

HIV and Syphilis Diagnosis at DSHS Laboratory

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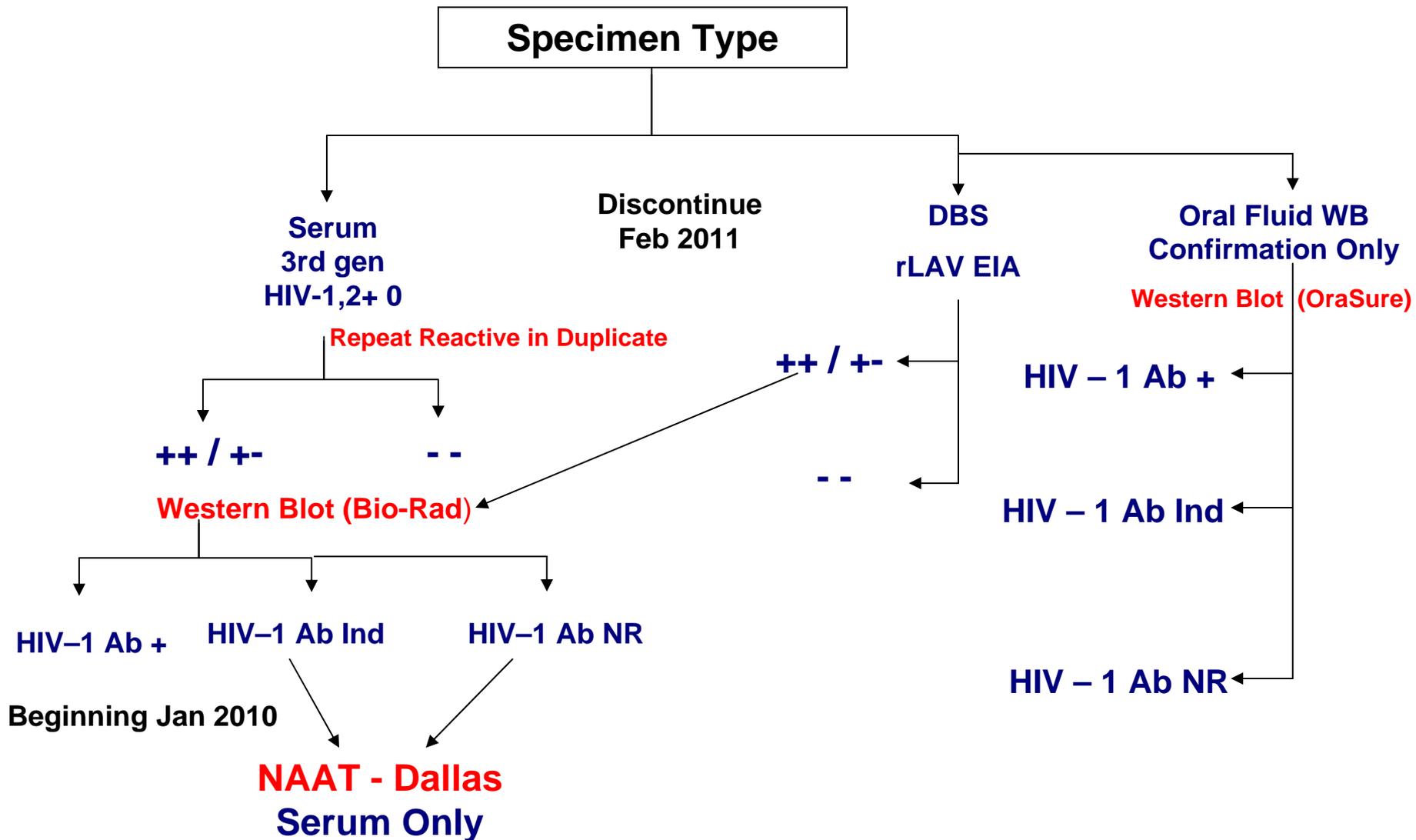
Manager, Serology Analysis Group

Laboratory Services Section

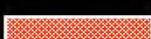
November 4, 2010

2010 HIV Prevention Contractor Summit
AT&T Conference Center

Current HIV Testing Algorithm - DSHS

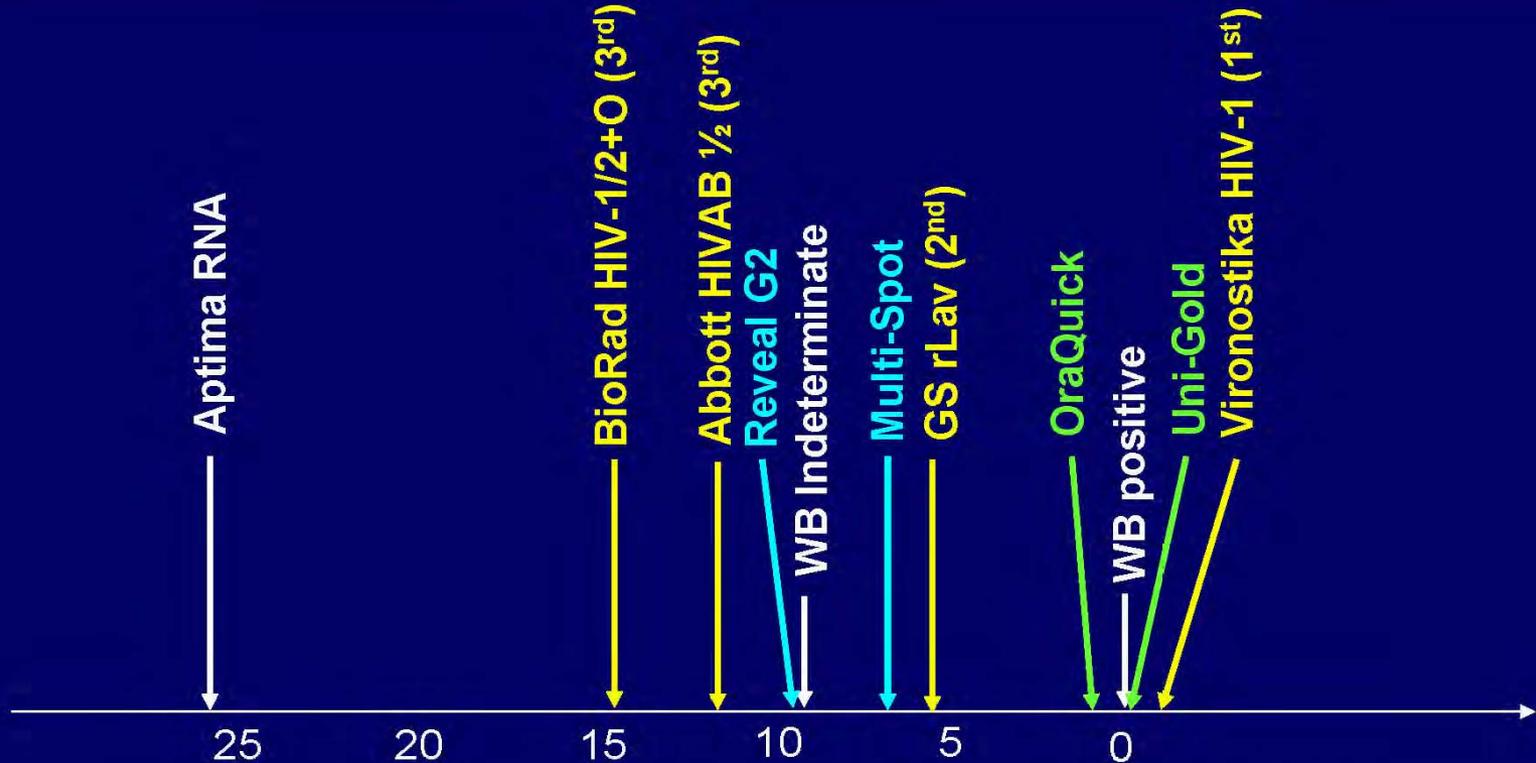


Change in Availability of EIAs

	FDA approval date		% used by PHL labs, 2006
<i>Vironostika HIV-1 Microelisa</i>	1987		58%
Abbott HIVAB HIV-1/2	1992		11%
Genetic Systems rLAV	1998		11%
Gen Sys HIV-1/HIV-2	2000		
Gen Sys HIV-1/2 Plus O	2003		20%
Siemens 1/O/2 eHIV	2006		
Ortho Vitros Anti-HIV 1+2	2008		

