Clinical Seminar Series

New Diagnostic Technologies

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http://dmdp.org

Kathmandu, Nepal, 2018

I-TECH
International
Training & Education
Center for Health

Overview

- How are new technologies implemented – in developed countries/in resource limited countries.
- Quality – what guidelines/standards are being addressed for new technologies and POC testing.
- Examples


CDC
SAFER • HEALTHIER • PEOPLE™


FDA
U.S. Food and Drug Administration
Protecting and Promoting Your Health


CMS
Centers for Medicare & Medicaid Services


AMERICAN
SOCIETY FOR
MICROBIOLOGY


APHL
ASSOCIATION OF
PUBLIC HEALTH LABORATORIES


CLINICAL AND
LABORATORY
STANDARDS
INSTITUTE


AACC
Improving healthcare
through laboratory medicine


AAB
American Association
of Bioanalysts


ASCP
American Society for Clinical Pathology


College of American Pathologists

Point-of-care tests for diagnosing infections in the developing world

R. W. Peeling and D. Mabey
London School of Hygiene and Tropical Medicine, London, UK

- Lack of regulation of diagnostic tests in many countries has resulted in the widespread use of sub-standard POC tests
- Many countries do not have established criteria for licensing and introducing new diagnostic tests, and many clinicians in developing countries have become disillusioned with diagnostic tests and prefer to rely on clinical judgment.
- POC tests can be used to improve global health, but **only if they are rigorously evaluated, correctly used, and effectively regulated.**

Clin Microbiol Infect 2010; 16: 1062–1069

Economic evaluation of point-of-care diagnostic technologies for infectious diseases.

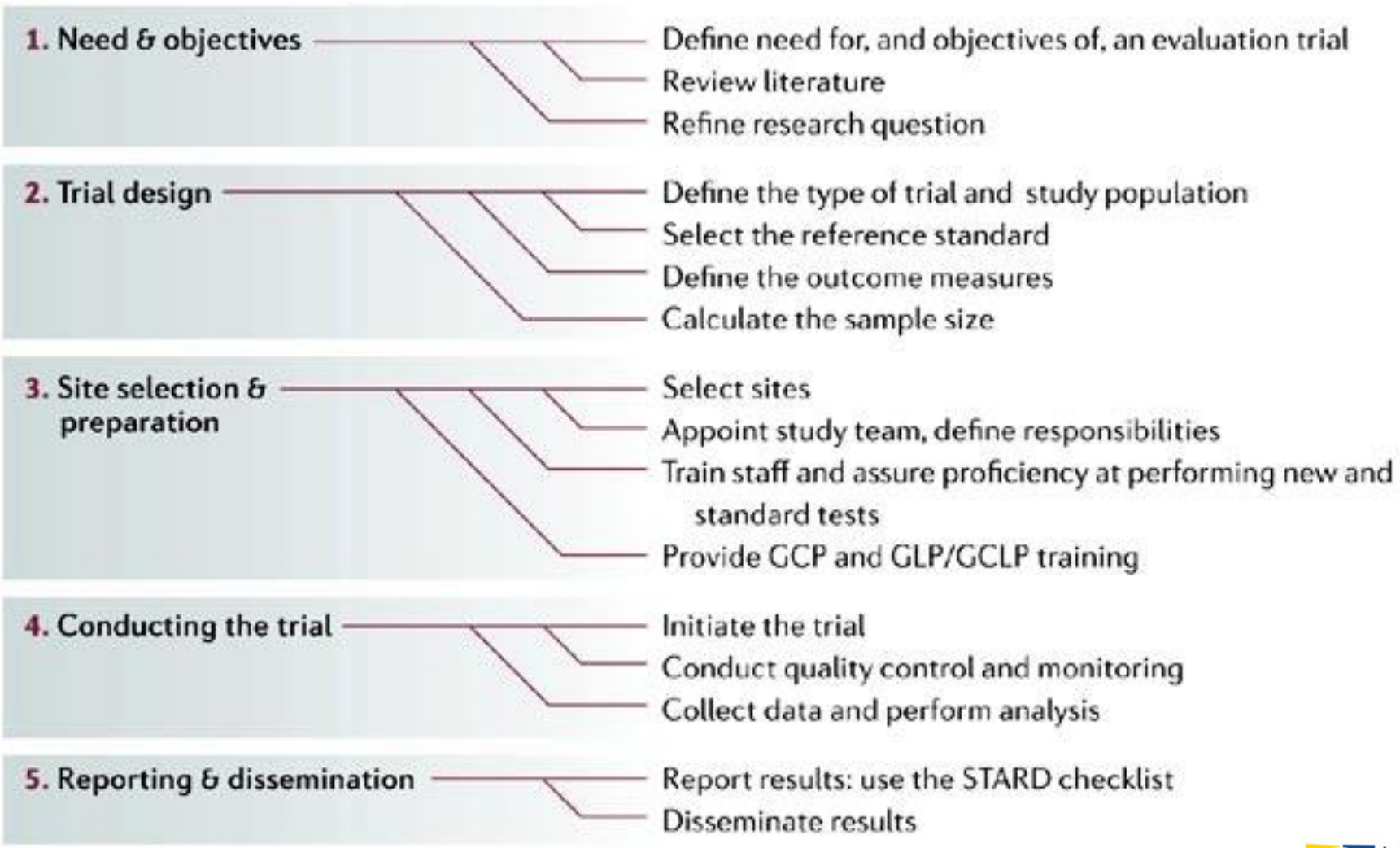
S. Loubiere and J.-P. Moatti

Clinical Microbiology and Infection, Volume 16 Number 8, August 2010

- Highly sensitive and specific POC diagnostic tests, given their ease of use and interpretation, could potentially be deployed at a peripheral level in epidemics, if a minimal degree of **personnel training, logistics and quality assurance** is guaranteed.

Evaluation of diagnostic tests for infectious diseases: general principles

TDR Diagnostics Evaluation Expert Panel (WHO/TDR)
Nature Reviews Microbiology (6)S2-S6, November 2008



Evaluation of diagnostic tests for infectious diseases: general principles

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Test under evaluation	Reference standard test		Total
	Positive	Negative	
Positive	a	b	a + b
Negative	c	d	c + d
Total	a + c	b + d	

