

HIV Diagnosis: New Tests and New Algorithms

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The views expressed in this presentation are those of the author and do not necessarily represent those of the Centers for Disease Control and Prevention



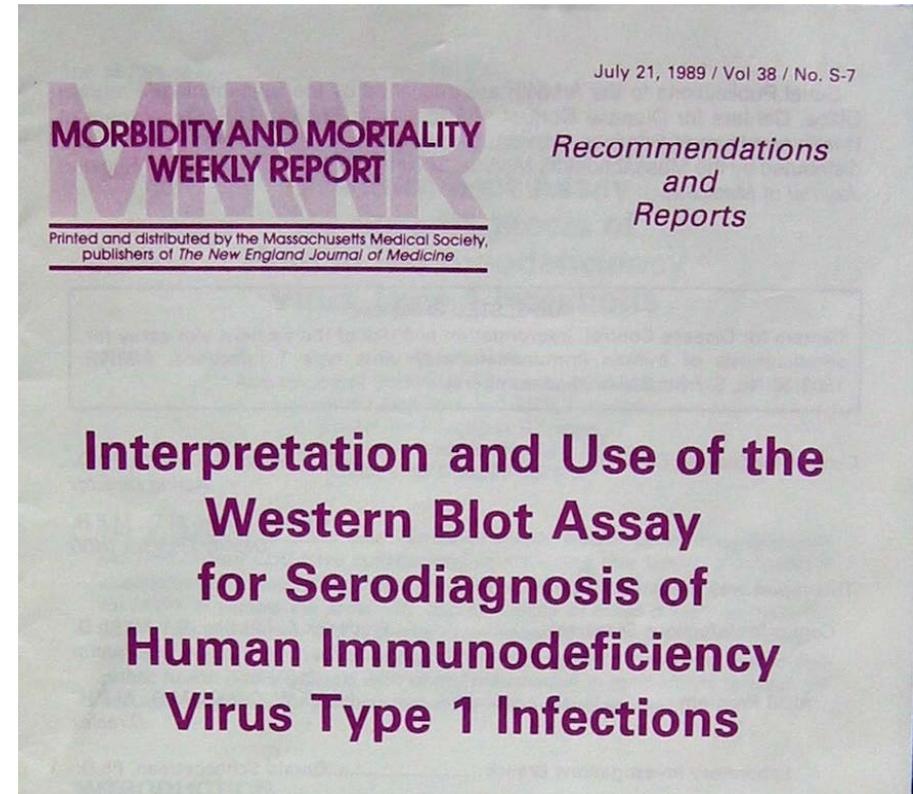


Outline

- Changes in HIV testing technology
- Detecting Acute HIV Infection
- HIV-2
- New HIV diagnostic testing algorithm: emphasis on sensitivity

Diagnostic Algorithm since 1989

- The Public Health Service recommends that no positive test results be given to clients/patients until a **screening test has been repeatedly reactive** on the same specimen **and a supplemental, more specific test such as the Western blot** has been used to validate those results.