 viral lysate EIA
  2nd generation EIA
  3rd generation, IgM-sensitive EIA

Current Assays with 15 Seroconverter Panels

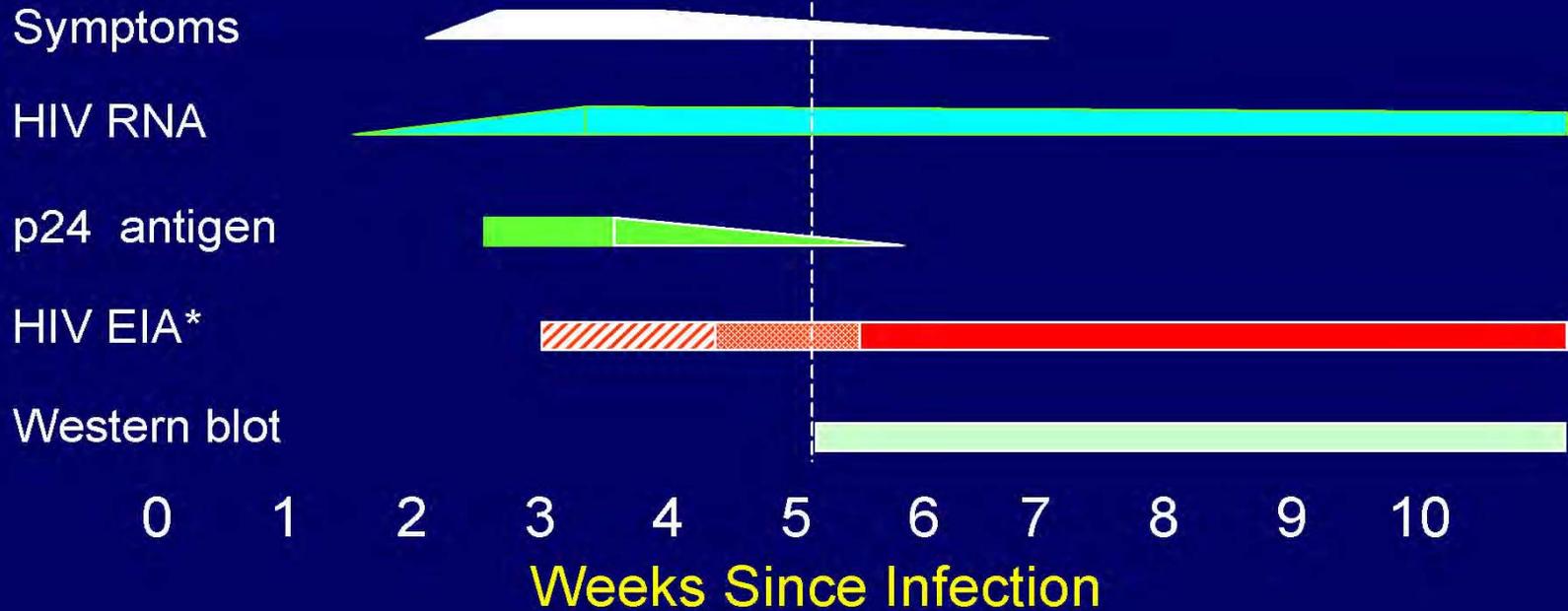


Days before Western blot positive when 50% of Specimens Reactive

185 specimens from 15 seroconverters
- Owen et al, J Clin Microbiol 2008



Detection of HIV by Diagnostic Tests



-  *3rd generation, IgM-sensitive EIA
-  *2nd generation EIA
-  *viral lysate EIA

After Fiebig et al, AIDS 2003;
17(13):1871-9

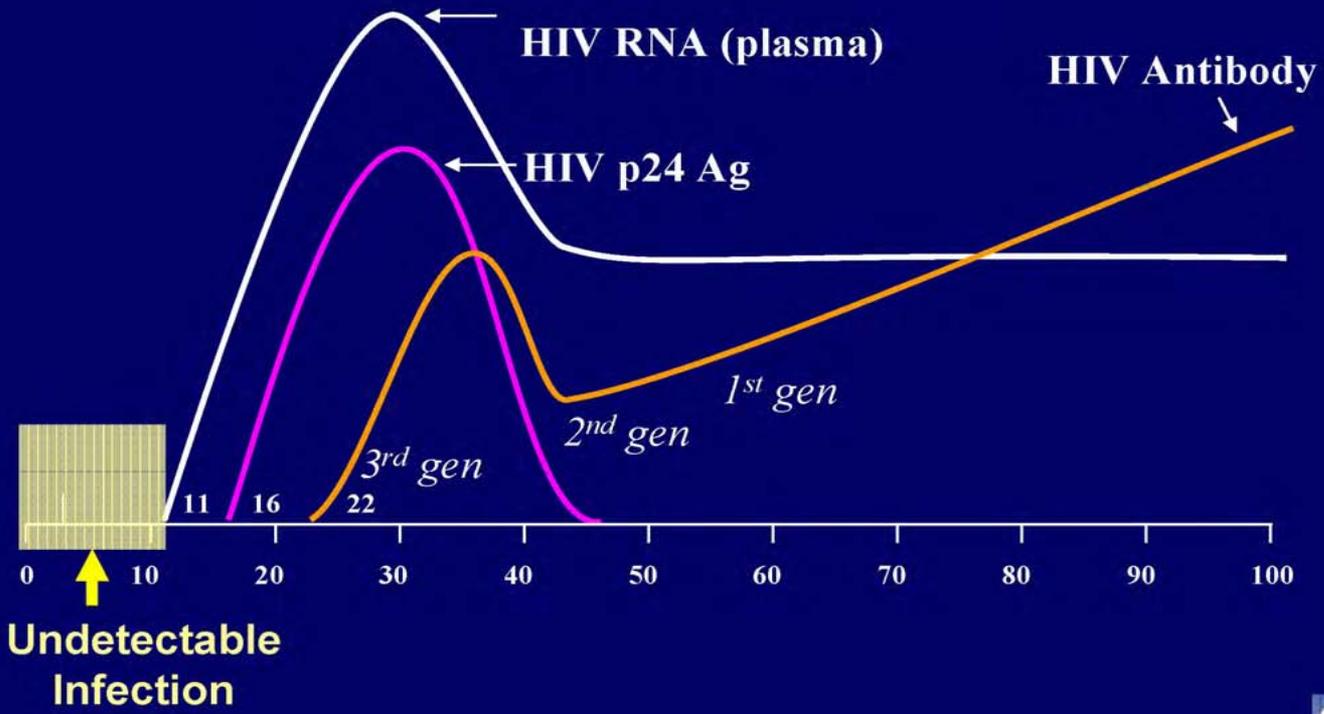


Why changing technologies?

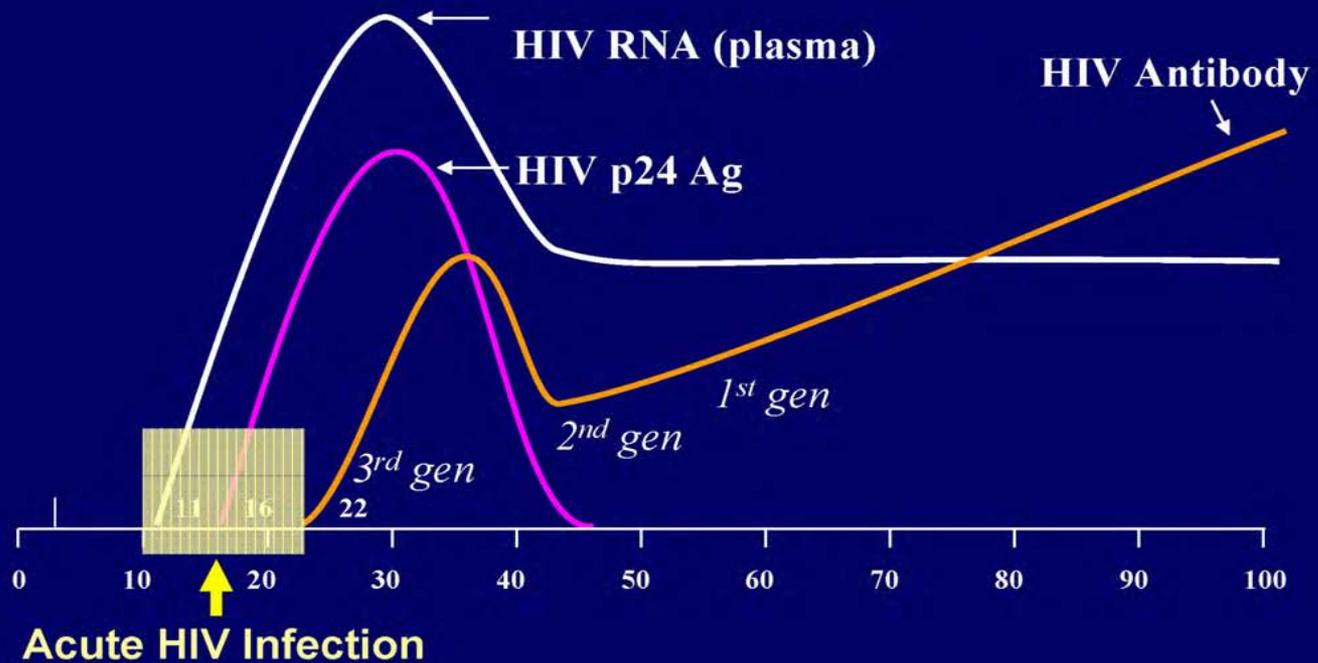
~10% of infected persons at the highest risk of transmission are not detected by Antibody tests



