

# The Changing Landscape of HIV Testing

Patricia Slev, PhD, D(ABCC)

June 21, 2012

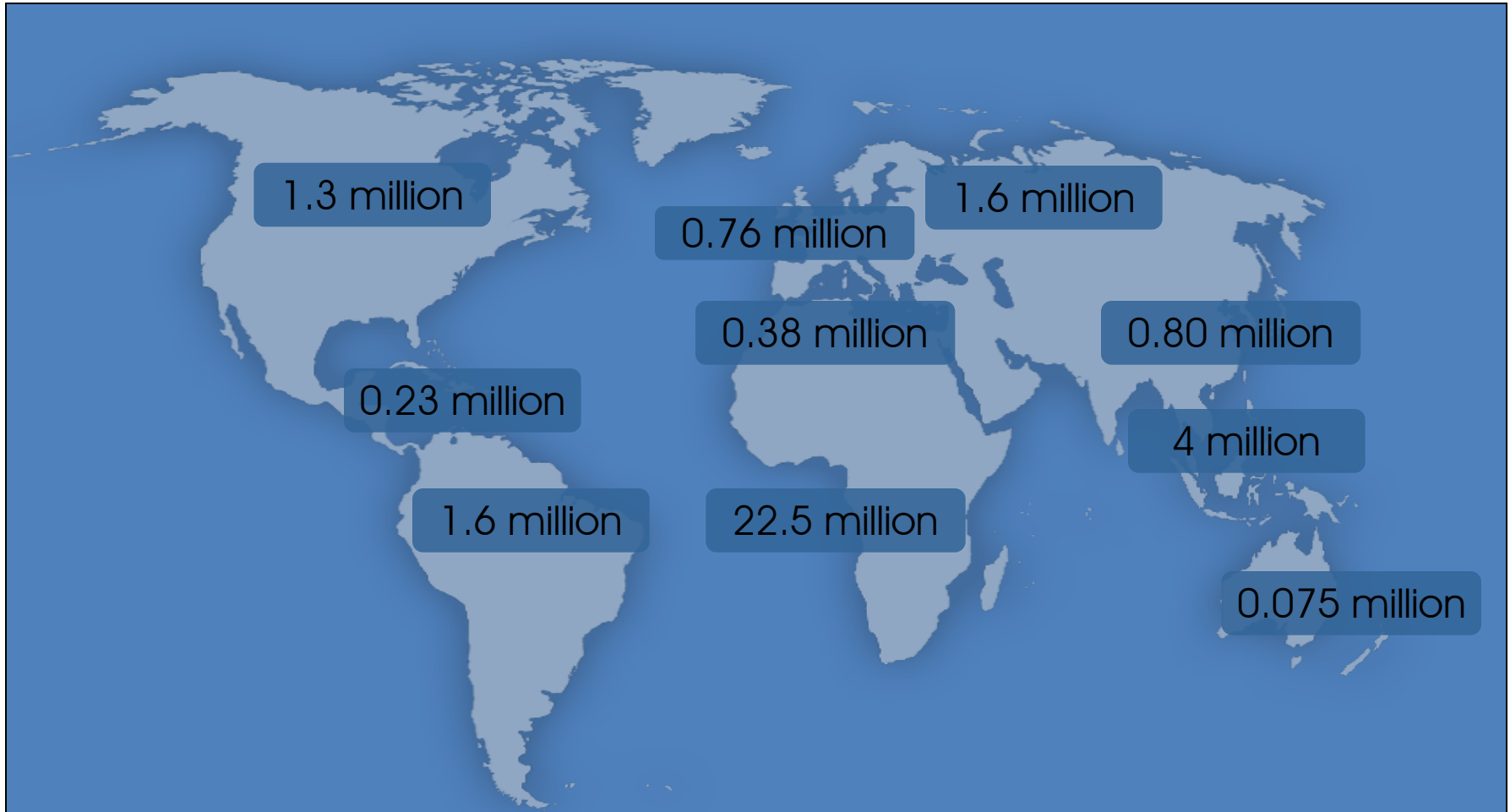
# Disclosure

- The speaker has nothing to disclose

# Objectives

- Explain the principles and benefits of Ag/Ab combination HIV screening assays.
- Describe the new CDC HIV diagnostic algorithm.
- Understand the limitations of Western blot confirmation.
- Use screening and follow-up confirmatory tests appropriately.

