Evolving Diagnostic Technologies and Emerging Issues Related to HIV Testing

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Outline

- Changing Landscape for HIV diagnostics and diagnostic algorithms
- Reporting preliminary positive results
- Good laboratory practice, evolving public health and clinical practice recommendations, and inconsistencies with package inserts

Algorithm

- Overall test sensitivity or specificity may be improved by using test combinations under one or more decision rules for resolving discordant results.
  - Sensitivity optimized: Positive if either of two tests in algorithm is positive
  - Specificity optimized: Positive if both of two tests in algorithm are positive

Albritton et al. HIV testing for patient-based and population-based diagnosis. J Infect Dis. 1996

July 21, 1989 / Vol 38 / No. S-7

MMWR

MORBIDITY AND MORTALITY WEEKLY REPORT

Printed and distributed by the Massachusetts Medical Society, publishers of *The New England Journal of Medicine*

Recommendations and Reports

Interpretation and Use of the Western Blot Assay for Serodiagnosis of Human Immunodeficiency Virus Type 1 Infections

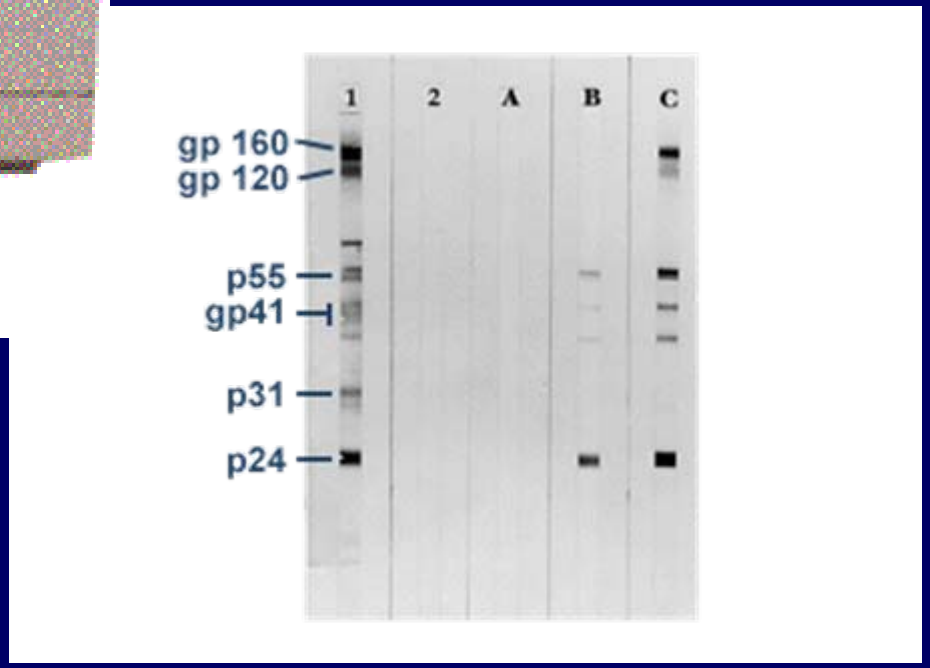
Diagnostic Algorithm: 1989

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

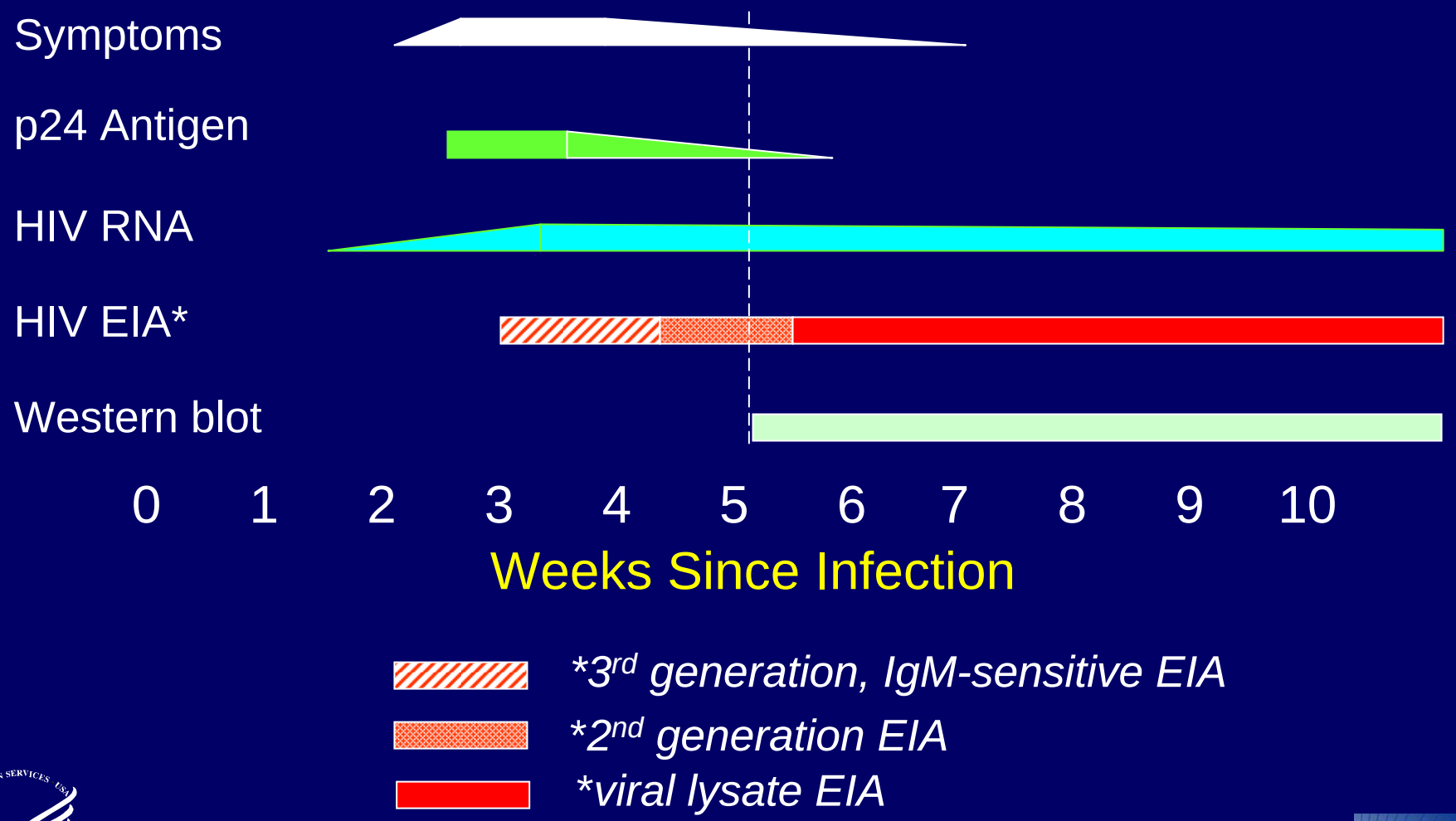
1989: State of the Art

EIA

Western blot



Detection of HIV by Diagnostic Tests



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

EIAs Used by Public Health Labs - 2004

	FDA approval date	*% used by PHL labs, 2004*
Vironostika HIV-1 Microelisa	*1987*	*63%*
Abbott HIVAB HIV-1/2	1992	20%
Genetic Systems rLAV	1998	20%
Gen Sys HIV-1/HIV-2	2000	18%
Gen Sys HIV-1/2 Plus O	2003	10%
Siemens 1/O/2 eHIV	2006	
Ortho Vitros Anti-HIV 1+2	2008	