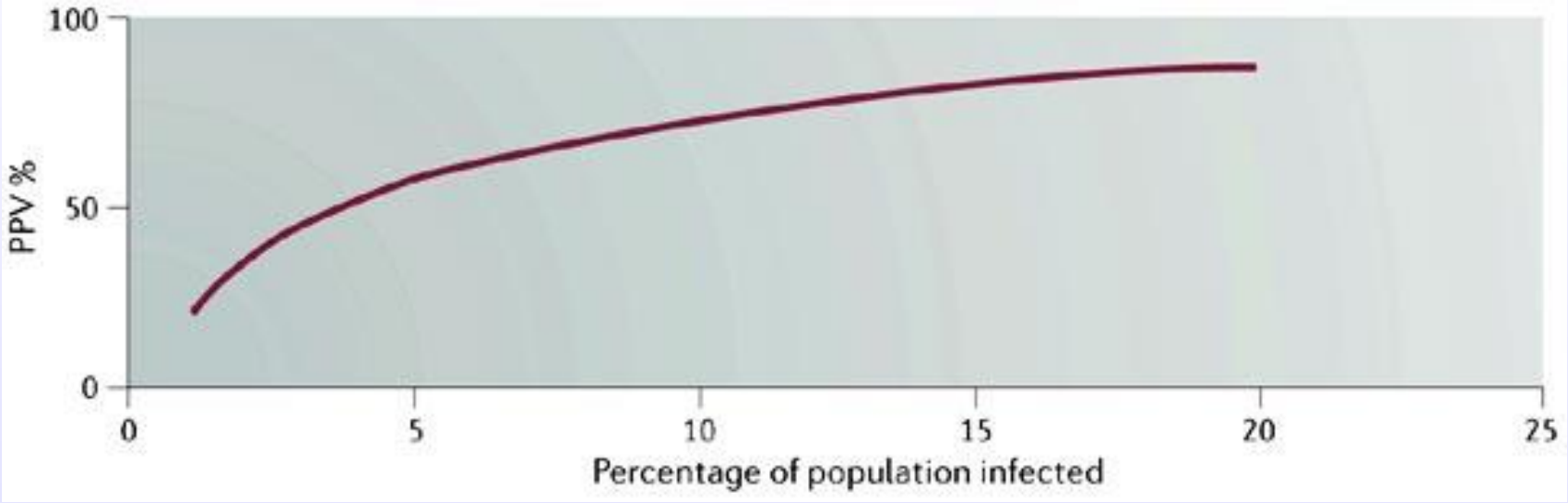
Test sensitivity = a/(a + c); test specificity = d/(b + d); PPV = a/(a + b); NPV = d/(c + d).
a = true positive, b = false positive; c = false negative; d = true negative

Evaluation of diagnostic tests for infectious diseases: general principles

TDR Diagnostics Evaluation Expert Panel (WHO/TDR)

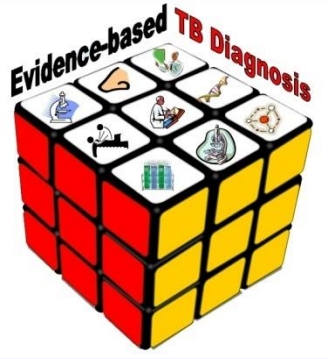
Nature Reviews Microbiology (6)S2-S6, November 2008

- The positive predictive value (PPV) of a test will depend not only on the sensitivity of the test but also on the prevalence of the condition within the population being tested. The figure below shows how the positive predictive value for a test with 96% sensitivity varies according to the prevalence of infection in the population.

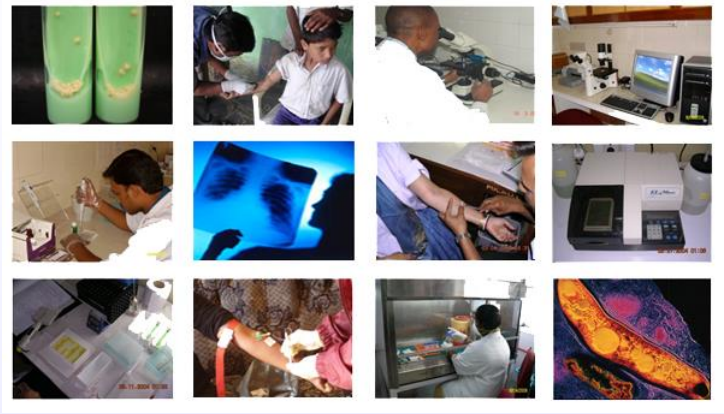


Evidence-Based Tuberculosis Diagnosis

A comprehensive resource for evidence syntheses, policies, guidelines and research agendas on TB diagnostics



www.tbevidence.org



Developed with the support of:

Stop TB Partnership's New Diagnostics Working Group (NDWG)
World Health Organization (WHO)
Foundation for Innovative New Diagnostics (FIND)
Special Programme for Research and Training in Tropical Diseases (TDR)
Global Laboratory Initiative (GLI)
Public Health Agency of Canada (PHAC)
Francis J. Curry National Tuberculosis Center, UCSF
McGill TB Research Group

Stop TB Partnership

FIND foundation for innovative new diagnostics

TDR For research on diseases of poverty UNICEF • UNDP • World Bank • WHO

gli

World Health Organization

Francis J. Curry National Tuberculosis Center

McGill

Public Health Agency of Canada

Towards more compassionate and effective care for drug resistant TB: improving diagnosis and case detection.

Theo Smart *HATiP | Issue 164 | 27 August 2010*

"Indeed, it makes little sense to put new laboratory infrastructure in place, especially expensive infrastructure, without making certain that the rest of the screening, transport and treatment system for people with drug-resistant TB is in proper working order. And even in settings without functioning computer systems linking the laboratory to the clinic, given the ubiquity of cell phones/SMS technology, there really is no excuse not to develop a system for the rapid return of results."

Evolution of technologies for TB & M(X)DR as per WHO endorsement

Year	Technology	Turnaround Time	Sensitivity Gain
Before 2007 	ZN Microscopy Solid Culture	2-3 Days 30-60 Days	Baseline
2007	Liquid Culture Rapid Speciation	15-30 Days	+10% compared to LJ
2008 	Line Probe Assay (1st line, RIF &INH)	2-4 Days	At this time for 3+ only
2009 	LED-based FM	1-2 Days	+10% compared to ZN
2010 	Integrated NAAT (TB, Rif)	90 minutes	+40% compared to ZN

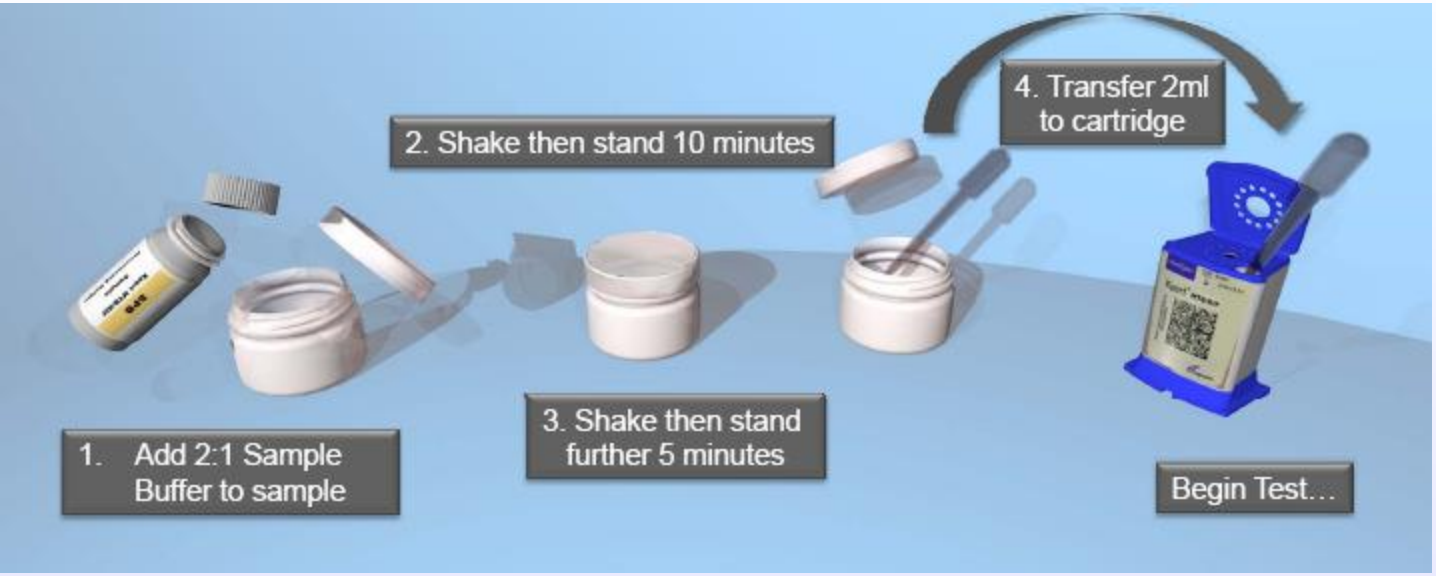
LED Fluorescence Microscopy

Advantages:

- increase in performance
- increase in lamp lifetime
- reduces initial, operating and maintenance costs
- No need for dark room

WHO recommended LED microscopy as an alternative to conventional microscopy.