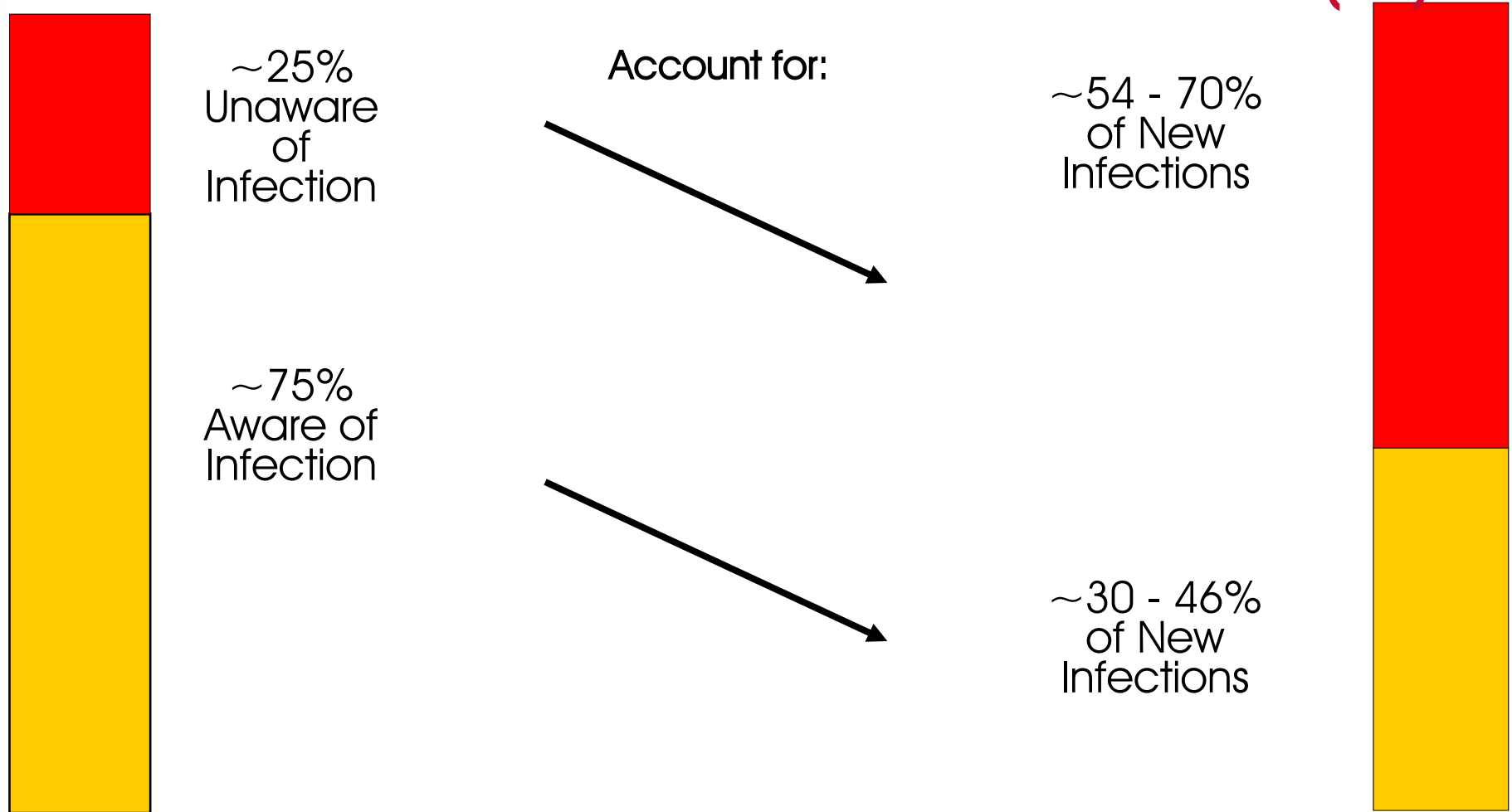
# Global HIV Epidemiology



WHO, UNAIDS. 07 AIDS epidemic update. [http://data.unaids.org/pub/EPISlides/2007/2007\\_epiupdate\\_en.pdf](http://data.unaids.org/pub/EPISlides/2007/2007_epiupdate_en.pdf).

Adapted from Siemens slide

# Awareness of Serostatus Among People with HIV and Estimates of STD Transmission (US)



Marks et al. AIDS 2006

People Living with HIV/AIDS: 1,039,000-1,185,000

New Sexual Infections Each Year: ~32,000

# CDC: Revised HIV Screening Recommendations



## Morbidity and Mortality Weekly Report

---

Recommendations and Reports

September 22, 2006 / Vol. 55 / No. RR-14

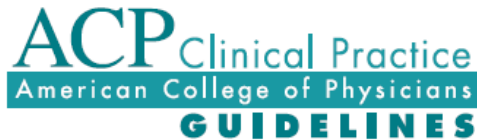
---

### Revised Recommendations for HIV Testing of Adults, Adolescents, and Pregnant Women in Health-Care Settings

# 2006 CDC Guidelines “Universal Testing”

- Routine HIV  
voluntary, not based on risk
- Opt-Out  
option to decline, general consent for care includes HIV testing
- Prevention Counseling  
no longer required
- Population  
13 -64 years old
- Venue  
inpatient services, ER, urgent care, STD clinics,  
substance abuse and correctional facilities

# HIV Screening: American College of Physicians-2009



CLINICAL GUIDELINES

## Screening for HIV in Health Care Settings: A Guidance Statement From the American College of Physicians and HIV Medicine Association

Amir Qaseem MD, PhD, MHA; Vincenza Snow, MD; Paul Shekelle, MD; Robert Hopkins Jr., MD; and Douglas K. Owens, MD, MS, for the Clinical Efficacy Assessment Subcommittee of the American College of Physicians\*

**Description:** The American College of Physicians (ACP) developed this guidance statement to present the available evidence on screening for HIV in health care settings.

**Methods:** This guidance statement is derived from an appraisal of available guidelines on screening for HIV. Authors searched the

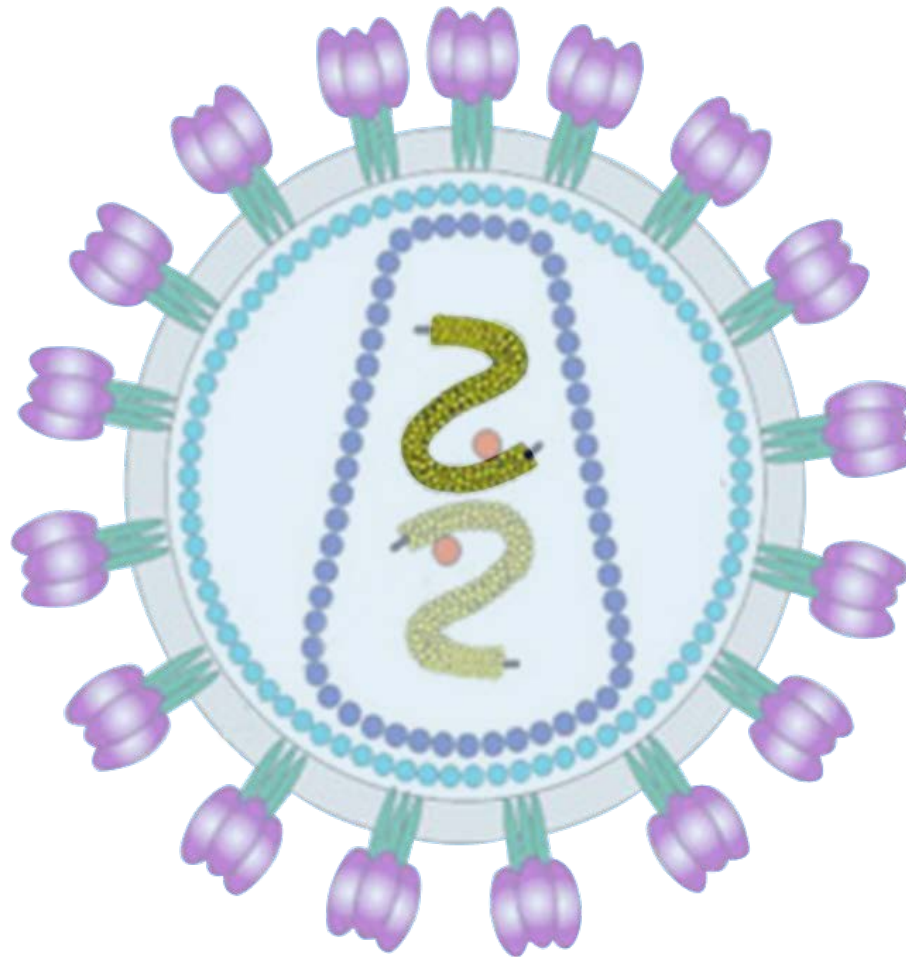
**Guidance Statement 1:** ACP recommends that clinicians adopt routine screening for HIV and encourage patients to be tested.