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

EIAs Used by Public Health Labs - 2006

	FDA approval date	*% used by PHL labs, 2006*
Vironostika HIV-1 Microelisa	*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	14%
Siemens 1/O/2 eHIV	2006	
Ortho Vitros Anti-HIV 1+2	2008	

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

EIAs Used by Public Health Labs - 2006

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Ortho Vitros Anti-HIV 1+2	2008	

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

Random Access Chemiluminescent Assays

- Advia Centaur eHIV
  - *3rd generation*
  - *approved July 2006*

- Ortho Vitros Anti HIV 1+2
  - *3rd generation*
  - *approved March 2008*

- *Both detect HIV 1/2/O*

Random Access to Different Assays On-Board

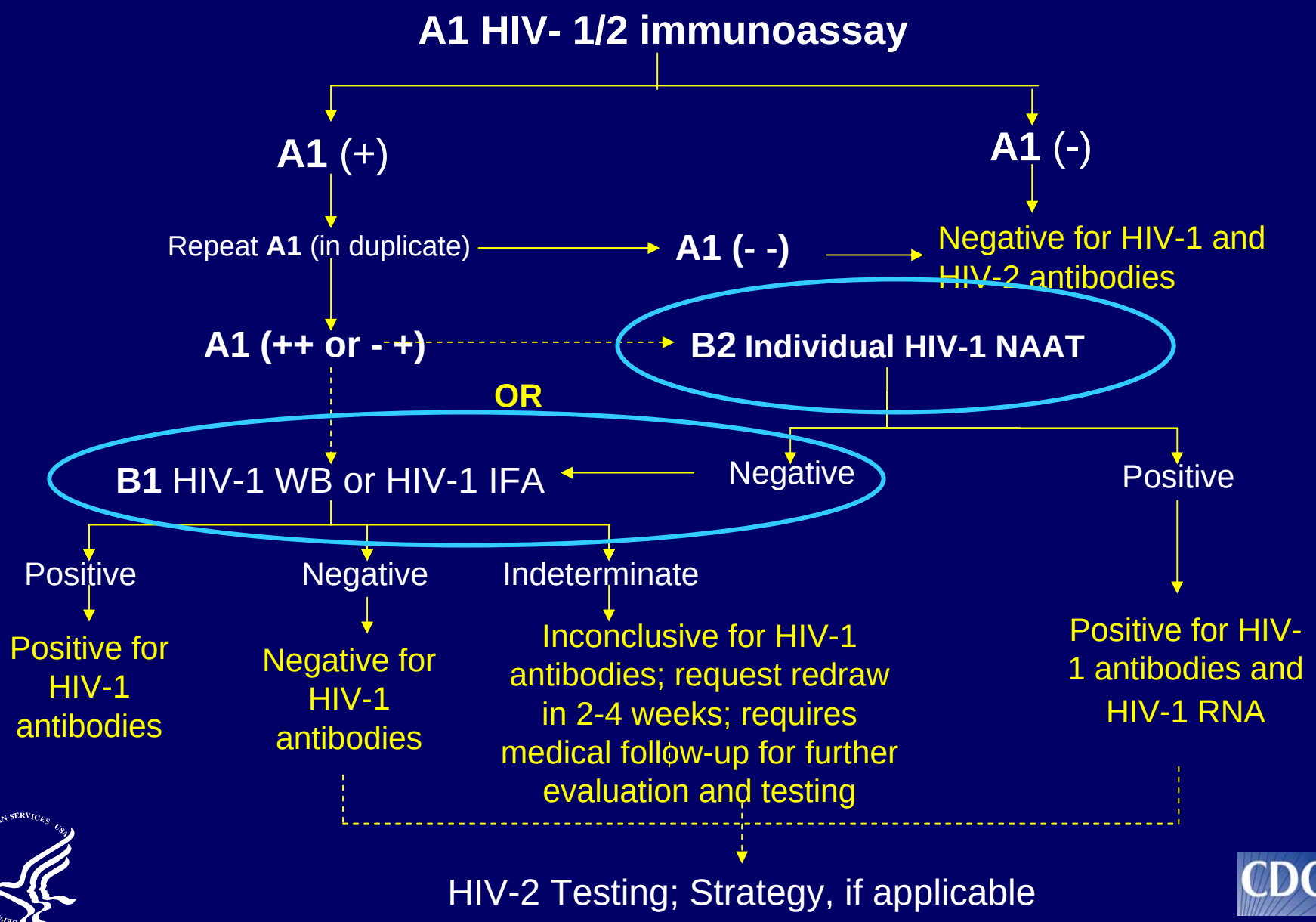
STAT sample requests without pausing

Results in ~60 minutes

APTIMA HIV-1 Qualitative RNA Assay

- Approved September 2006
- Aid to HIV-1 diagnosis
- Diagnosis of acute HIV-1 infection in antibody-negative persons
- Confirmation of HIV-1 infection in antibody-positive persons <u>when it is reactive</u>
  - *5% - 7% of EIA+/WB+ specimens may test negative for HIV-1 RNA*

Lab Strategy 2: HIV-1/2 EIA/WB/IFA/NAAT



CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

March 27, 1998 / Vol. 47 / No. 11

MMWR™

MORBIDITY AND MORTALITY WEEKLY REPORT

209 Imported Dracunculiasis — United States, 1995 and 1997
211 Update: HIV Counseling and Testing Using Rapid Tests — United States, 1995
215 Strategies for Providing Follow-Up and Treatment Services in the National Breast and Cervical Cancer Early Detection Program — United States, 1997
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219 Notices to Readers

Update: HIV Counseling and Testing Using Rapid Tests — United States, 1995

Approximately 25 million persons each year in the United States are tested for antibody to human immunodeficiency virus (HIV). Publicly funded counseling and testing (CT) programs conduct approximately 2.5 million of these tests each year. CT can have important prevention benefits (*1*); however, in 1995, 25% of persons testing HIV-positive and 33% of persons testing HIV-negative at publicly funded clinics did not return for their test results (*2*). Rapid tests to detect HIV antibody can be performed in an average of 10 minutes (*3*), enabling health-care providers to supply definitive

Recommendation …and a Promise

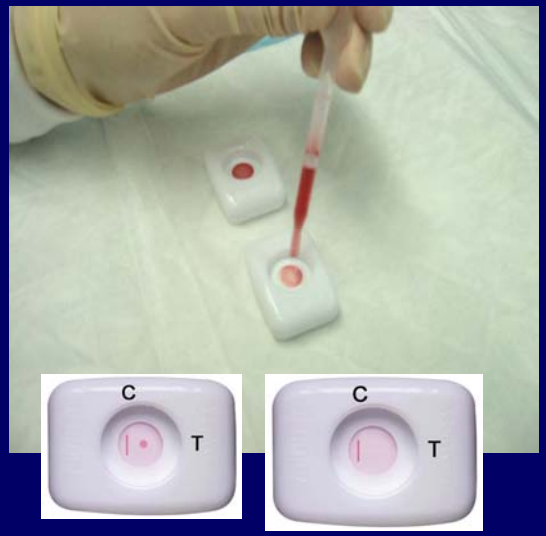
- Health-care providers should **provide preliminary positive test results** before confirmatory results are available in situations where tested persons benefit.

- When additional rapid tests become available for use in the United States, the **PHS will re-evaluate algorithms using specific combinations** of two or more rapid tests for screening and confirming HIV infection.