GeneExpert - Detection of *M. tuberculosis* and drug resistance



Integrated NAAT (TB, Rif)

90 minutes

+40%
compared to ZN

	Sensitivity S+C+	Sensitivity S-C+	Specificity Non-TB	Sensitivity in phenotypic Rif resistant cases	Specificity in phenotypic Rif sensitive cases
[95% CI]	**99.5% (564/567) [98.5 – 99.8]**	**90.2% (157/174) [84.9 – 93.8]**	**98.1% (604/616) [96.6 – 98.9]**	**97.5% (199/204) [94.4 - 99.0]**	**98.1% (504/514) [96.5 - 98.9]**

Xpert™ MTB performance for case detection and Rifampicin resistance detection compared to conventional methods
Including results from 5 sites: Peru, Azerbaijan, India and South Africa (2)
* C: culture; S: AFB smear

Xpert MTB/Rif: Low biosafety requirements

Biosampler measurements (108 cfu/ml):

1. No aerosol release during sample preparation
2. Aerosol generation < than during preparation of direct smear

- Spiking studies:7 log killing after 15 min SR incubation
- Clinical studies: >90% MGIT neg after 15 min SR incubation

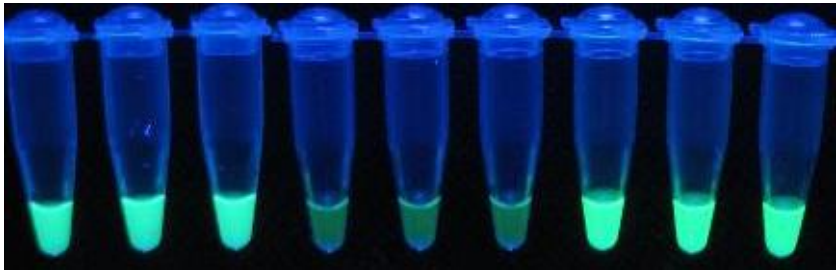
Closed system
Sample treatment buffer (SR) high inactivation activity

Issues

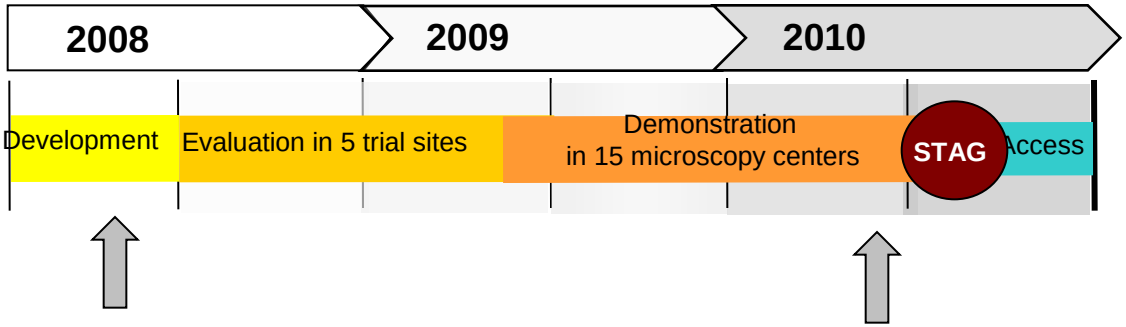
- Expensive equipment
  ($30,000 – $50,000)
- Expensive tests - $20+ per test (if three negative tests is the standard, then $60+)
- Availability of consistent power
- Availability of repair
- Quality assurance material

Molecular meets Microscopy: TB LAMP

LAMP demonstration at microscopy center in Dharavi slum, Mumbai, India



https://en.wikipedia.org/wiki/Loop-mediated_isothermal_amplification



Challenges upstream:
Reaching required sensitivity & simplicity

Challenges downstream:
Implementation of disruptive technology
Laboratory preparedness

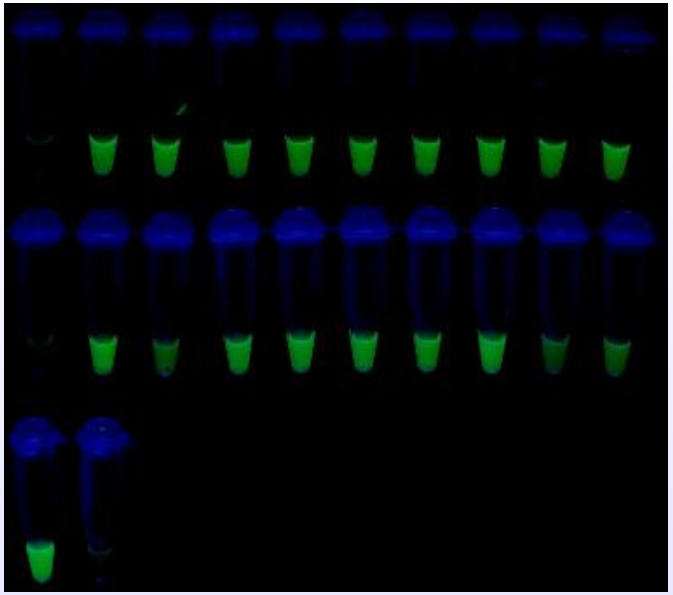
A technology platform:

- TB
- Malaria
- HAT
- Potential for …

LAMP NAAT Tests

A technology platform for:

- TB
- Malaria
- HAT
- HIV - early infant diagnosis



On the way to a POC: The search for TB antigens

Urinary LAM assay: Potential application in HIV positive patients?