Windows



Window Period & Acute HIV Infection



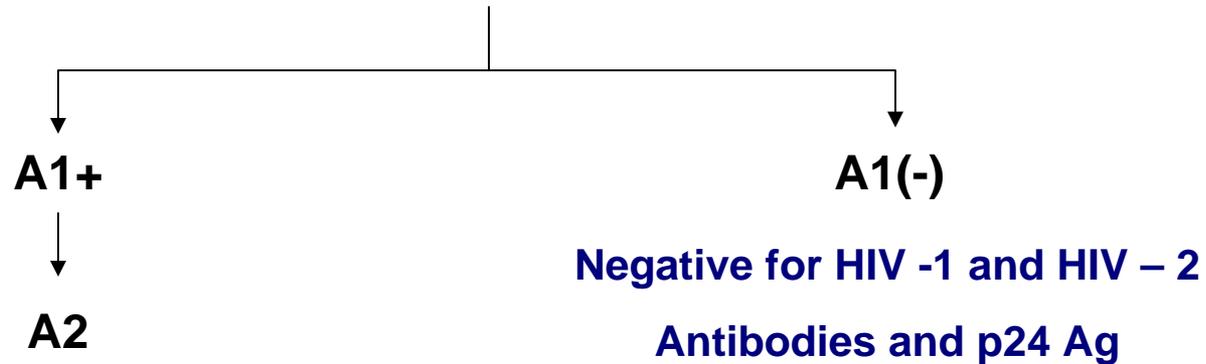
4th Gen EIAs

- **4th Generation Immunoassays – Ag/Ab combo**
 - **Abbott (Architect)**
 - **Bio-Rad (Evolis?)**
 - **Ortho**

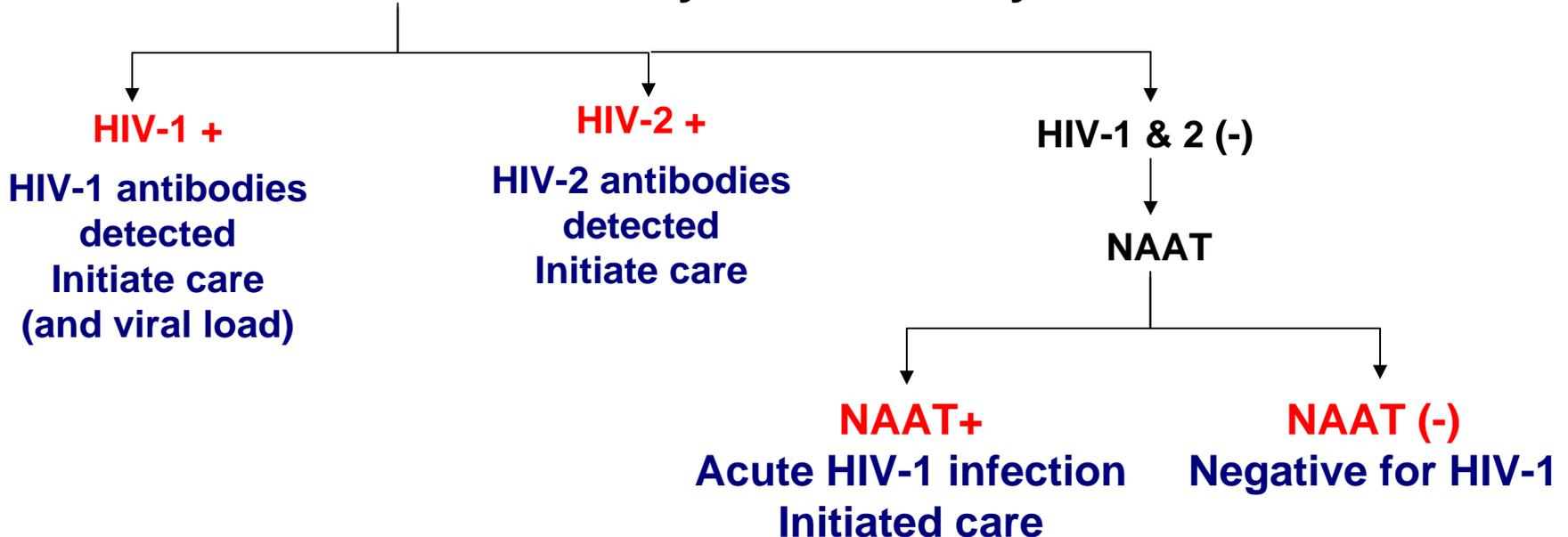
When ? End of 2010 or early 2011?

(Abbott (AxSym-Canada) approved test)

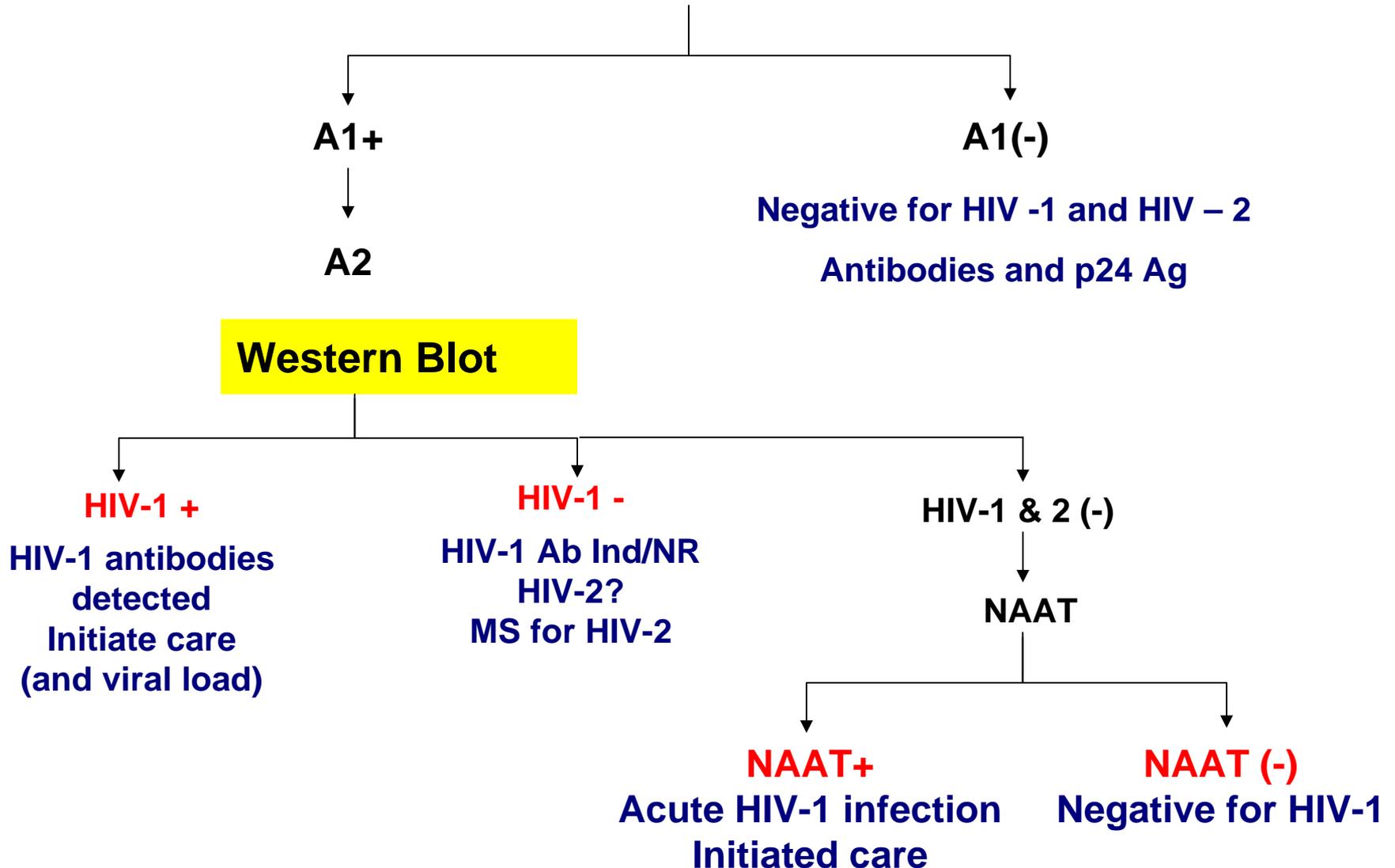
A1: 4th generation HIV-1/2 immunoassay - Proposed



HIV-1 / HIV-2 discriminatory immunoassay



A1: 4th generation HIV-1/2 immunoassay - Suggested



Current Algorithm Suggested-DBS/Oral

By March 2011

Avioq EIA
HIV-1

Validate for Serum
(Back-up for testing)

Oral Fluid

DBS

Repeat Reactive in Duplicate

Repeat Reactive in Duplicate

++ / +-

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++ / +-

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Western Blot (OraSure)

Western Blot (Bio-Rad)