Uni-Gold Recombigen

Multispot HIV-1/HIV-2

Clearview Complete HIV 1/2



Reveal G3

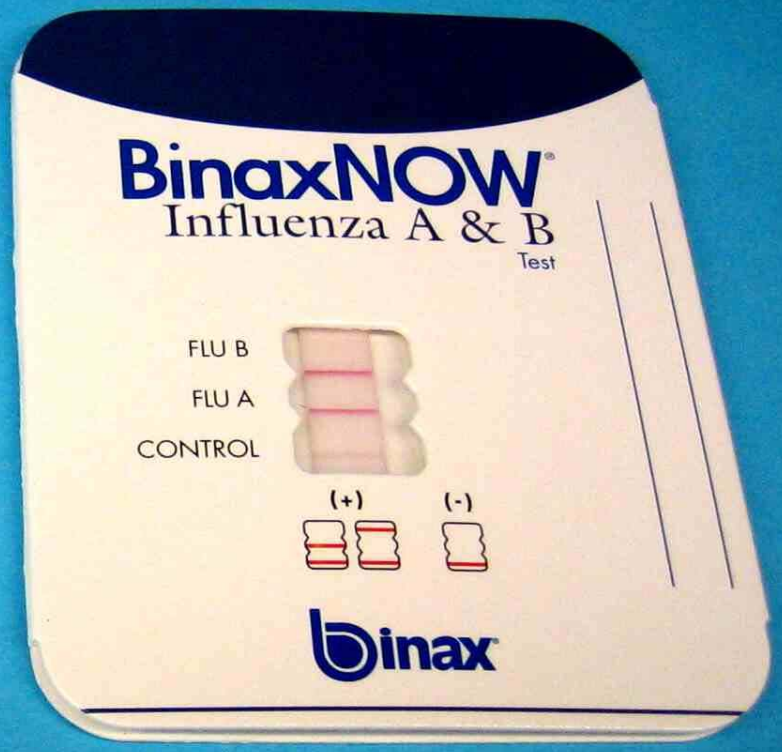
OraQuick Advance

Clearview HIV 1/2 Stat Pak

FDA-Approved Rapid HIV Tests

	Sensitivity (95% C.I.)	Specificity (95% C.I.)
Oral fluid		
OraQuick Advance	99.3 (98.4 - 99.7)	99.8 (99.6-99.9)
Whole blood		
Un-Gold Recombigen	100 (99.5 – 100)	99.7 (99.0 – 100)
Clearview Stat-Pak	99.7 (98.9 – 100)	99.9 (98.6 – 100)
Clearview Complete	99.7 (98.9 – 100)	99.9 (98.6 – 100)
Serum/plasma		
Reveal G3	99.8 (99.2 – 100)	99.9 (98.6 – 100)
Multispot	100 (99.9 – 100)	99.9 (99.8 – 100)

Senitivity: Influenza Rapid Tests



A: 92.5%

B: 95%

A: 77%

B: 82%

A: 81%

B: 65%

POC Strategy 1: Single Rapid Test for HIV Screening

A1

[HIV-1 or HIV-1/2 rapid test, Oral Fluid or Blood]

A1+
Preliminary positive for HIV-1 or HIV-2 antibodies; requires supplemental laboratory testing

A1 –
Negative for HIV-1 and HIV-2 antibodies*

If using an HIV-1 only rapid test*, **Negative for HIV-1 antibodies only

Developing Alternative HIV Testing Algorithms

- APHL/CDC HIV Steering Committee
  - Priority to develop new algorithms with directives from APHL Board of Directors, CDC
- Algorithm Workgroups
  - Point of care (POC)
  - Laboratory
- Data gathering –retrospective and prospective

www.aphl.org/hiv/statusreport

Organizations, Agencies and Groups Represented on Workgroups

- APHL
- ACLA
- ASM
- Blood Banks
- CAP
- CDC
- Commercial Labs
- DoD
- FDA
- HIV Program Staff

- Proposed algorithms:

www.aphl.org/hiv/statusreport

CDC Alternative Algorithm Study

- Infected and uninfected U.S. blood donors: 997
  - *621 HIV+, 513 HIV-, 41 Indeterminate*
- International: 178 total, 128 non-B subtypes
  - Blood donors: 64
  - CDC Cameroon study: 114
- Seroconversion panels:
  - *183 specimens from 15 pts*
- HIV-2 specimens: 32

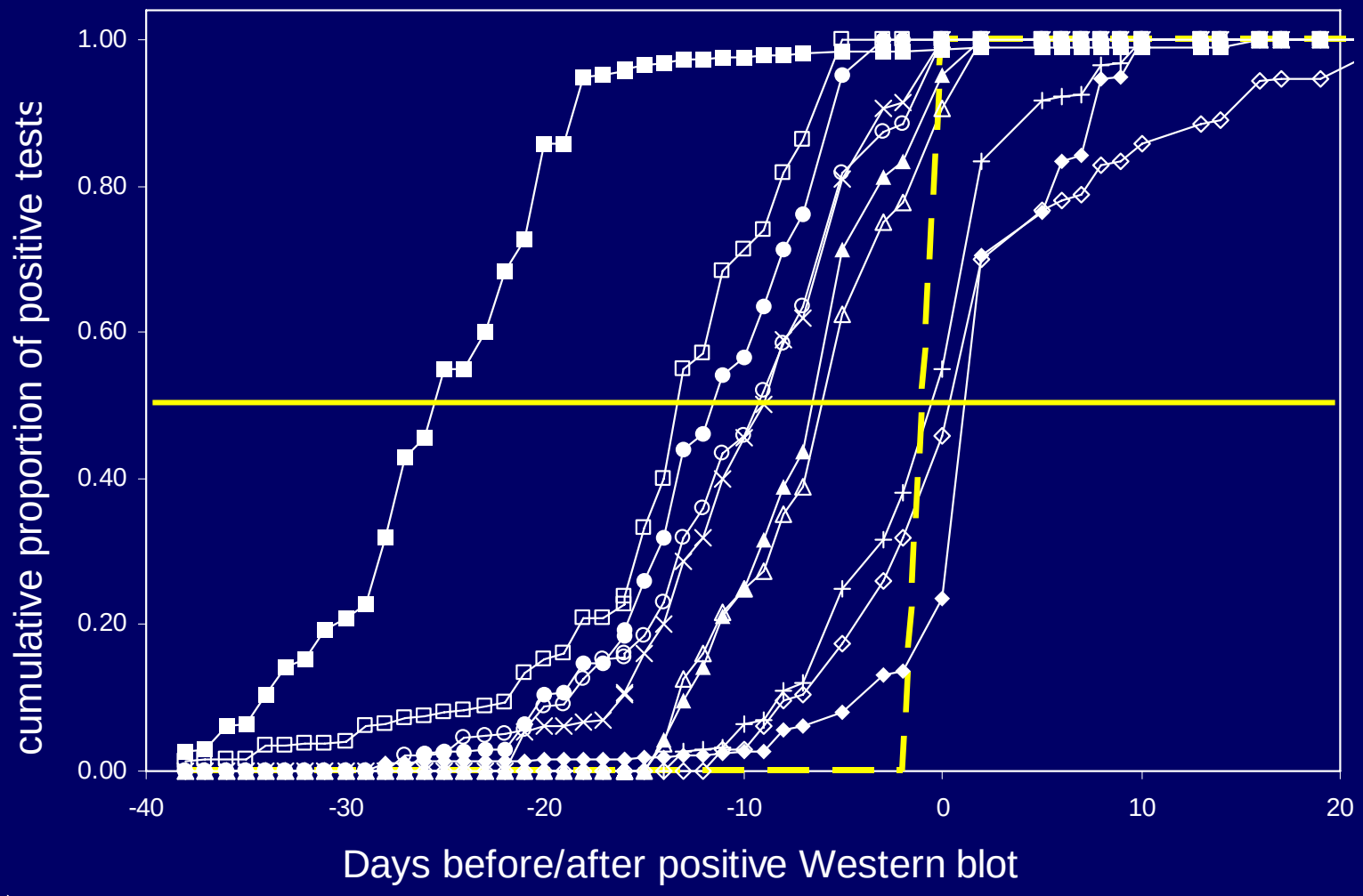
R z h q#hwdo/M#Fdq1#P lfurelrd1#P dufk#533;