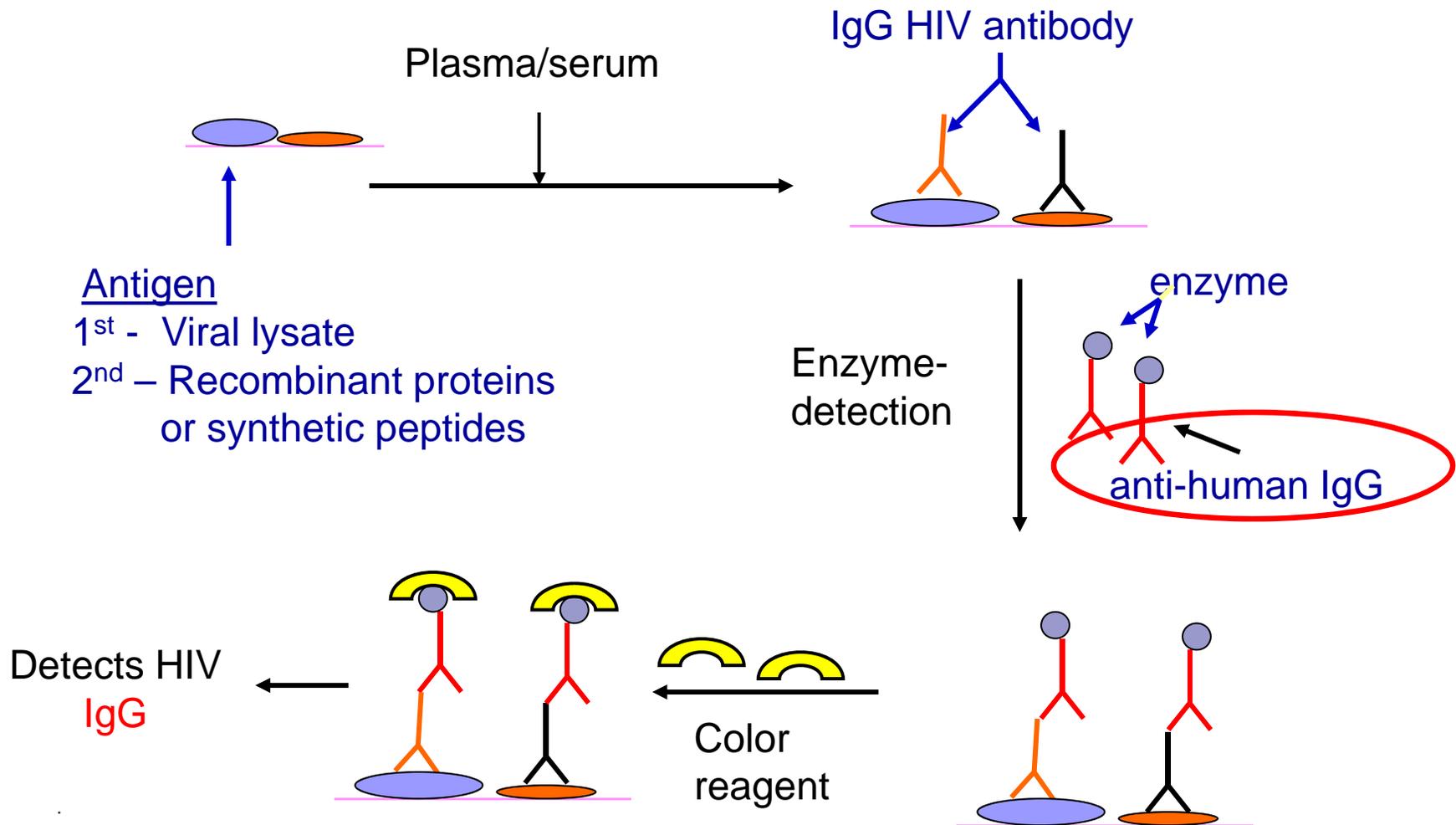
1989: State of the Art



Generations



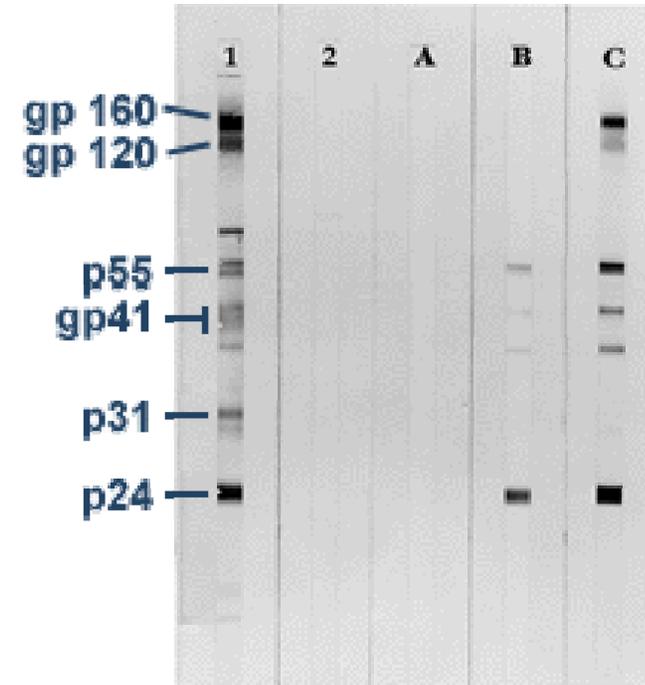
1st and 2nd Generation EIA



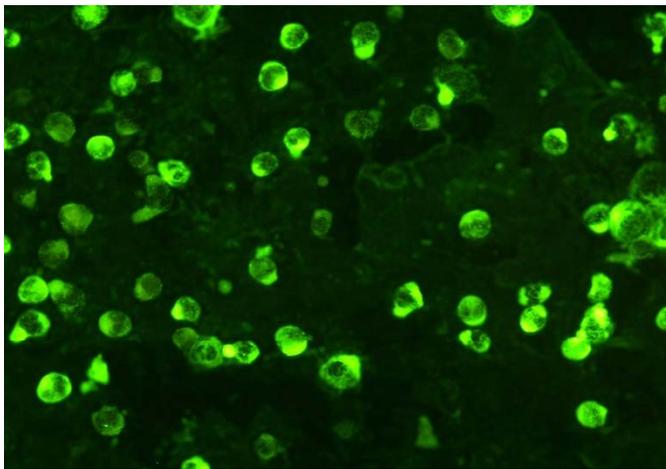
EIA



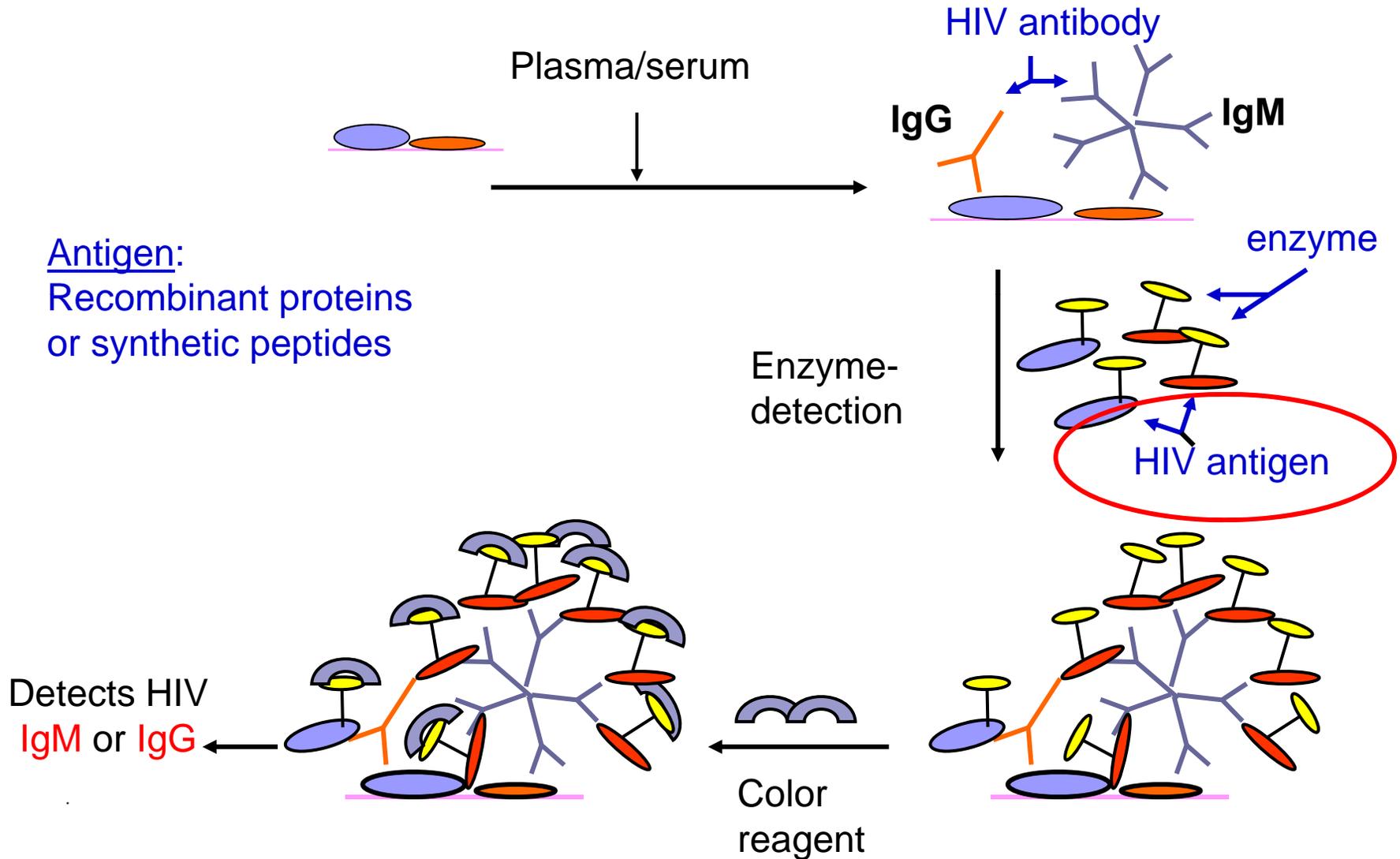
Western blot



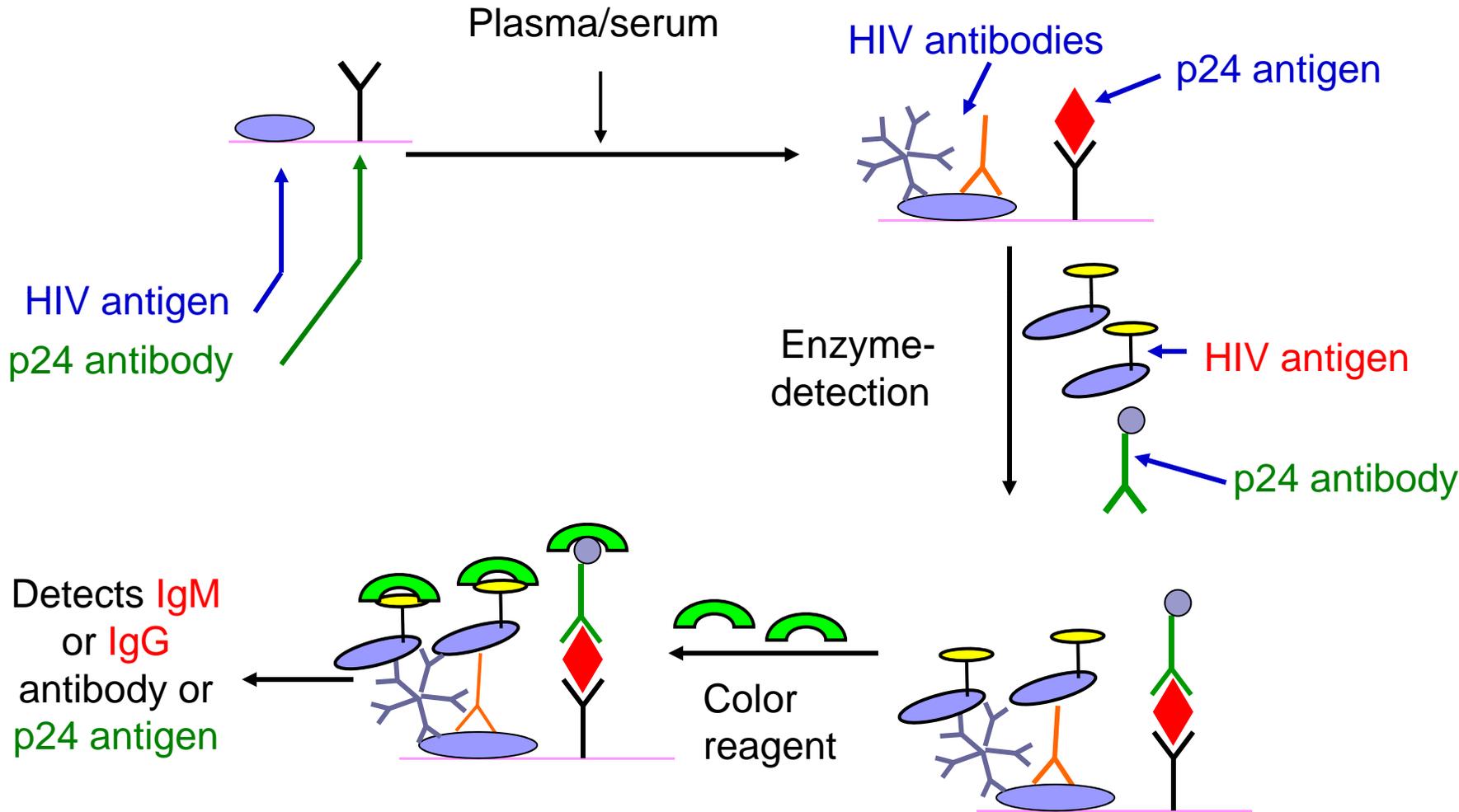
IFA



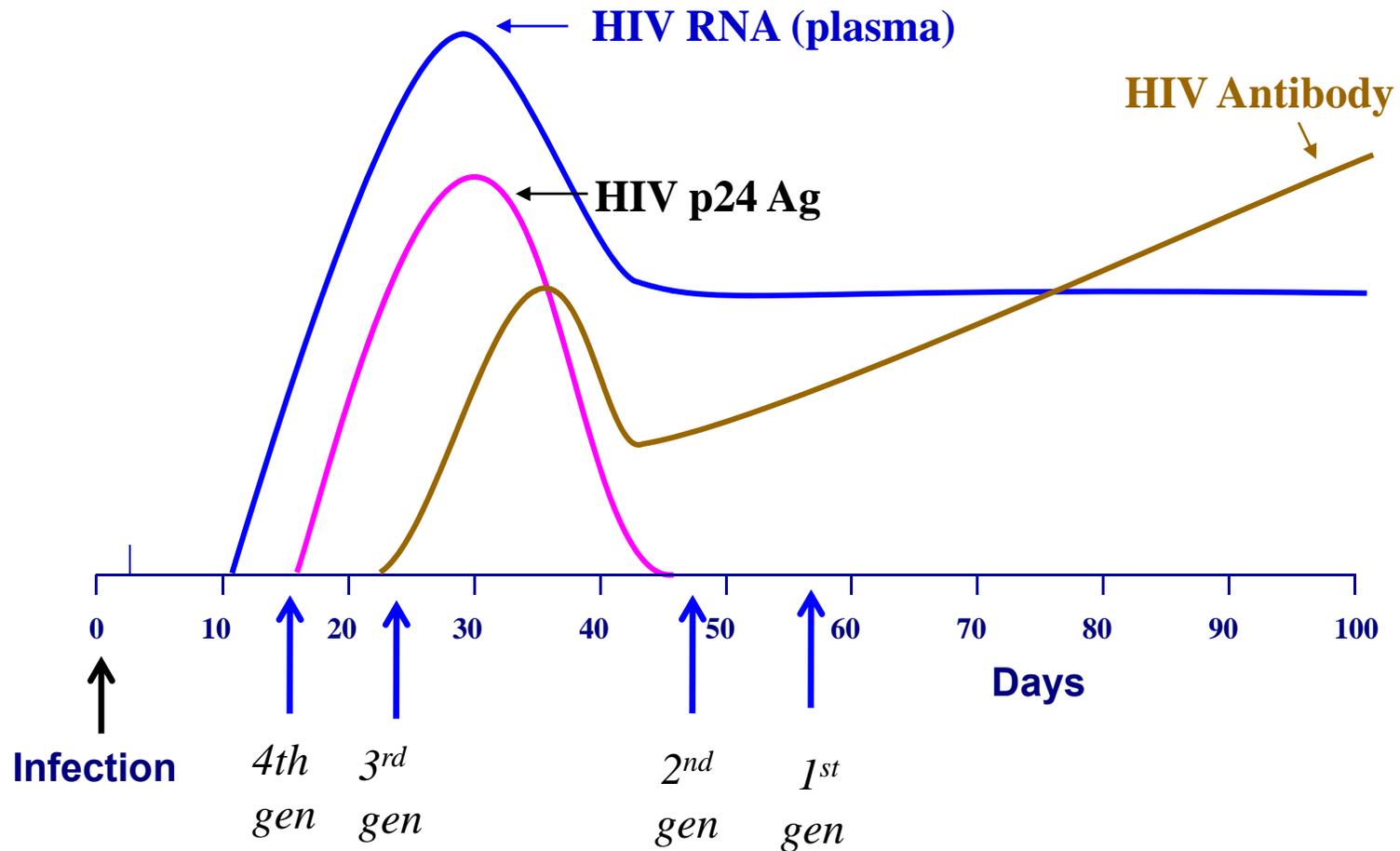
3rd Generation "Sandwich" EIA



4th Generation Combo EIA



HIV Infection and Laboratory Markers



Modified after Busch et al. Am J Med. 1997

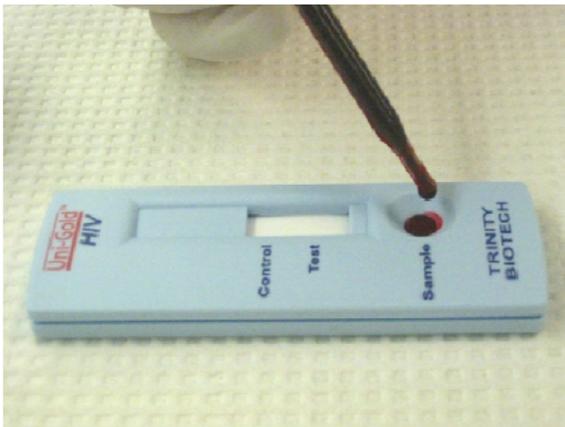


**OraQuick
Advance**

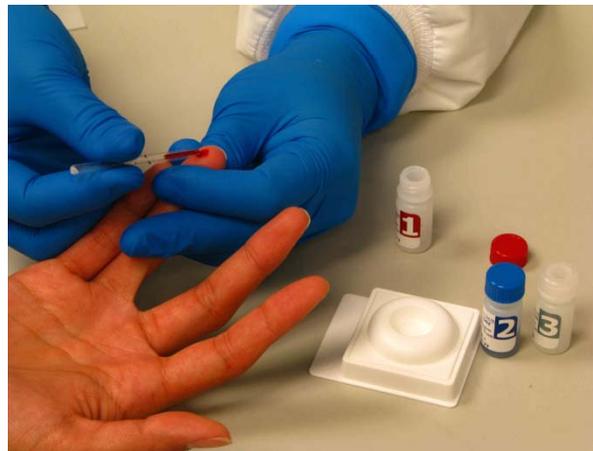
**CLIA-Waived
Point-of-Care
Rapid HIV Tests**



Clearview Complete



Uni-Gold Recombigen



INSTI

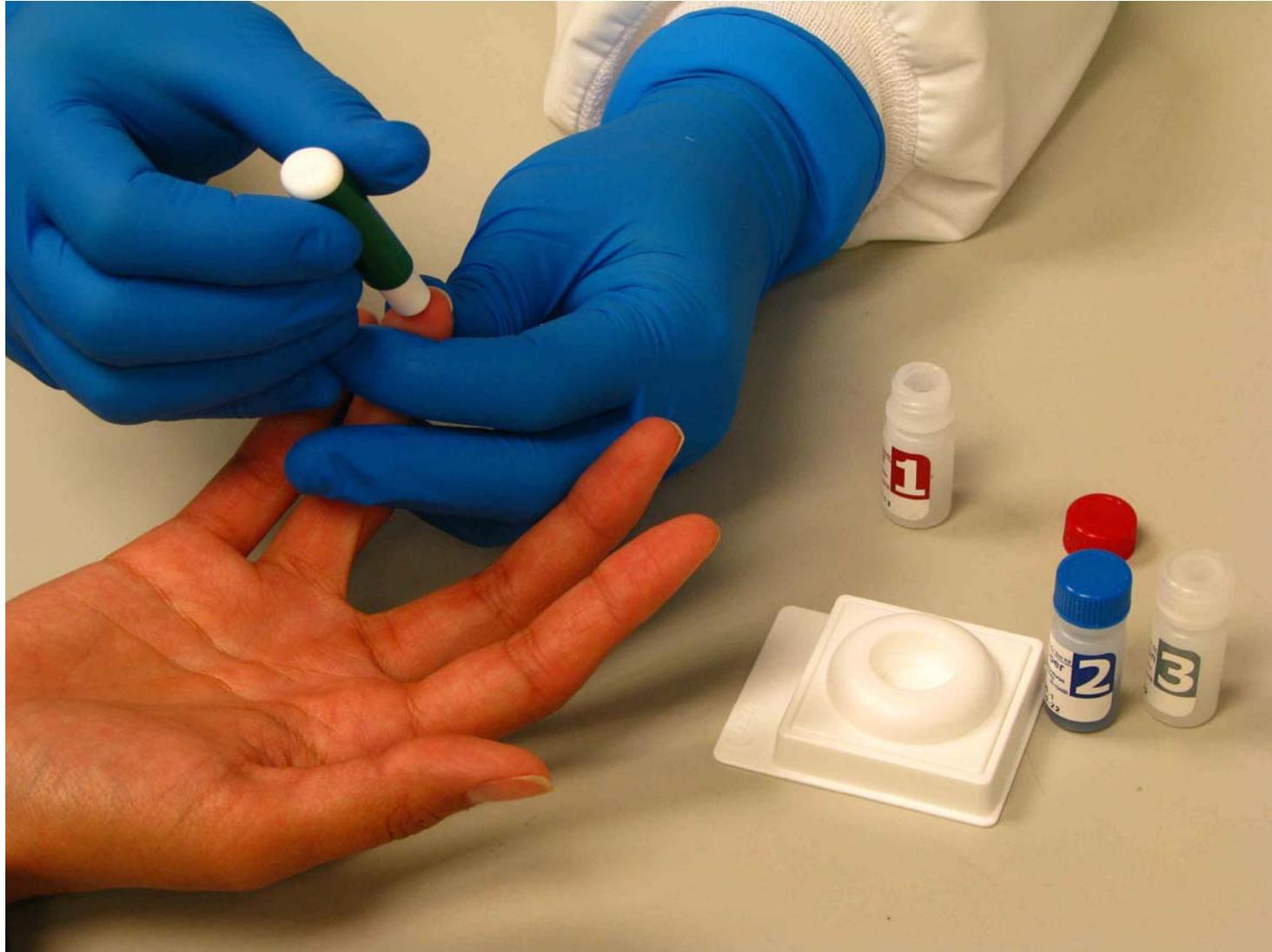


Clearview Stat Pak

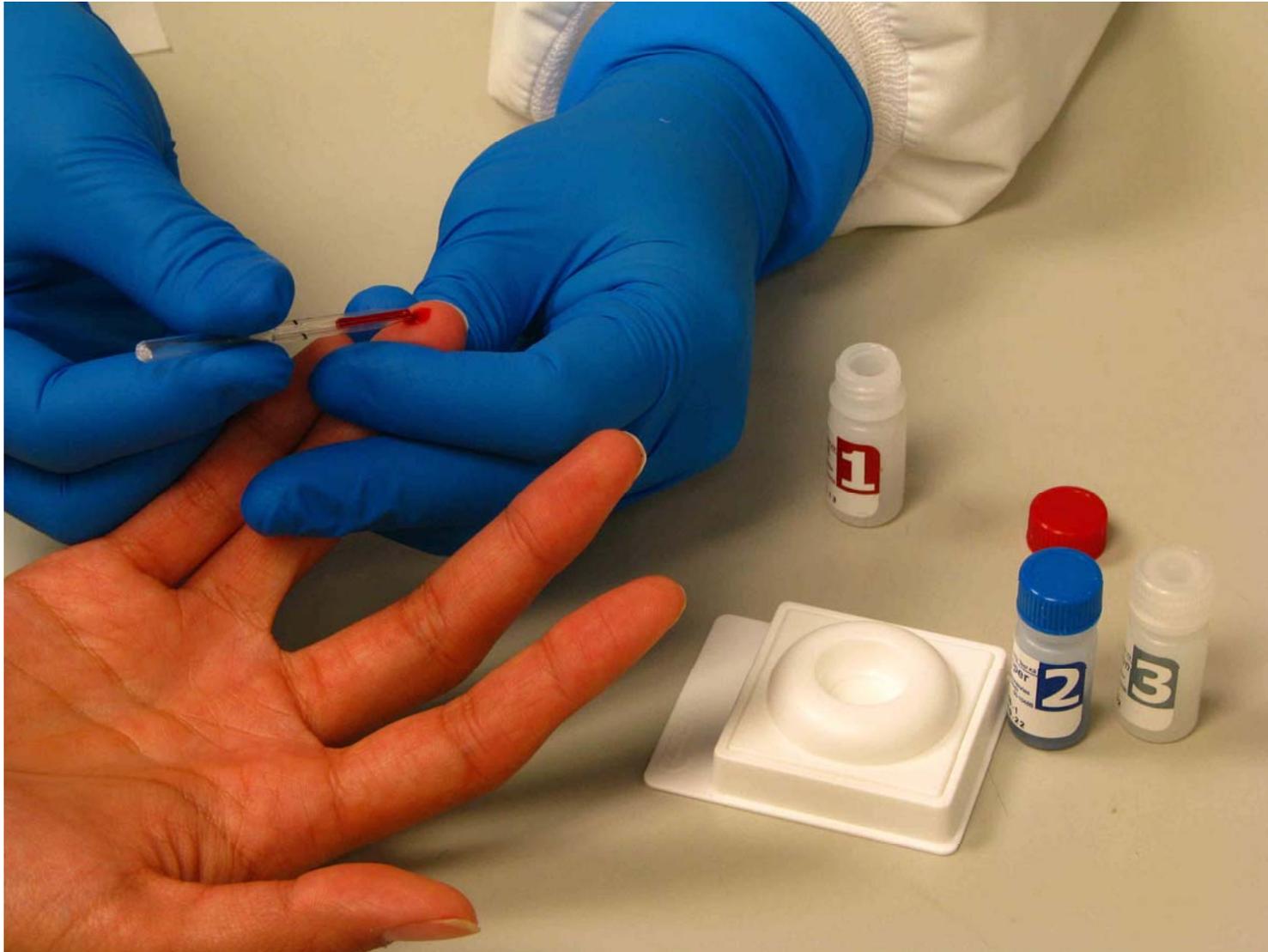
INSTI



- FDA-approved
November 29, 2010
- CLIA-waived
July 23, 2012
- Whole blood, serum, or
plasma
- Results <1 minute



