From the Laboratory Towards the Patient: Nucleic Acid Amplification-Based Diagnostics at the Point of Care

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PATH
Seattle
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APHL Meeting

PATH
A catalyst for global health

What is the Definition of POC Testing?

"Tests designed to be used at or near the site where the patient is located, that do not require permanent dedicated space, and that are performed outside the physical facilities of the clinical laboratories."

College of American Pathologists

"Testing at or near the site of patient care whenever the medical care is needed."

Louie et al. 2000. Laboratory Medicine, 31 (7)

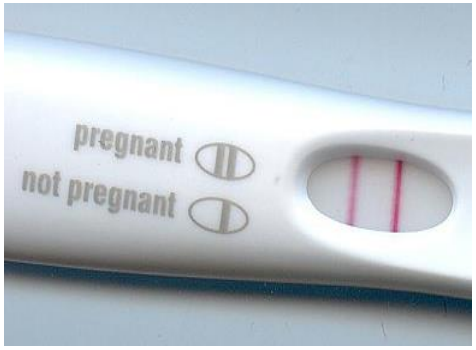
"A test that anyone can use by themselves in any setting, any ambulatory setting."

Ron Zwanziger, CEO, Alere

Critical View of NAAT Testing in LRS

"It is worth noting that there are no successfully marketed genuine POC nucleic acid tests anywhere in the world (for TB or other infectious diseases)"

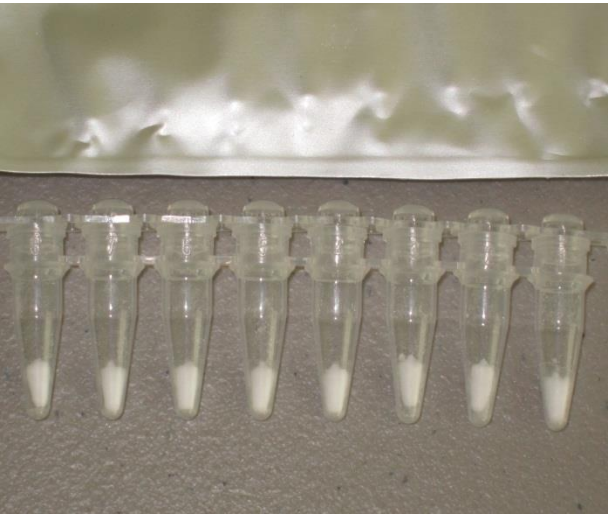
Batz et al. 2011. Towards Lab-Free Tuberculosis Diagnosis. A Report by TAG, the TB/HIV Working Group of the Stop TB Partnership, Imperial College and the MSF Access Campaign.





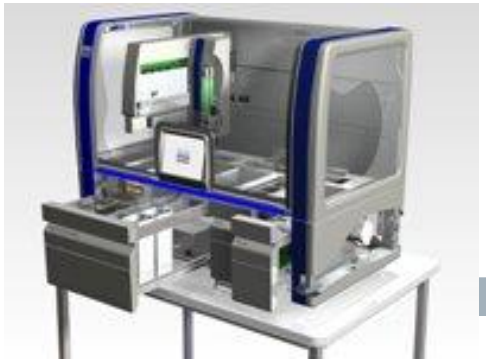
Benefits of NAAT as a Diagnostic Tool

1. Greater performance over traditional Dx tests
2. More rapid time to result
3. Capacity to multiplex tests
4. Reduced user training/skill
5. Move Dx closer to the patient population in LRS



Potential Disadvantages of NAAT as a Diagnostic Tool

1. Cost
2. Stability/robustness of tests/equipment
3. Adequate training
4. EQA and QC
5. Adequate supply/cold chain of materials/reagents
6. Maintenance
7. Dissemination of test results



"ASSURED"–The Accepted Norm(?)

The key components for a successful LRS Dx tool via ASSURED

	RDT	NAAT
Affordable	✓*	X
Sensitive	✓*	✓
Specific	✓*	✓
User-friendly	✓*	✓/?
Rapid and robust	✓	✓/?
Equipment-free	✓	X
Deliverable to end-users	✓ *	X/?

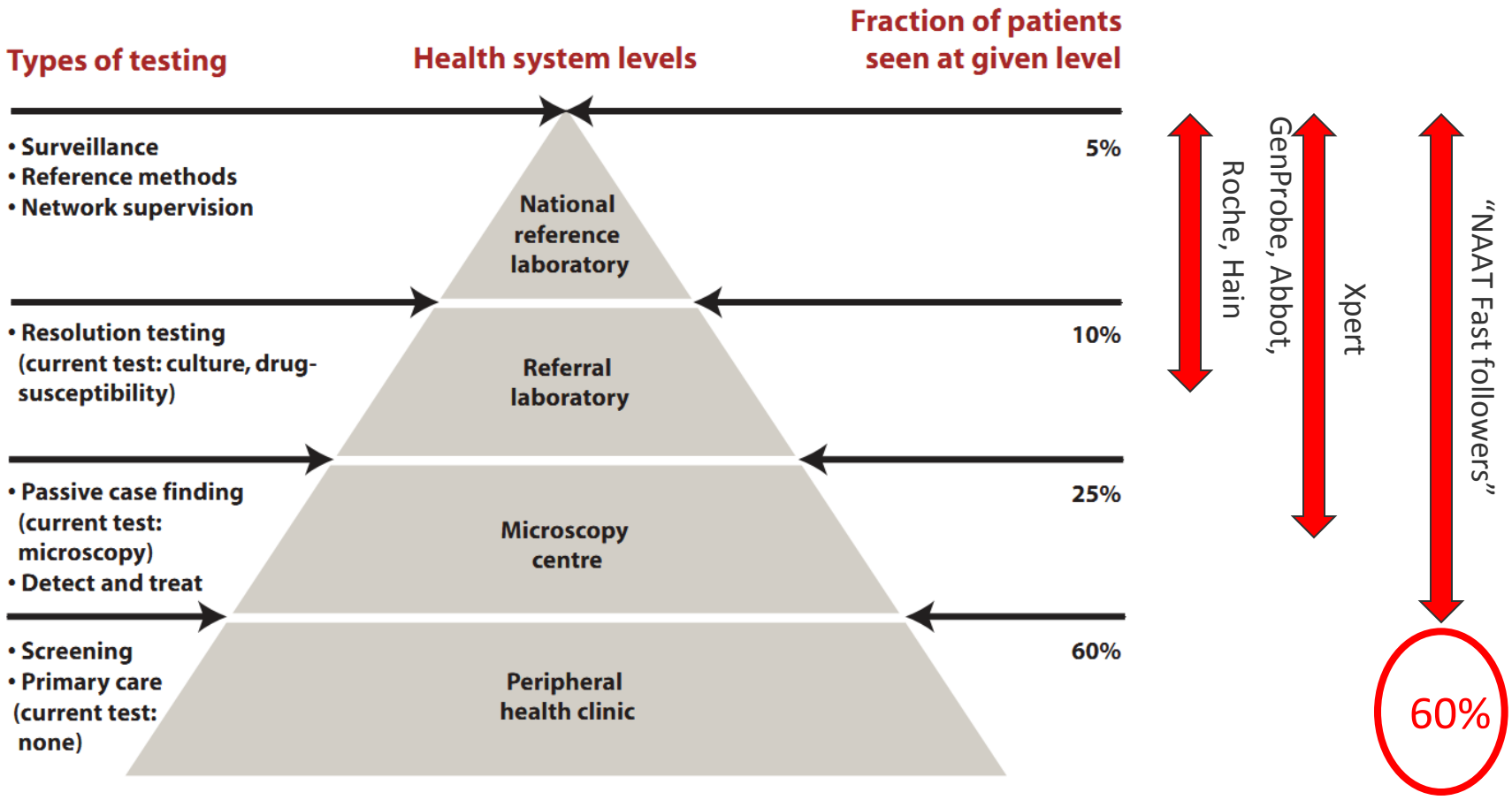
* "Poor testing procedure in the field can lead to exceedingly low levels of rapid HIV test sensitivity."

Wolpaw B. et al. BMC Health Service Res. 2010 10:73

Where Are NAATs Currently Performed?