CDC Alternative Algorithm Study

Test *621 HIV+, 513 HIV-*	Sensitivity	Specificity
Genetic Systems HIV-1/2+O	99.8	99.4
Abbott rDNA	99.4	97.7
Vironostika HIV-1 + O	99.7	99.0
Genetic Systems HIV-1/2 peptide	98.7	99.8
Genetic Systems rLAV	97.4	100.0
Vironostika HIV-1 microelisa	99.0	98.4
Oraquick Advance	98.6	99.8
Reveal G2	99.0	99.8
Uni-Gold Recombigen	98.4	99.4
Procleix *[Aptima]*	97.4	99.6
CDC RNA	95.8	99.4
Ampliscreen	92.6	96.9

Owen et al, J. Clin. Microbiol. March 2008

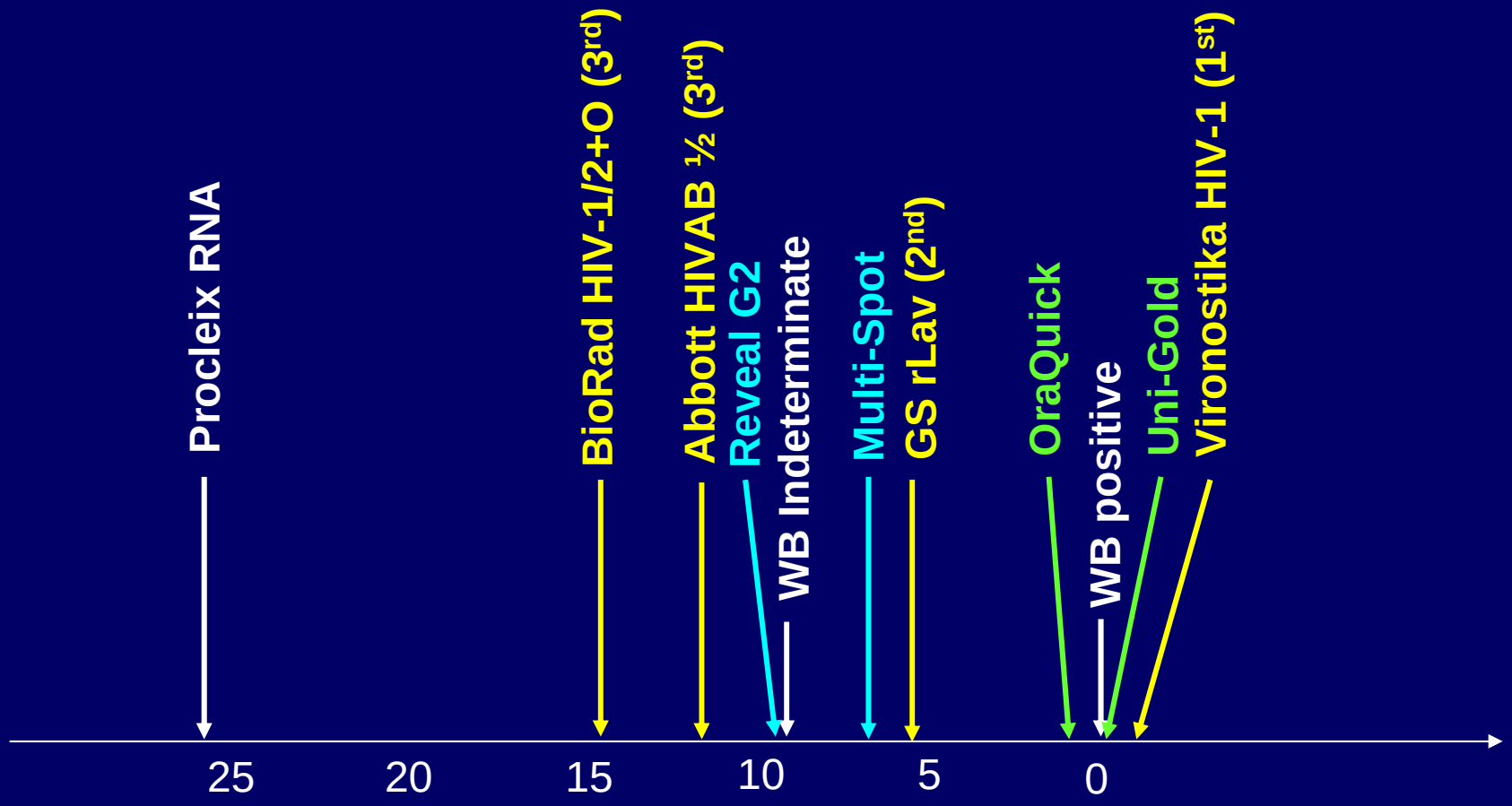
Current Assays with 15 Seroconverter Panels