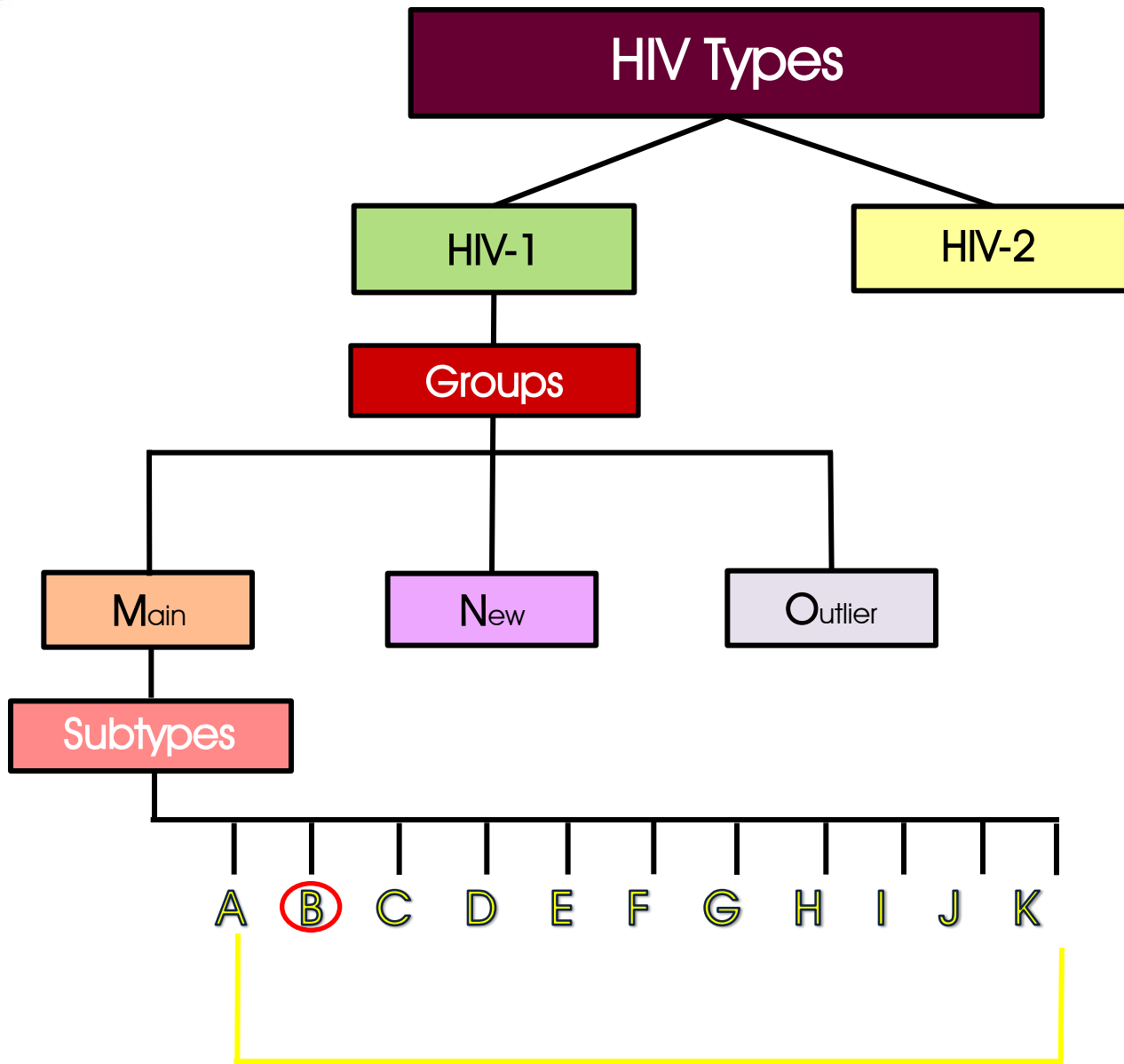
**Guidance Statement 2:** ACP recommends that clinicians determine the need for repeat screening on an individual basis.

- Screen all patients 13 years and older for HIV
- Retest high risk patients (per physician perception of need)
- Importance of screening pregnant women



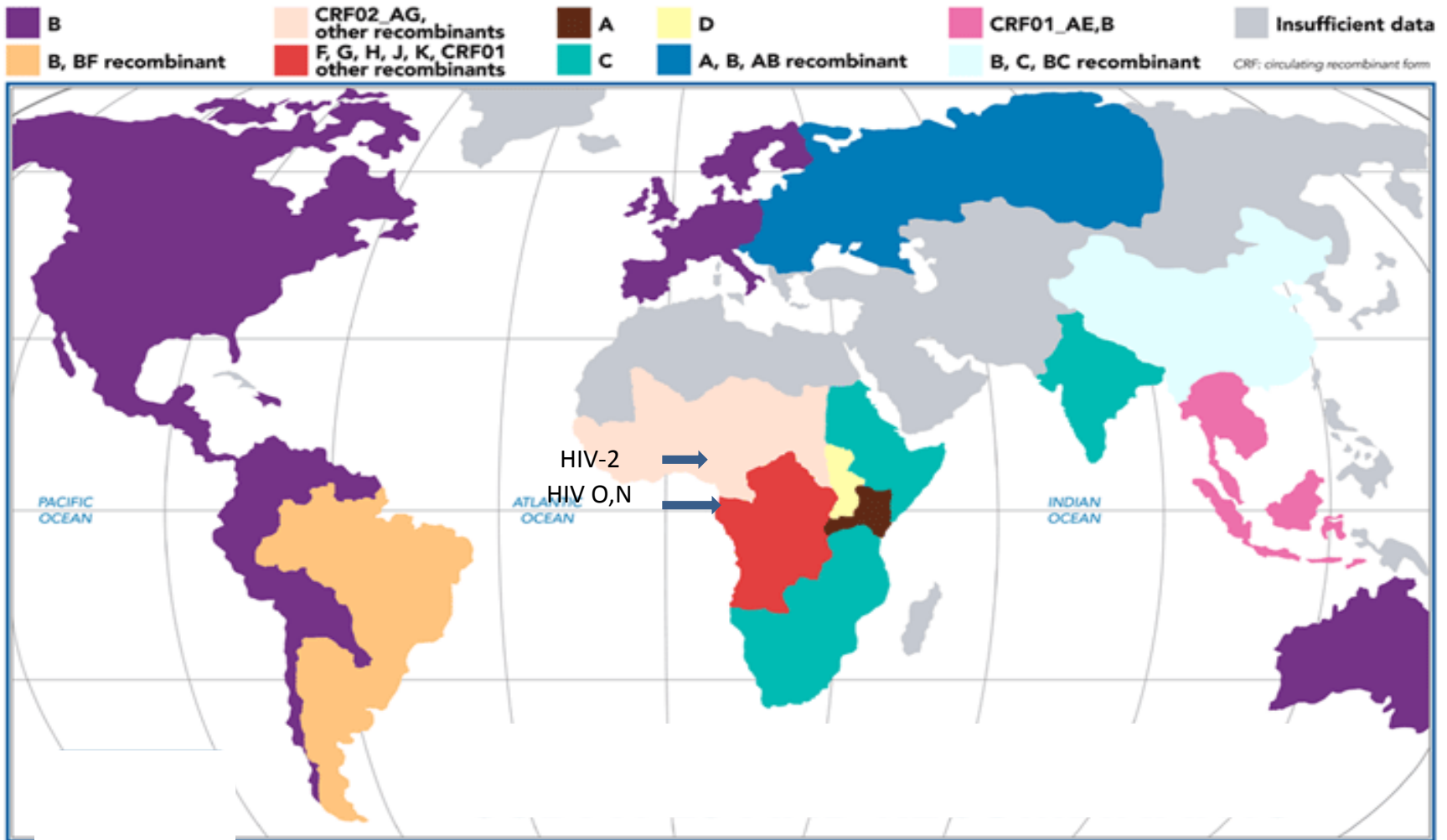
# Human Immunodeficiency Virus (HIV)





