	Device (Tt)	
1	scanty S1(4/100)	14.0	
	S2(2/100)	12.1	
	purulent P1(1+)	13.1	
	bloody B2(3+)	15.3	
2	S3(5/100)	13.4	
	S4(7/100)	13.1	
	scanty S5(6/100)	12.0	
	S6(4/100)	19.3	
	S7(7/100)	13.2	
	S8(6/100)	13.4	
	P2(1+)	12.5	
	P3(2+)	13.4	
	purulent P4(1+)	-	
	P5(1+)	12.0	
	P6(1+)	12.0	
	P7	10.5	
	bloody B1(1+)	10.5	
B3(8/100)	12.1		
		A	B
3	scanty S9(5/100)	18.4	14.0
	S10(8/100)	11.4	11.2
	P8(1+)	13.2	12.5
	purulent P9(2+)	11.5	11.0
	P10(1+)	11.4	11.1
	bloody B4(6/100)	14.4	14.5
B5(5/100)	12.5	12.3	

Decentralization of molecular diagnostics



Patient-centered approach / technological platforms

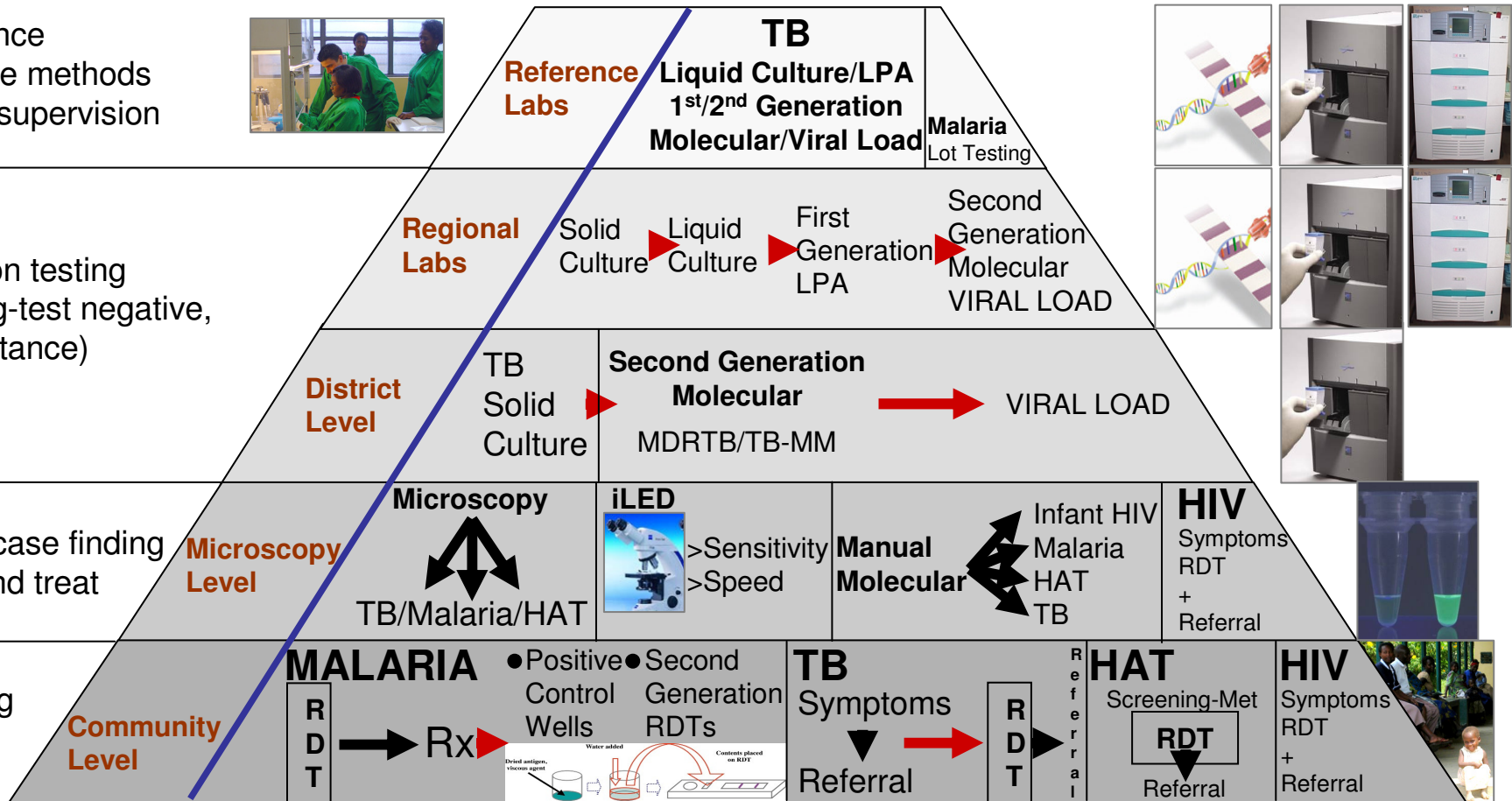
- Surveillance
- Reference methods
- Network supervision