HIV-1 Ab +
Initiate care
& Viral Load

HIV-1 Ab Ind

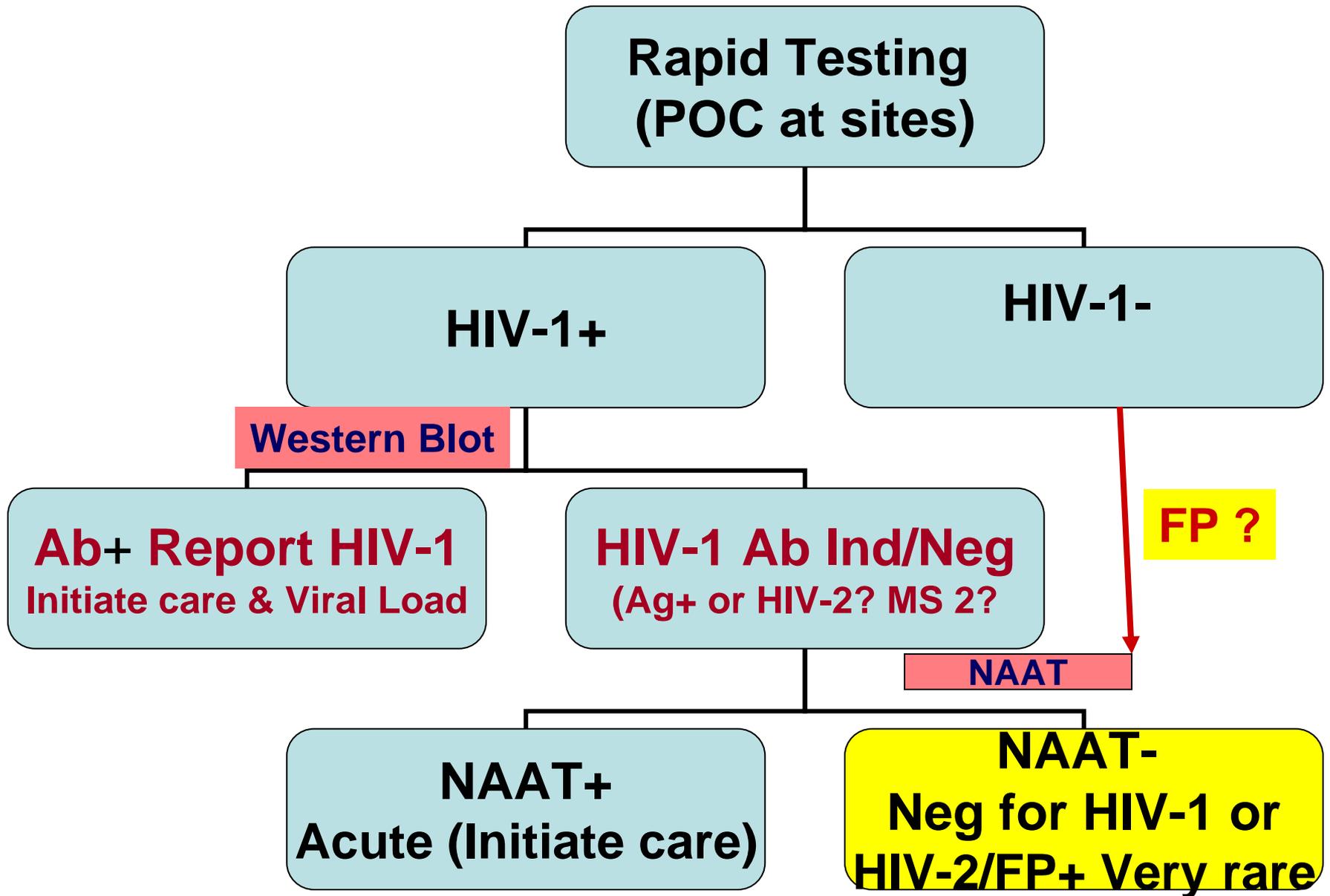
HIV-1 Ab Neg

HIV-1 Ab +
Initiate care
& Viral Load

HIV-1 Ab Ind

HIV-1 Ab Neg

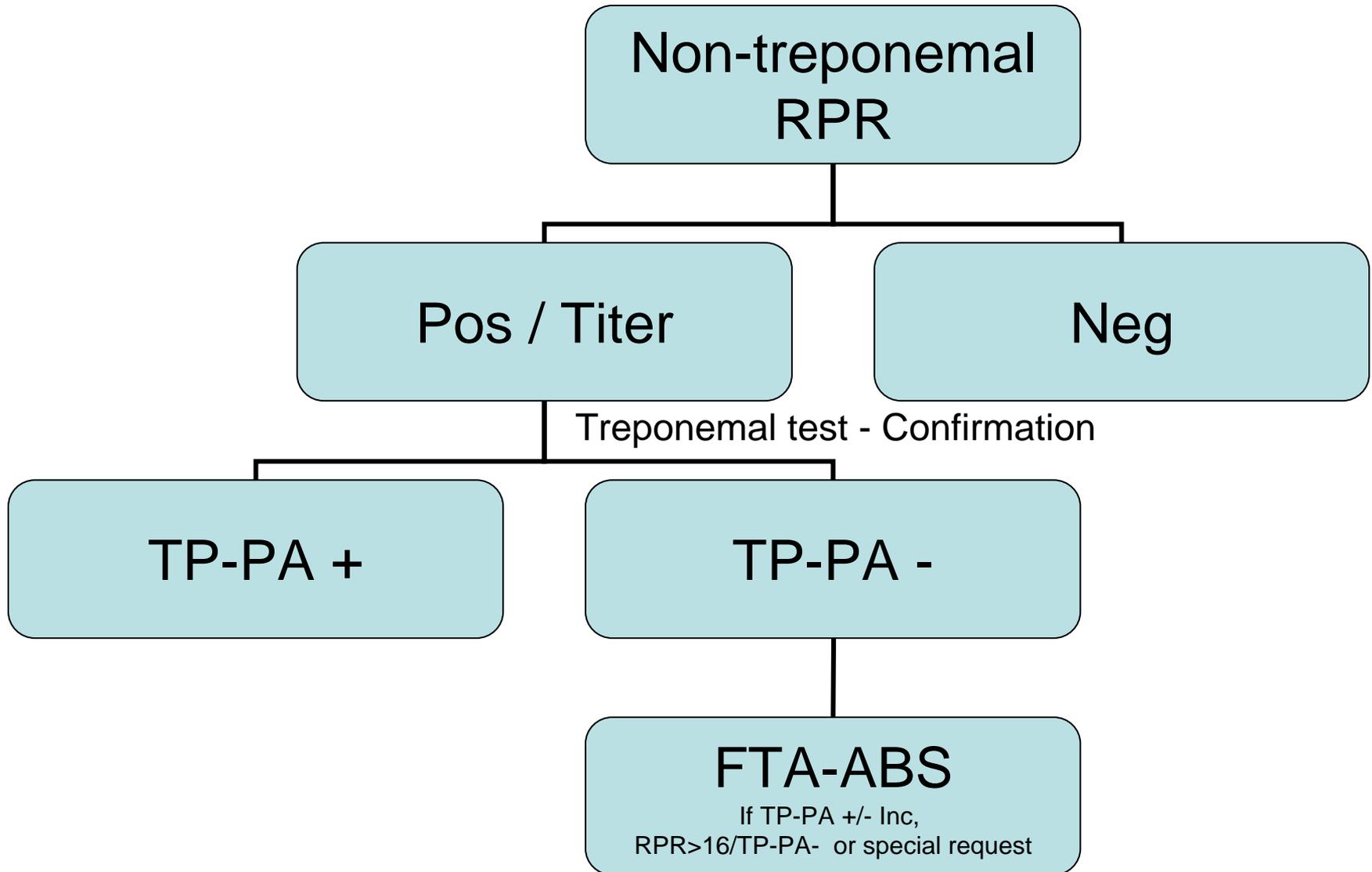
Rapid Testing Algorithm Suggested



Diagnosis of Syphilis

- Direct method
 - Darkfield microscopy
 - DFA
 - PCR
- Culture (rabbit testes inoculation)
- **Serology**
 - Non-treponemal tests
 - RPR, VDRL, TRUST
 - Treponemal tests
 - TPPA, FTA-ABS, TPHA, IgG/M EIA, CLIA, **Luminex Technology**