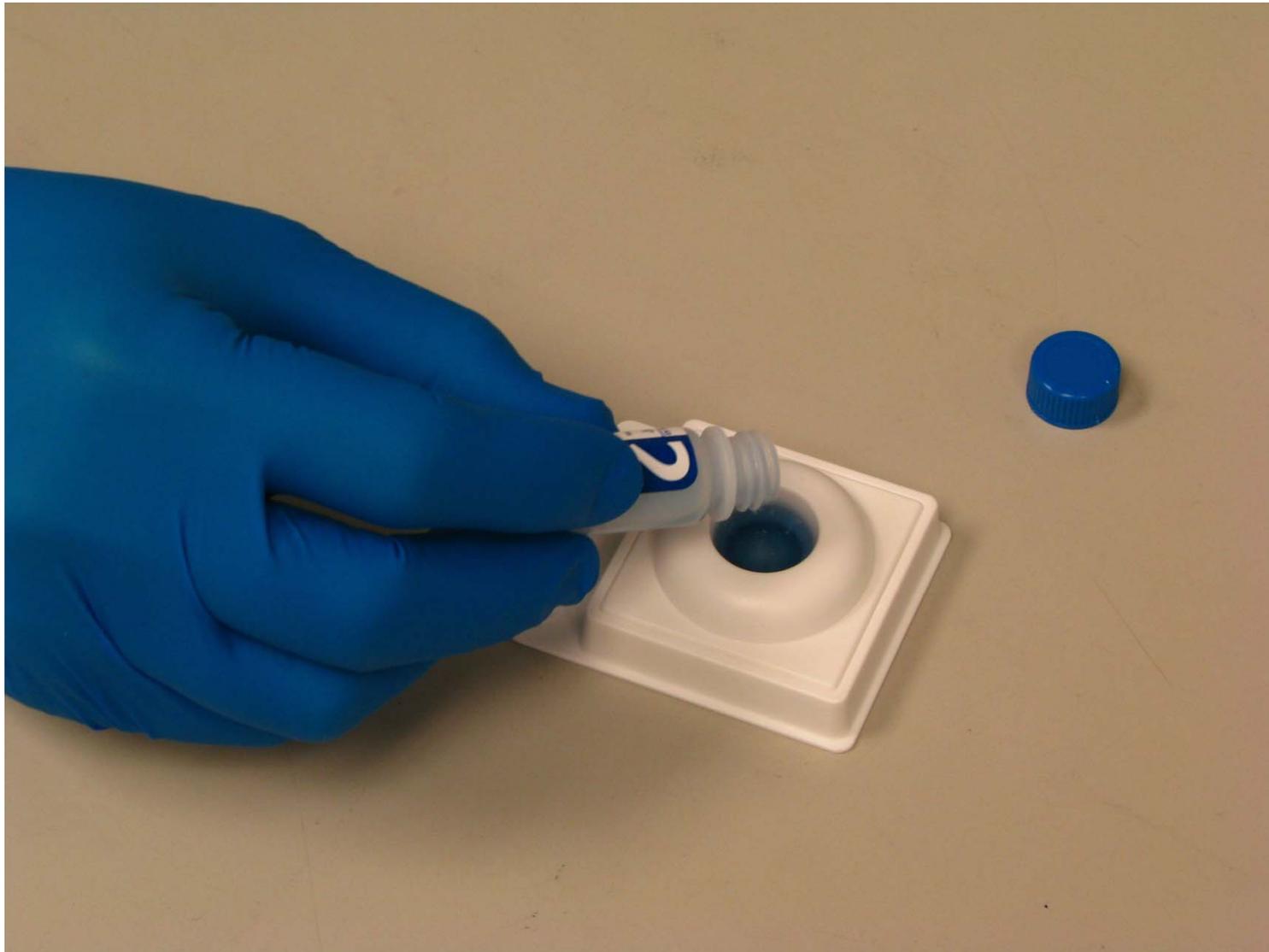
Perform fingerstick



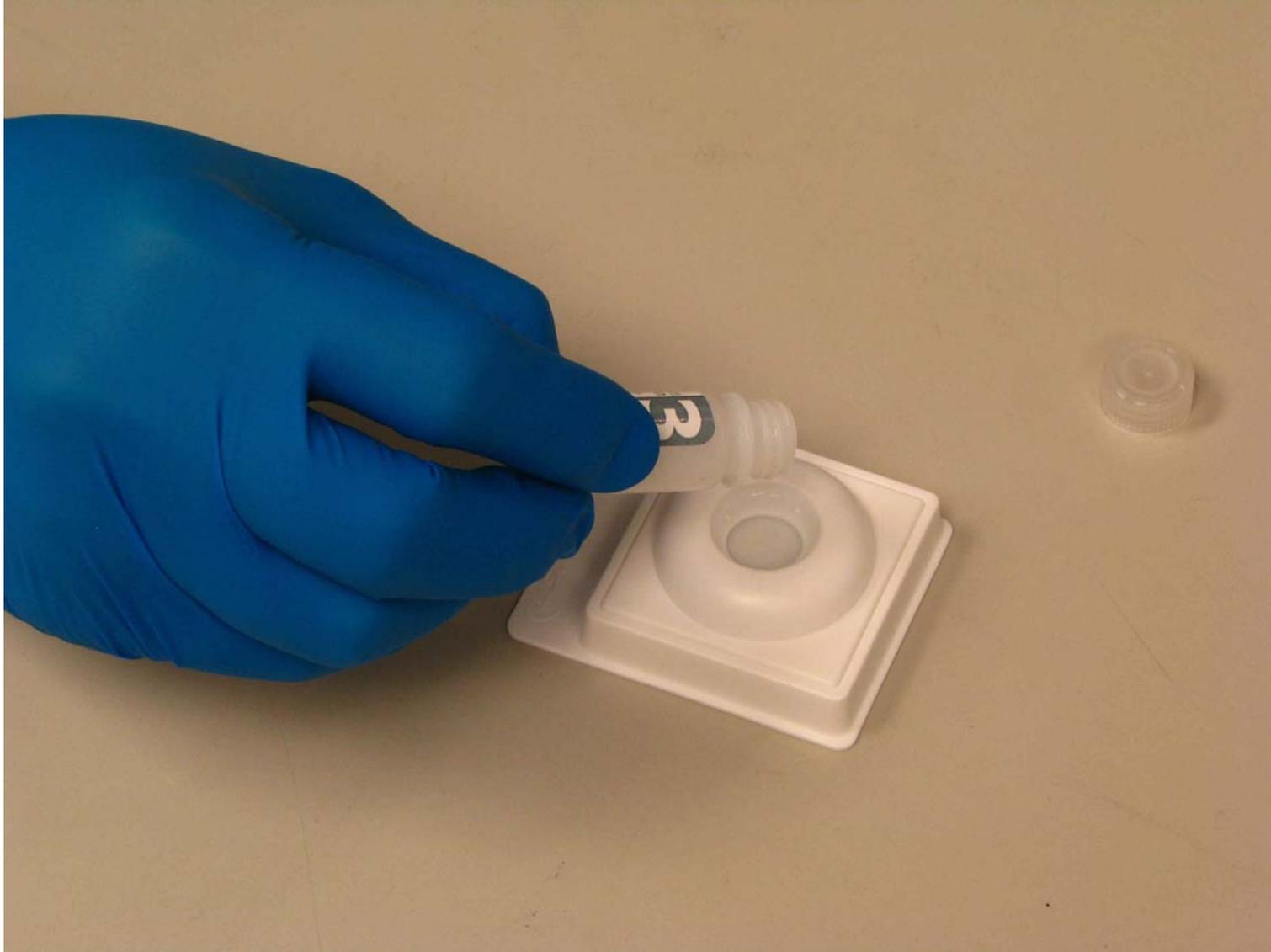
Add 1 drop (50 μ l) whole blood to diluent (vial 1)



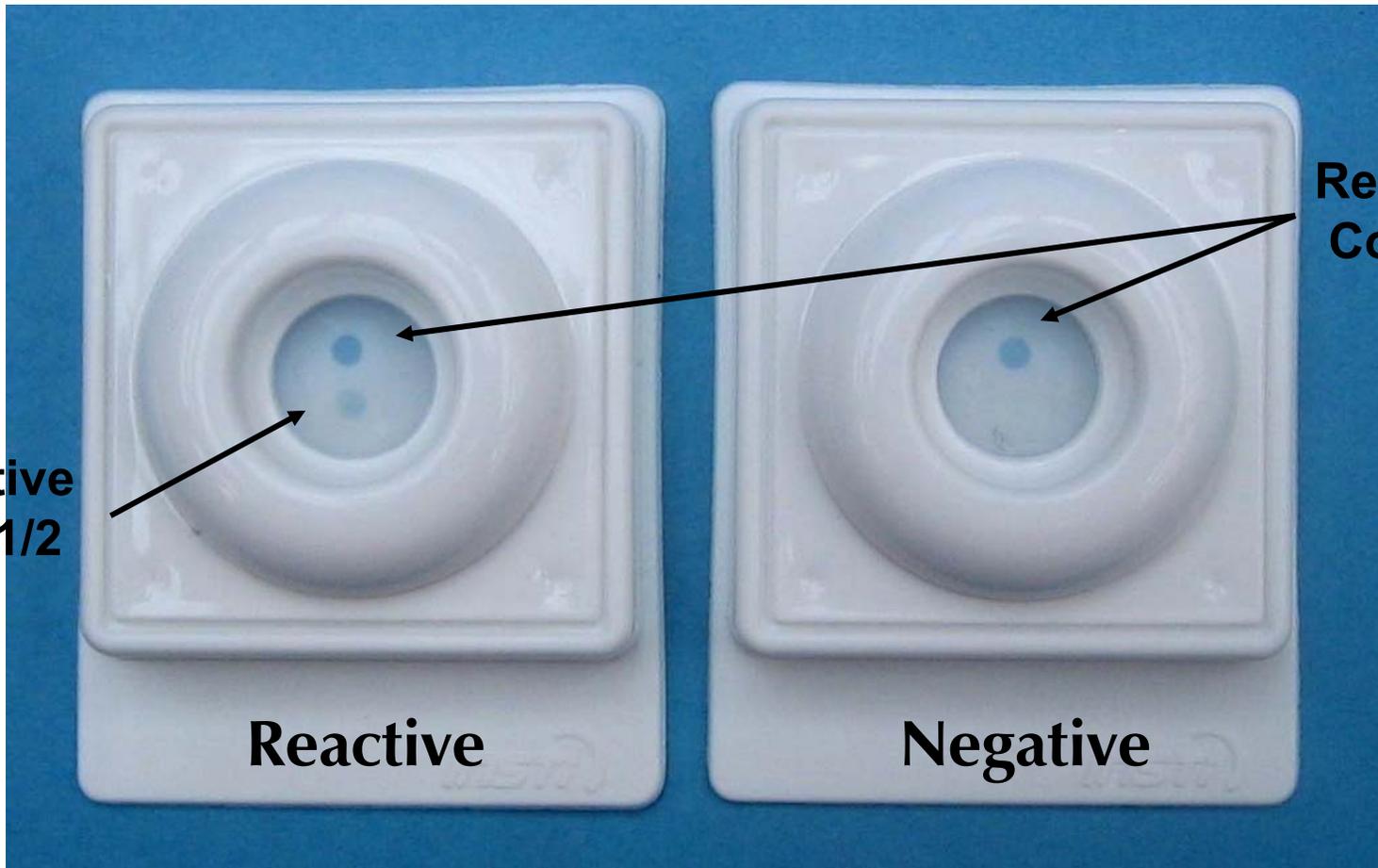
Add specimen to membrane unit



Add color developer solution



Add clarifier solution



**Positive
HIV-1/2**

Reactive

Negative

**Reactive
Control**

Read results immediately

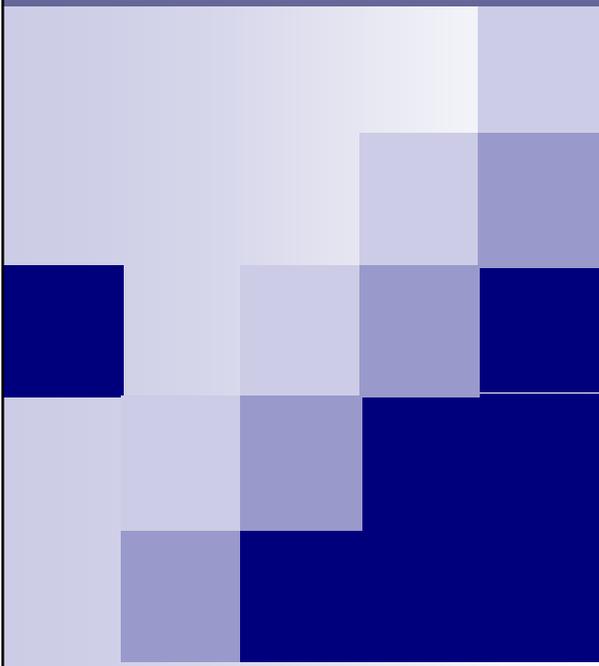
OraQuick In-Home HIV Test



Sensitivity: 91.7%
Specificity: 99.9%

\$39.99

FDA-approved July 3, 2012



Rapid HIV Test Results
without
Rapid Test Kits

A photograph showing a person's hand holding a white assay cartridge with a pink label. The cartridge is being held in front of a multiplatform analyzer, which has a row of similar cartridges in its tray. The analyzer is a white, multi-well plate format device. The background is slightly blurred, showing the depth of the tray.