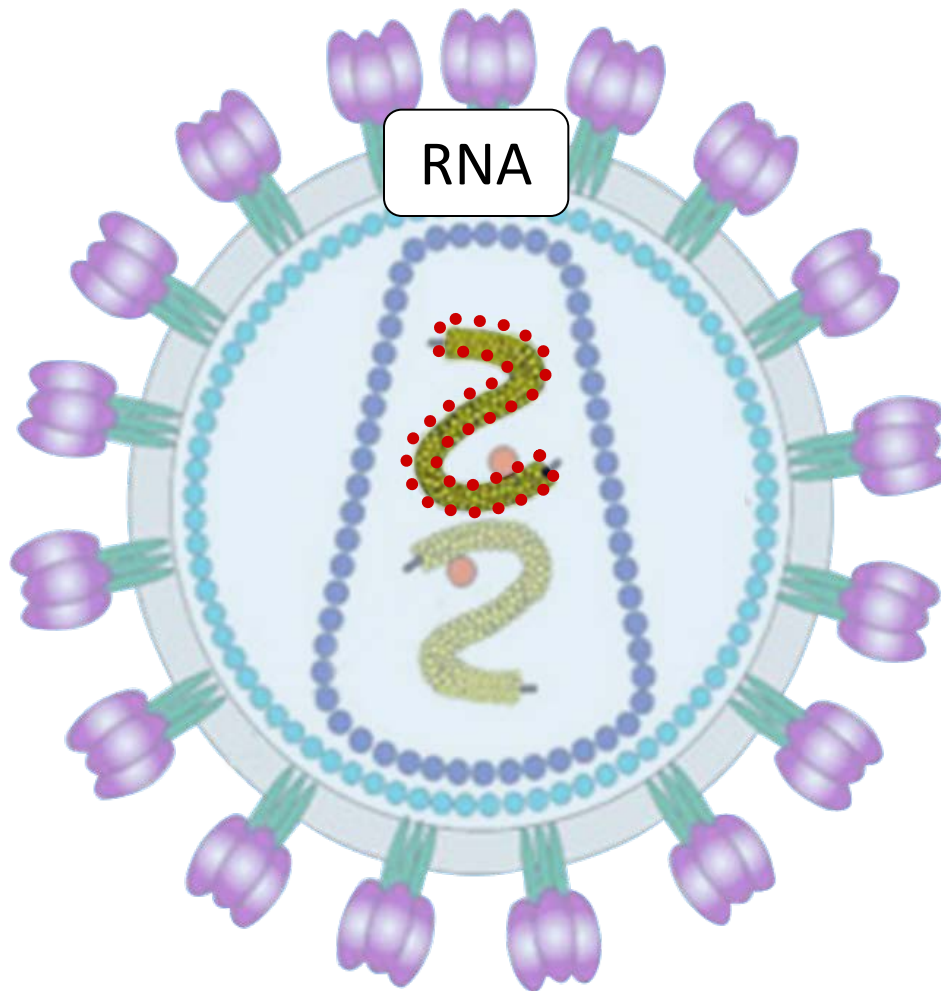
Circulating Recombinant Forms (CRF)

# HIV Distribution

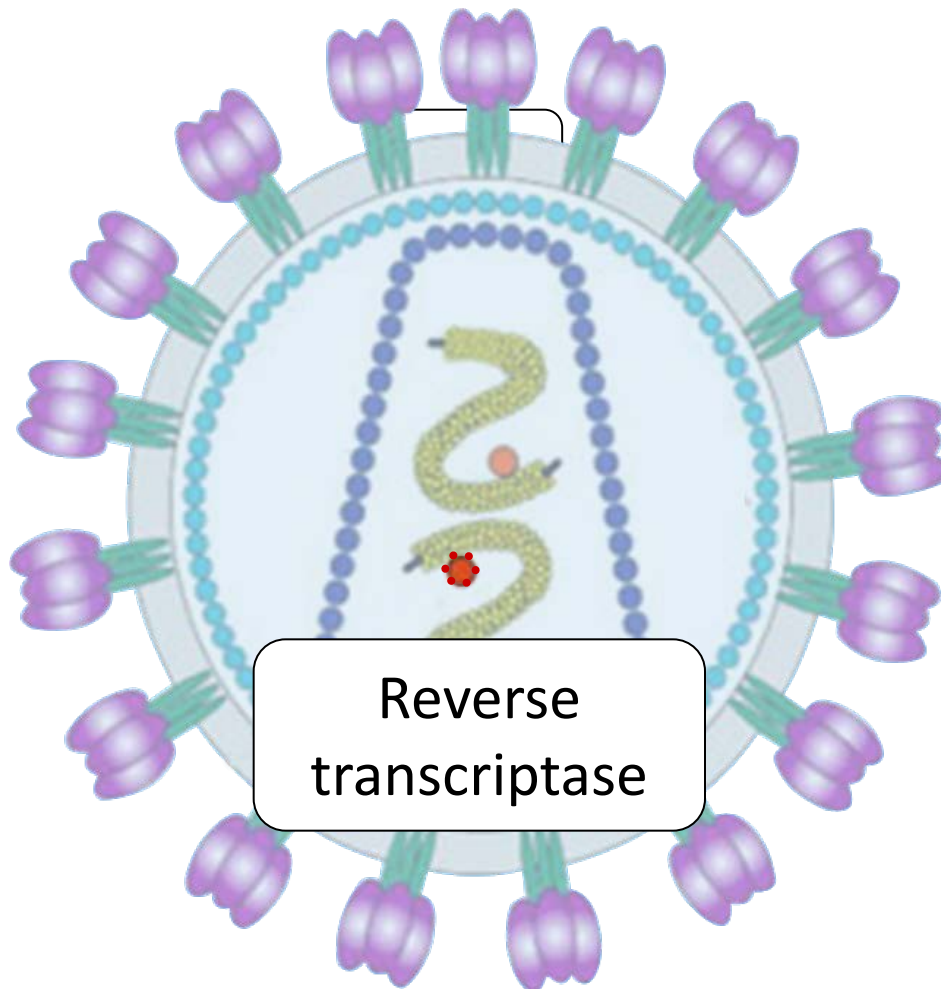


McCutchan, Henry M. Jackson Foundation (Rockville, Maryland). McCutchan and colleagues are indebted to the many international collaborators who helped develop the data used to generate this map.