Currently commercial NAATs are laboratory-based for TB and HIV

The GeneXpert is the first NAAT TB Dx outside of the large laboratory



Source: World Health Organization. 2006. Diagnostics for tuberculosis: Global demand and market potential.

What Are The Primary Obstacles for POC NAAT?

For an NAAT to be effective in low-resource settings, the following areas need to be addressed:

- Specimen collection
- Specimen preparation
- Amplification

Ease of use, safety, size, stability

- Detection
- Scoring
- Test data storage

Reduced complexity, robustness, size

- Equipment storage ?

Ideally these can be in a fully integrated device BUT costs and intended use case scenario do not dictate this to be essential

Specimen Collection

- An inadequate specimen negates a test result regardless of downstream technology
- The pathogen/symptoms dictate the specimen type
- Many specimens types exist: POC
  - Highly invasive: Amniotic fluid or CSF ×
  - Invasive: Whole blood (phlebotomy)/bronchial lavage ×/✓
  - Minimally invasive: Finger*/heel stick/Nasal swab* ✓
  - Noninvasive: Urine*/Stool/Sputum* ✓

* Even “simple” specimen collection can require trained users

Specimen Preparation

To release nucleic acids, remove confounding substances, and concentrate target analyte

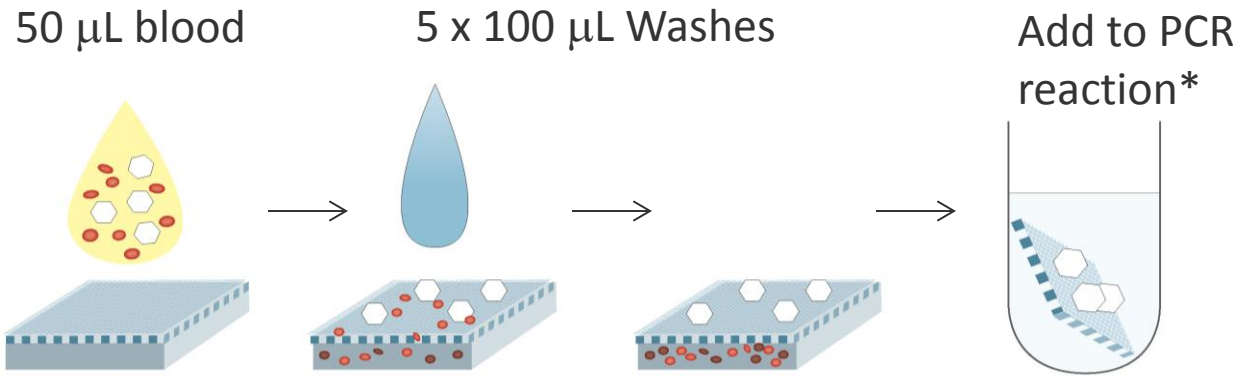
- Enzyme, chemical, physical or typically a combo

Several key obstacles:

- Pathogen physiology: Spores, cell wall, capsular
- Confounding substances: Stool, sputum, blood
- Low pathogen load: HIV, typhoid, MTB
- Stability of analyte: RNA

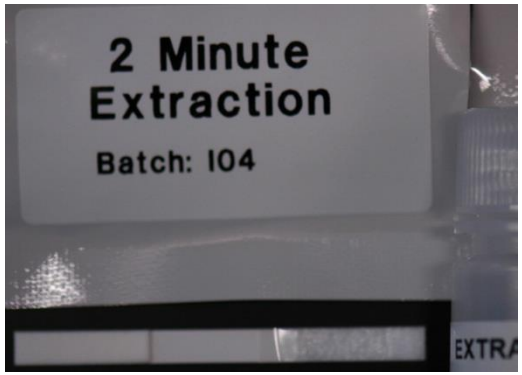
To Purify NA Or Not?

Some POC tests do not need “clean” NA



*NOTE: This may be integrated with any NAAT technology

Target Capture by (Very) Basic Chromatography



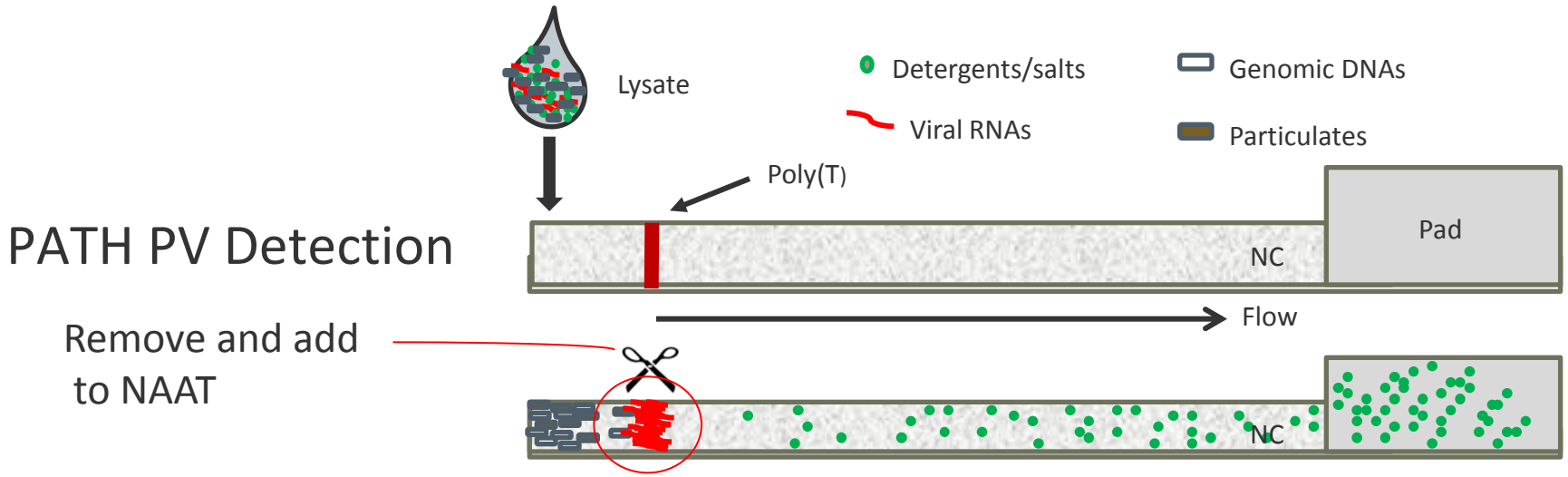
Rapid sample preparation for detection of a fungal pathogen by LAMP

Tomlinson et al., Plant Pathology (2010) 59

Malaria RDTs being used to capture and enrich for malarial DNA prior to real-time PCR.