Site	Overall Sensitivity	Sensitivity in HIV-infected	Sensitivity in HIV-uninfected	Specificity
Mbeya	51%	65%	34%	93%
Dar Es Salaam	65%	65%		86%
Harare	44%	52%	21%	89%
Cape Town 1	59%	67%	14%	96%
Cape Town 2	38%	38%		100%

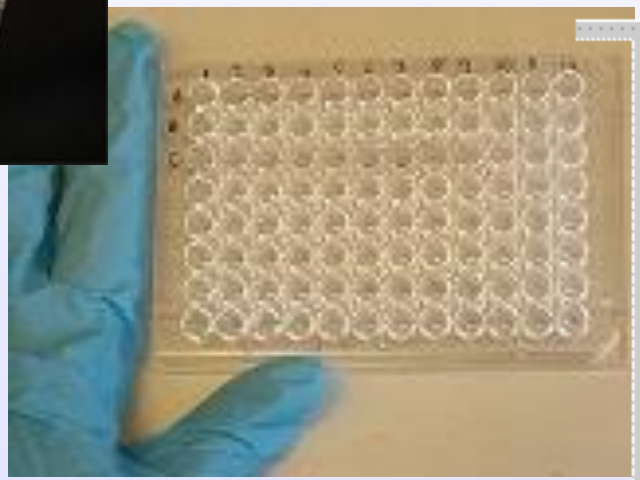
Courtesy FIND

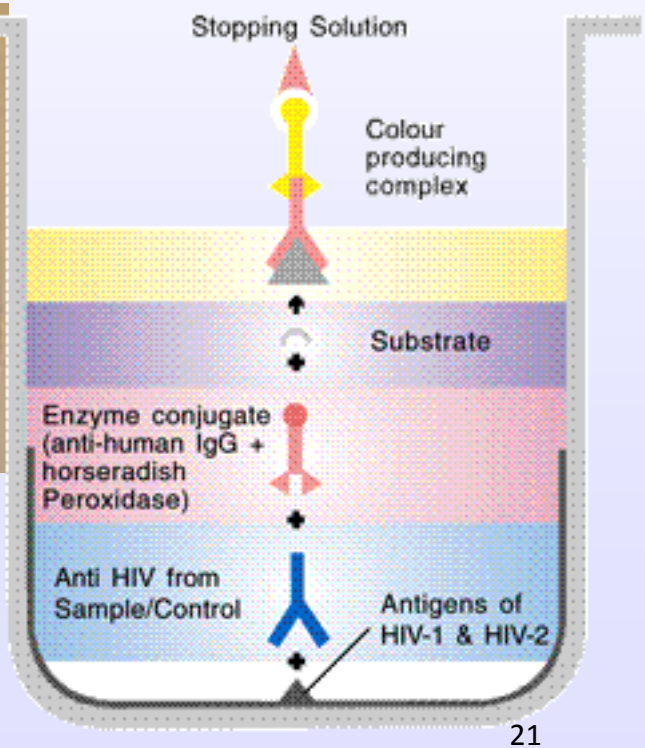
Evolution of HIV Tests

- First Generation - detection of antibody to viral antigens (viral lysate) and polyclonal antibody to human immunoglobulin.

- Second Generation - detection of antibody to recombinant antigens (HIV-1&+2)

Enzyme Immunoassay (EIA)





Test Principle

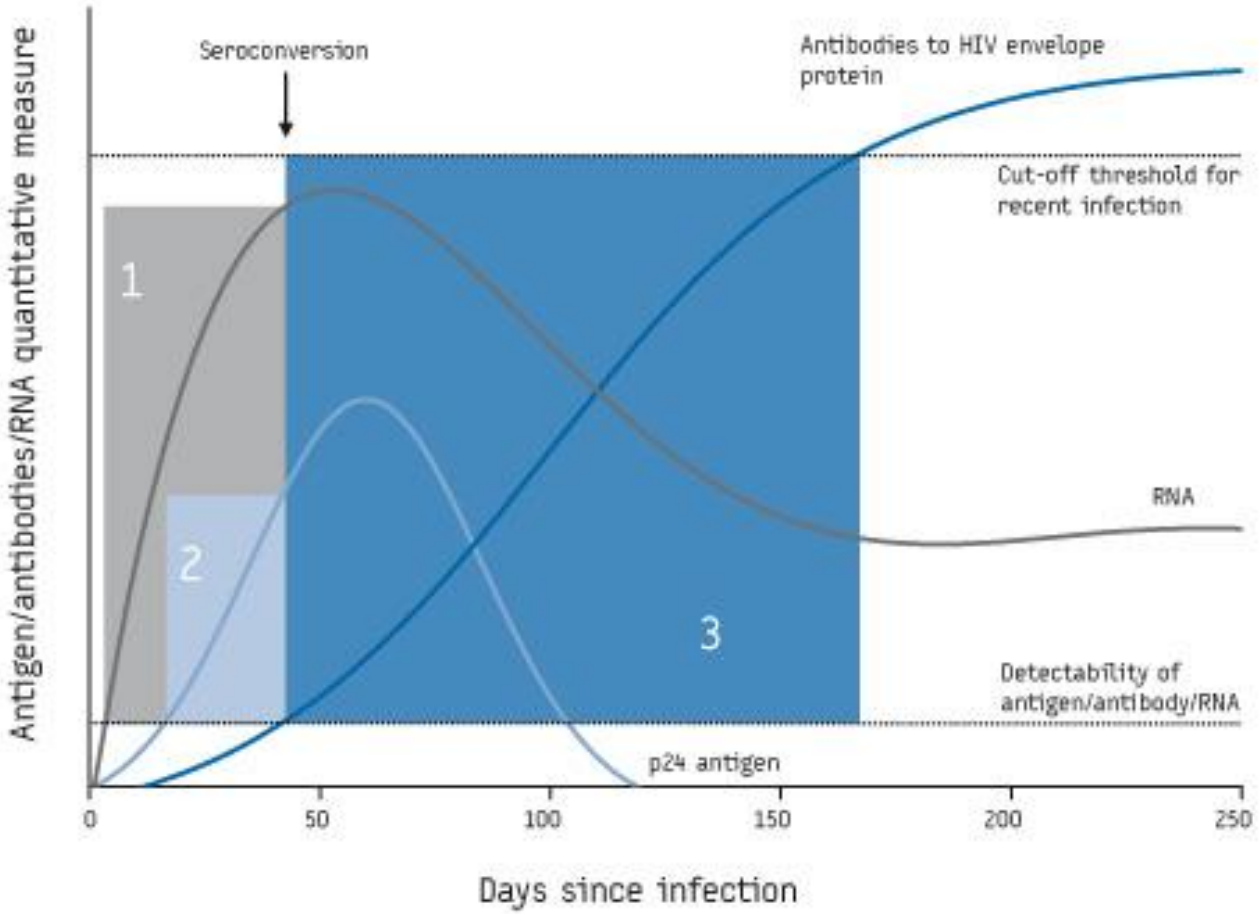
Complex sequence requiring hands on expertise
Wash steps required

Evolution of HIV Tests (2)