Random Access Multiplatform
analyzers for HIV testing

On-board Refrigeration of Multiple Different Assays

A close-up photograph of a person wearing a white lab coat and a yellow nitrile glove. The person is loading a sample into a multiplatform analyzer. The machine is white and has several sample slots. A purple text box is overlaid on the image, containing the text "Random Access Multiplatform analyzers for HIV testing".

Random Access Multiplatform
analyzers for HIV testing

STAT sample requests without pausing
Results in <60 minutes

ADVIA[®] Centaur[™] HIV 1/O/2 Enhanced (EHIV)



- Chemiluminescent immunoassay
- 3rd generation format
 - HIV-1: gp41, p24
 - HIV-2: gp36
 - group O
- Time to result <1 hour
- FDA-approved July 2006

Scale Up of HIV Screening in Houston



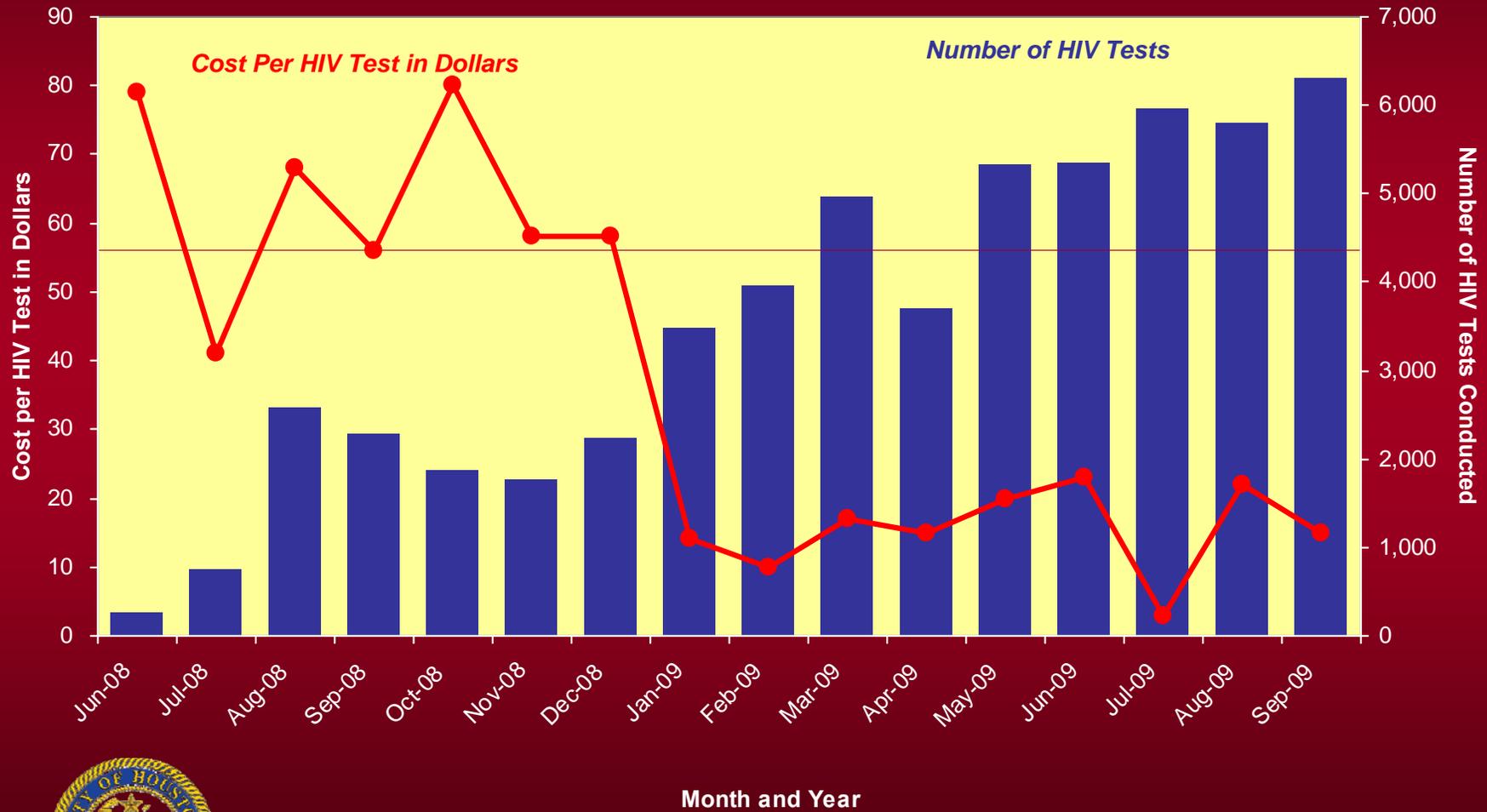
Marlene McNeese-Ward



Cost Analysis – Per HIV Test

Houston/Harris County, Texas

June 2008 – September 2009



Ortho VITROS ECi/ECiQ



- Chemiluminescent immunoassay
- 3rd generation format
 - HIV-1: gp41, gp120, p24
 - HIV-2: gp36
 - group O
- Time to result <1 hour
- Repeat only borderline results
- FDA-approved March 2008

Abbott Architect 4th Generation Ag/Ab Combo Assay

- Chemiluminescent immunoassay
- Detects p24 antigen and HIV antibody
- Time to result: 29 minutes
- FDA-approved June 22, 2010



APTIMA Qualitative HIV-1 RNA Assay



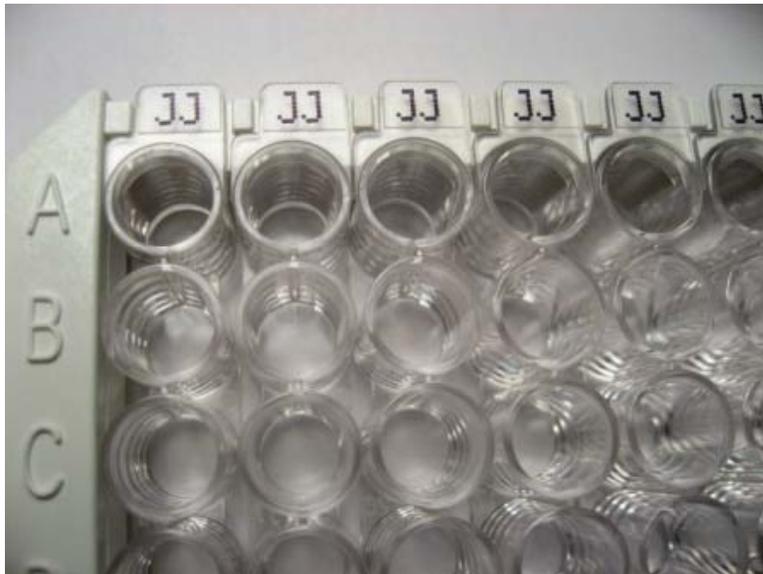
- Aid to HIV-1 diagnosis
- Diagnosis of acute HIV-1 infection in antibody-negative persons
- Confirmation of HIV-1 infection in antibody-positive persons when it is reactive
- FDA-approved July 2006



Avioq HIV-1 Microelisa

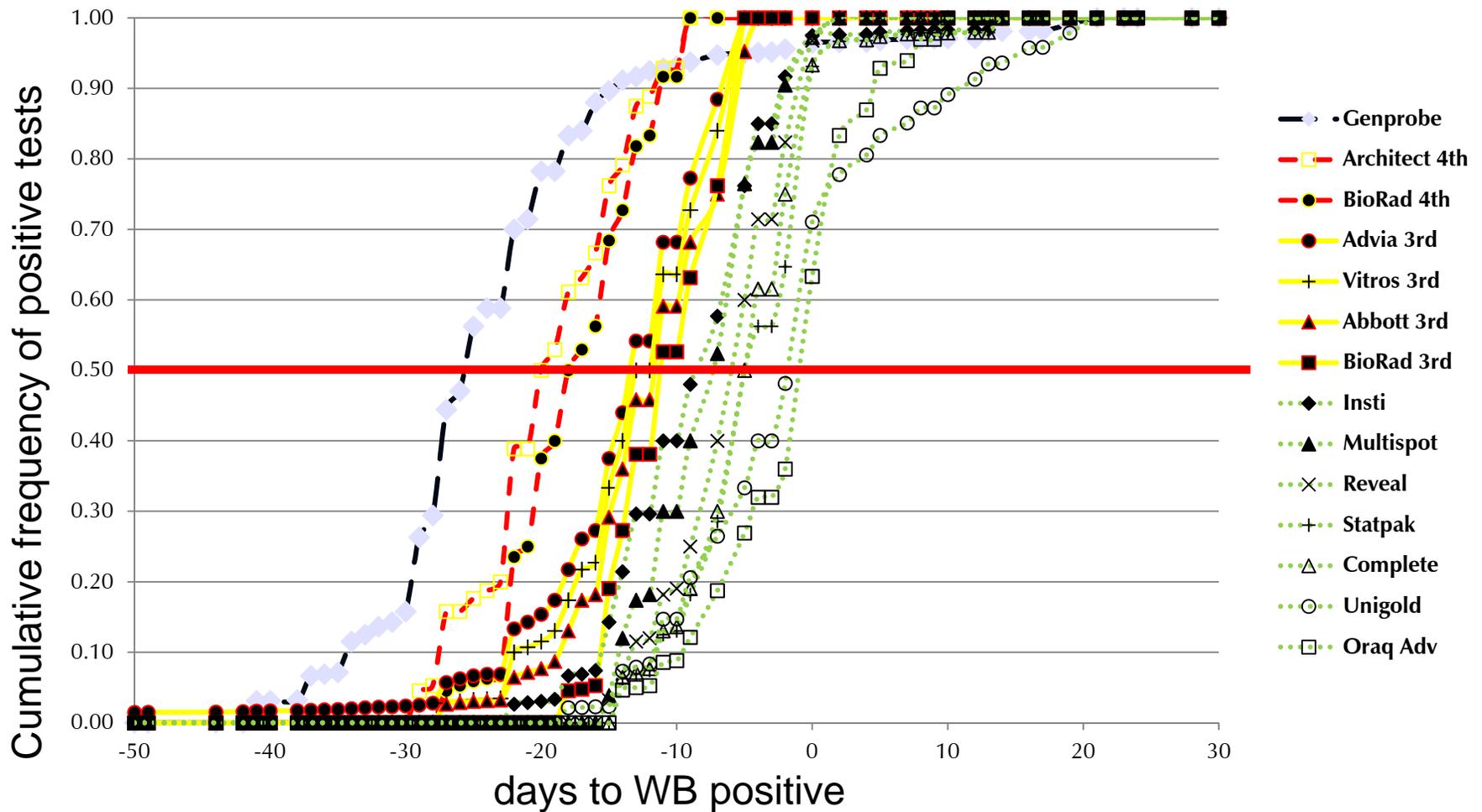
- Whole viral lysate (1st generation) HIV-1 EIA
- Only EIA for oral fluid, dried blood spots
- HIV-1 only
- FDA-approved August 10, 2009

Bio-Rad GS HIV Combo Ag/Ab EIA



- Microwell plate EIA
- 4th generation:
 - HIV-1: gp160
 - HIV-2: gp36
 - Group O
- p24 antigen
- FDA-approved July 25, 2011

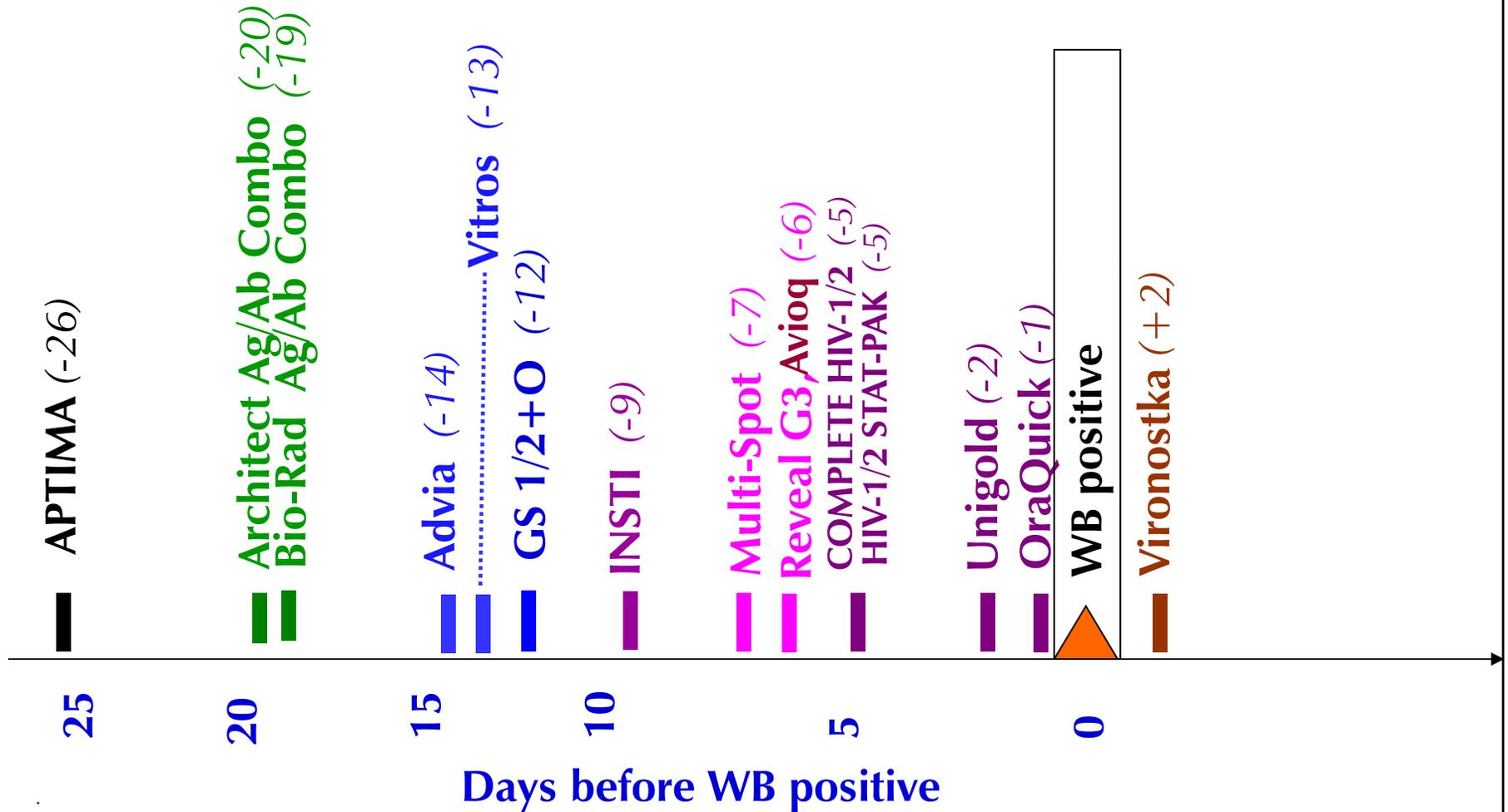
Relative Seroconversion Sensitivity



- 26 seroconverters were analyzed with 14 tests
- 17 seroconverters with WB positive used for cumulative frequency analysis