Cnops et al., Malaria Journal (2011) 10,67

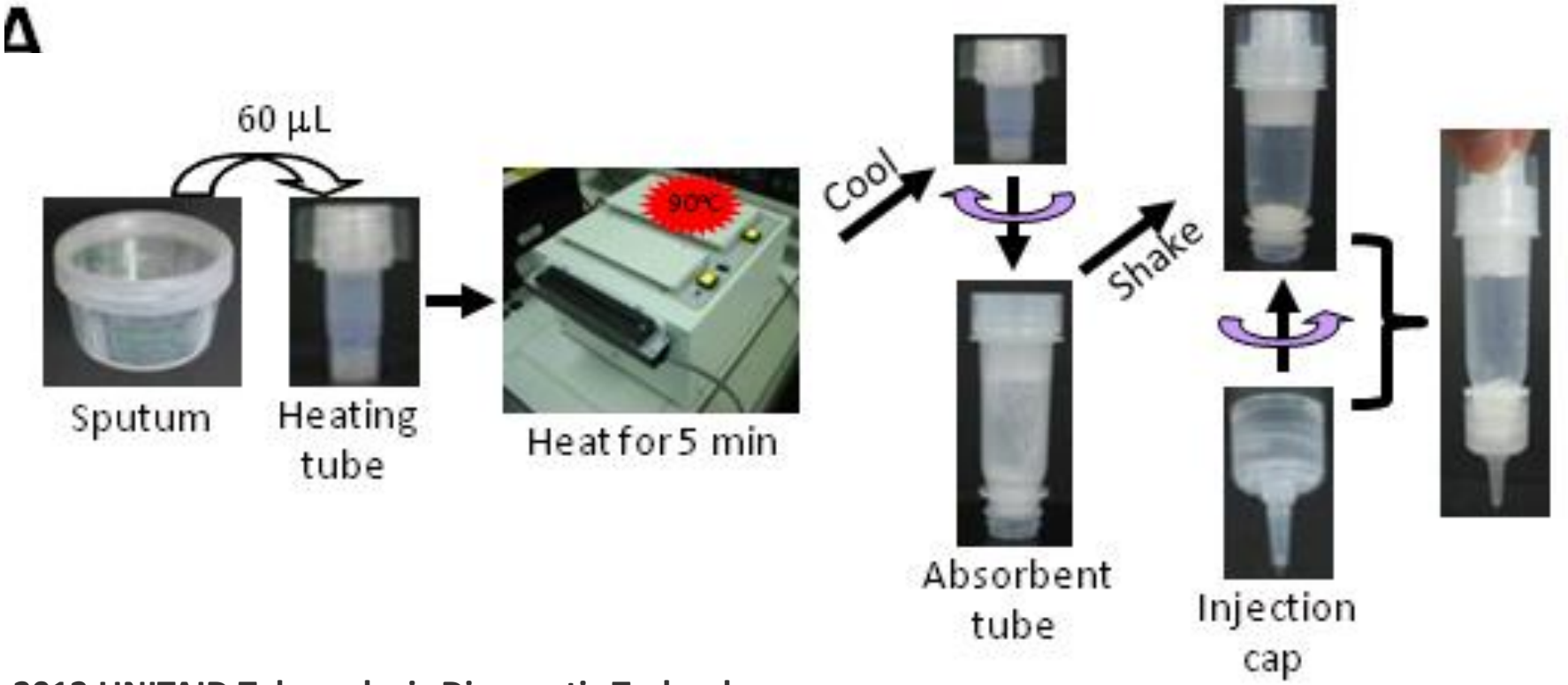
PATH PV Detection



Removal of Inhibitory Compounds

The Eiken/FIND Loopamp® TB assay

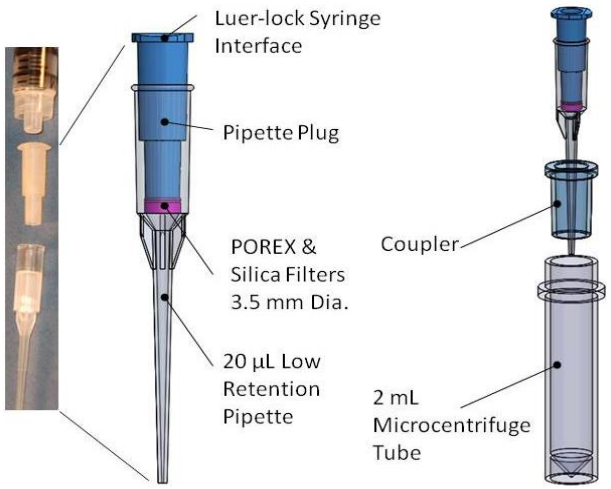
Sample lysis and removal of inhibitors rather than concentration of DNA

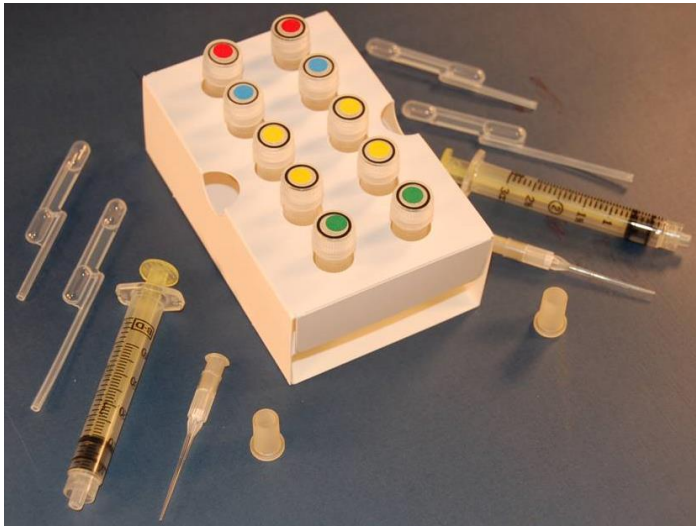


2012 UNITAID Tuberculosis Diagnostic Technology Landscape Semi-annual Update November 2012

Simplified SP Extraction

- Boom chemistry to purify/concentrate NA on silica membranes
- Non instrumented





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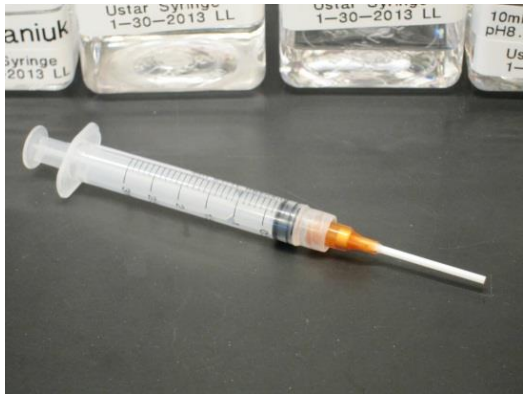
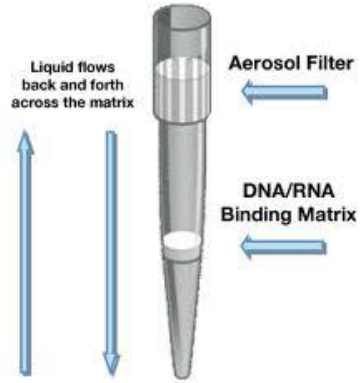
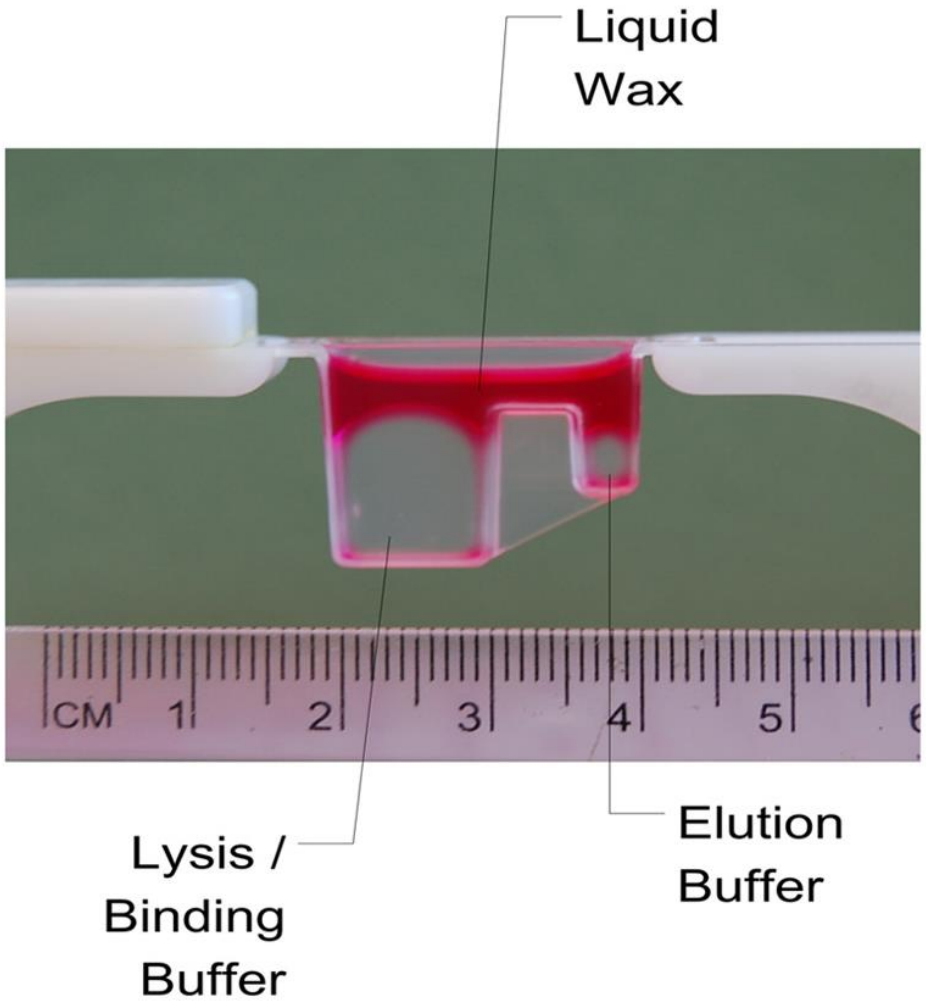