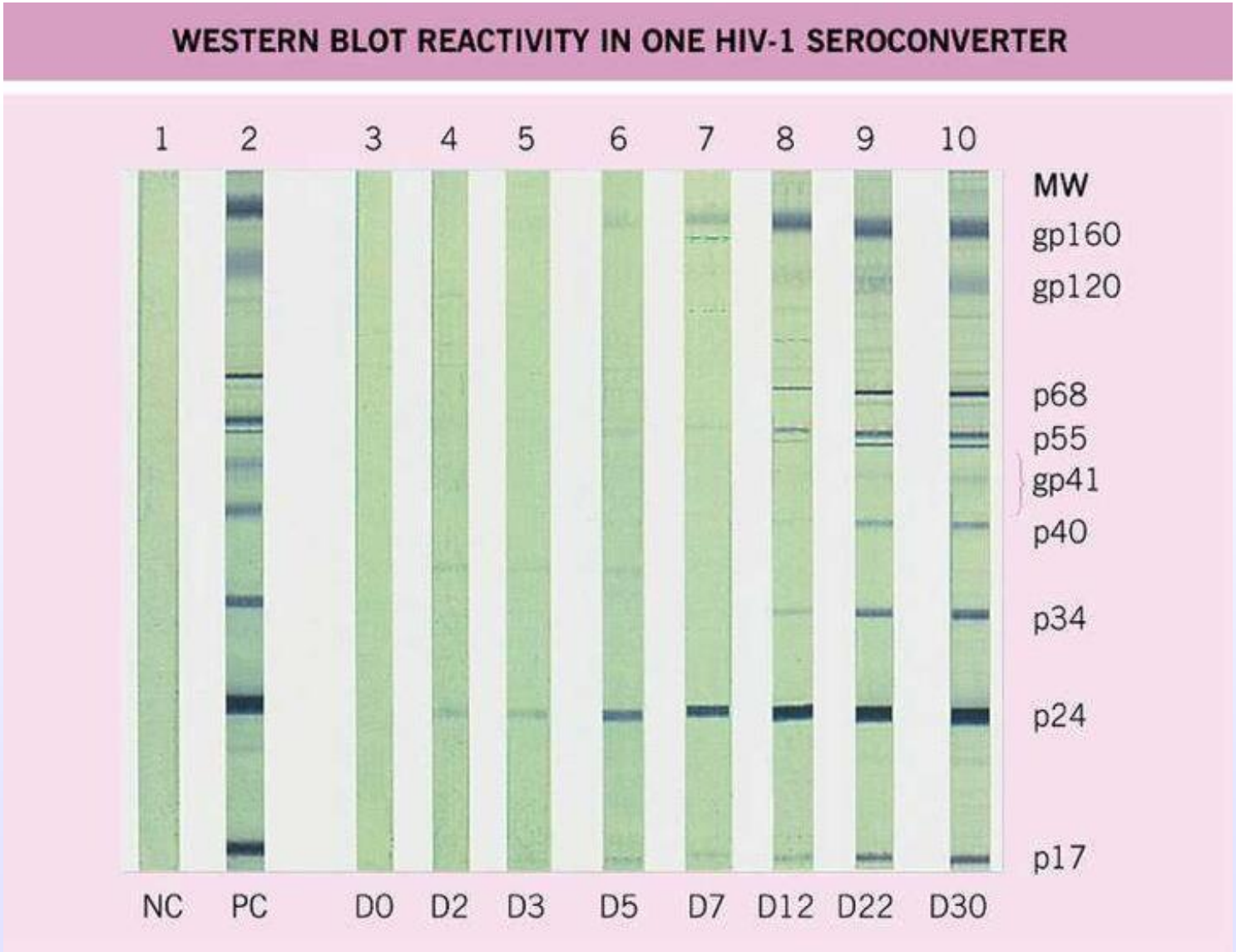
- Third Generation - recombinant antigens and/or peptides and similar antigens & peptides conjugated to a detection enzyme that could detect HIV-specific ab (including IgM) bound to a solid phase.
- Fourth Generation - HIV-1 +2 antibody and p24 antigen of HIV1in a single test
- Fifth Generation- HIV-1 +2 Nucleic acid detection (NAT)

FIGURE 1

Kinetics of virological markers and host immune response used to define transient states in the early phase of HIV infection



1: RNA-to-seroconversion transient state as defined by Busch *et al.*, 2005 [2]
2: p24-to-seroconversion transient state as defined by Brookmeyer *et al.*, 1995 [1]
3: Antibody-based mean window period as defined by Janssen *et al.*, 1998 [4]



Detecting patient antibodies directed against specific HIV antigens (p24 etc)

Summary of assay performance

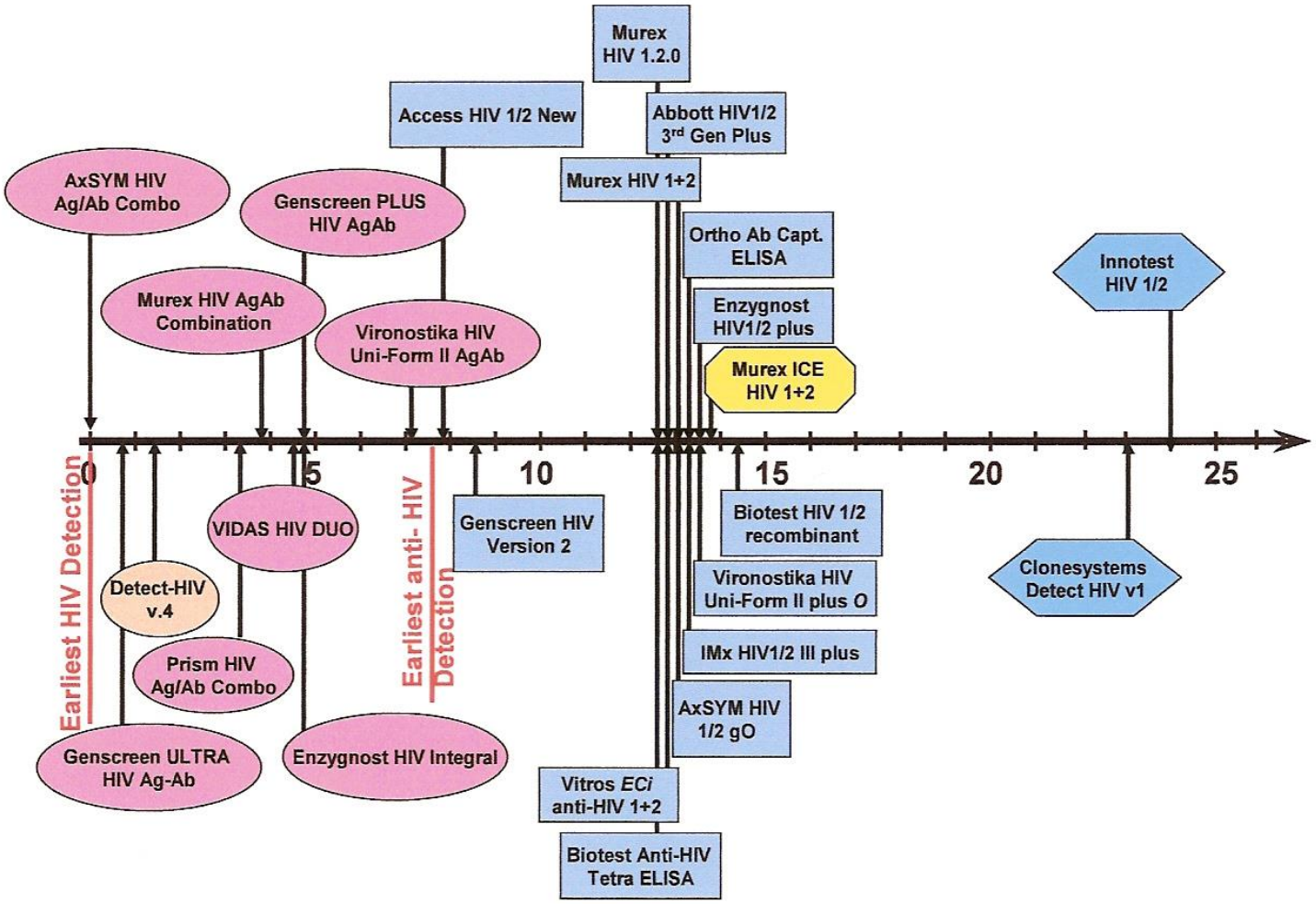
# Positive samples detected by:	Seroconversion (n=133)	HIV Ab (n=50)	HIV Ag variants (n=34)	Total (n=217)
AxSYM	80	49	22	151
Murex	2	43	25	140
Vidas Duo Ultra	73	46	19	138
Vidas Duo	62	46	9	117
Genscreen Plus	67	46	7	130
Enzygnost	54	47	1	102
Vironostika	50	44	0	94

Ly, TD; et al. J Clin Microbiol V39(9)3122-28. Seven Human Immunodeficiency Virus (HIV) Antigen-Anibody Combination Assays: Evaluation of HIV Seroconversion Sensitivity and Subtype Detection

Evaluation of Abbott Architect HIV Ag/Ab Combo Assay Burgess, C. et. al. NHS Blood and Transplant.