Current Syphilis Serology Algorithm





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MMWR

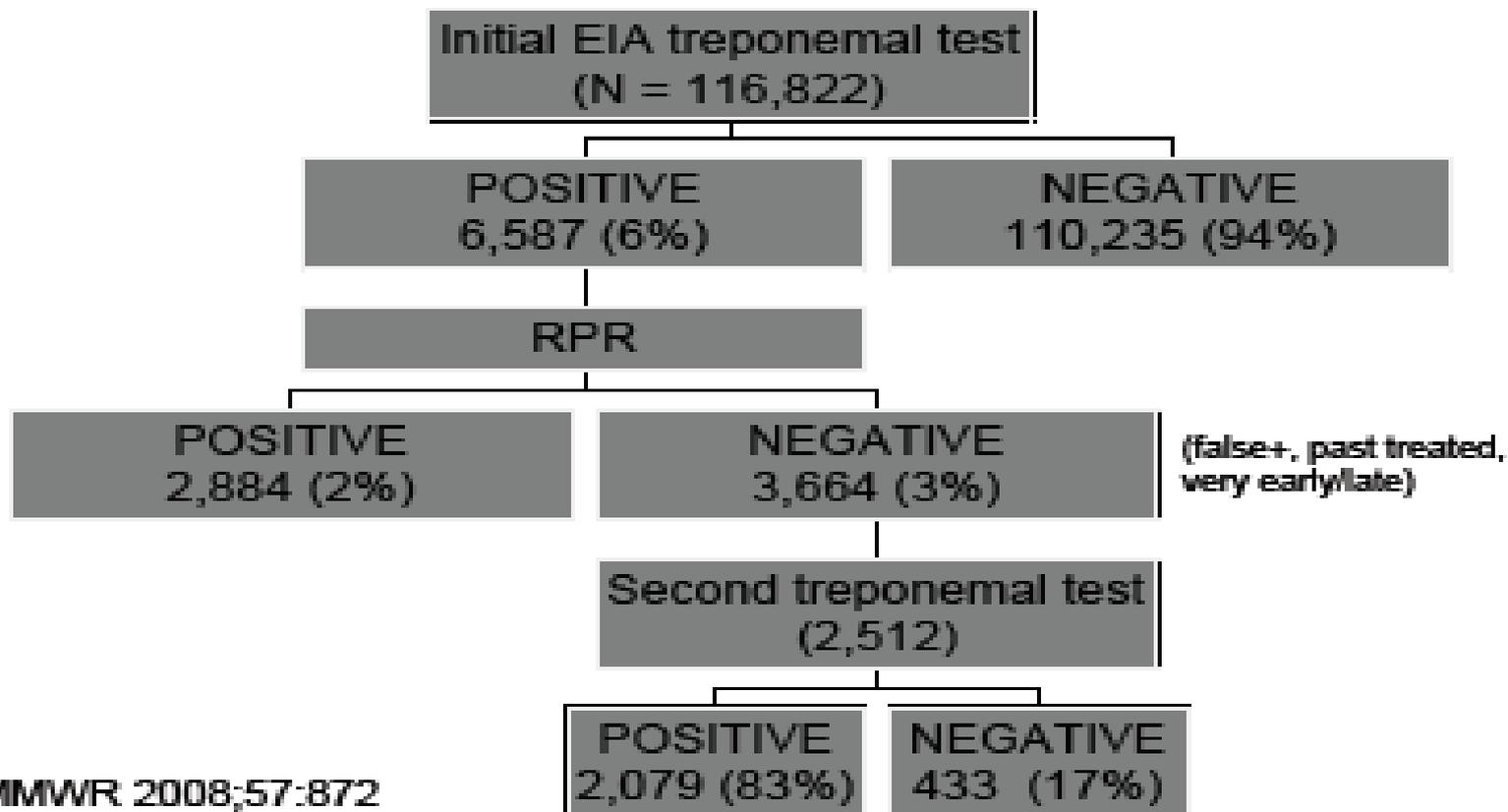
Weekly

August 15, 2008 / 57(32);872-875

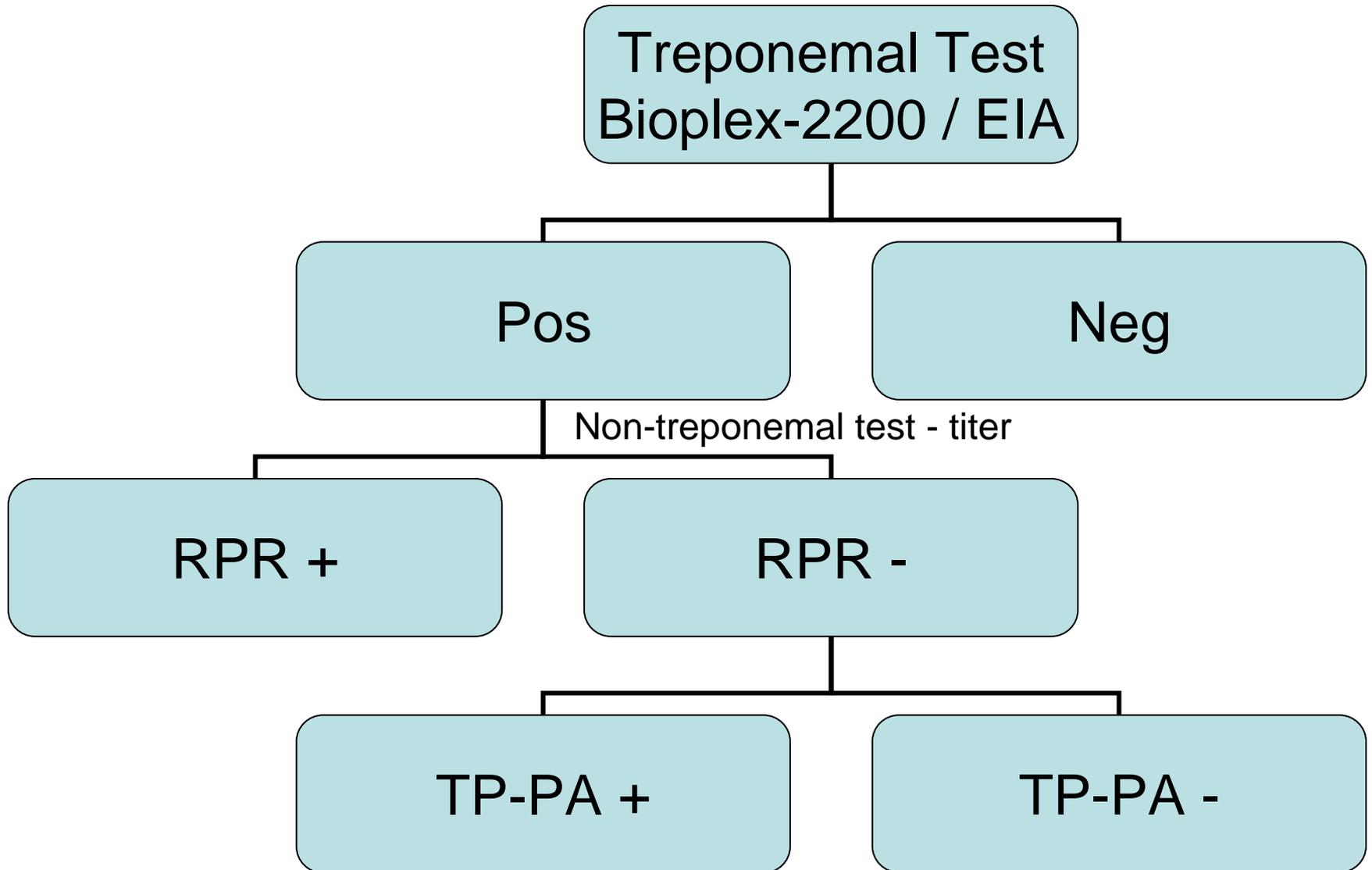
Syphilis Testing Algorithms Using Treponemal Tests for Initial Screening --- Four Laboratories, New York City, 2005--2006

New Serological Testing Algorithm

Implementation of New Testing Algorithm



Proposed Syphilis Serology Algorithm



Future of HIV Diagnostic Testing

- 4th Generation Ag/Ab Combo EIA
- SAMBA-Simple **a**mplification-**b**ased nucleic acid test for HIV-1, Dipstick-based visual detection
- MICT-**M**agnetic **I**mmuno-**C**hromatography **T**esting for rapid HIV-1 p24 Ag
- Rapid confirmation for HIV-1/HIV-2 gp105 band-Brazil
- Next generation POC-handheld microfluidic based
- Battery powered instrumentation for reading

Diagnostics for the Real World: SAMBA Device and Point of Care Machine



Front view



Rear view

Simple technology

- Sample preparation module in development
- Cartridge with breakable seals
- Isothermal NA amplification ~1 hr
- Dipstick-based visual detection

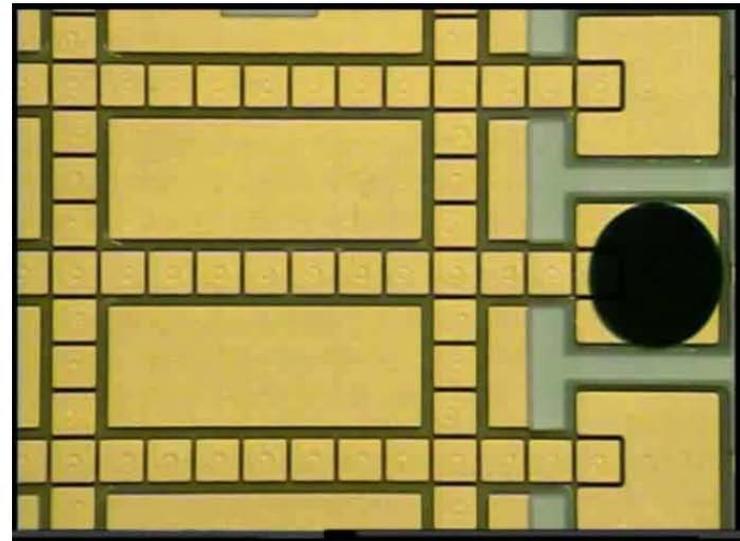
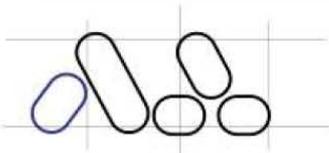
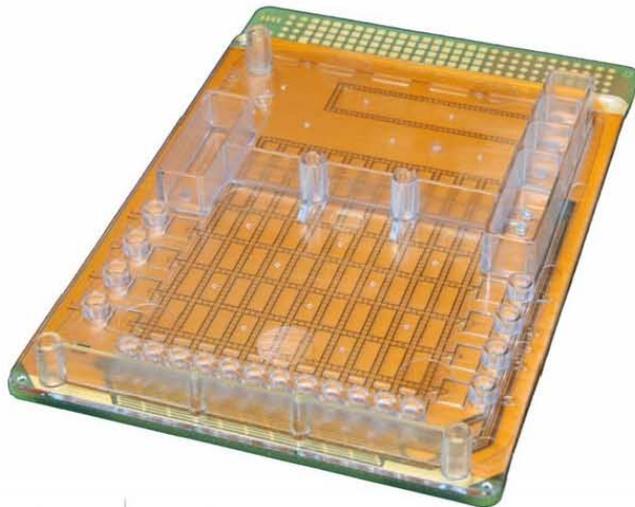
SAMBA (Simple Amplification Based nucleic acid test) machine