Ustar



Akonni

Surface Tension Valves: The iFAST



Similar performance to columns

BUT

Small

Based on well established tech

Much faster

No need for centrifuge

No need for wash buffers

Integrated

Licensed to Quidel

Source: Sur K. et al. J Mol. Diagn. 2010 12(5)

Amplification Technologies

PCR-based methods have led the way:

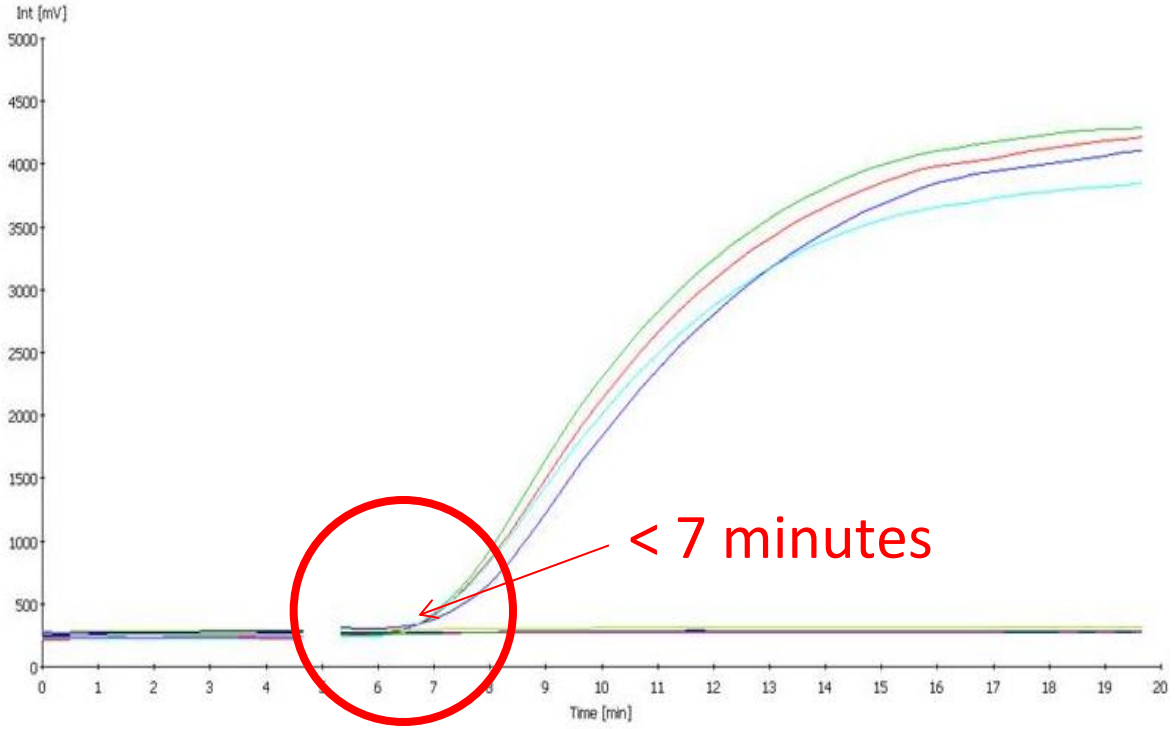
HAI and respiratory pathogens: FDA approved >12 PCR, 2 HDA, and 1 LAMP

Fully to partially integrated with sample preparation

Highly multiplexed	Biofire, Apollo
Moderate	GeneXpert, ICubate, Enigma ML, others
Low	BD Max, Genedrive, Uno

Advantages Offered by Isothermal Amplification

- Reduced reactor complexity (↓cost, ↓size, ↓power needs, ↑robustness)
- Greater tolerance to inhibitory compounds
- Faster time to result