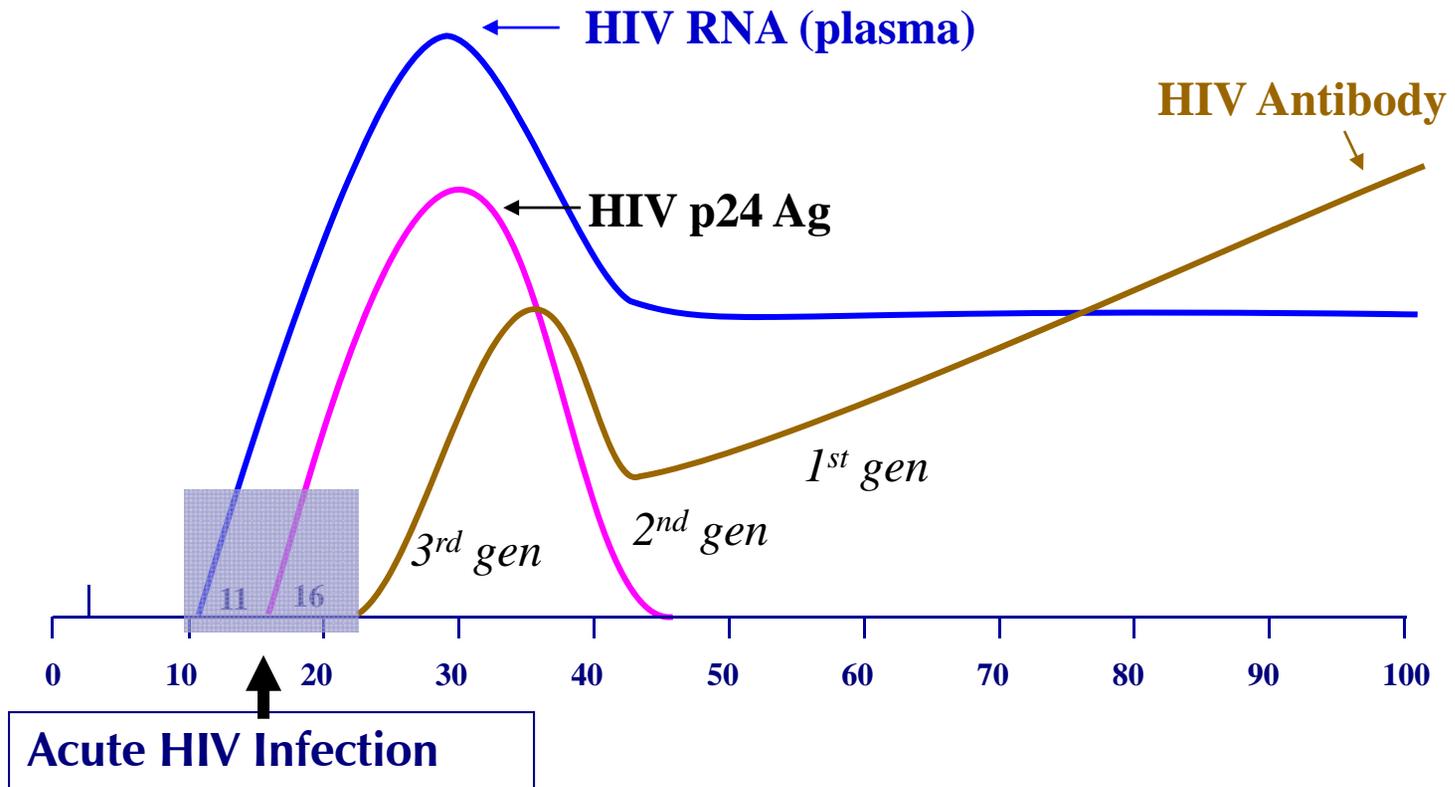
Sequence of Test Positivity Relative to WB

166 specimens, 17 Seroconverters - 50 % Positive Cumulative Frequency



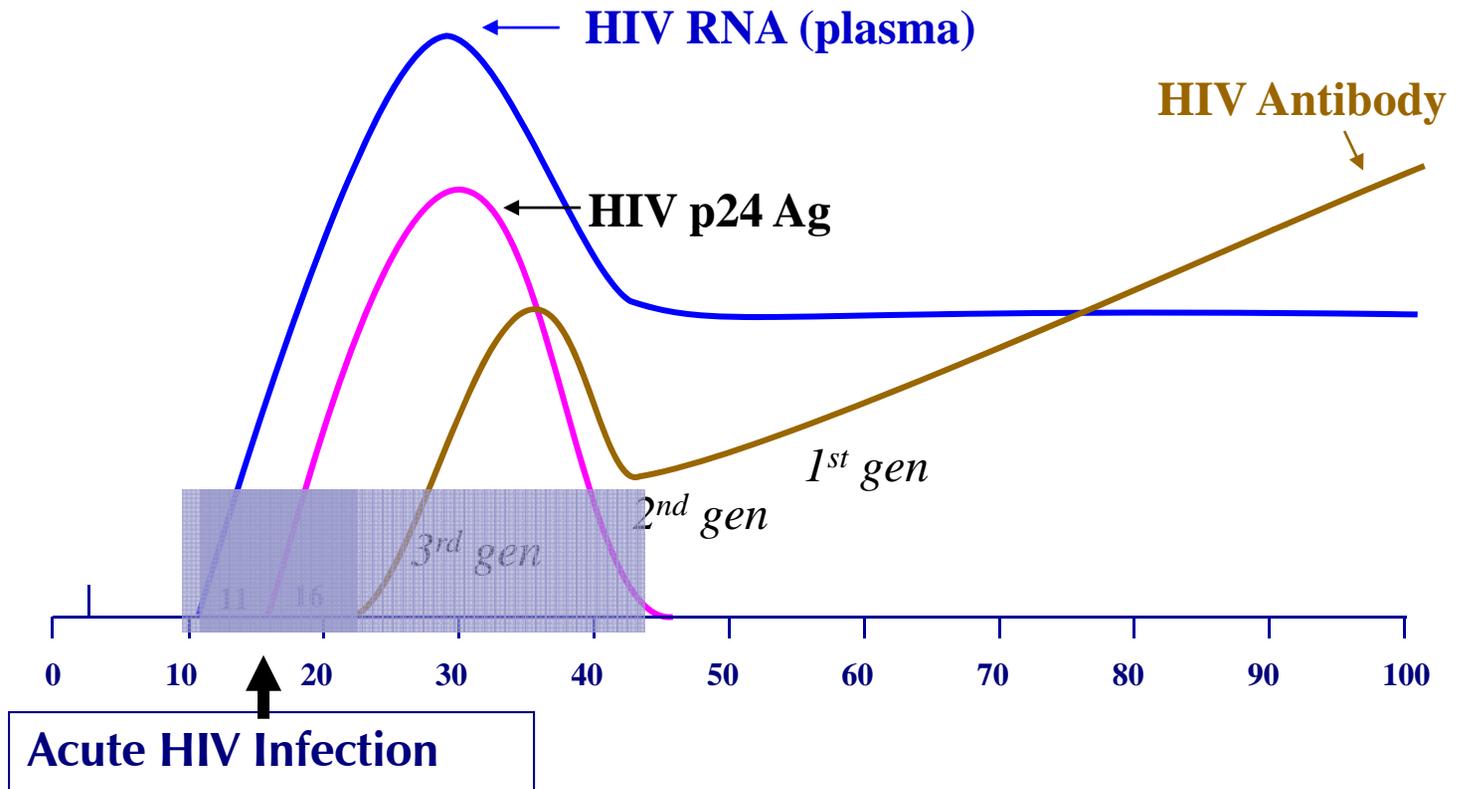
Modified from Masciotra et al, *J Clin Virol* 2011
and Owen et al, *J Clin Micro* 2008