Advanced Liquid Logic

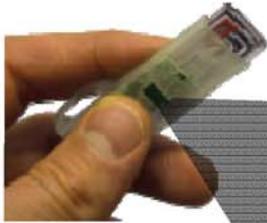
Digital microfluidics

- Cartridge is fabricated using low-cost printed-circuit-board technology
- No pipes, pumps or valves
- Discrete droplets are manipulated electrically (electrowetting) within an oil-filled cartridge
- Use whole blood with a magnetic bead capture protocol



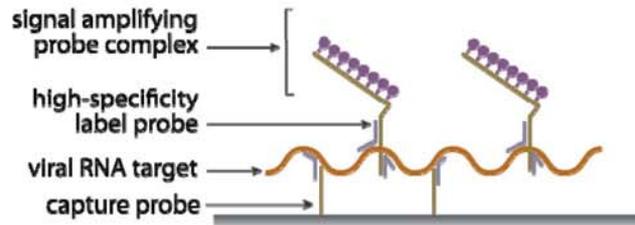
WAVE 80 Biosciences

L. Mazzola: Poster #40



- Continuous-flow microfluidics
- Onboard lyophilized reagents
- No fluid exchange
- Disposable
- Finger-stick sampling
- Licensed microchip and assay technology
- Wave 80 proprietary IP

Branched DNA-like Nucleic Acid Signal Amplification



- Highly sensitive signal amplification
- No risk of amplifying non-targeted RNA
- No temperature or stability issues



- Flexible instrument design
- Luminescent readout
- Robust operation
- Low maintenance

wave 80

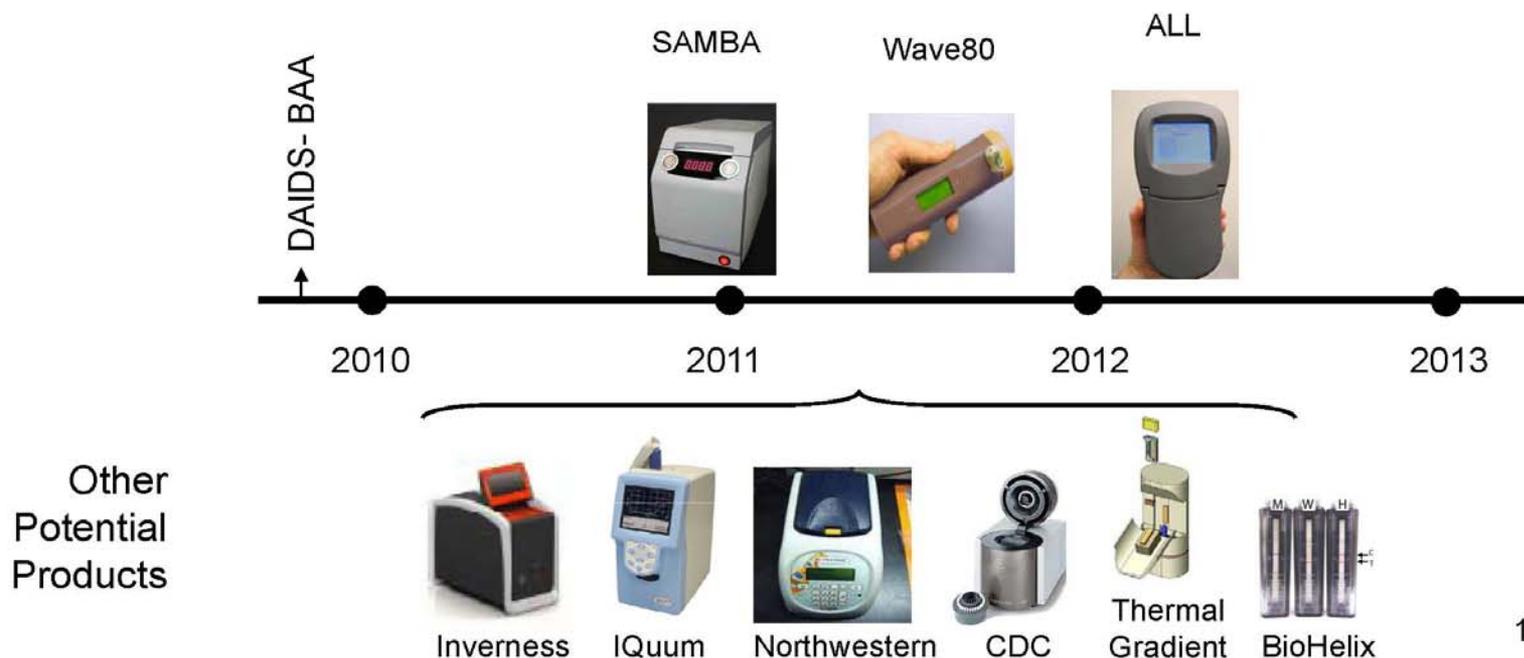
POC Technology Pipeline

Near POC

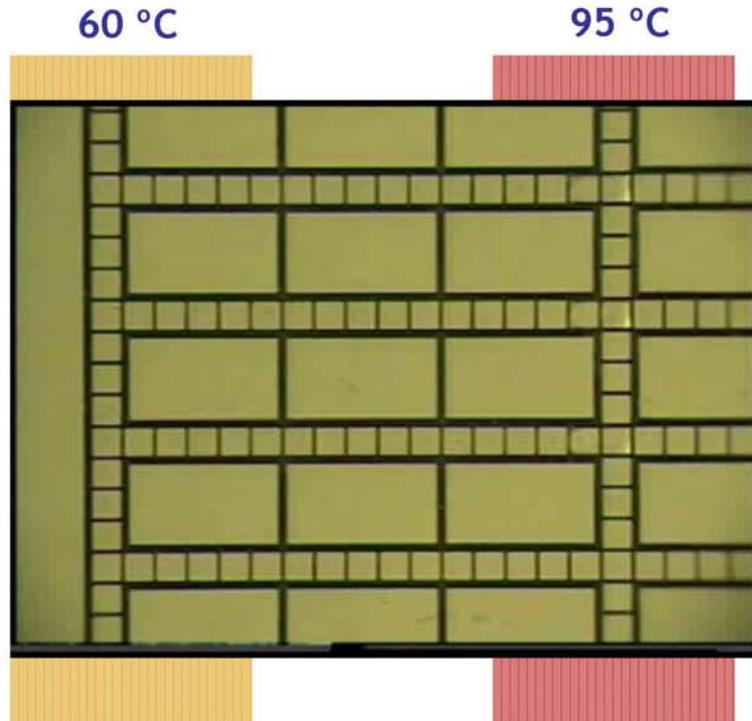
- Tabletop assays using finger-stick blood will be evaluated in clinical trials over the next year (DAIDS can assist in evaluation)

Next generation POC

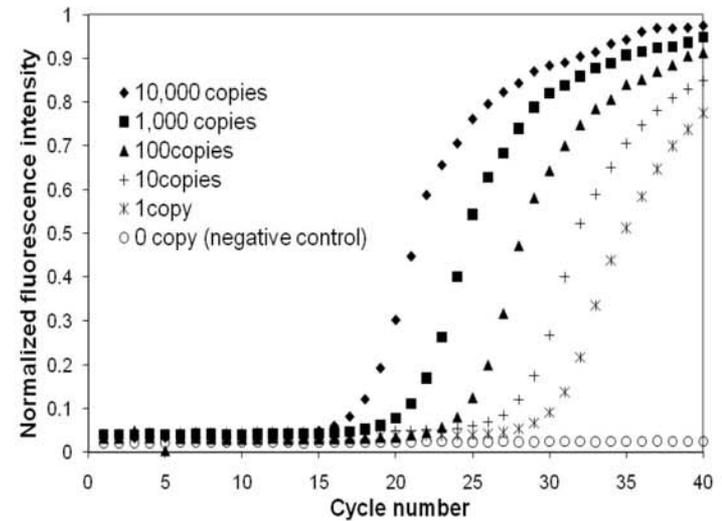
- Handheld microfluidic-based battery powered assays require an additional year of development before clinical trials