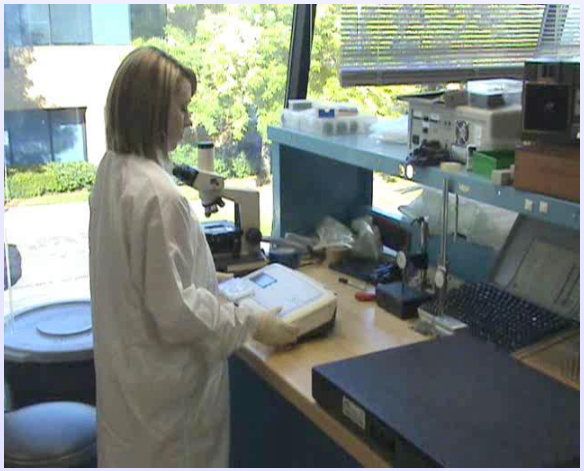
HPA-MiDAS and NBS-NTMRL, April 2008.

Figure 2: Comparative timing of detection of primary HIV infection following seroconversion.

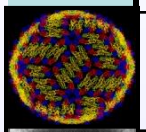
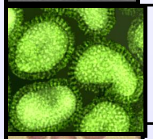
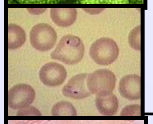
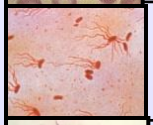
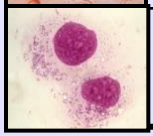


The DxBox (Diagnostics Box)

- A platform for rapid differential diagnosis of disease states
- Ability to detect 6 pathogens in a single sample by 2 methods
- PCR for amplification of nucleic acids
- Immunoassay for detection of antigens and antibodies
- Turnaround time of ~30 minutes per sample
- Disposable contains all reagents
- On-card calibration and test validation
- Small, lightweight, rugged, battery-powered instrument
- Simple operation
- Low cost
- Low maintenance

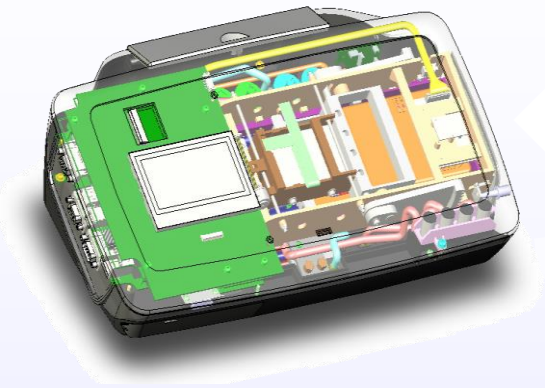


DxBox Initial Targets: Fever-Causing Pathogens in Whole Blood

	Pathogen class	Disease	DxBox analytes
	RNA virus	Dengue	Pan-dengue (serotypes1-4) RNA and IgM
	RNA virus	Measles	Measles RNA and IgM
	RNA virus	Influenza	Pan-Influenza A & B RNA and IgM
	Protozoan Parasite	Malaria	*Plasmodium falciparum* & pan-*Plasmodium* *RNA, DNA and 2 antigens*
	Bacterium	Typhoid	Pan-*Salmonella* & *Salmonella typhi* *RNA,* DNA and IgM
	Bacterium	Rickettsia	Pan-*Rickettsia* (incl. *Orientia-tsutsugamushi*) RNA, DNA and IgM

Challenge: Sample to be 2-3 drops of blood from a finger stick

End of Year 4 DxBox Summary



DxBox

NA card (Micronics & Epoch)

ELISA-like IA card (Micronics)

Membrane-based IA cards (Yager)

2° Generation membrane-based cards (direct assay, new reagents)

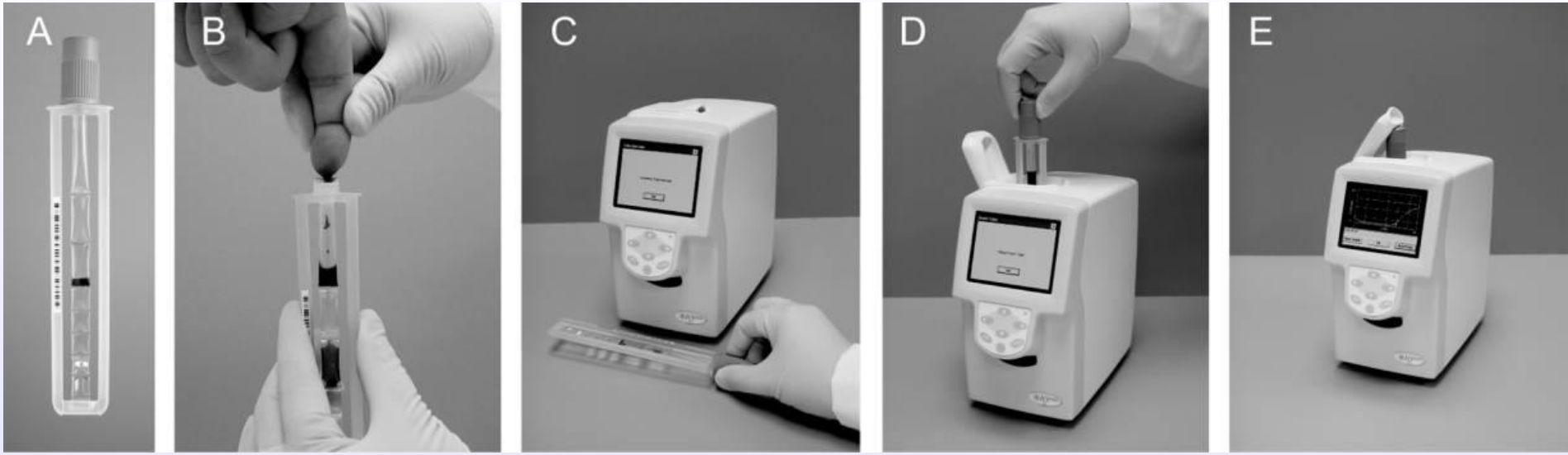
Stayton technologies

Completion of integration

Micronics PanNAT

- **Integrated closed test cartridge**: all reagents and controls integrated into the test cartridge; room temperature storage with minimal waste
- **Ease of use**: no sample pre-conditioning or treatment with minimal sample prep steps and unique sample transfer accessory
- **Portable**: lightweight for use across a range of settings; battery backup, Wi-Fi, no external computer required
- **Flexible**: low to high multiplexing using end point PCR with capability for real-time PCR and melt curves
- **Multiple Markets**: Designed to meet needs in CLIA highly and moderately complex sites as well as CLIA waived environments