Window Period and HIV Infection



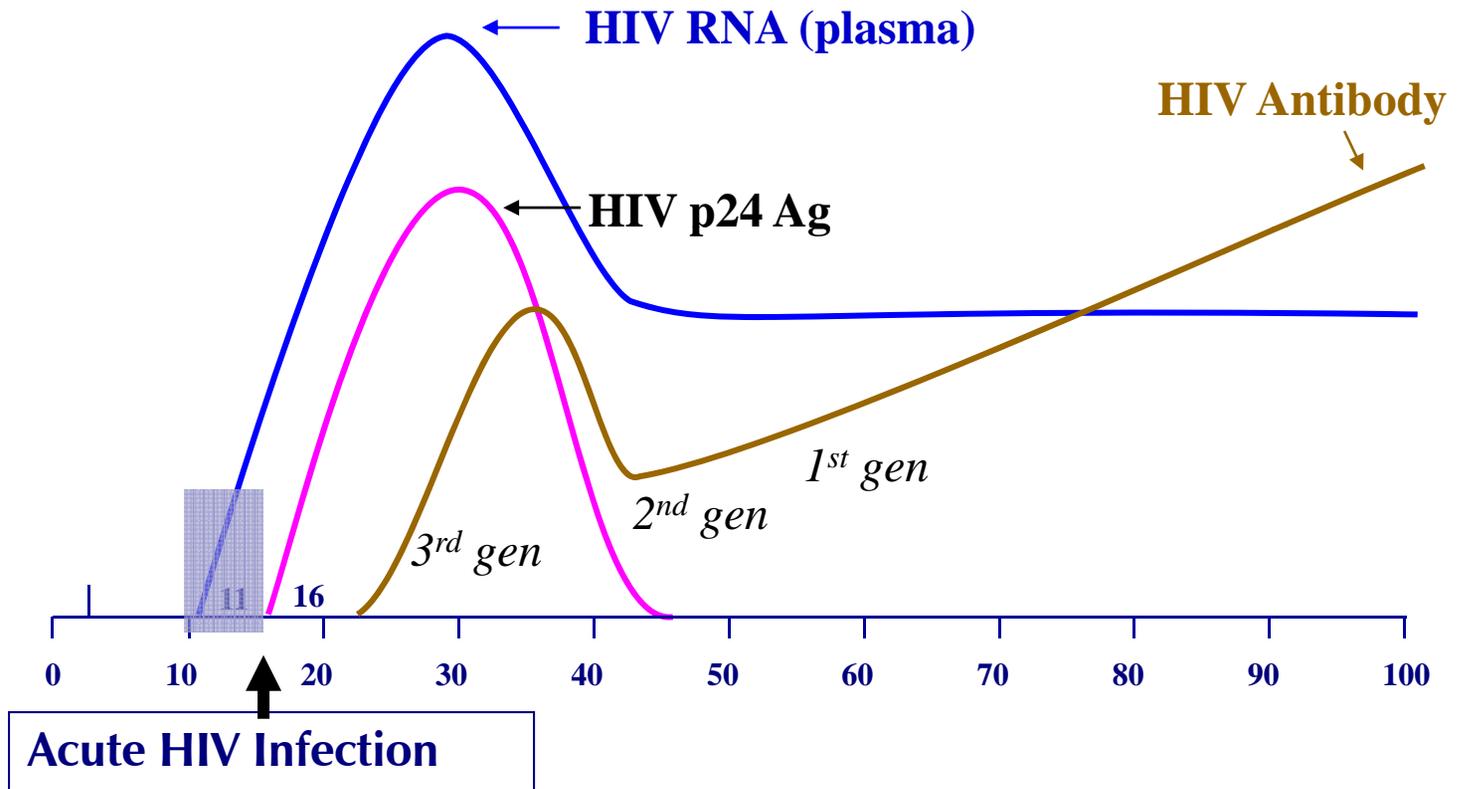


Windows

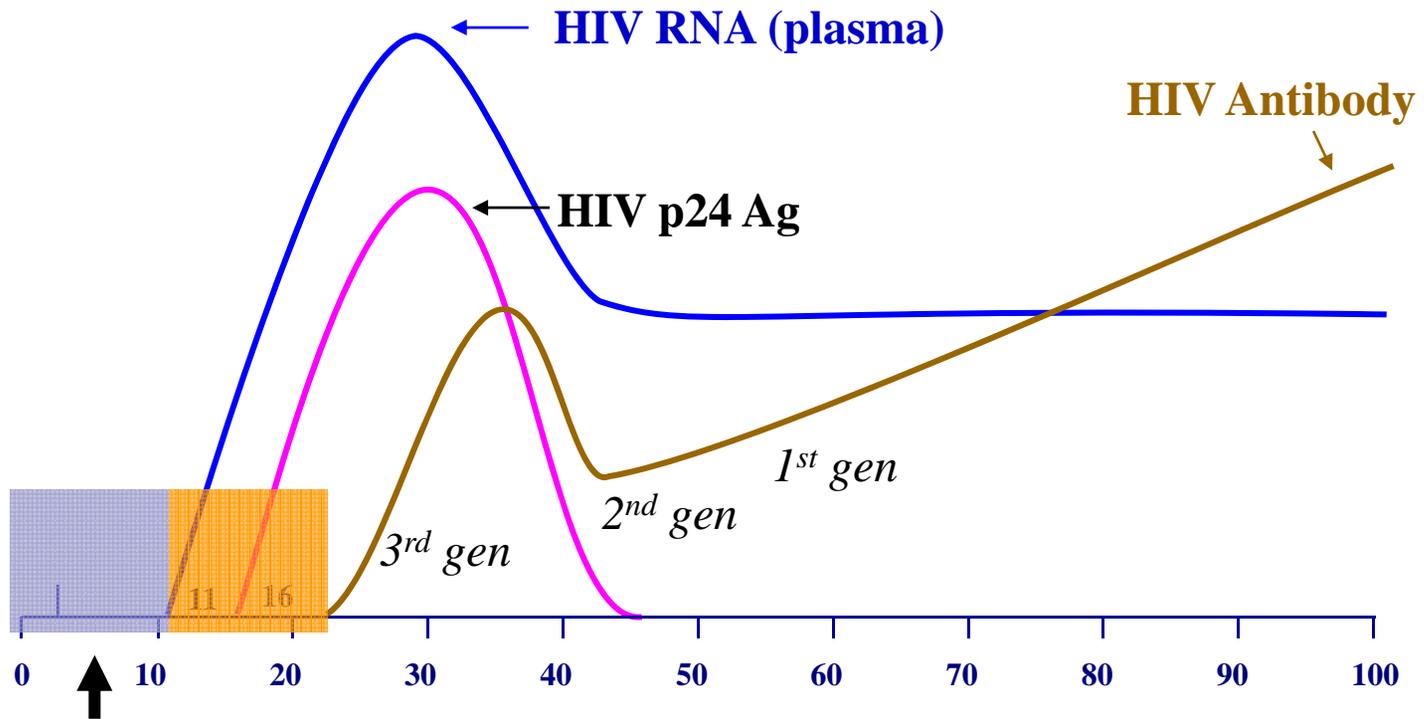




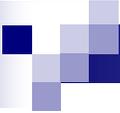
Windows



We Can't Close the Window



Infection
Undetectable



Clinical Syndrome of Acute HIV

- 40-90% develop symptoms of Acute HIV
- 50%-90% with symptoms seek medical care
- Of those diagnosed with Acute HIV, 50% of patients seen at least 3 times before diagnosis

- Kahn et al, NEJM 1998

- Weintrob et al, Arch Int Med 2003

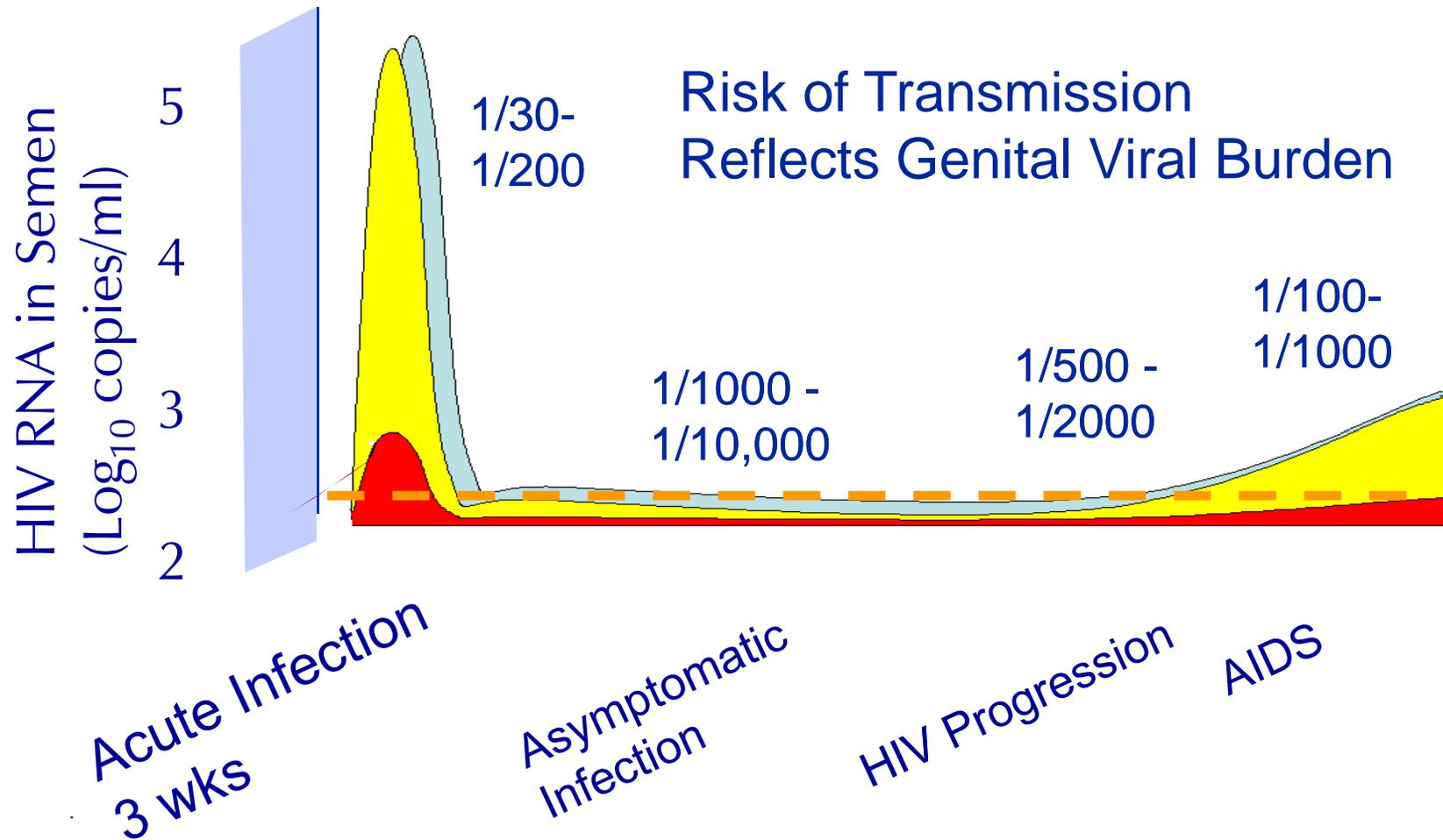
Clinical Manifestations

101 seroconverters, HIVNET cohort 1995-98

Symptom	Percent	Median Duration
Any symptom	85%	Days (IQR)
Fatigue	56%	9 (5-29)
Fever	55%	5 (4-10)
Pharyngitis	43%	7 (5-10)
Lymphadenopathy	36%	7 (4-14)
Rash	16%	8 (6-14)

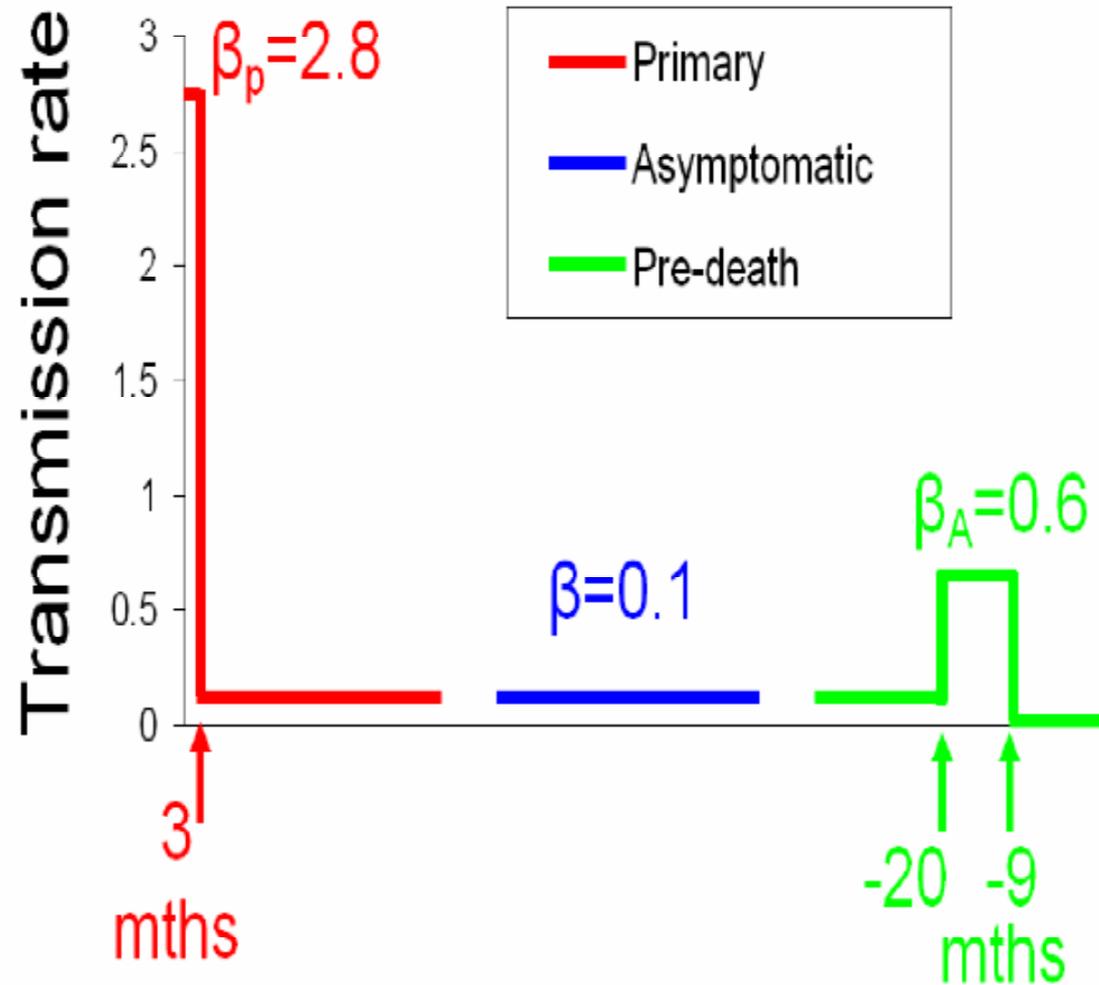
- Celum et al, JID 2001

Risk of Sexual Transmission of HIV



Cohen MS, et al. *J Infect Dis.* 2005

Transmission Rate by Stage of Infection



Hollingsworth et al, JID 2008

HIV-1 Transmission, by Stage of Infection and Behavior Pattern

Infection Stage	Transmission Hazard per Person-year	Mean Duration, Years (%)	No. (%) New Transmissions, by Sexual Behavior	
			Serial Monogamy	Random Mixing
Acute	2.76	0.24 (2%)	0.10 (9%)	0.67 (31%)
Asymptomatic	0.106	8.38 (82%)	0.77 (71%)	0.91 (42%)
AIDS	0.760	0.75 (16%)	0.21 (20%)	0.57 (27%)

Hollingsworth et al, JID 2008

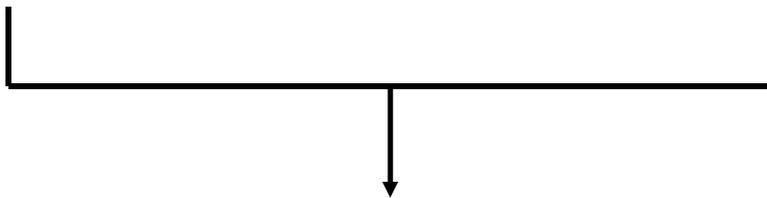
Pooled RNA Screening for Acute HIV Infection



CDC Acute HIV Infection Study

1-Stage RNA Pooling, antibody-negative specimens

16 Specimens



1 Master Pool

- 80 HIV testing clinics in Florida
- 14 STD clinics and 1 MSM clinic in Los Angeles
- 3 STD clinics in New York

- Patel et al, Archives Int Med 2010

Yield from Pooled RNA Screening after EIA

<u>Site</u>	<u>Number tested</u>	<u>HIV Ab+</u>	<u>RNA+ / Ab-</u>
Florida – 2006-08	54,948	663 (1.2%) [†]	9 (0.02%)
L.A. - 2006-08	37,012	427 (1.2%)*	35 (0.09%)

[†]Screened with Bio-Rad GS HIV1/HIV-2 PLUS O EIA

*Screened with Vironostika HIV-1 Microelisa System

- Patel et al, CDC , Archives Int Med 2010

Yield from Pooled RNA Screening after EIA

<u>Site</u>	<u>Number tested</u>	<u>HIV Ab+</u>	<u>RNA+/ Ab-</u>
Florida – 2006-08	54,948	663 (1.2%) [†]	9 (0.02%)
L.A. - 2006-08	37,012	427 (1.2%)*	35 (0.09%)
L.A. - 2006-08	37,012	441 (1.2%) [†]	18 (0.05%)

[†]Screened with Bio-Rad GS HIV1/HIV-2 PLUS O EIA

*Screened with Vironostika HIV-1 Microelisa System

- Patel et al, CDC , Archives Int Med 2010

Yield from Pooled RNA Screening after Rapid Test

<u>Site</u>	<u>Number tested</u>	<u>HIV Ab+</u>	<u>RNA+/ Ab-</u>
Florida	604	17 (2.8%)*	1 (0.2%)
New York	6,547	29 (0.4%)*	7 (0.1%)

**Screened with OraQuick ADVANCE Rapid HIV-1/2 Antibody Test*

- Patel et al, CDC , Archives Int Med 2010

Yield from Pooled RNA Screening after Rapid Test

<u>Site</u>	<u>Number tested</u>	<u>HIV Ab+</u>	<u>RNA+ / Ab-</u>
Florida	604	17 (2.8%)*	1 (0.2%)
Florida	604	17 (2.8%) †	1 (0.2%)
New York	6,547	29 (0.4%)*	7 (0.1%)
New York	6,547	35 (0.5%) †	1 (0.02%)

*Screened with OraQuick ADVANCE Rapid HIV-1/2 Antibody Test

†Screened with Bio-Rad GS HIV-1/HIV-2 PLUS O EIA

- Patel et al, CDC , Archives Int Med 2010

Acute HIV Screening: 99,111 tested

<u>EIA-RR/WB+</u>	<u>1,136 (1.1%)</u>
RNA+	1,094 (96.3%)
RNA-	42 (3.7%)
<u>EIA-RR/WB-ind</u>	<u>30 (0.03%)</u>
RNA+	3 (10.0%)
RNA-	27 (90.0%)
<u>EIA-neg/RNA+</u>	<u>52 (0.05%)</u>
Acute HIV	48 (92%)
False-pos RNA	4 (8%)