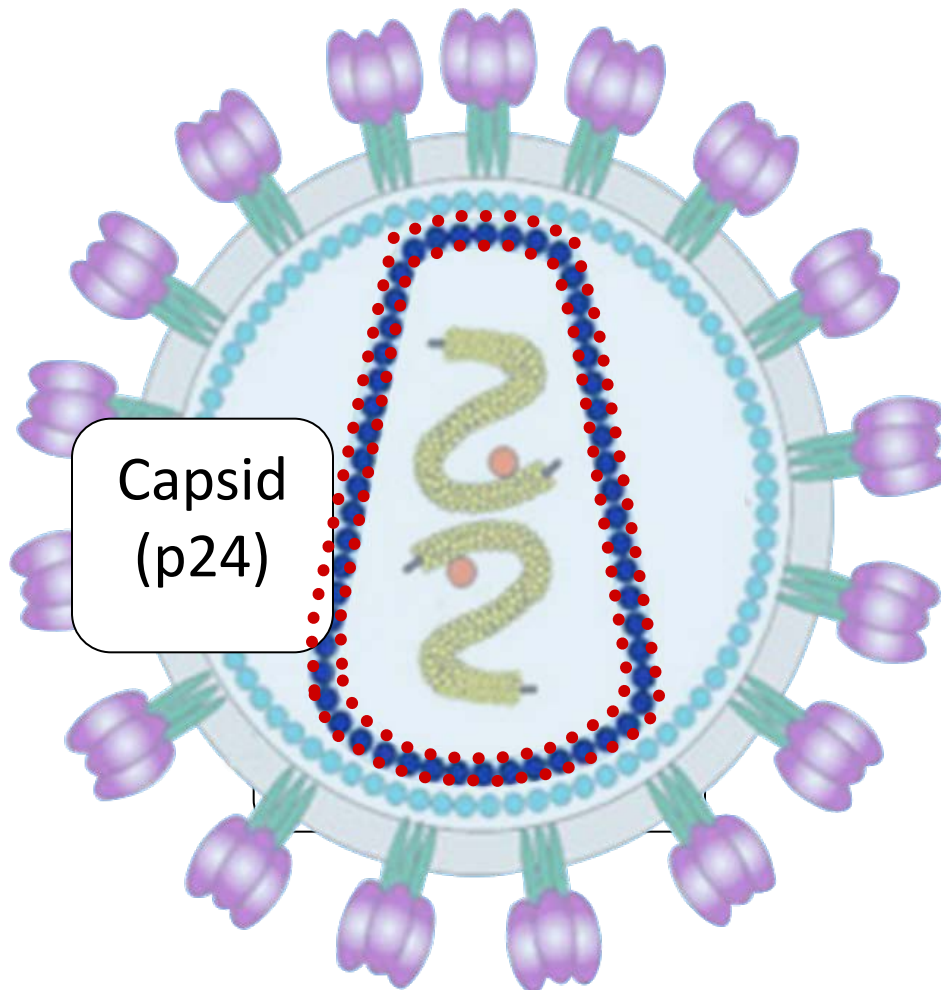
# Organization of HIV-1



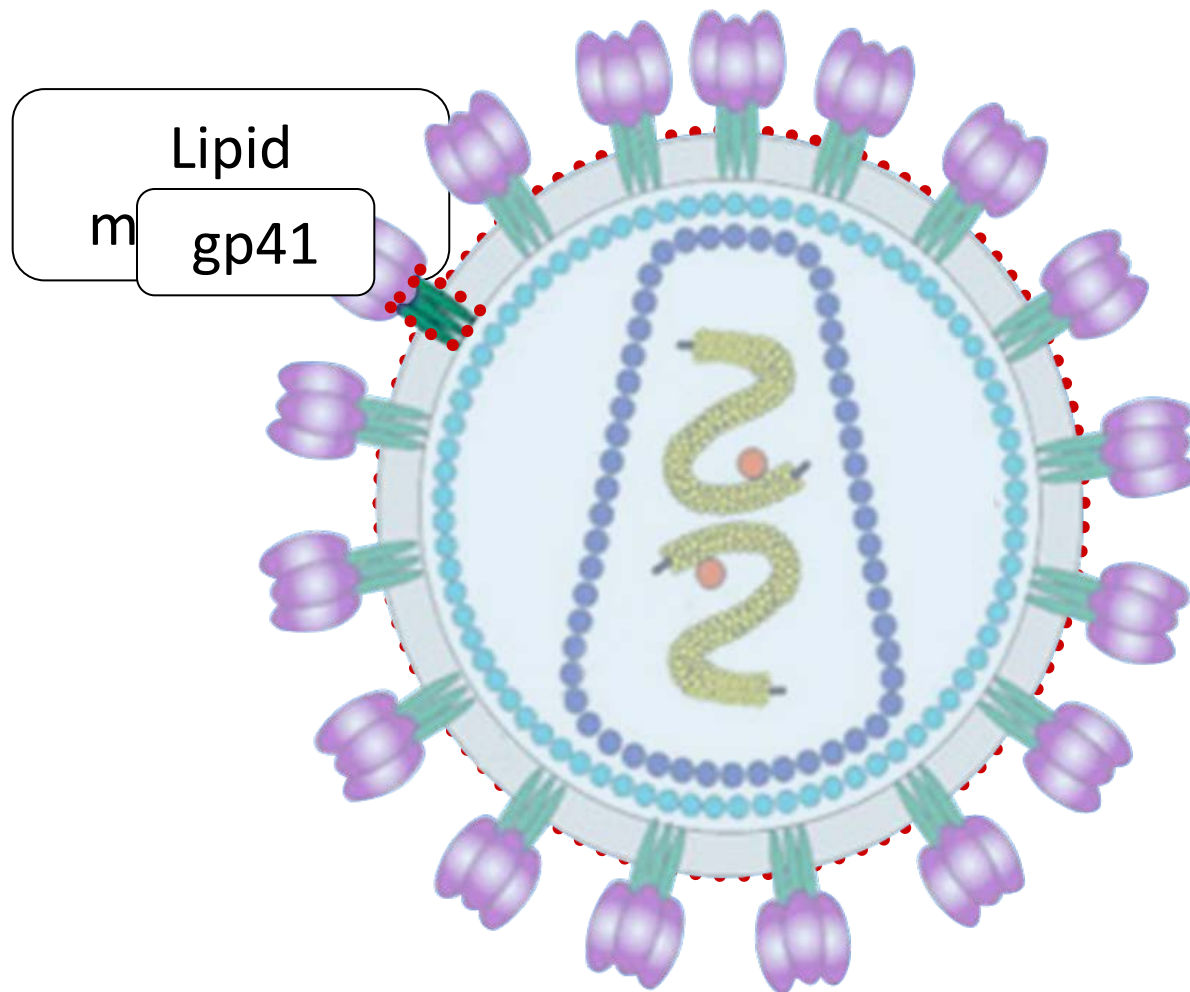
# Organization of HIV-1



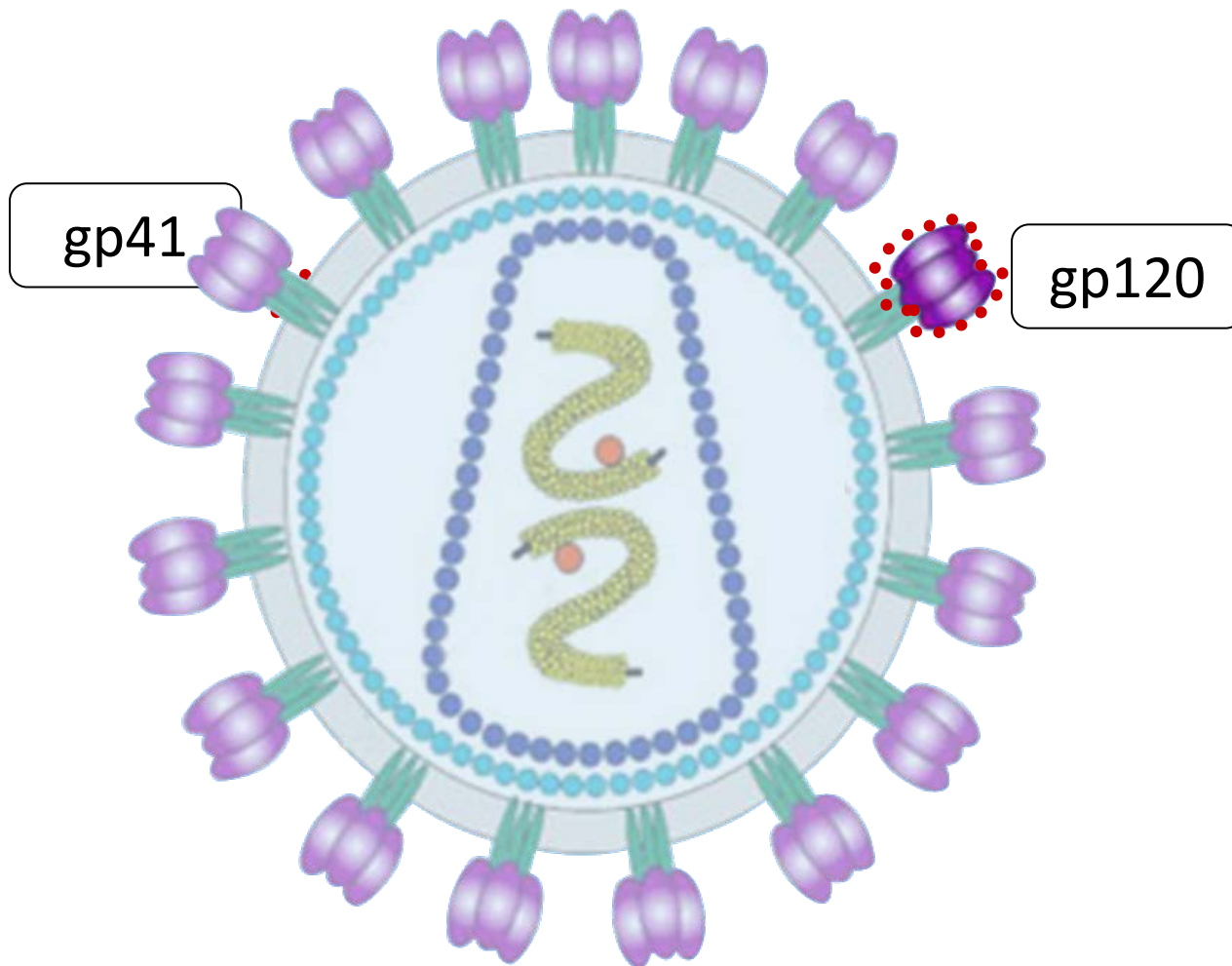