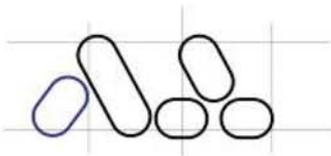
Flow-Through Real-Time PCR



MRSA Titration

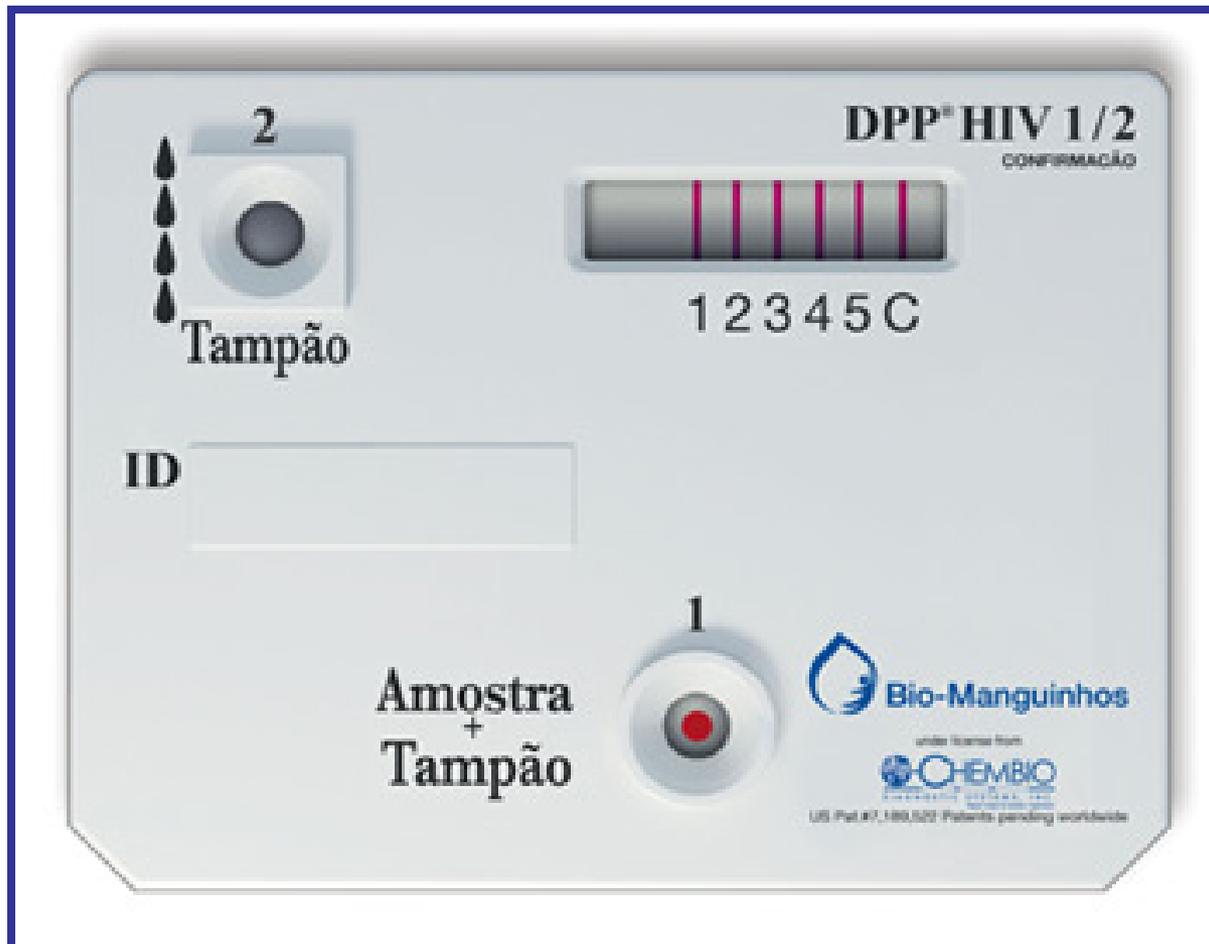


Hua et al., Analytical Chemistry, 2010

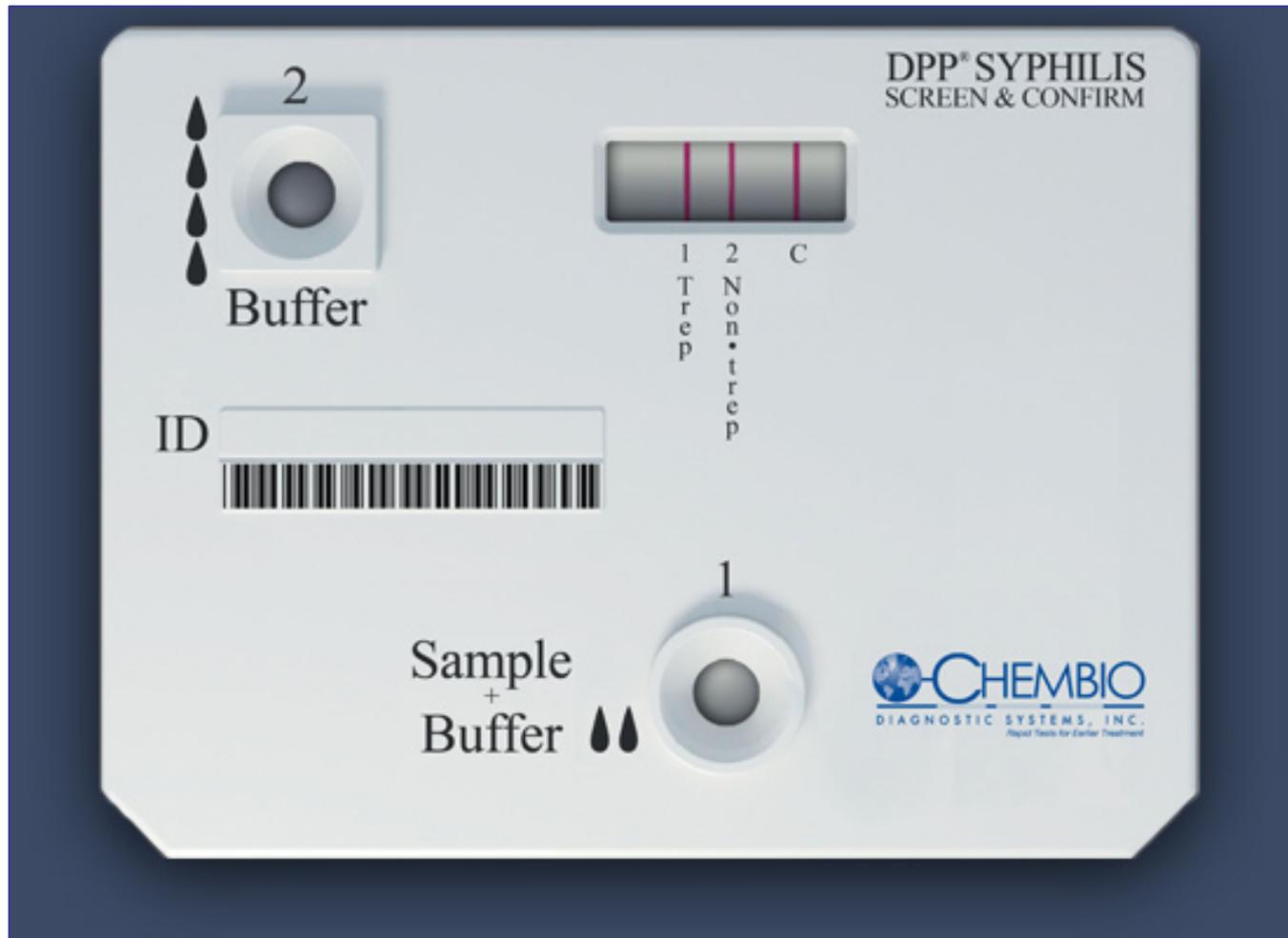


Multiplex POC Test for Confirmation of HIV 1&2

Chembio Diagnostic Systems, INC.-The Dual Path Platform (DPP®) Technology



DPP–Syphilis Screen & Confirmation





BioPlex™ 2200

from Bio-Rad Laboratories





Multiplex features



- Multiplexing is
 - The ability to perform and record multiple reactions occurring simultaneously in a single reaction vessel
- Multiplex features include:
 - Improve workflow efficiencies
 - Generate up to 22 simultaneous results from a single sample
 - Incorporate real-time assay integrity checks
 - Warehouse and retrieve unordered results
 - Reduces need to collect and run second patient sample





Multiplex science



8 micron beads

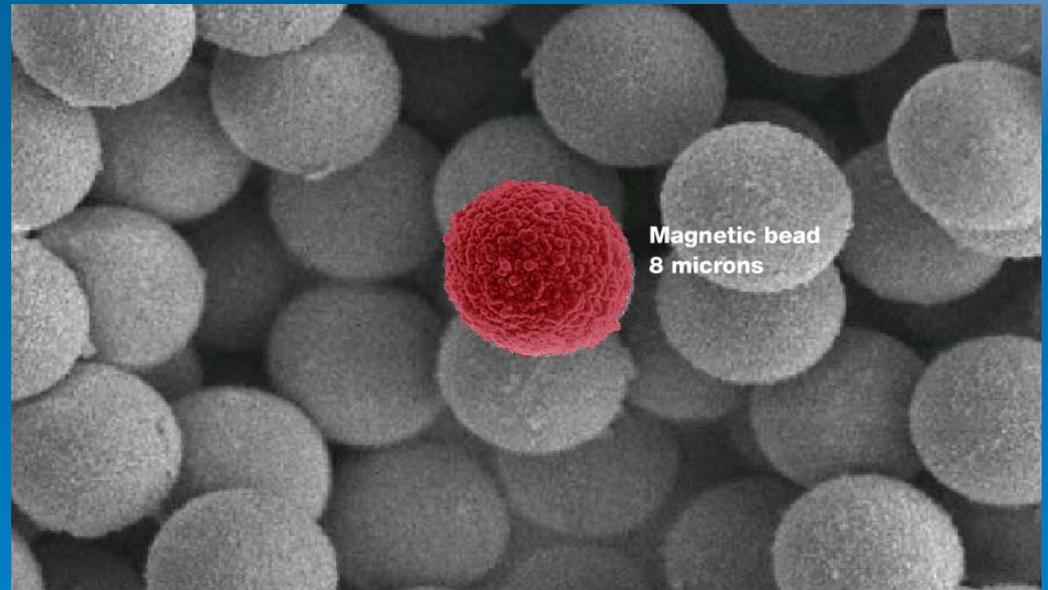
Electron micrograph of pre-dyed beads

3 coating layers

Each bead is coated with three distinct layers, internal latex core, iron crystal coating and external reactive surface