- Patel et al, CDC , Archives Int Med 2010

Acute HIV Screening: 99,111 tested

<u>EIA-RR/WB+</u>	<u>1,136 (1.1%)</u>
RNA+	1,094 (96.3%)
RNA-	42 (3.7%)
<u>EIA-RR/WB-ind</u>	<u>30 (0.03%)</u>
RNA+	3 (10.0%)
RNA-	27 (90.0%)
<u>EIA-neg/RNA+</u>	<u>52 (0.05%)</u>
Acute HIV	48 (92%)
False-pos RNA	4 (8%)

- Patel et al, CDC , Archives Int Med 2010



Receipt of Pooled RNA Results

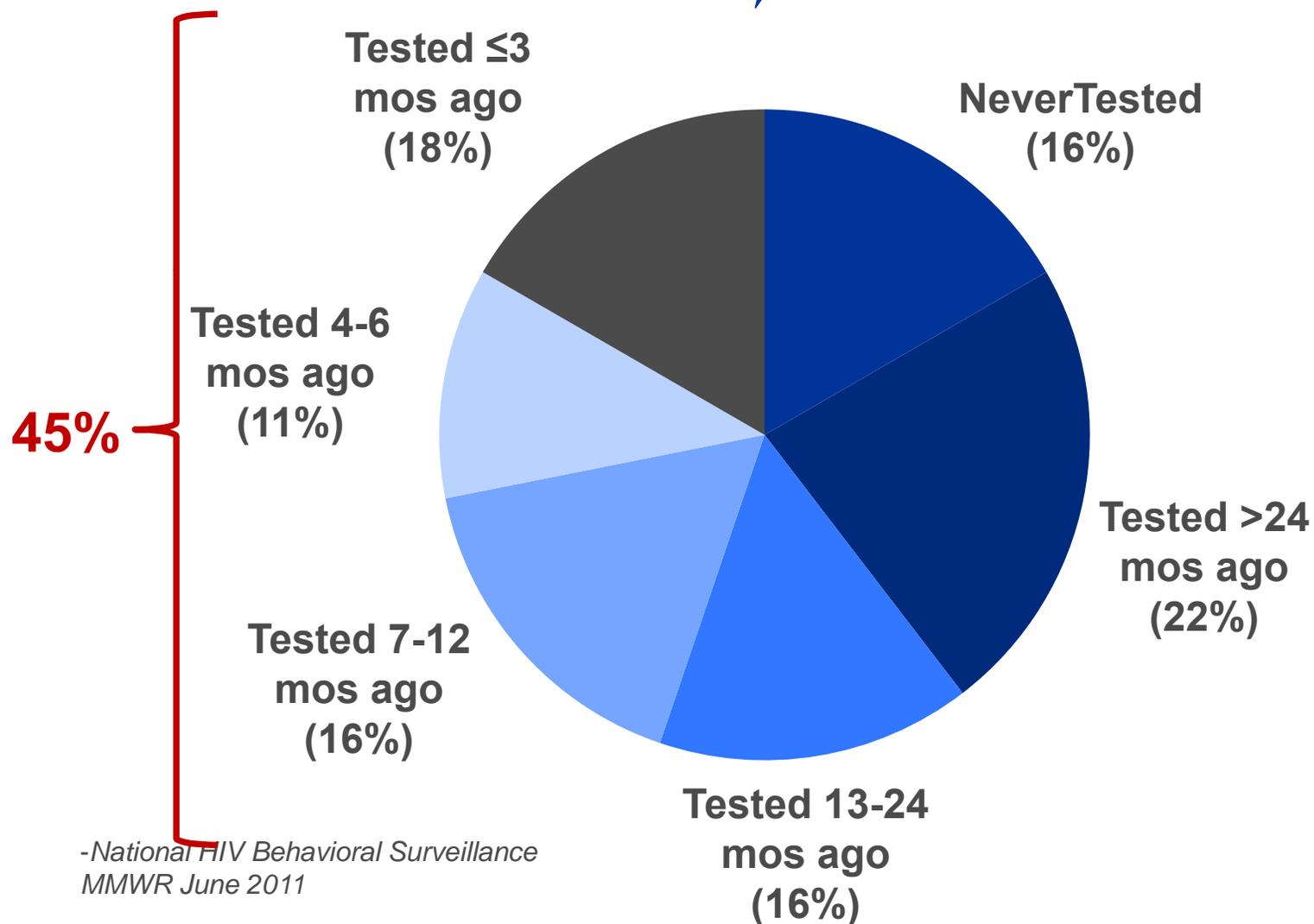
- 7/55 (13%) never received their results
- 48/55 (87%) AHI received their results:
 - 11 (23%) within 7 days of testing
 - 23 (48%) within 8-14 days of testing
 - 12 (29%) more than 14 days after testing
- **19/55 (42%) too late for optimal intervention**

Acute Infections in MSM detected by RNA only

- 0.3% of 14,005 frequently tested MSM in Seattle STD clinic; **20% of all HIV infections detected**
- Stekler et al, Clin Infect Dis 2009
- 26 (74%) of 35 AHI cases detected in LA at MSM clinic; **25% of all HIV infections detected**
- Patel et al, Archives Int Med 2010
- 0.08% of 21,425 STD clinic patients in New York City; **9% of all HIV infections detected**; 94% were MSM
- Shepard et al, MMWR 2009

Time Since Most Recent HIV Test among 680 HIV-Infected MSM Unaware of Their Infection

21 cities, 2008



-National HIV Behavioral Surveillance
MMWR June 2011

RNA vs. 4th Generation Ag/Ab Assay

- RNA+/ 3rd gen-negative specimens detected by 4th generation EIA:
 - 38 of 46 (83%) – *Australia**
 - 10 of 14 (71%) – *CDC AHI study***
 - 51 of 61 (84%) – *CDC panel****

 - 4 days after RNA – 9 seroconversion panels***

* *Cunningham P, HIV Diagnostics Conf 2007*

** *Patel P, CROI 2009*

*** *Owen M, CROI 2009*

HIV-2 Infection

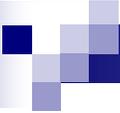
- ❑ **Remains uncommon in U.S., but**
 - Does not respond to NNRTIs, some PIs (first line therapy)
 - Undetectable by HIV-1 viral load tests

- ❑ **Misclassification by HIV-1 Western blot:**
 - 54/58 (93%) HIV-2 patients tested had positive HIV-1 WB (NYC)*
 - 97/163 (60%) HIV-2 cases reported had positive HIV -1 WB (CDC)**

- ❑ **HIV-2 often diagnosed after immunologic deterioration in patient with negative viral load**

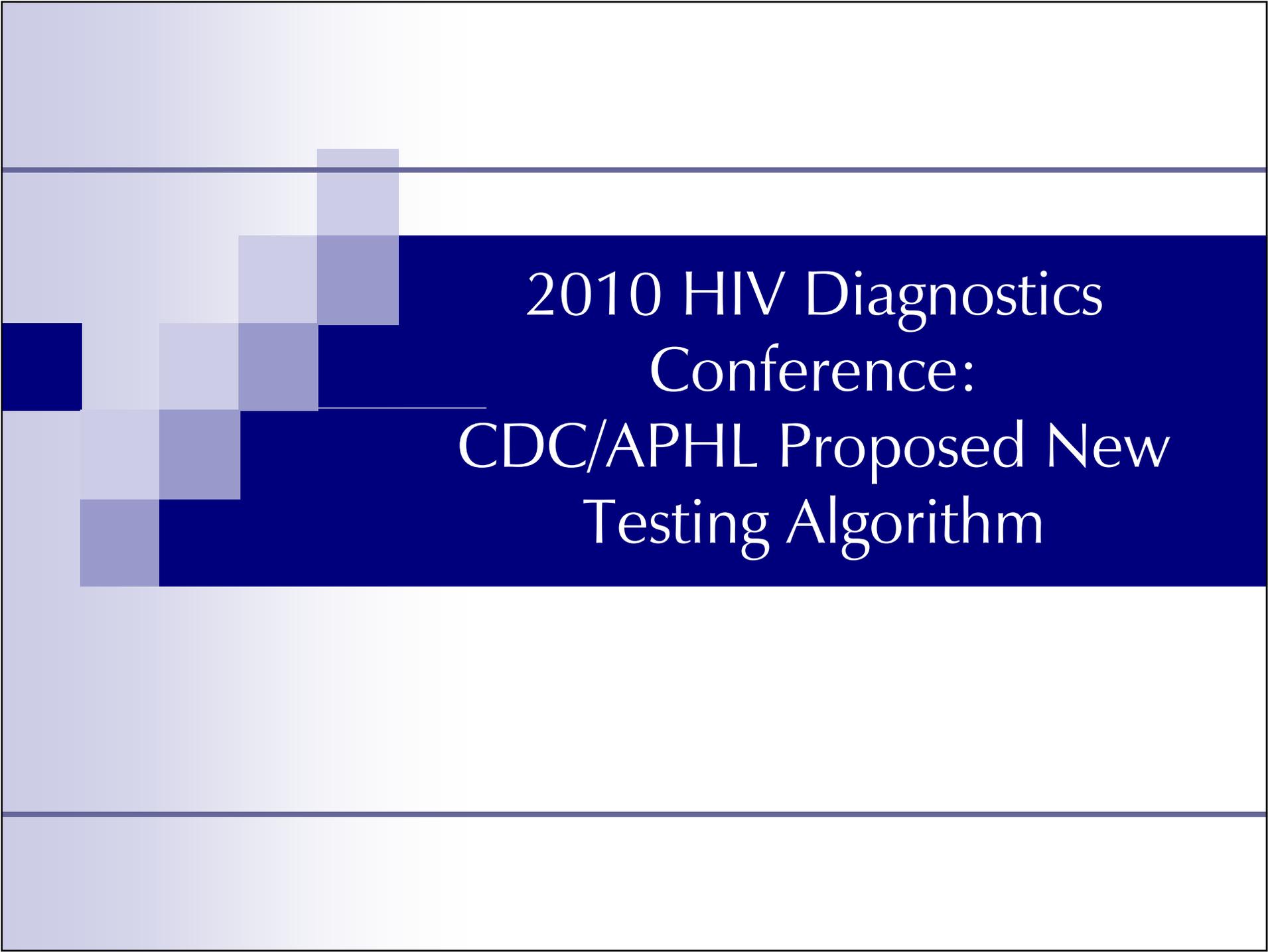
**Torian et al, Clinical Infectious Disease 2010*

***MMWR July 2011*



Limitations of the 1989 Algorithm

- Western blot is less sensitive during early infection than most screening tests in current use
- Antigen/antibody combo tests now FDA-approved, that can detect most antibody-negative persons during highly infectious acute infection stage
- Because of cross-reactivity, >60% of persons with HIV-2 infection misclassified as HIV-1 by Western blot



2010 HIV Diagnostics
Conference:
CDC/APHL Proposed New
Testing Algorithm

New HIV Diagnostic Algorithm

A1: 4th generation HIV-1/2 immunoassay

A1+

A1(-)

Negative for HIV-1 and HIV-2
antibodies and p24 Ag

A2: HIV-1/HIV-2 differentiation immunoassay

HIV-1 +

HIV-1 antibodies
detected
Initiate care
(and viral load)

HIV-2 +

HIV-2 antibodies
detected
Initiate care

HIV-1&2 (-)

RNA

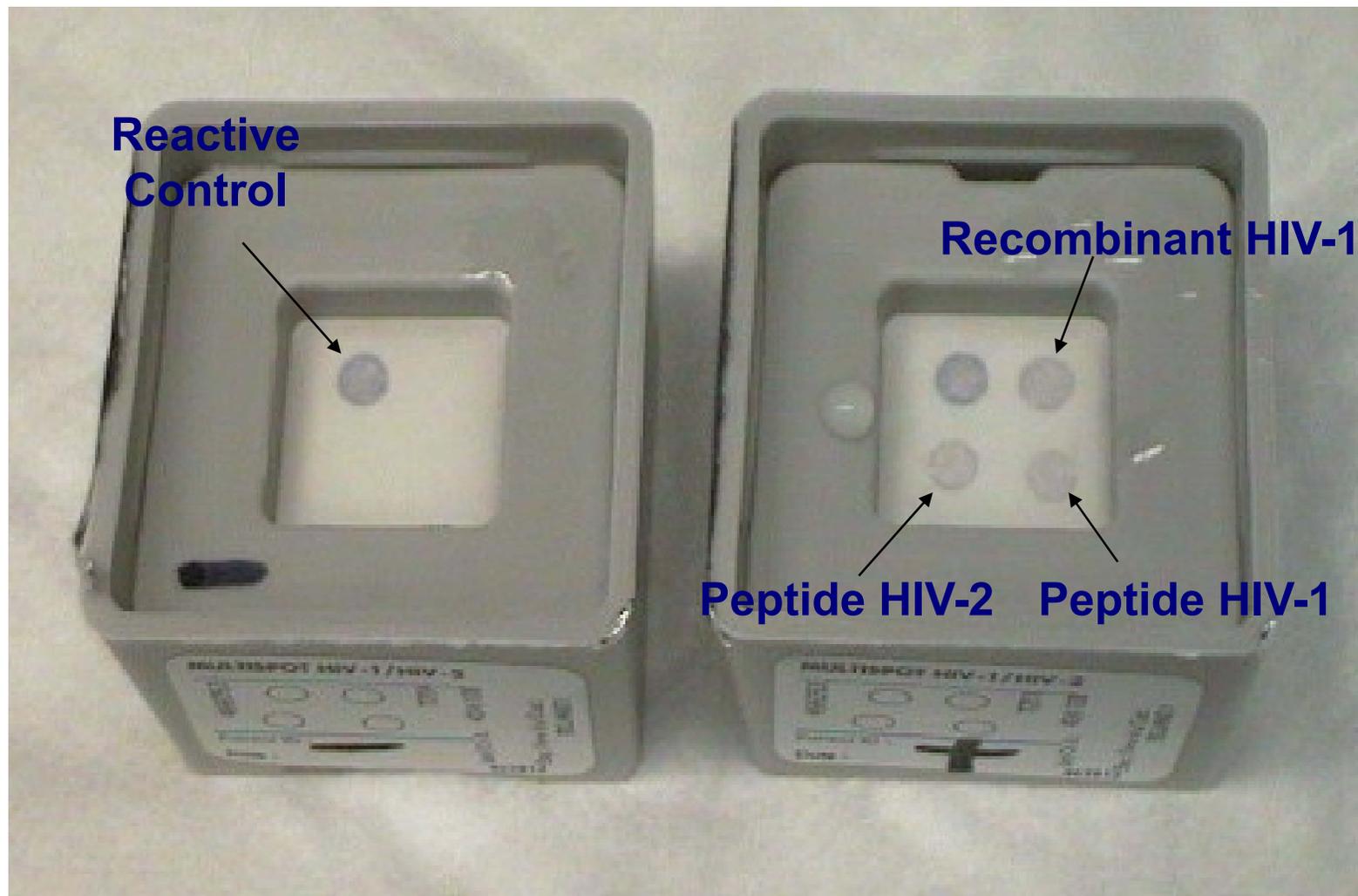
RNA

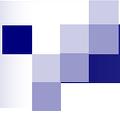
Acute HIV-1 infection
Initiate care

RNA

Negative for HIV-1

FDA-approved HIV-1/HIV-2 Antibody Differentiation Assay





Validation Results – NY State

38,257 specimens – 1,659 GS HIV-1/2 Plus O (3rd gen) EIA repeatedly reactive

Current Algorithm

- 1,546 HIV-1 positive
 - 32 discrepant specimens:
 - 28 indeterminate
 - 4 negative