# Organization of HIV-1



# Organization of HIV-1

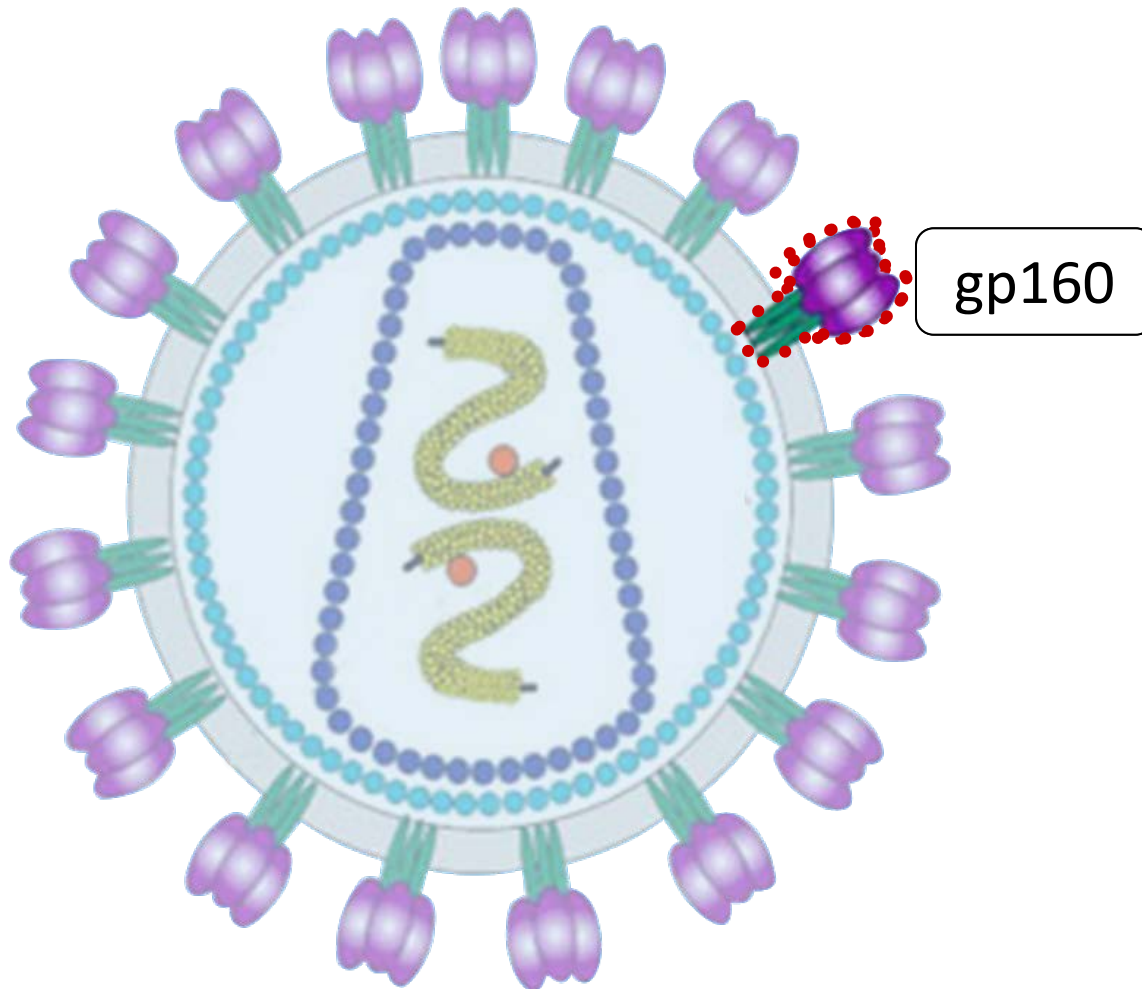


# Organization of HIV-1

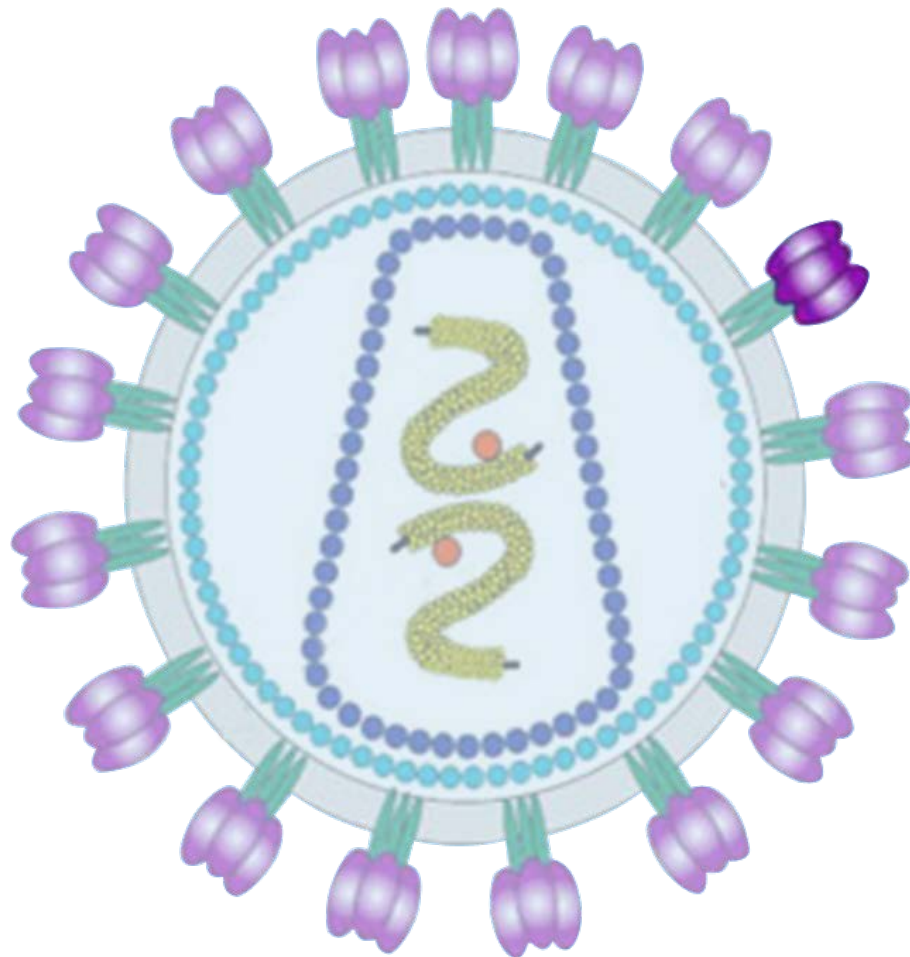




# Organization of HIV-1



# Organization of HIV-2