Dying process

Each bead is dyed with a ratio of yellow and red dye to create a distinct bead color. Each colored bead is unique to its respective analyte



Multiplex chemistry

Addition of patient sample

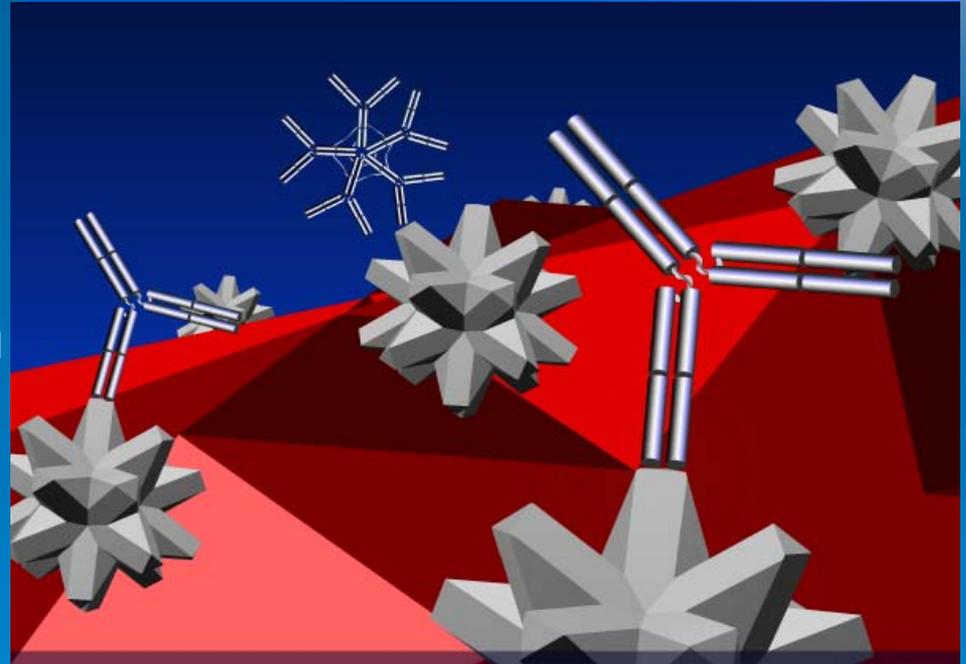
If antibodies are present in patient sample, they bind to their respective bead antigen in the reaction vessel.

Washing step

Two wash steps are employed to eliminate unbound material. First after addition and incubation with primary sample and second after addition and incubation of conjugate.

Addition of conjugate

Conjugate is added after first wash step and incubated. Second wash step removes unbound conjugate material.



Multiplex detection

Beads aspirated into probe

Aliquot of bead solution from reaction vessel is aspirated into the flow cell

Beads lined up in single file

Beads move single file through a flow cytometer

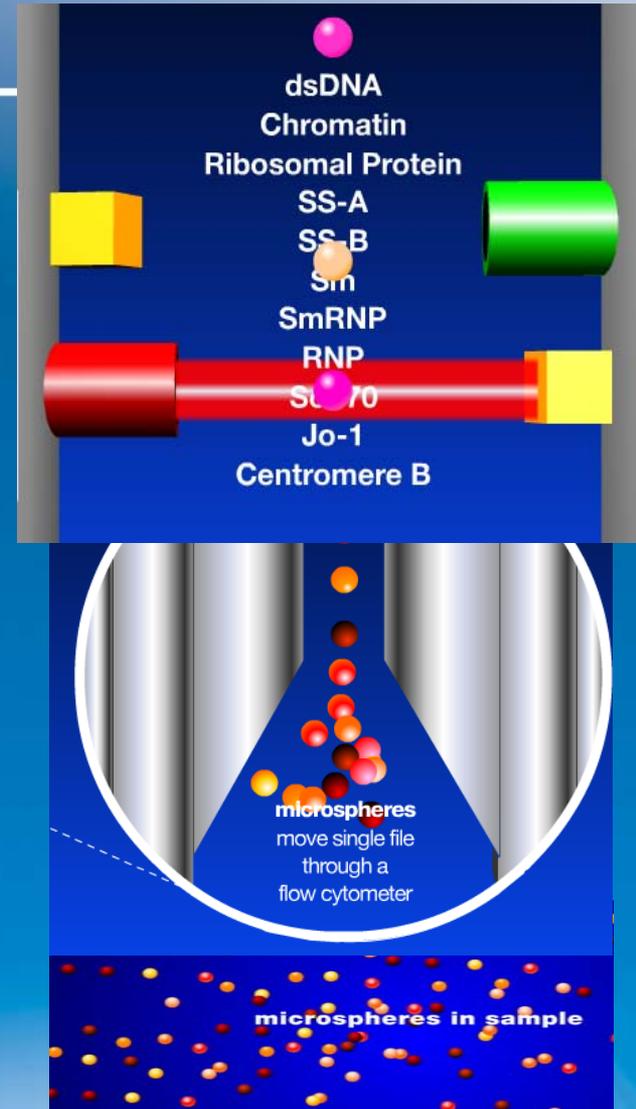
Each bead subjected to two lasers

Red or classification laser determines which bead is present

Green or reporter laser determines if bead is negative or positive and level of intensity for positive beads

Characterizes 150 beads

The BioPlex 2000 classifies a minimum of 150 beads from each analyte prior to making a determination



Multiplex IQ (internal quality) beads

Employ 3 IQ beads with each specimen

To ensure assay integrity for each patient result

Serum Verification Bead

SVB confirms that the sample is plasma or serum, flags for short sample and sample dilution

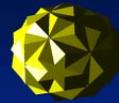
Internal Standard Bead

Real time measurement to standardize detector voltage fluctuation

Reagent Blank Bead

Identifies samples with non-specific binding antibodies

Internal Quality Control



Serum Verification Bead (SVB)

Confirms adequate sample pickup and verifies dilutions



Internal Standard Bead (ISB)

Appears in every tube to standardize detector performance



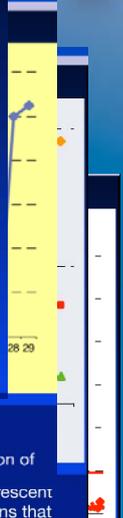
Reagent Blank Bead (RBB)

Identifies sample problems arising from non-specific binding

The Serum Verification Bead (SVB) ensures that a sufficient volume of non-dilute serum or plasma is present in samples. It does so by measuring Free Factor XIIIb, whose concentration exhibits minimal variation. This characteristic allows verification of undiluted sample as serum/plasma, and allows detection of unexpected dilutions.

The Internal Standard Bead (ISB) performs fluorescence intensity data-correction. The inherent fluorescent signal of the Internal Standard Bead is used to detect and compensate for Detector Module fluctuations that may occur during sample analysis.

The Reagent Blank Bead (RBB) is a non-protein coated bead that identifies sample problems arising from non-specific binding. Using pre-established acceptance limits, the instrument checks for elevated background results arising from non-specific binding.



BioPlex-2200



- **Advantages**

- CDC recommendation of new syphilis algorithm
- Advanced technology with interfacing to LIS
- Random access platform
- Avoid transcription error thru automation
- Limited biohazard exposure
- Reduced TAT
- Workflow advantage and savings?
- BioPlex expandable serology menu
- Space saving – no need of old EIA equipments

- **Disadvantages**

- Cost
- New algorithm interpretation ?



BioPlex 2200 Current Menu

510(k) cleared

- **Syphilis IgG**
 - r15, r17, r47
- **ToRC IgG/IgM**
 - Toxoplasma
 - Rubella
 - CMV
- **MMRV IgG**
 - Measles
 - Mumps
 - Rubella
 - Varizella zoster
- **HSV 1 and 2**
 - HSV 1
 - HSV 2



BioPlex 2200 Future Menu

• Other Panels –2011 +

- HIV Combo (Ag/Ab)
- Hepatitis A, B, C Panels
- Lyme Panel

- Cardiac Damage
- Cardiac Risk Assessment
- Diabetes (Hemoglobin A1c)
- Urine Toxicology
- Gastrointestinal
- Phospholipids

Future Workflow at DSHS Lab

		Serology	
	STD	VPD	Inf Dis Ser
Current	Syphilis, HIV, Hep C	MMRV, Hep B & A	Arbo, Toxo, CMV, Lyme QuantIFERON-TB Rick/Ehrl, CSD, QF, Legion Tular, Bruc, Plag Fungal
	RPR, TP-PA, IFA, and EIA	EIA, IFA	MIA, EIA, IFA, Agg, ID/CFT
2010-2011	BioPlex Syphilis IgG	MMRV - IgG	
2011-2012	BioPlex Hep C, Syphilis IgM	R - IgM, MM - IgM?	Toxo/CMV IgG, IgM
2012-2013	BioPlex HIV-1/2 Ag/Ab	Hep B & Hep A	Lyme, Arbo? as needed ?
Future	Syphilis HIV Hep C	MMRV Hep B Hep A	Arbo Toxo CMV Lyme
		HSV 1 and 2 IgG ???	