Source: Boyle DS et al. 2013 mBio

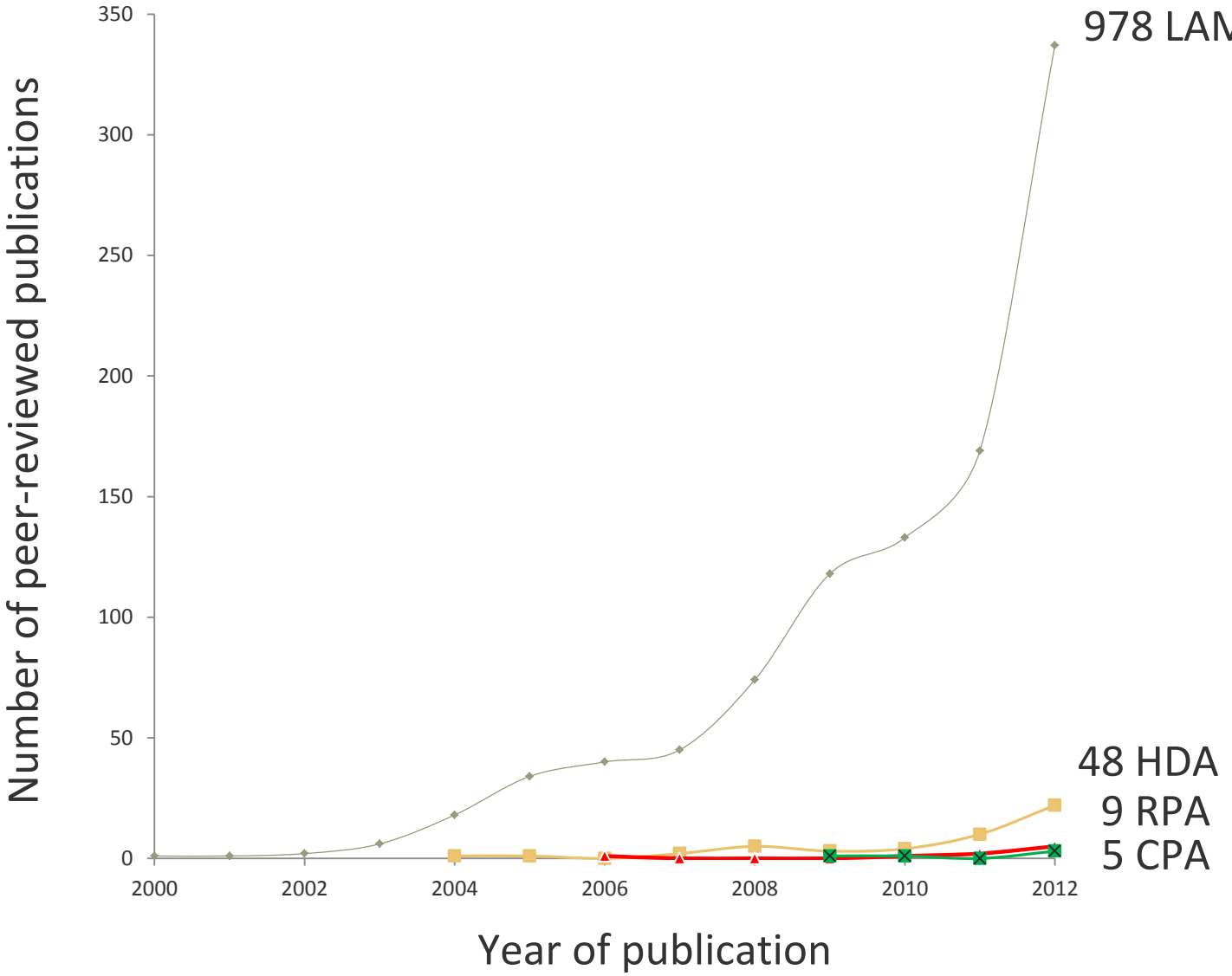
2011 Review of Isothermal Methods

Assay	Reaction temperature (°C)[a]	Reaction duration (min)[a]	Multiplex[b]	Rapid detection formats[c]	Target	Amplification product
Methods based on RNA transcription						
NASBA	41[d]	105	Y	RTF, NALF	RNA (DNA)	RNA, DNA
TMA	60[d]	140	Y	RTF	RNA (DNA)	RNA, DNA
SMART	41[d]	180	N/A	RTF	RNA, DNA	RNA
Methods based on DNA replication with enzymatic duplex melting/primer annealing						
HDA	65	75-90	Y	RTF, NALF	DNA[e]	DNA
RPA	30-42	20	Y	RTF, NALF	DNA[e]	DNA
Methods based on DNA-polymerase-mediated strand displacement from linear or circular targets						
LAMP	60-65[d]	60-90	N/A	RTF, NALF, RTT, TE	DNA[e]	DNA
CPA	65	60	N/A	RTF, NALF	DNA	DNA
SMART-AMP	60	45	N/A	RTF	DNA[e]	DNA
RCA	65	60	N/A	RTF	DNA[e]	DNA
RAM	63[d]	120-180	N/A	RTF	DNA[e]	DNA
Methods based on polymerase extension/strand displacement, plus a single strand cutting event						
SDA	37	120	Y	RTF, NALF	DNA[e]	DNA
NEAR	55	10	Y	RTF, NALF	DNA[e]	DNA
NEMA	65	30	N/A	NALF	DNA	DNA
ICA	60	60	N/A	RTF	DNA	DNA
EXPAR	55	10-20	Y	RTF, NALF	DNA	DNA
BAD AMP	40	40	N/A	RTF	DNA	DNA
PG-RCA	60	60-120	N/A	RTF	DNA	DNA

Niemz et al., 2011 TiBtech, 29(5)

At least 5 more as of 2013…

The Evolution of Selected Isothermal Methods



Improving Performance via Novel Enzymology

For improved performance of PCR:

- New sources of DNA polymerases
- Mutational modifications of Taq (e.g. KlenTaq1)
- Phage-based enzymes (e.g. Pyrophage)

The same is now happening with strand displacing DNA polymerases:

NEB Bst v2.0, Lucigen pyrophage, and Optigene GspF, M, SSD, and E

- Like Tth, some Bst-like enzymes have reverse transcriptase activity

Novel Polymerases: DNA Amplification

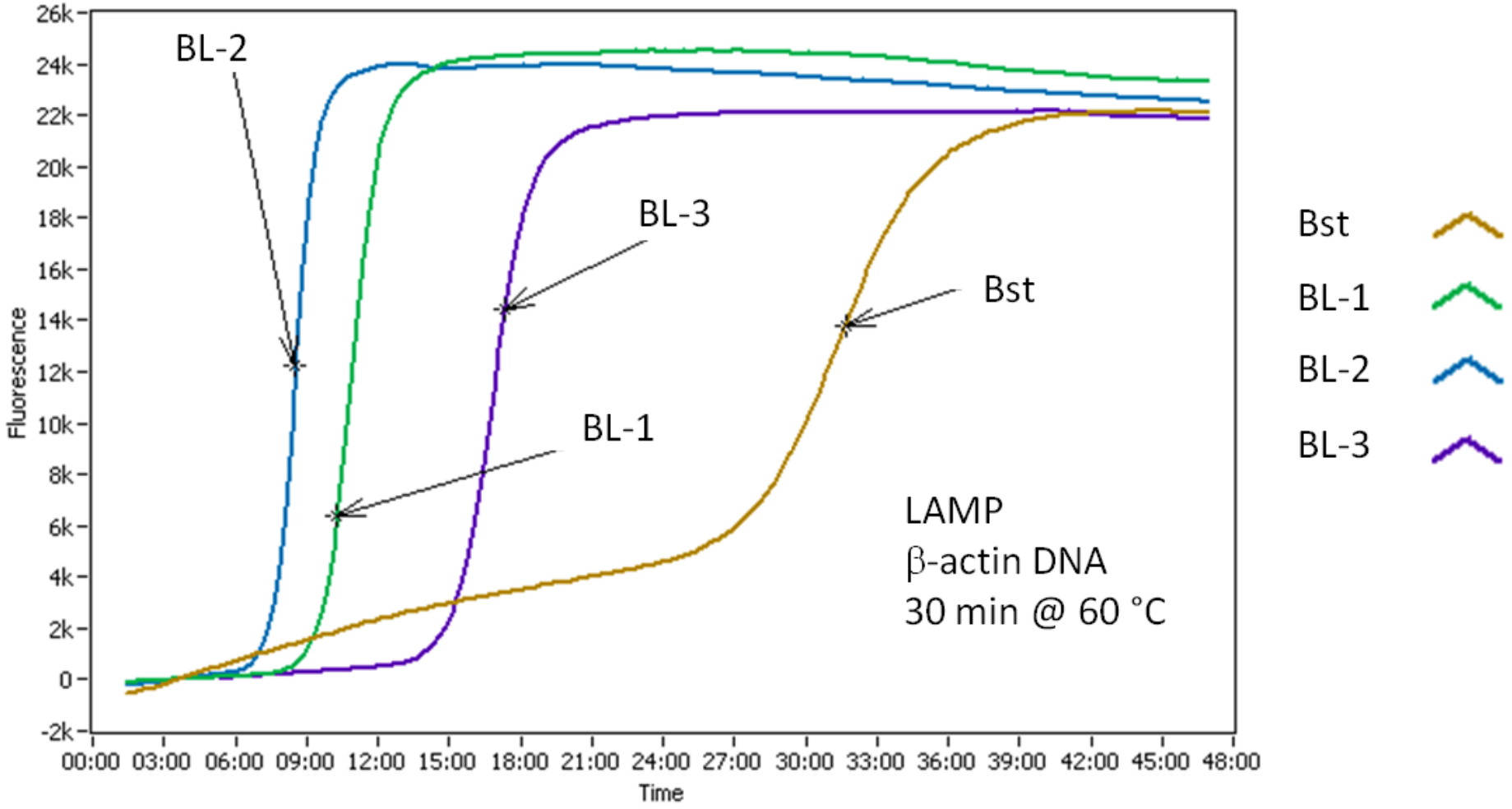


Image courtesy of Optigene (UK)