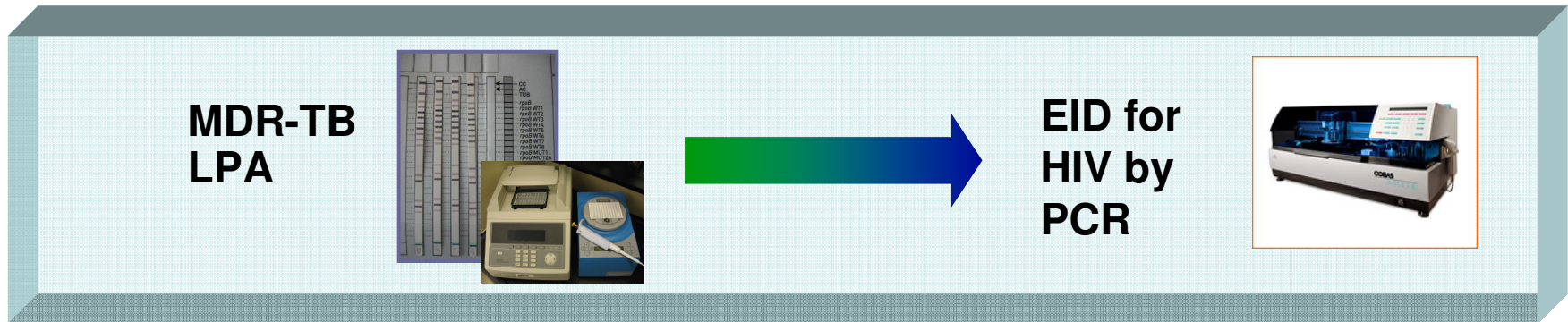
- Resolution testing (screening-test negative, drug resistance)

- Passive case finding
- Detect and treat

- Screening
- Primary care



Integrating HIV-TB diagnostics platforms: Lesotho



Molecular laboratory in Maseru

**Demonstration
(Evidence)**



**WHO
(Policy)**

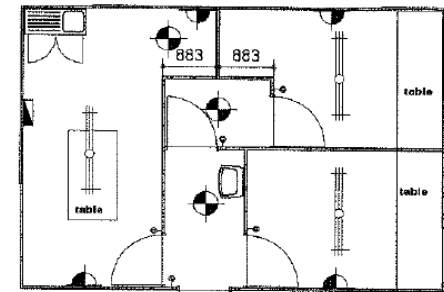


**Implementation
(Practice)**

March 2007 LC
April 2008 LPA

June 2007 LC
June 2009 LPA

November 2007 LC
November 2008 LPA
April 2009 EID for HIV by PCR



Partners
•WHO
•PIH
•MOH (Lesotho)



Thank you