183 specimens from 15 seroconverters

Owen et al, J. Clin. Microbiol. doi:10.1128/JCM.02196-07 March 2008

Current Assays with 15 Seroconverter Panels

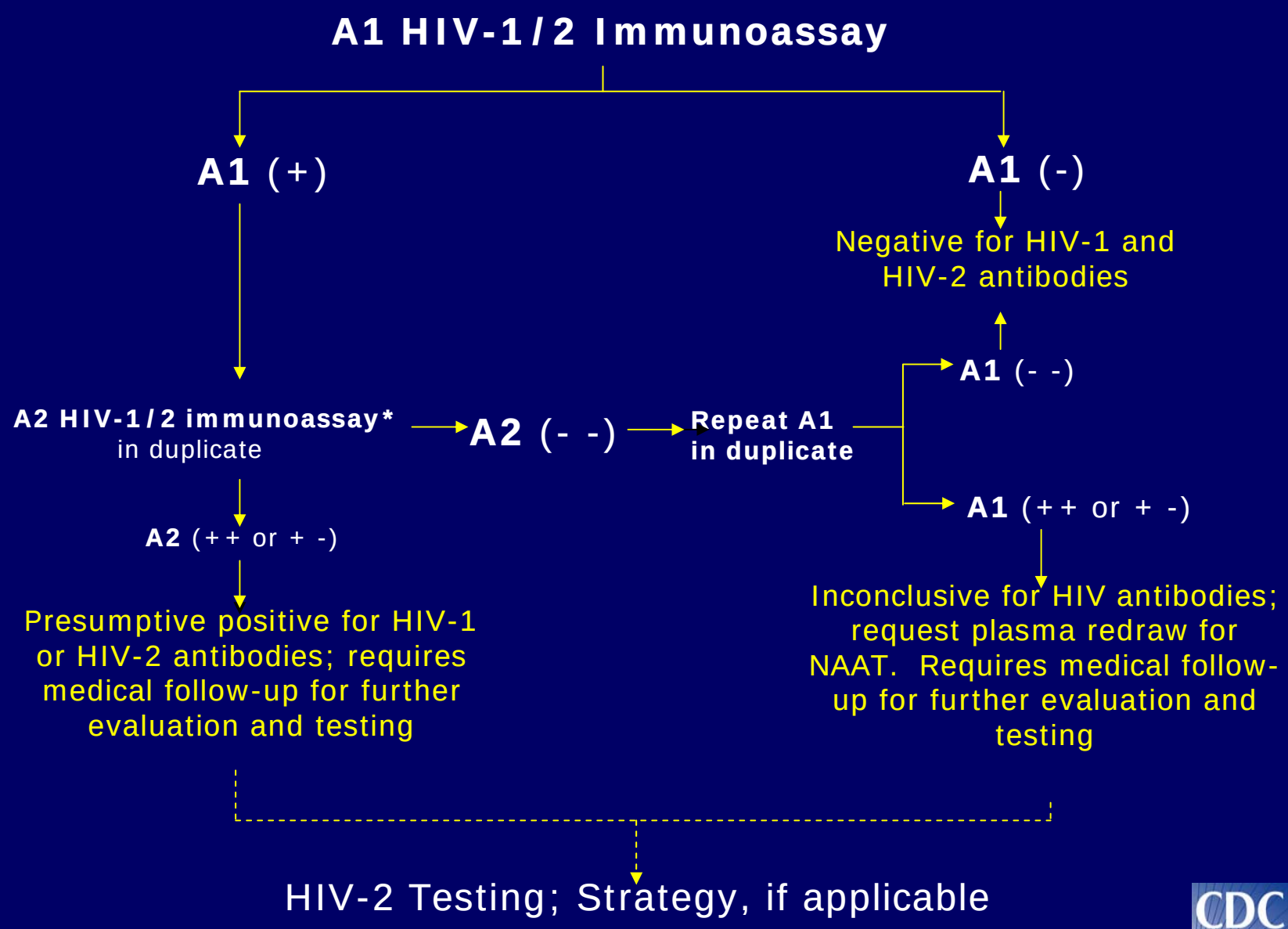


Days before Western blot positive when 50% of Specimens Reactive

185 specimens from 15 seroconverters

Owen et al, J. Clin. Microbiol. doi:10.1128/JCM.02196-07 March 2008

Lab Strategy 3. HIV-1/2 Dual Immunoassay



Specificity – Optimized Algorithm (both tests positive)

	GS HIV-1/2+O	Abbott	Vir HIV-1+O	GS HIV-1/2 peptide	GS rLAV	Vir HIV-1	Ora-quick	Reveal	Uni-Gold	Procleix	CDC RNA	Ampli-screen
GS HIV-1/2+O	99.8 / 99.4	99.4	99.7	98.7	97.4	99.0	98.6	99.0	98.4	97.3	95.6	92.4
Abbott	99.6	99.4 / 97.7	99.2	98.6	97.1	98.9	98.4	98.9	98.2	97.3	95.7	92.4
Vir HIV-1+O	100.0	100.0	99.7 / 99.0	98.7	97.4	99.0	98.6	99.0	98.4	97.3	95.7	92.4
GS HIV-1/2 peptide	100.0	100.0	100.0	98.7 / 99.8	97.3	98.4	98.6	98.7	98.0	97.1	95.7	92.4
GS rLAV	100.0	100.0	100.0	100.0	97.4 / 100	97.3	97.1	97.3	96.8	95.7	94.2	91.0
Vir HIV-1	100.0	99.8	99.8	100.0	100.0	99.0 / 98.4	98.2	98.7	98.2	97.1	95.5	92.2
Oraquick	100.0	100.0	100.0	100.0	100.0	100.0	98.6 / 99.8	98.6	97.8	96.9	95.5	92.2
Reveal	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.0 / 99.8	98.0	97.3	95.7	92.4
Uni-Gold	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	98.4 / 99.4	96.4	94.8	91.2
Procleix	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	97.4 / 99.6	95.7	92.6
CDC RNA	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	95.8 / 99.4	91.43
Ampliscreen	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	92.6 / 96.9

Sensitivity – Optimized Algorithm (either test positive)

	GS HIV-1/2+O	Abbott	Vir HIV-1+O	GS HIV-1/2 peptide	GS rLAV	Vir HIV-1	Oraquick	Reveal	Uni-Gold	Procleix	CDC RNA	Ampli-screen
GS HIV-1/2+O	99.8 / 99.4	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	100.0	100.0	100.0
Abbott	97.5	99.4 / 97.7	99.8	99.5	99.7	99.5	99.5	99.5	99.5	99.5	99.5	99.5
Vir HIV-1+O	98.4	96.7	99.7 / 99.0	99.7	99.7	99.7	99.7	99.7	99.8	99.8	99.8	99.8
GS HIV-1/2 peptide	99.2	97.5	98.8	98.7 / 99.8	98.9	99.4	98.7	99.0	99.1	99.0	98.9	98.9
GS rLAV	99.4	97.7	99.0	99.8	97.4 / 100	99.2	98.9	99.2	98.9	99.2	99.0	99.0
Vir HIV-1	97.9	96.3	97.7	98.2	98.4	99.0 / 98.4	99.4	99.4	99.3	99.4	99.4	99.4
Oraquick	99.2	97.5	98.8	99.6	99.8	98.2	98.6 / 99.8	99.0	99.1	99.0	98.9	98.9
Reveal	99.2	97.5	98.8	99.6	99.8	98.2	99.6	99.0 / 99.8	99.5	99.2	99.2	99.2
Uni-Gold	99.0	97.3	98.8	99.2	99.4	97.8	99.2	99.2	98.4 / 99.4	99.3	99.1	99.1
Procleix	99.0	97.3	98.6	99.4	99.6	98.1	99.4	99.4	99.0	97.4 / 99.6	97.6	97.4
CDC RNA	98.8	97.1	98.4	99.2	99.4	97.9	99.2	99.2	98.8	99.0	95.8 / 99.4	96.9
Ampliscreen	96.3	94.5	95.9	96.7	96.9	95.3	96.7	96.7	96.3	96.5	96.3	92.6 / 96.9

Additional Validation Studies

- False-positive EIA in pregnant vs non-pregnant
  - 3.3 million tested (BioRad HIV 1/2 Plus O)
  - 921,500 pregnant
    - 0.06% HIV+
    - 0.14% False Positive
  - 2.4 million non-pregnant or unknown
    - 1.3% HIV+
    - 0.21% False Positive

Additional Validation Studies

- Prospective evaluation
  - Screen with BioRad HIV 1/2 Plus O
    - Identify 4,000 EIA reactive, WB-neg or indeterminate
    - Perform all rapid tests, Ortho Vitros, Advia
  - Seroconversion panels, 1200 HIV+, 1500 HIV-
    - Rapid tests performed at CDC
    - Perform Ortho Vitros, Advia
- Solicit validation data from public health laboratories

POC Strategy 2: Two Rapid Tests in Sequence on Blood

[A1 and A2 must be different rapid tests]

A1

A1+

A2

A1-
Negative for HIV-1 and HIV-2 antibodies*

A1+ A2+
Presumptive positive for HIV-1 or HIV-2 antibodies; requires medical follow-up for further evaluation and testing