Novel Polymerases: RNA Amplification

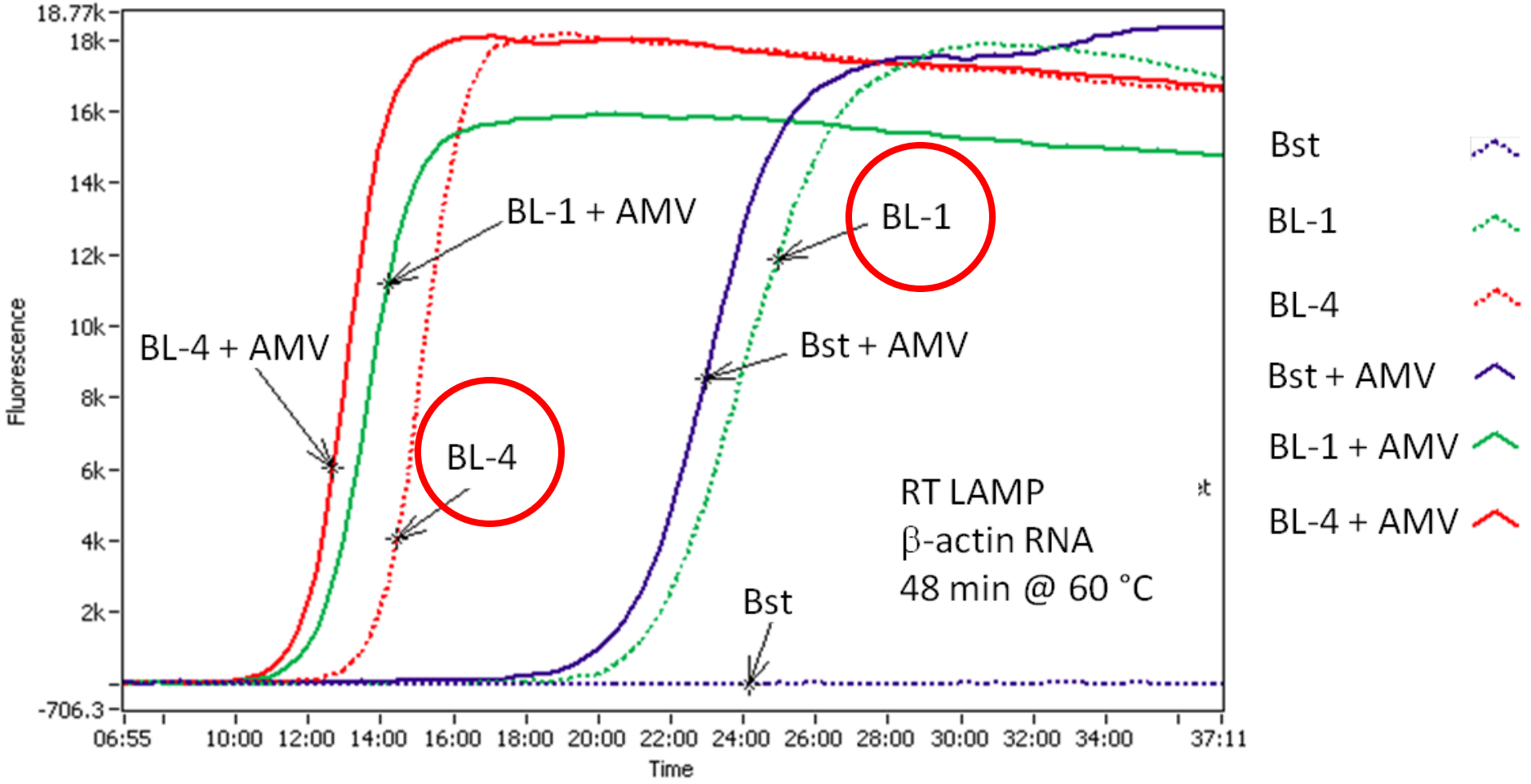


Image courtesy of Optigene (UK)

Improved Oligonucleotide Chemistry

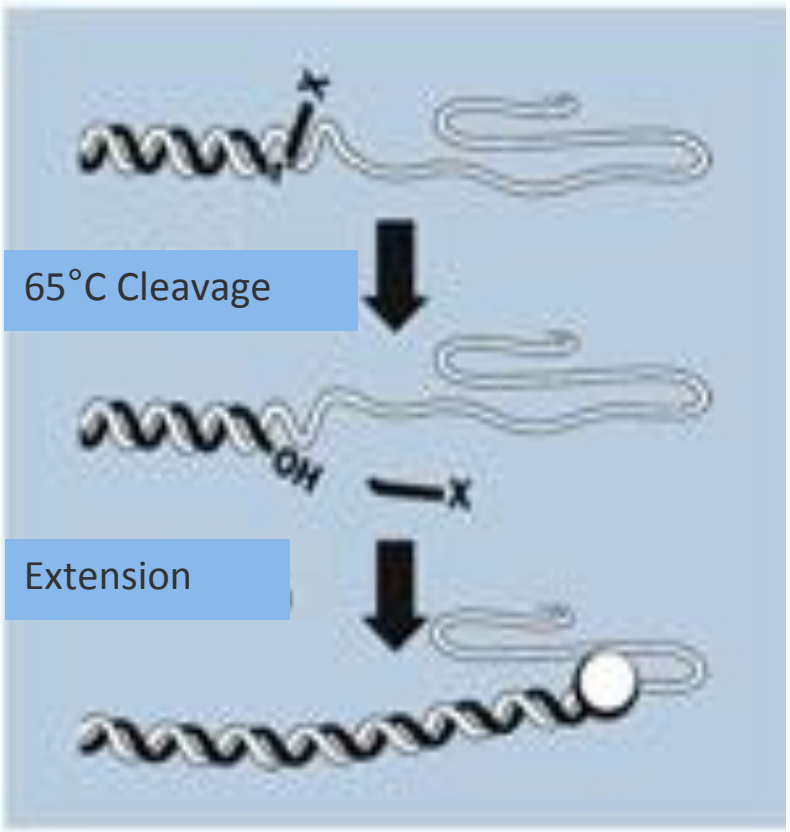
Chemical modifications to oligonucleotides improves test performance:

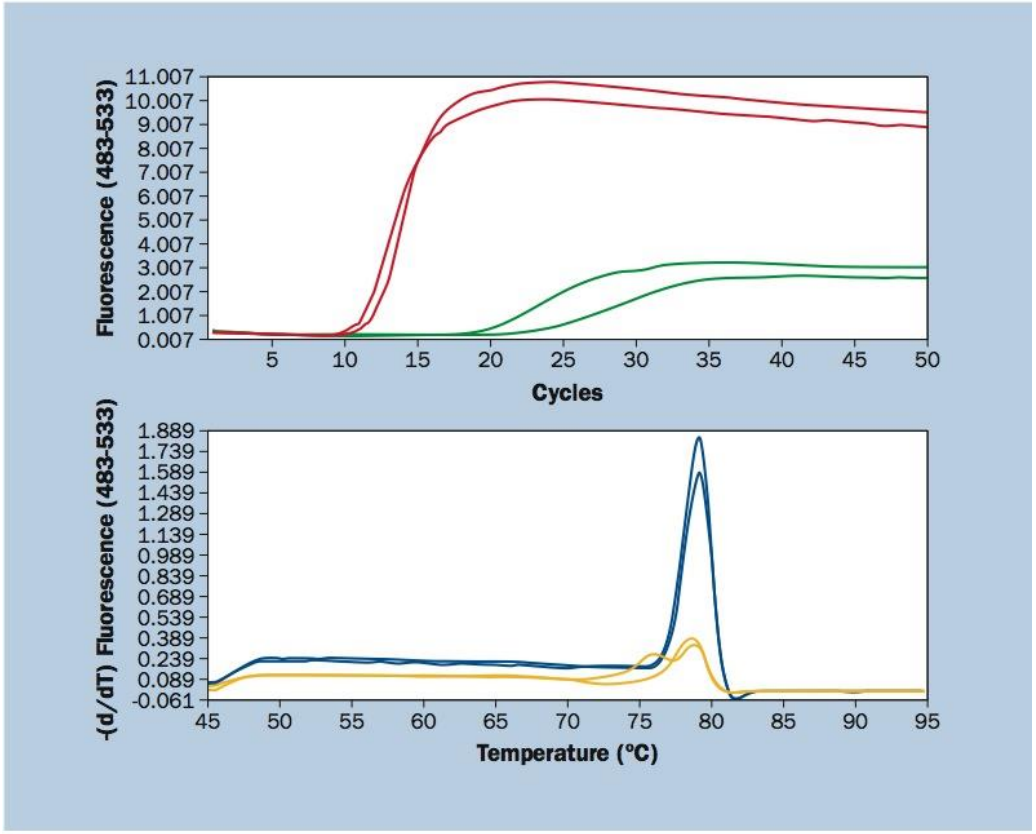
1. The minor groove binder MGB - Higher fidelity, yet shorter Taqman probes
2. Locked nucleic acids
   Super bases
   Peptide nucleic acids
   } Improve binding to targets
3. Spermidylated primers/probe (zipDNAs)
4. Blocked primer/probe technology (e.g. bpHDA and RPA)
5. Dual Priming Oligonucleotides (DPO)

Chemistry and Enzymology

Blocked primer HDA - Developed by Great Basin Corp. (Utah)

Thermostable RNase H2 and *Bst* polymerase





Rea *et al.*, 2012, IVD Technology

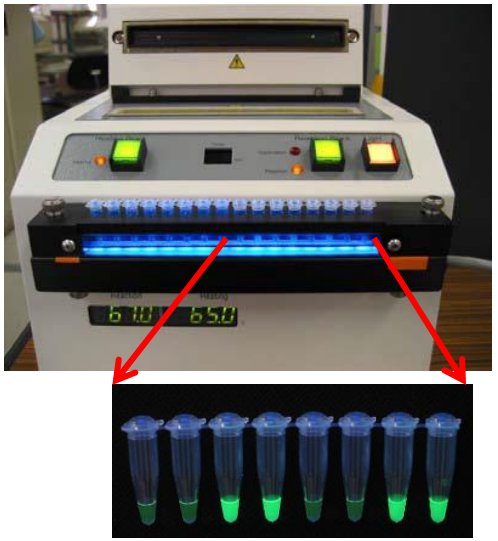
Reaction Incubation and Detection of Amplicons