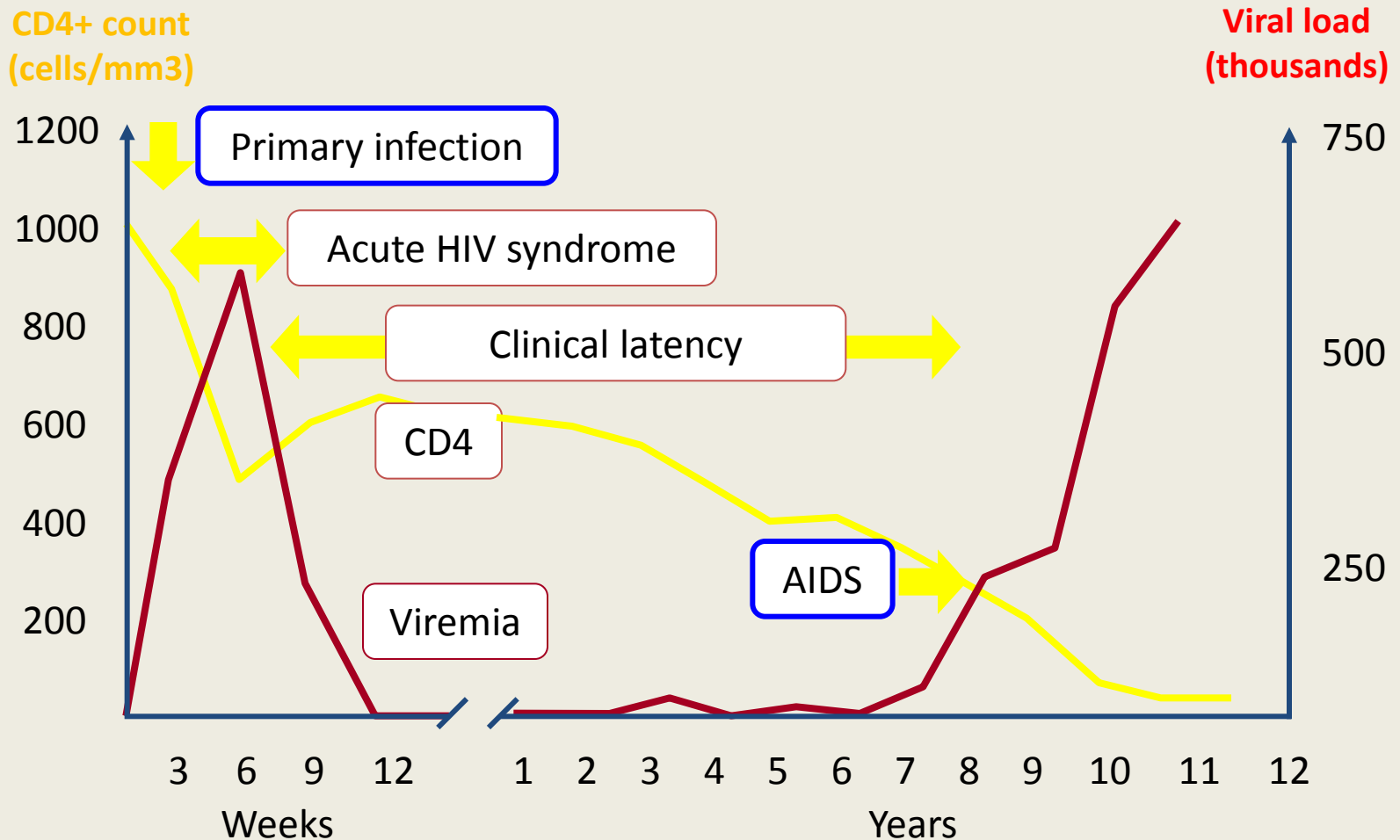
gp105

gp36

HIV-2 causes AIDS  
but disease progression  
is slower, virus is less fit,  
and does not respond to  
certain drugs used to  
treat HIV-1 infection