A1+ A2-
Inconclusive rapid test result; requires additional testing

**If using an HIV-1 only rapid test, Negative for HIV-1 antibodies only*

Results – 2 Rapid Tests, Sequential

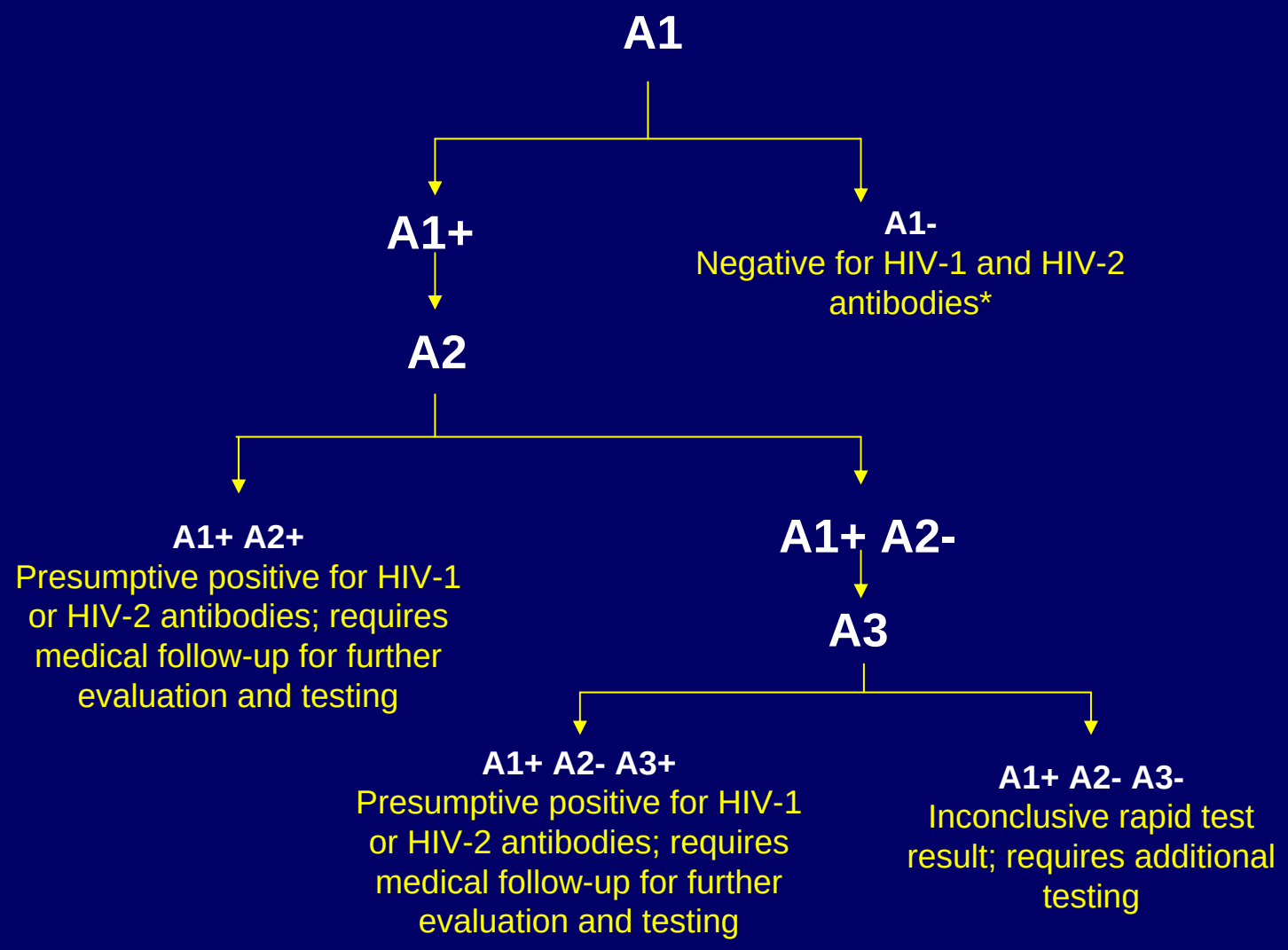
Prospective, 222 HIV+, 4288 HIV- High-Risk Patients

Tests		Algorithm Results				
1st test	2nd test	Further testing	True Positive	False Negative	True Negative	False Positive
CLIA-Waived						
OQ-b	SP-b	2	221	1	4288	0
OQ-o	UG-b	4	149	4	4287	0
SP-b	OQ-b	1	221	2	4288	0
CLIA- Moderate Complexity						
OQ-b	UG-pl	8	215	1	4288	0
SP-b	MS-PL	2	220	2	4288	0
MS-PL	Re-PL	35	219	1	4256	1

Combination Rapid Test Validations

- 46,404 persons at 113 testing sites in 4 states
  - 581 reactive on initial rapid test
  - 345 agreed to 2nd rapid test
    - 287 positive on 2nd test = WB positive
    - 56 negative on 2nd test = WB negative
    - 2 negative on 2nd test = WB positive (but positive on same 2nd rapid test in lab)