Isothermal reactions:

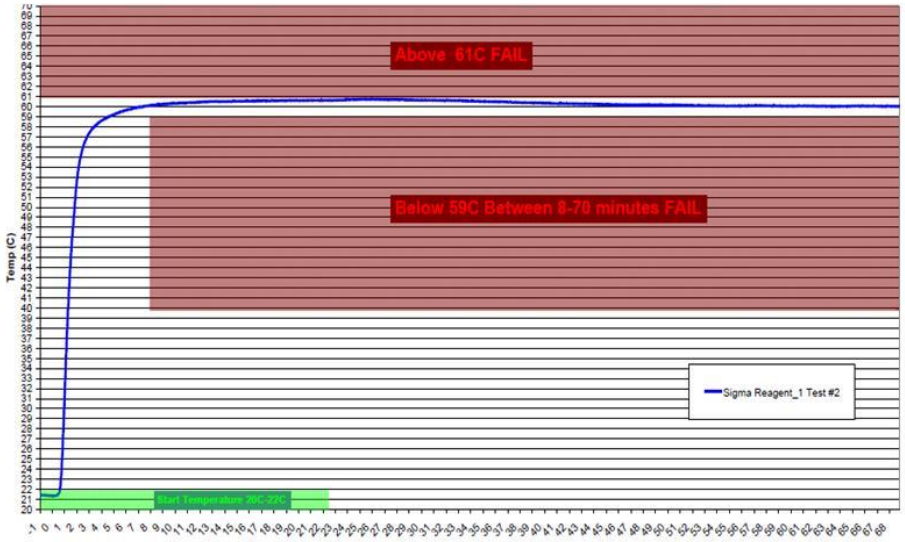
Dedicated platform heating with visual detection (turbidity, Calcein, Hydroxyl napthol blue

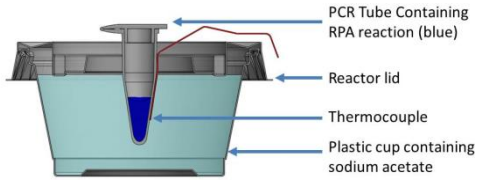
OR

Non instrumented Heater (NINA)



Images from C. Boehme, FIND





Images from P LaBarre, PATH

PATH

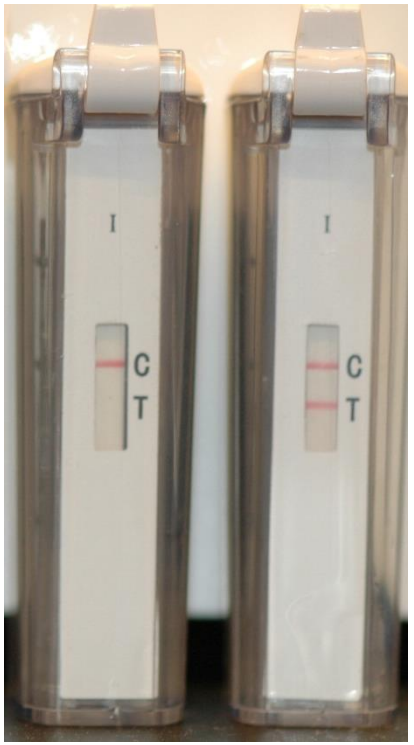
Or No Heater!!!

Non-Instrumented Incubation of Recombinase Polymerase Amplification for the Sensitive and Rapid Detection of HIV Infection in Low Resource Settings…

Temp (°C)	Thermocycler				Ambient Temperature			
	HIV		NTC		HIV		NTC	
	20’	30’	20’	30’	20’	30’	20’	30’
29	1/3	3/3	0/1	0/1	0/3	2/3	0/1	0/1
30	2/3	3/3	0/1	0/1	0/3	3/3	0/1	0/1
31	3/3	3/3	0/1	0/1	3/3	3/3	0/1	0/1
33	3/3	3/3	0/1	0/1	3/3	3/3	0/1	0/1
35	3/3	3/3	0/1	0/1	3/3	3/3	0/1	0/1
37	3/3	3/3	0/1	0/1	3/3	3/3	0/1	0/1
39	3/3	3/3	0/1	0/1	3/3	3/3	0/1	0/1
40	3/3	3/3	0/1	0/1	3/3	3/3	0/1	0/1
42	3/3	3/3	0/1	0/1	3/3	3/3	0/1	0/1
43	3/3	3/3	0/1	0/1	3/3	3/3	0/1	0/1
44	1/3	0/1	0/1	0/1	2/3	2/3	0/1	0/1

End Point Analysis via LFS Detection

Detection of hapten-labeled amplicons via LFS with (Ustar: BioHelix, Quidel, TwistDx, and other comm. groups)



Detection of amplicon via hapten-labeled probes targeting single stranded target.

SAMBA.

Lee et al., JID, 2010; 201(S1)

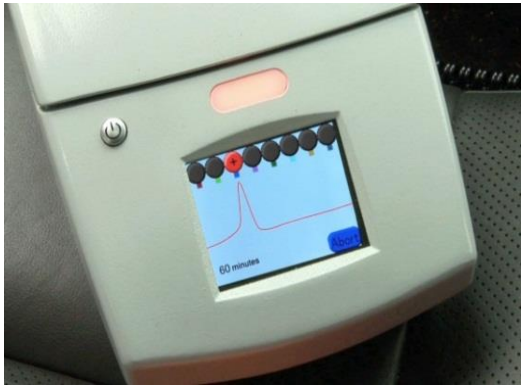
Detection in Real Time

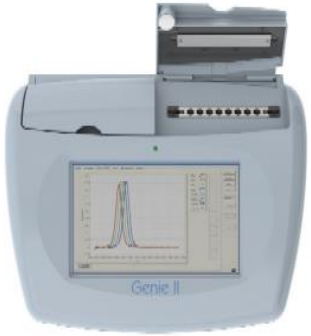
Real-time detection via fluorescence/bioluminescence

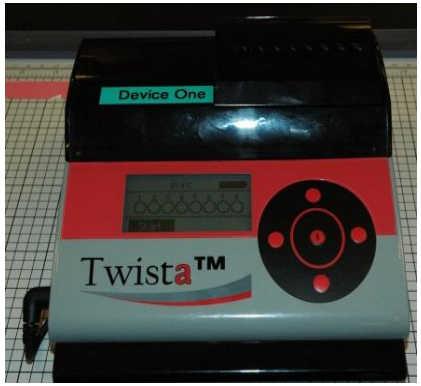
CPA, HDA, LAMP, GEAR, NEAR, PCR, and RPA amplicon detection probe-based and intercalatory dyes