New Algorithm

- 1,579 HIV-1 positive (MS)
 - 32 discrepant specimens:
 - 29 RNA+
 - 3 follow-up specimens +

Validation Results – NY State

38,257 specimens – 1,659 GS HIV-1/2 Plus O (3rd gen) EIA repeatedly reactive

Current Algorithm

- 1,546 HIV-1 positive
 - 32 *discrepant specimens*:
 - 28 *indeterminate*
 - 4 *negative*

New Algorithm

- 1,579 HIV-1 positive (MS)
 - 32 *discrepant specimens*:
 - 29 *RNA+*
 - 3 *follow-up specimens +*
 - 75 required RNA
 - 3 RNA detected
 - 63 RNA-negative

Validation Results – NY State

38,257 specimens – 1,659 GS HIV-1/2 Plus O (3rd gen) EIA repeatedly reactive

Current Algorithm

- 1,546 HIV-1 positive
 - 32 discrepant specimens:
 - 28 indeterminate
 - 4 negative

- 48 indeterminate

New Algorithm

- 1,579 HIV-1 positive (MS)
 - 32 discrepant specimens:
 - 29 RNA+
 - 3 follow-up specimens +
- 75 required RNA
 - 3 RNA detected
 - 63 RNA-negative

- 9 indeterminate (RNA unsuitable)

Validation Results – NY State

38,257 specimens – 1,659 GS HIV-1/2 Plus O (3rd gen) EIA repeatedly reactive

Current Algorithm

- 5 HIV-2
 - 112 HIV-2 EIA on WB-neg or indeterminate specimens
 - 5 Multispot supplemental

- 36,649 (99.95%) correctly reported negative

New Algorithm

- 5 HIV-2 (Multispot)

- 36,661 (99.98%) correctly reported negative

Abbott Architect Ag/Ab Evaluation: Specimens from 4 CDC studies

Result	HIV-1 infected n=3386	Sensitivity	HIV-1 uninfected n=7551	Specificity
Repeatedly Reactive	3384	99.94%	38	99.5%
	Acute Infections n=58			
Positive	48	82.76%		
Negative	10			

-Chavez et al, J Clin Virol 2011



Implications

- Ability to detect acute HIV infection
 - Focus for partner services and intervention efforts
 - New surveillance case definition
- Ability to detect HIV-2 infections
 - False-Negative results with viral load tests
 - Do not respond to many ARVs, e.g. NNRTI's and several protease inhibitors

Using Rapid HIV Testing Algorithms to Improve the Accuracy of HIV Testing, Receipt of Test Results, and Linkage to Care

- *Delaney et al, CROI 2011*



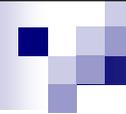


Intervention

- **Rapid test algorithm**

- Clients with a preliminary-positive test have blood drawn for standard (offsite) confirmatory testing
- Up to 2 additional rapid blood tests
- **2 positive rapid tests = same day referral** for HIV care

- Los Angeles: 4 sites San Francisco: 5 sites



Comparison

- **Rapid test with laboratory confirmation**

- Clients with a preliminary-positive test had blood drawn for standard offsite confirmatory testing
- Appointment scheduled (usually for 7 days later) to receive confirmatory test results
- **Referral if confirmatory test positive**

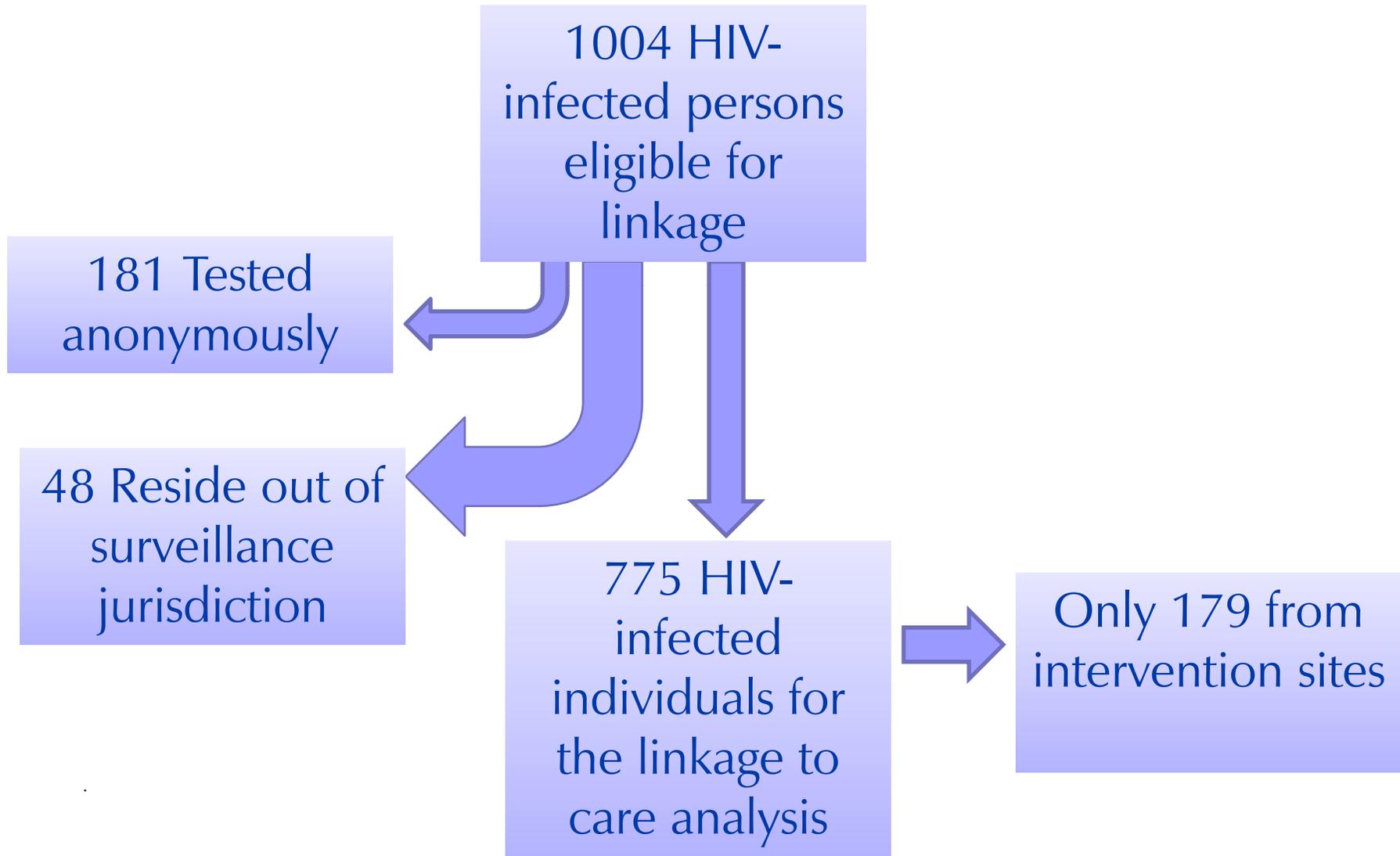
- Los Angeles: 12 sites San Francisco: 11 sites

Results

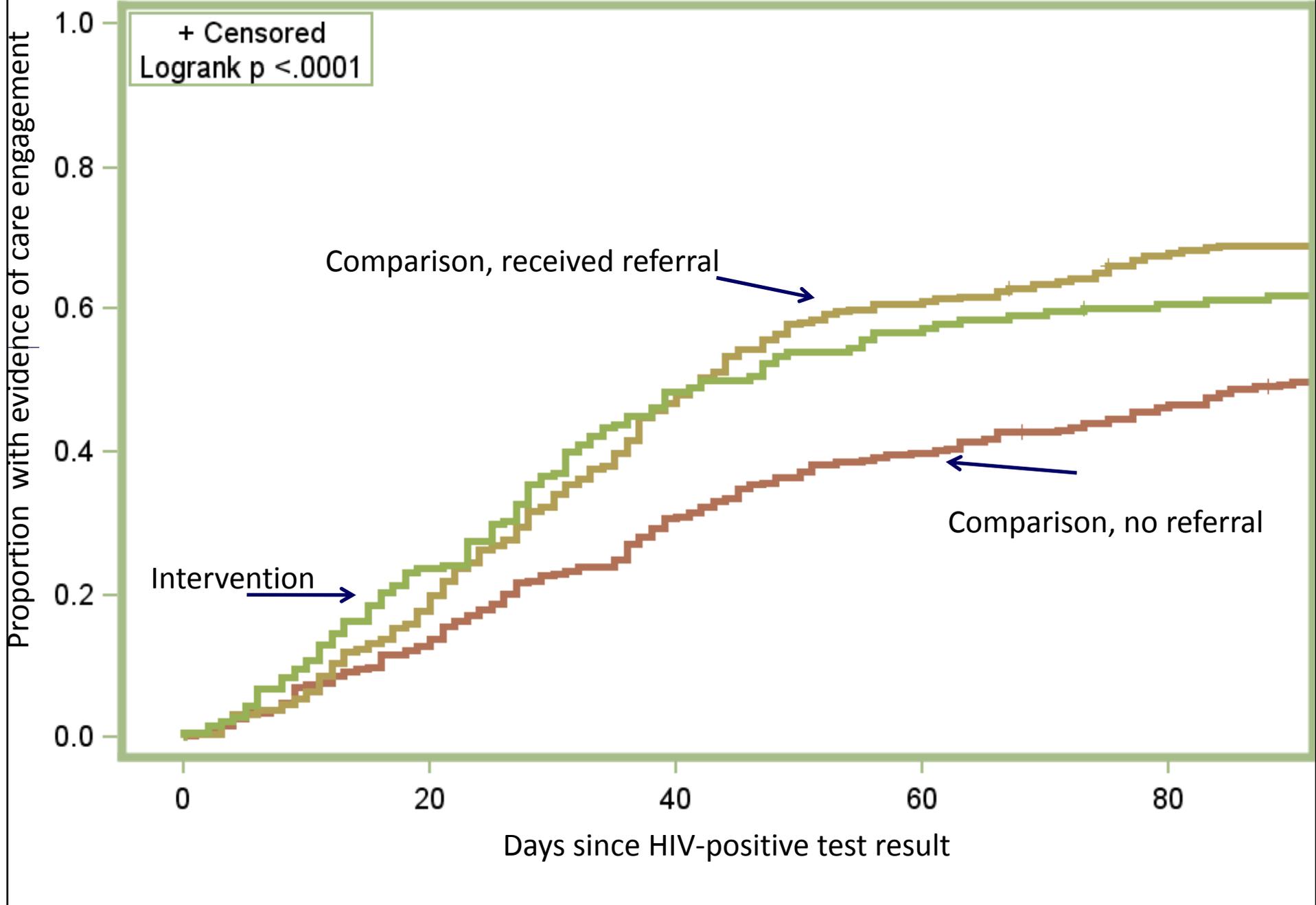
	Intervention Sites		Comparison Sites	
	N	%	N	%
False-positive rapid test	37	14.8%	124	13.6%
Confirmed positive	213	85.2%	791	86.4%
Positive on multiple rapid tests	213* 100.0%			
Received results	250	100.0%	430 47.0%	

*Includes one client who tested (false) negative on the 2nd test before testing positive on a third rapid test

Engagement in Care within 3 Months



Estimates of Time from Diagnosis to First Reported CD4 or Viral Load



Conclusions

- ❑ PPV: rapid test algorithm 100%; single rapid test 85%
- ❑ Engaged in care <90 days:
 - 67% of clients who received referral
 - 50% of clients who did not return for confirmatory results or receive referral
- ❑ Referral to care after reactive rapid test is essential

On the Horizon...



Rapid HIV 1/2 Ag-Ab Test



HIV 1/2 Rapid Line Assay



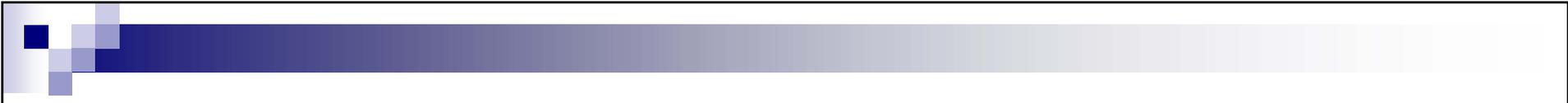
This product is developed for Brazil ONLY and product under registration and recommended for National Algorithm instead of Western Blot

Test result can be read visually or using digital reader (with mobile printer or connectivity to laboratory information system)



Point-of-care finger-stick viral load and CD4 analyzer





Additional Information

- 2011 Journal of Clinical Virology Supplement
 - *Open access:*
 - *www.journalofclinicalvirology.com*
- Guidance for Supplemental HIV Testing
 - *CLSI M-53A Criteria for Laboratory Testing and Diagnosis of HIV, 2011*
 - *Updated CDC Recommendation anticipated 2012*



The findings and conclusions in this presentation are those of the author and do not necessarily represent the views of the Centers for Disease Control and Prevention