Strategy 4: Three Screening Tests Performed in Sequence



If using an HIV-1 only rapid test, Negative for **HIV-1 antibodies only*

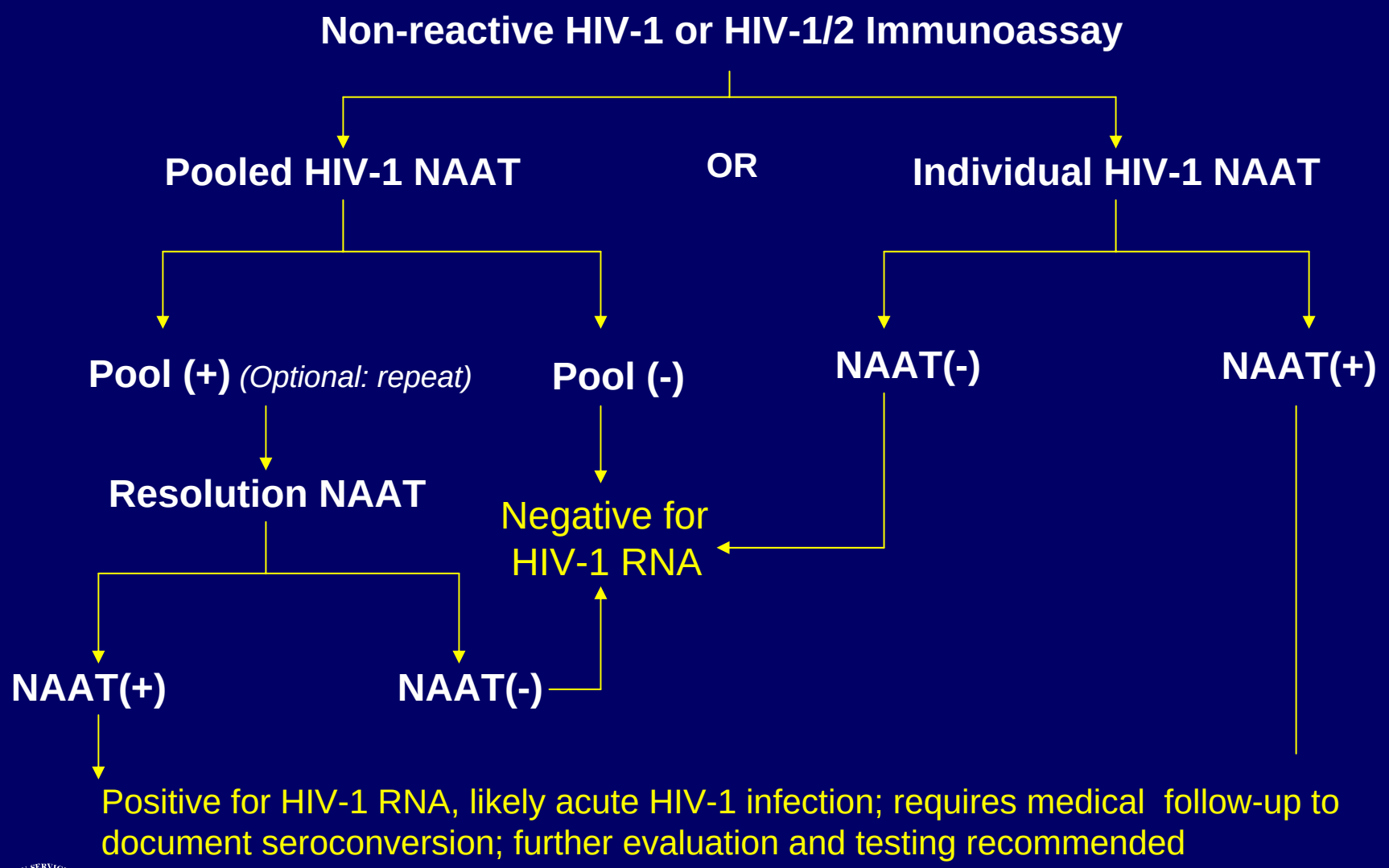
CDC Alternative Algorithm Study

621 HIV+, 513 HIV-

			3-test combination	
Test1	Test2	Test3	sensitivity	specificity
GS HIV-1/2+O	Abbott	Vir HIV-1 + O	99.8	99.6
GS HIV-1/2+O	Vir HIV-1 + O	Abbott	99.8	99.6
Abbott	Vir HIV-1 + O	GS HIV-1/2+O	99.8	99.6
GS HIV-1/2+O	Abbott	Procleix	99.4	99.6
GS HIV-1/2+O	Procleix	Abbott	99.4	99.6
Abbott	Procleix	GS HIV-1/2+O	99.4	99.6
OraQuick	Reveal	Uni-Gold	98.7	100.0
OraQuick	Uni-Gold	Reveal	98.7	100.0
Reveal	Uni-Gold	OraQuick	98.7	100.0

Pooled RNA Screening for Early HIV Infection

Strategy 4. Acute HIV Infection Testing

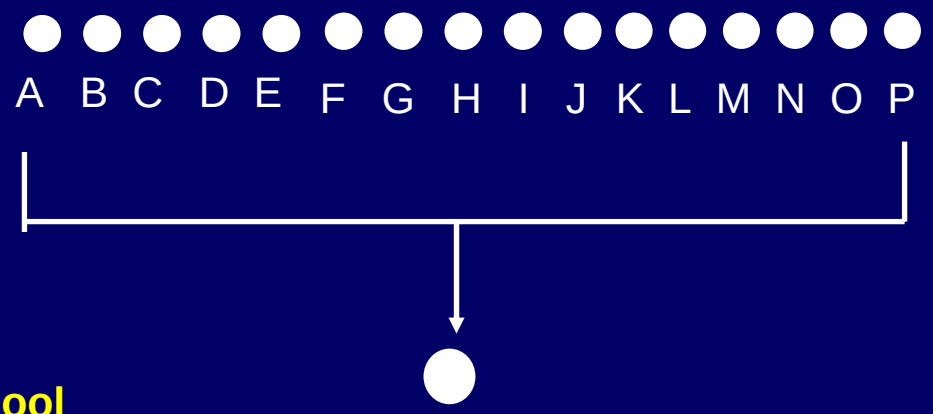


CDC Acute HIV Infection Study

- Florida: Counseling and testing sites statewide
  - NAAT testing at FL state laboratory after negative 3rd gen EIA
- Los Angeles: STD clinics and LA Gay/Lesbian Center clinic
  - NAAT testing and 3rd gen EIA at NY state laboratory after negative 1st gen EIA in Los Angeles

1-Stage Pooling

16 Specimens



1 Master Pool

Yield from Pooled RNA Screening, 2006-08

Site	Number tested	HIV Ab+	RNA+/ Ab-
Florida	54,948	663 (1.2%)†	12 (0.02%)
L.A.	37,012	424 (1.1%)*	35 (0.09%)

†Screened with Bio-Rad 1-2 Plus O
*Screened with Vironostika EIA

Patel et al, CDC unpublished data, 2009

Yield from Pooled RNA Screening, 2006-08

Site	Number tested	HIV Ab+	RNA+/ Ab-
Florida –	54,948	663 (1.2%)†	12 (0.02%)
L.A.	37,012	424 (1.1%)*	35 (0.09%)
L.A.	37,012	441 (1.2%)†	18 (0.05%)

†Screened with Bio-Rad 1-2 Plus O
*Screened with Vironostika EIA

Patel et al, CDC unpublished data, 2009

Acute HIV Screening: 92,564 tested

EIA-RR/WB+	1,107 (1.2%)
NAAT+	1,065 (96.2%)
NAAT-	42 (3.8%)
EIA-RR/WB-ind	30 (0.03%)
NAAT+	3 (10.0%)
NAAT-	27 (90.0%)
EIA-neg/NAAT+	47 (0.05%)
Acute HIV	43 (91%)
False-pos NAAT	4 (9%)

Patel et al, CDC unpublished data, 2009

Acute HIV Screening: 92,564 tested

EIA-RR/WB+	1,107 (1.2%)
NAAT+	1,065 (96.2%)
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Patel et al, CDC unpublished data, 2009

On the Horizon: Antibody-p24 Antigen Combo Assays

Abbott Architect 4th Generation EIA

Determine Ag/Ab
Rapid Test

4th Generation EIA vs. RNA

- 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 seroconversion panels****

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

** Cunningham P, HIV Diagnostics Conf 2007*

*** Patel P, CROI 2009*

**** Owen M, CROI 2009*

Combination Test Validations

- CDC and APHL soliciting data from validations conducted by PHL labs with new tests
- CDC funding comparative evaluations of tests by commercial laboratories
- Data on combination test algorithms to be reviewed at 2010 HIV Diagnostics Conference

Questions for CLIAC

- Does the Committee agree that a menu of combination-test algorithms is appropriate for different testing settings and circumstances?
- Can the Committee comment on the proposed plans for central validation of combination test algorithms?

Questions for CLIAC

- What would CLIAC suggest for making laboratories, testing sites, and clinicians aware of the revised algorithms and result reporting requirements?
- What tools could be used to assist laboratories and testing sites make the appropriate selection of one or more algorithm(s) that would serve the needs of their population?

Reporting Preliminary Results

Rapid Test Package Inserts

- Specify “Preliminary Positive” interpretation
- “This test is suitable for use in multi-test algorithms designed for the statistical validation of rapid HIV test results. When multiple rapid HIV tests are available, this test should be used in appropriate multi-test algorithms.:

EIA Package Inserts

- “Repeatedly reactive specimens must be investigated using supplemental tests for HIV-1 and/or HIV-2. “
- “Repeatedly reactive specimens may contain antibodies to either HIV-1 or HIV-2. Therefore, additional, more specific or supplemental tests for antibodies to both HIV-1 and HIV-2 such as Western blot or immunofluorescence must be performed to verify the presence of antibodies to HIV-1 or HIV-2. “

New York Clinical Laboratory Evaluation Program Advisory, April 2009

- No clinical laboratory shall notify a physician… that an HIV test is positive solely on the basis of HIV antibody screening, except that a clinical laboratory may report a preliminary finding of HIV infection pursuant to the written request of a physician.