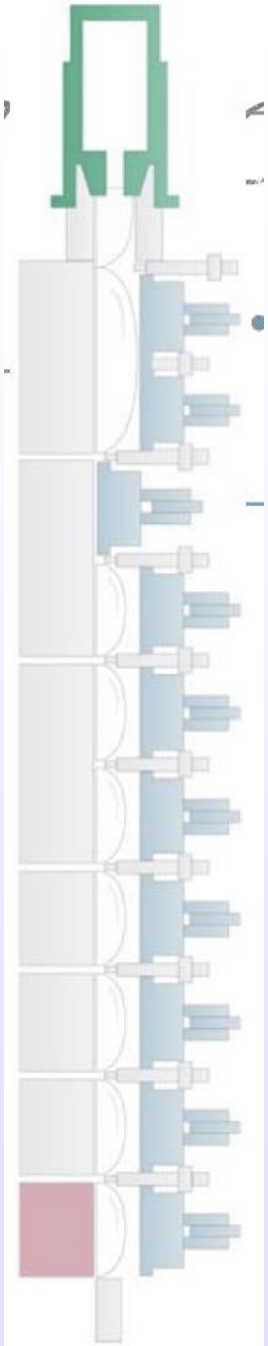
Lab in a tube (LIAT) Analyzer (Roche)



Whole blood or plasma collected into tube (A and B), analyzer scans bar cord (C), and tube inserted in analyzer (D), with results reported in 1 h.

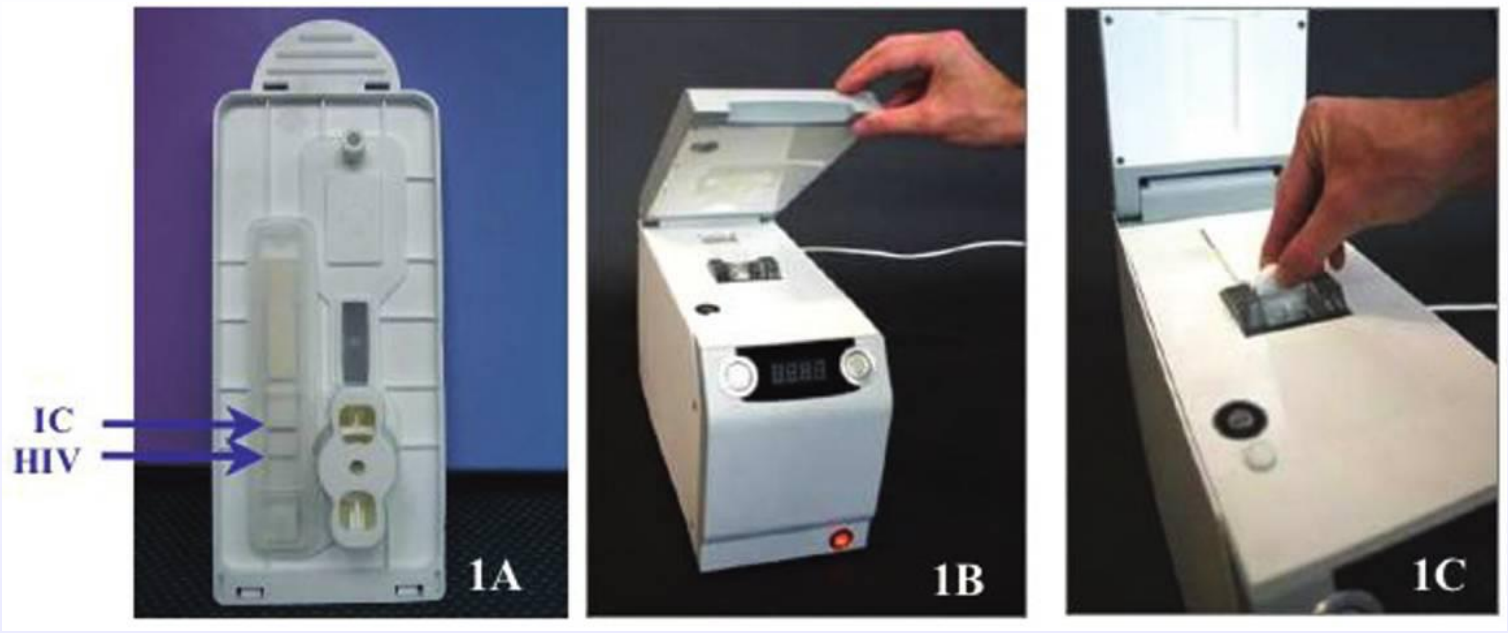
A Rapid and Automated Sample-to-Result HIV Load Test for Near-Patient Application
S. Tanriverdi, L. Chen and S. Chen JID 2010:201 (Suppl 1) S52-S58

LIAT Assay Tube



Actuator compressing assay tube liberates reagents and mixes reaction etc.

https://www.youtube.com/watch?v=WGNH7NaGqiw



Simple amplification-based assay (SAMBA) cartridge and prototype point-of-care (POC) machine. A, SAMBA cartridge showing an incorporated dipstick with a positive signal at the target (HIV) and internal control (IC) lines. *B,* POC machine. C, Loading of the SAMBA cartridge into the POC machine.

Simple Amplification-Based Assay: A Nucleic Acit-Based Point-of-Care Platform for HIV-1 Testing. *H.H. Lee et al. JID 2010:201 (Suppl 1) S65-72*

MALDI Biotyper

An example of rapid and accurate detection and identification but increasingly sophisticated technology and instrumentation.

https://en.wikipedia.org/wiki/Matrix-assisted_laser_desorption/ionization

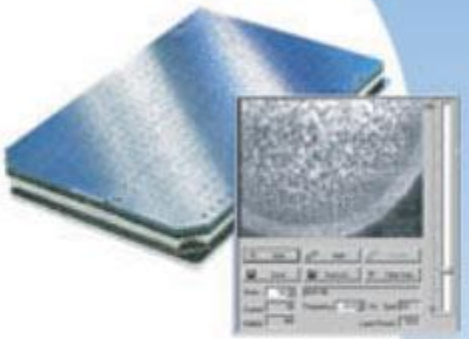
Bruker Mass Spectrometer

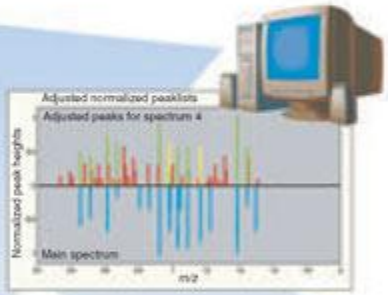
How MALDITOF is changing diagnostic microbiology: https://www.youtube.com/watch?v=5bluoI6AQ_I

Unknown
'single colony'

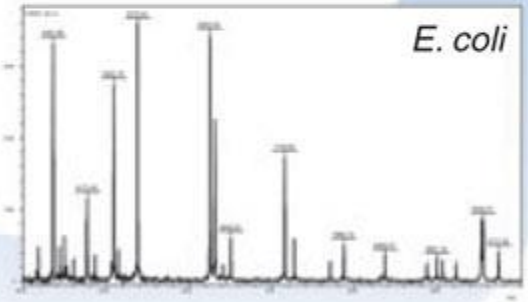
Sample preparation:
-direct thin layer without any treatment
-direct measurement of cell extracts