Alere, Epistem, Lumora, MolBio, Optigene, Qiagen (ESE), and others

Battery powered, small, result scoring, on-board data storage

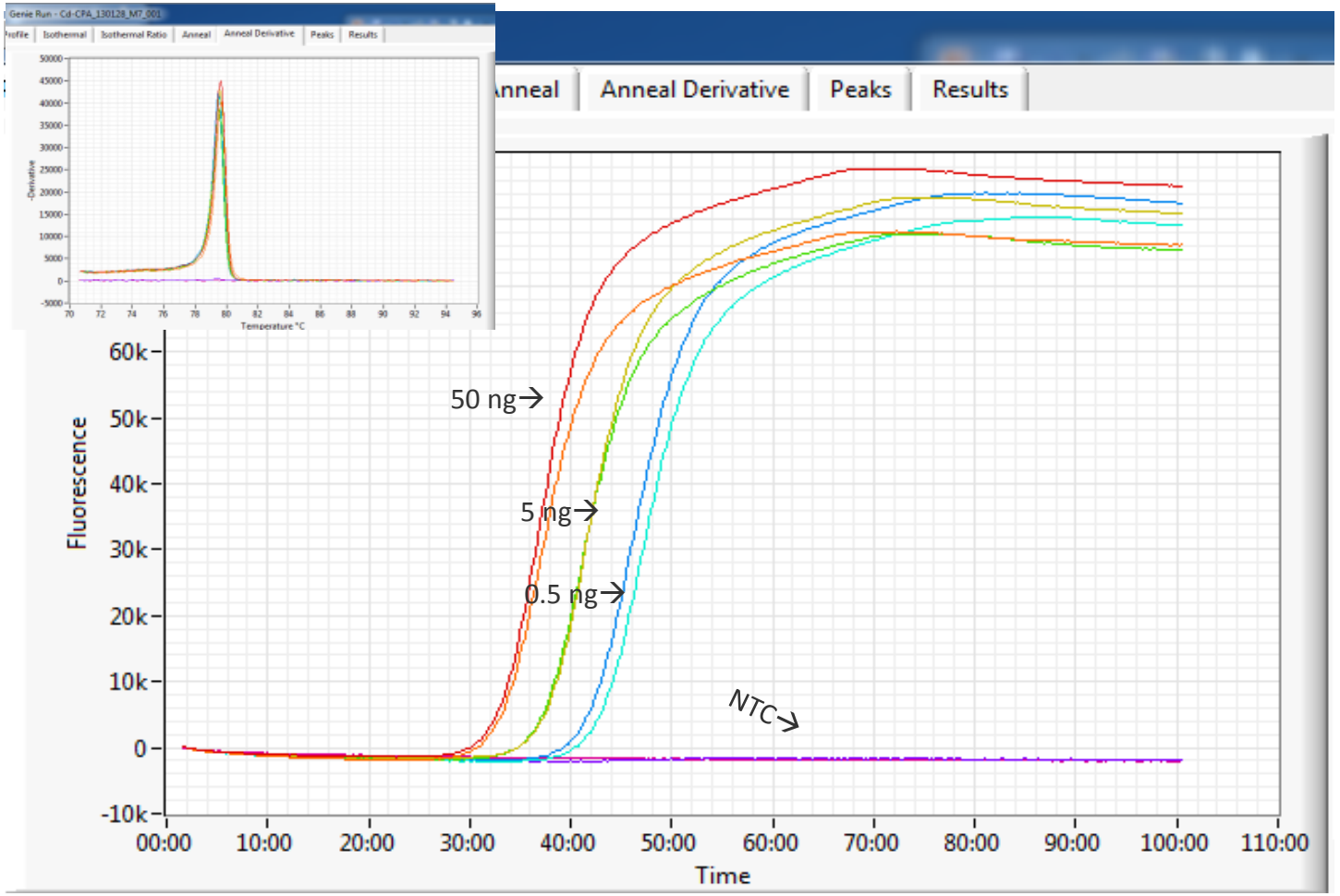






Optigene Genie II

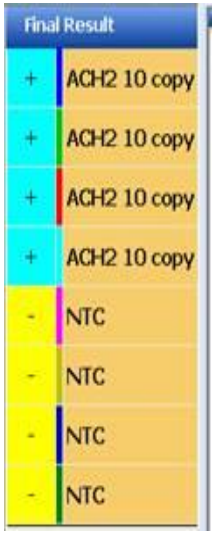
Single channel fluorescent analysis, reverse melt curve analysis confirms amplicon



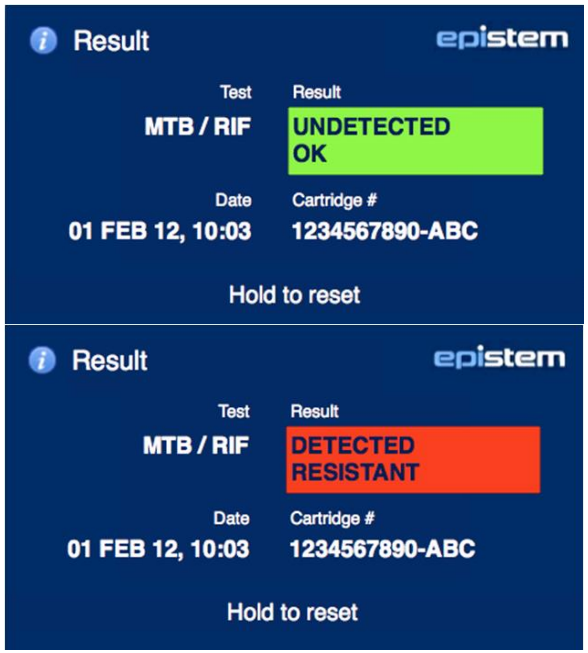
PATH

Automated Scoring of Results

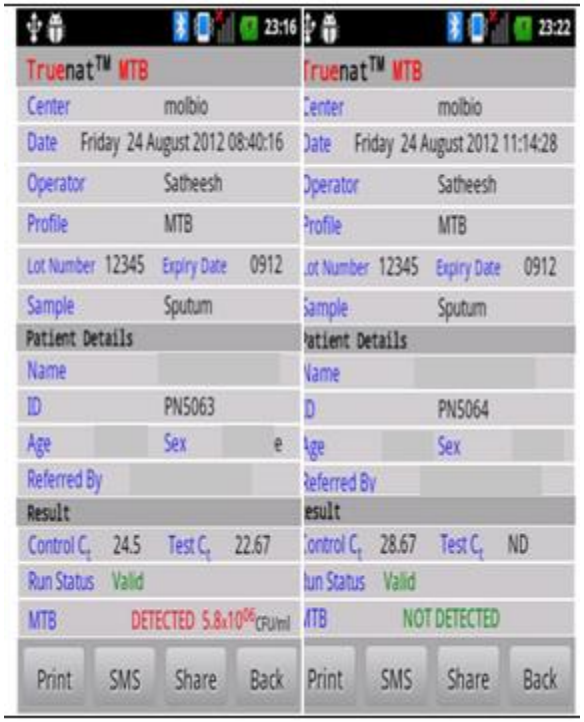
Fast followers can interpretate data and give results via simple user interface



ESE Twista



Epistem Genedrive



MolBio Truelab

Summary

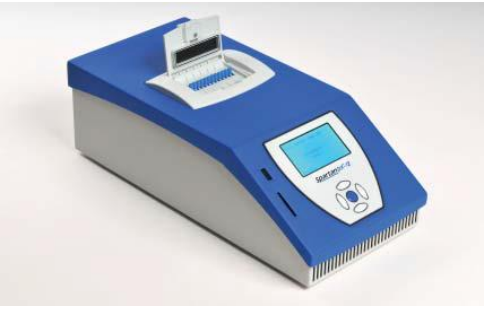
1. A wide variety of sample preparation methods are in use
2. Many ‘new’ amplification methods’ have potential
3. Test performance can be improved via the evolution of core components
4. Innovative engineering approaches to reaction monitoring and co-integration
5. Integrated to varying degrees in current technology
6. Level of integration is a trade of with cost/# of tests/location

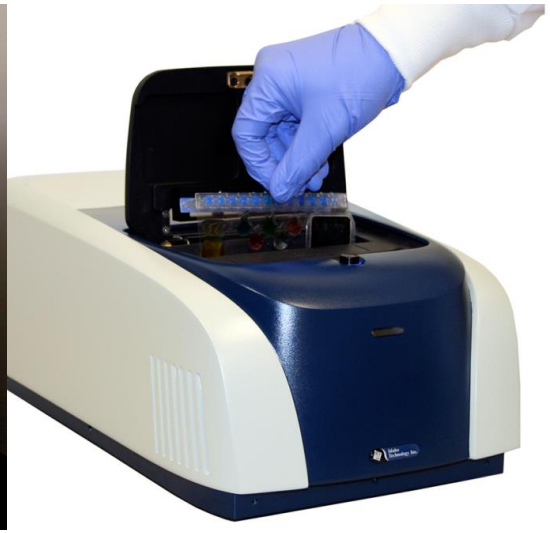
PCR is Dead!

The history and perception…

Large, expensive, power heavy, complex, not robust, and needs PC

Long Live PCR!
The reality…after engineering and innovation





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