- A report of a preliminary finding of HIV infection shall prominently and clearly state that the finding is preliminary, and that results of confirmatory testing will follow.

Circumstances that Warrant Reporting of Preliminary Results Before Confirmation

- Pregnant women in labor for whom ARV prophylaxis would be beneficial
- Evaluation after occupational exposures
- Urgent clinical management of patients with symptoms of HIV-associated conditions
- Inpatients that may be discharged before completion of confirmatory testing
- Persons who are likely to be lost to follow-up

Question to CLIAC

- Does the Committee agree that clinical laboratories should be permitted to report reactive immunoassay results in situations where tested persons benefit?

- How can clinical laboratories accommodate the provider's role in selection of follow-up tests after a reactive immunoassay result?

Evolving Public Health and Clinical Practice Recommendations and Good laboratory Practice

Example: Rapid Testing of Newborns

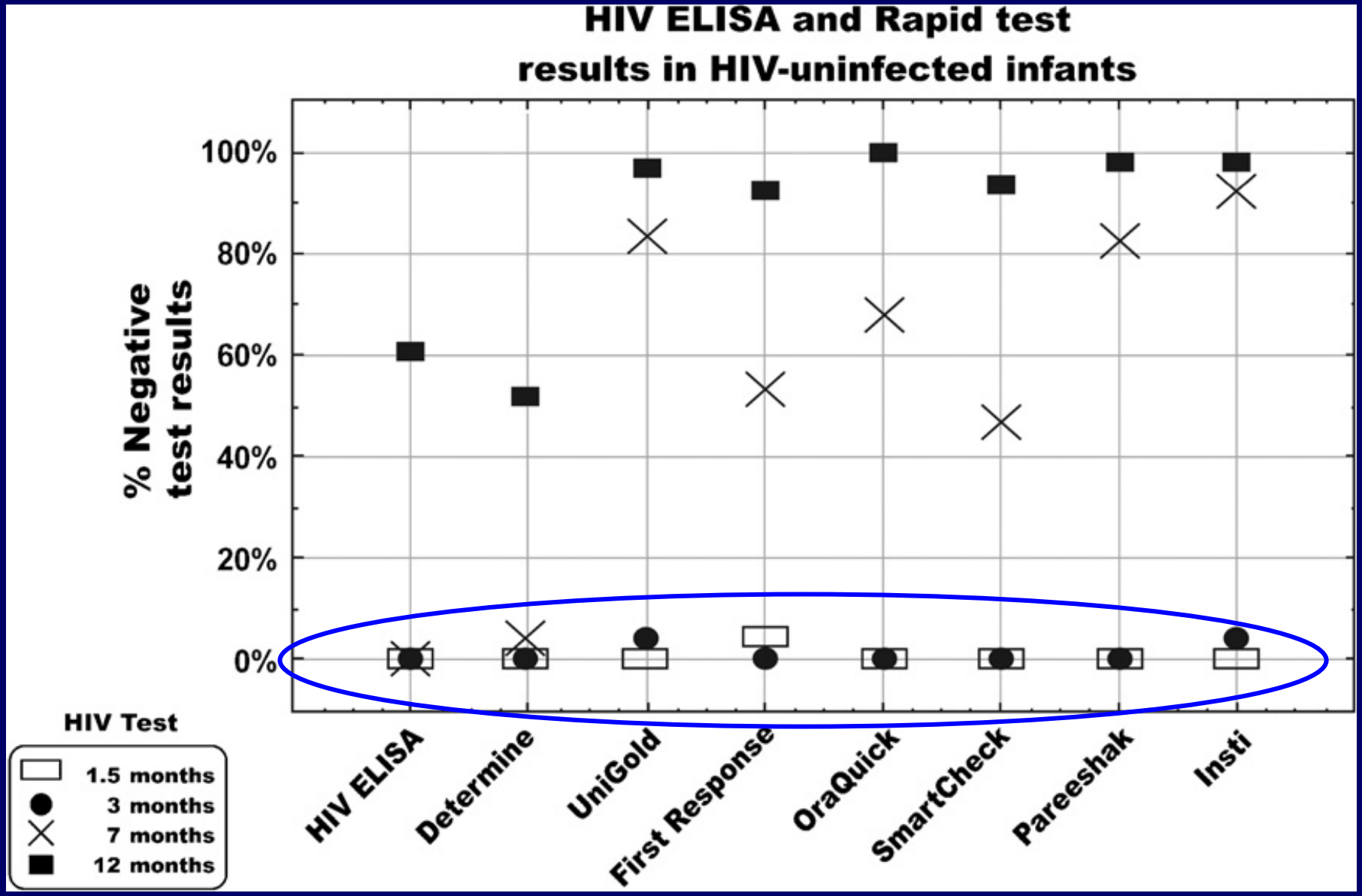
- In 2006, 74% of women giving birth had documented prenatal HIV test results
  - 22% of infants were born to women with undocumented HIV status
- In 2006, an estimated 8,650 – 8,900 HIV-infected women gave birth
  - 1.4% of HIV-exposed infants were born to women with unknown HIV status before delivery, and 1.2% had unknown timing of maternal or neonatal diagnosis

CDC Recommendation

- Because ARVs given to HIV-exposed neonates can reduce HIV transmission by up to 50%, CDC recommends:
  - When the mother's HIV status is unknown postpartum, rapid testing of the newborn as soon as possible after birth is recommended so antiretroviral prophylaxis can be offered to HIV-exposed infants.

Good Clinical Practice

- Detection of maternal antibodies identifies HIV-exposed infants in need of ARV prophylaxis
- Multiple studies from international settings demonstrate tests detect passively transferred maternal antibodies



Sherman et al, J Clin Virol 2008

Dilemma for Laboratories

- "Limitations" statements, rapid test package inserts:
  - "Clinical data has not been collected to demonstrate the performance… in persons under 12 years of age."
  - "This assay has not been evaluated for newborn screening, cord blood specimens, or individuals less than 18 years of age."

Dilemma for Laboratories

- Insufficient demand for manufacturers to seek indication for detection of maternal antibodies in HIV-exposed infants
- Too few HIV-exposed infants in any institution to allow performance validation
- Difficult to perform validations on limited-volume specimens from infants

Other Examples

- Oral fluid EIA when FDA-approved product withdrawn from market
- HIV-2 validation and confirmation

Question for CLIAC

- Please comment on mechanisms to facilitate implementation of evolving public health and clinical practice recommendations in good laboratory practice

Questions for CLIAC

- Does the Committee agree that a menu of combination-test algorithms is appropriate for different testing settings and circumstances?
- Can the Committee comment on the proposed plans for central validation of combination test algorithms?

Questions for CLIAC

- What would CLIAC suggest for making laboratories, testing sites, and clinicians aware of the revised algorithms and result reporting requirements?

- What tools could be used to assist laboratories and testing sites make the appropriate selection of one or more algorithm(s) that would serve the needs of their population?

Question to CLIAC

- Does the Committee agree that clinical laboratories should be permitted to report reactive immunoassay results in situations where tested persons benefit?

- How can clinical laboratories accommodate the provider's role in selection of follow-up tests after a reactive immunoassay result?

Question for CLIAC

- Please comment on mechanisms to facilitate implementation of evolving public health and clinical practice recommendations in good laboratory practice