Add HCCA matrix solution

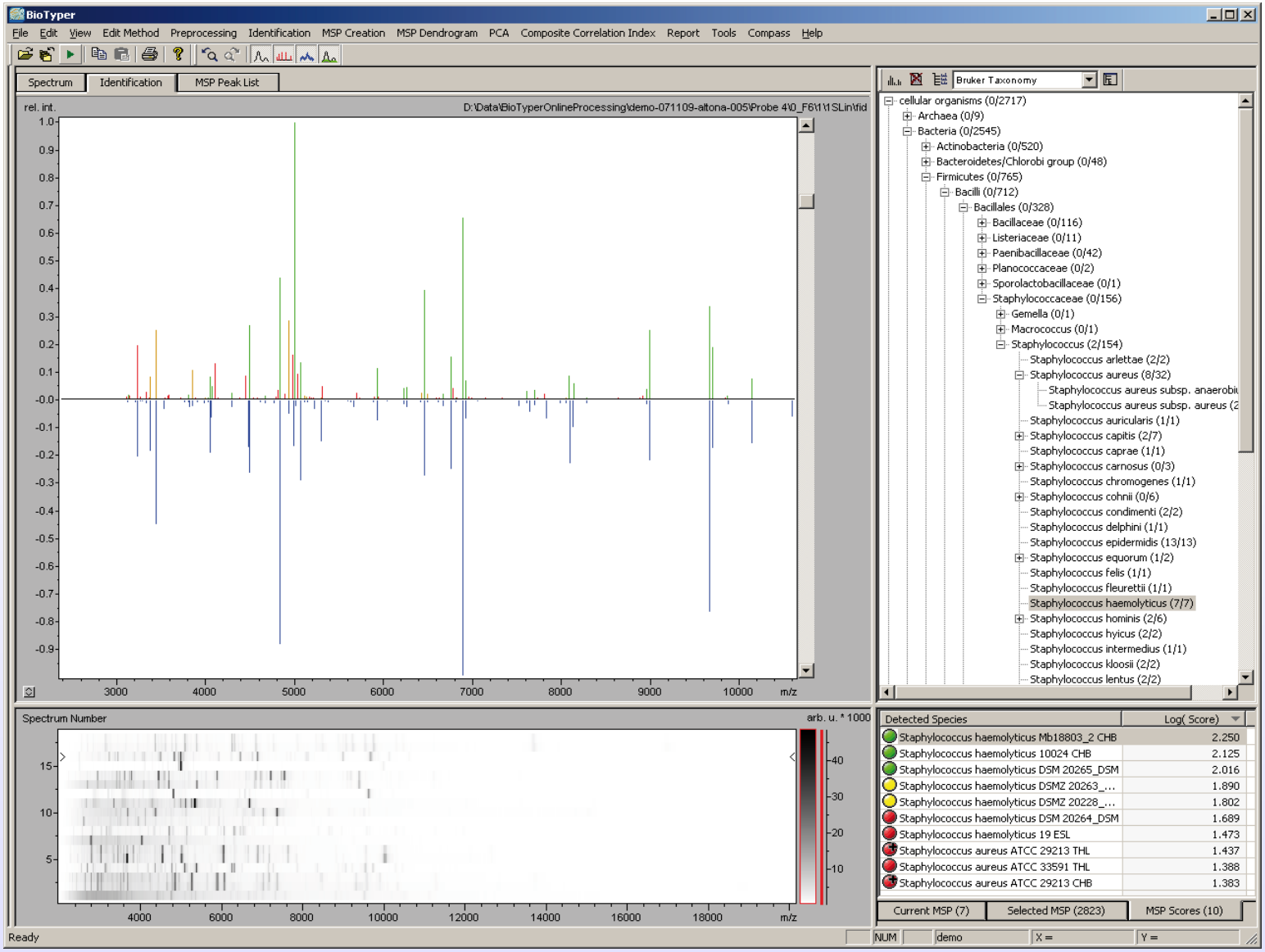




Data evaluation
identification and classification



Acquisition of MALDI-TOF MS spectra
Detection limit:
- 10^5 cells (ground steel target)
→ approx. 0.5 µg biological material
- 5 x 10^3 cells (400) µm AnchorChip™ target)
→ ~25 ng biological material


BioTyper
File Edit View Edit Method Preprocessing Identification MSP Creation MSP Dendrogram PCA Composite Correlation Index Report Tools Compass Help
Spectrum Identification MSP Peak List
rel. int.
D:\Data\BioTyperOnlineProcessing\demo-071109-altona-005\Probe 4\0_F6\1\1SLin\fid
m/z
Spectrum Number
arb. u. * 1000
Bruker Taxonomy
cellular organisms (0/2717)
Archaea (0/9)
Bacteria (0/2545)
Actinobacteria (0/520)
Bacteroidetes/Chlorobi group (0/48)
Firmicutes (0/765)
Bacilli (0/712)
Bacillales (0/328)
Bacillaceae (0/116)
Listeriaceae (0/11)
Paenibacillaceae (0/42)
Planococcaceae (0/2)
Sporolactobacillaceae (0/1)
Staphylococcaceae (0/156)
Gemella (0/1)
Macrococcus (0/1)
Staphylococcus (2/154)
Staphylococcus arlettae (2/2)
Staphylococcus aureus (8/32)
Staphylococcus auricularis (1/1)
Staphylococcus capitis (2/7)
Staphylococcus caprae (1/1)
Staphylococcus carnosus (0/3)
Staphylococcus chromogenes (1/1)
Staphylococcus cohnii (0/6)
Staphylococcus condimenti (2/2)
Staphylococcus delphini (1/1)
Staphylococcus epidermidis (13/13)
Staphylococcus equorum (1/2)
Staphylococcus felis (1/1)
Staphylococcus fleurettii (1/1)
Staphylococcus haemolyticus (7/7)
Staphylococcus hominis (2/6)
Staphylococcus hyicus (2/2)
Staphylococcus intermedius (1/1)
Staphylococcus kloosii (2/2)
Staphylococcus lentus (2/2)
Detected Species
Log(Score)
Staphylococcus haemolyticus Mb18803_2 CHB 2.250
Staphylococcus haemolyticus 10024 CHB 2.125
Staphylococcus haemolyticus DSM 20265_DSM 2.016
Staphylococcus haemolyticus DSMZ 20263_... 1.890
Staphylococcus haemolyticus DSMZ 20228_... 1.802
Staphylococcus haemolyticus DSM 20264_DSM 1.689
Staphylococcus haemolyticus 19 ESL 1.473
Staphylococcus aureus ATCC 29213 THL 1.437
Staphylococcus aureus ATCC 33591 THL 1.388
Staphylococcus aureus ATCC 29213 CHB 1.383
Current MSP (7)
Selected MSP (2823)
MSP Scores (10)
Ready
NUM
demo
X =
Y =

Biofire Respiratory Panel

The FILMARRAY® Respiratory Panel is incredibly comprehensive, with **simultaneous testing for 20 of the most common pathogens involved in UTRI**.

Viruses	Bacteria	
Adenovirus	Influenza A/H1	*Bordetella pertussis*
Coronavirus 229E	Influenza A/H1-2009	*Chlamydophila pneumoniae*
Coronavirus HKU1	Influenza A/H3	*Mycoplasma pneumoniae*
Coronavirus OC43	Influenza B	
Coronavirus NL63	Parainfluenza 1	
Human Metapneumovirus	Parainfluenza 2	
Human Rhinovirus/Enterovirus	Parainfluenza 3	
Influenza A	Parainfluenza 4	
	RSV	

Biofire Testing Procedure

- See demonstration:

https://www.youtube.com/watch?v=V5fUwjodmz4