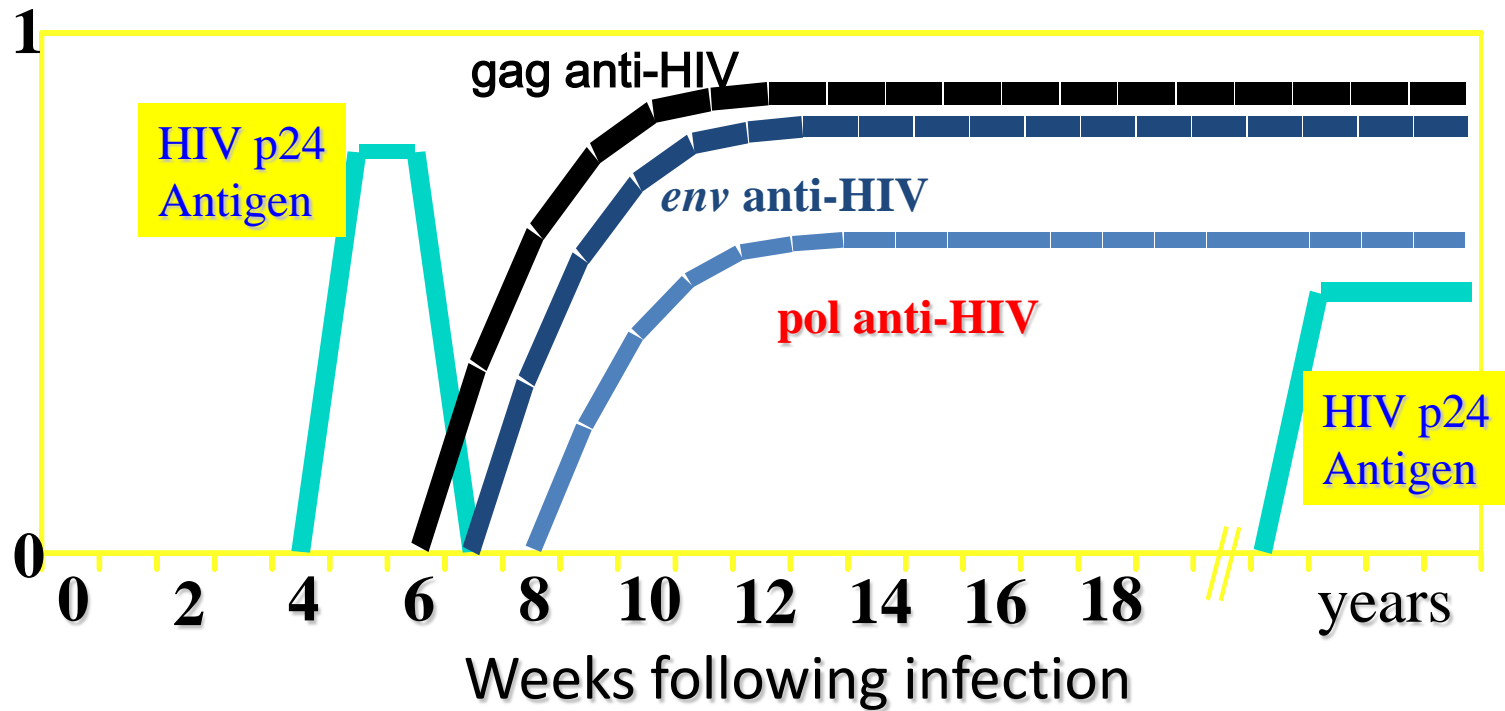
Adapted from Siemens slide

# HIV Infection Course



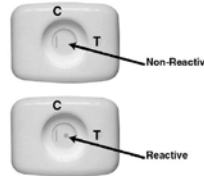
# HIV Serological Response

Typical response following infection

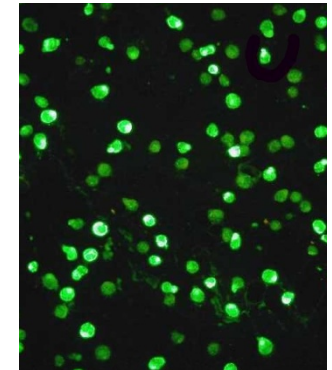
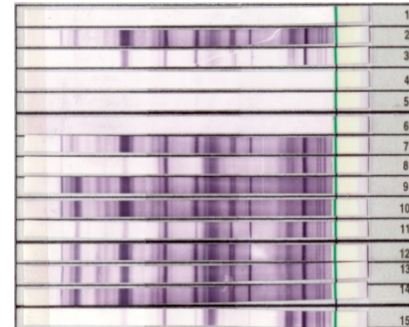


# HIV Diagnostic Algorithm

- Screen  
traditional EIA/CIA  
rapid tests



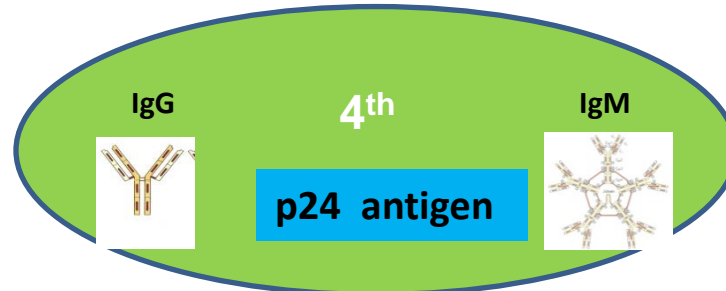
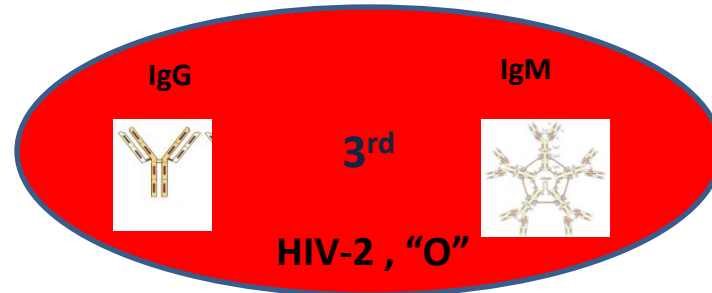
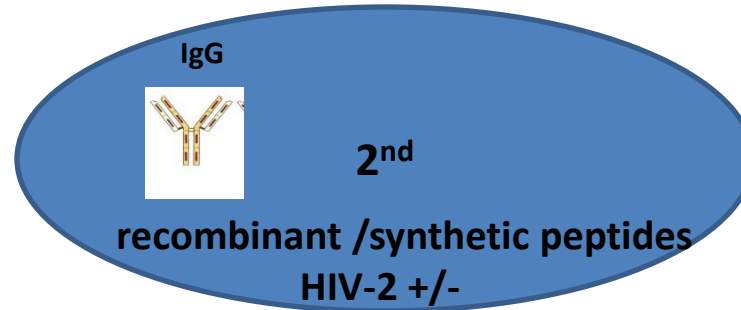
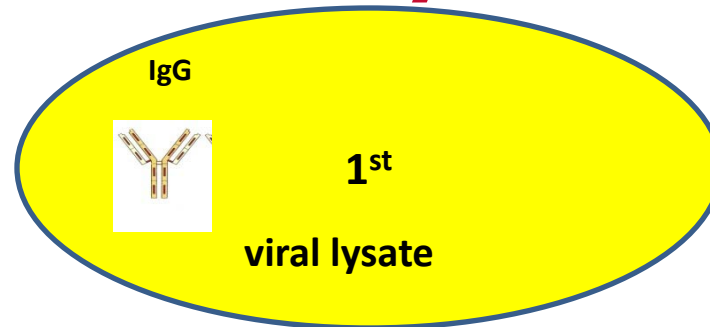
- Confirmation  
Western blot (98%)  
IFA



- Prognosis and monitoring  
viral load – nucleic acid amplification test (NAAT)

Note: APTIMA, Genprobe (TMA format) approved in 2006 for diagnosis and confirmation

# Immunoassay Generations



# US FDA-Cleared Rapid Antibody Tests

Table 1  
**FDA-Approved Rapid HIV Antibody Tests**

| Test   | Manufacturer   | Sample Type                               | Sensitivity | Specificity |
|--|--|---|-------------|-------------|
| OraQuick ADVANCE Rapid HIV-1/2 Antibody Test | OraSure Technologies (Bethlehem, Pa.)                          | oral fluid                                | 99.3%       | 99.8%       |
|  |  | whole blood (fingerstick or venipuncture) | 99.6%       | 100%        |
|  |  | plasma                                    | 99.6%       | 99.9%       |
| Clearview COMPLETE HIV ½                     | Inverness Medical Professional Diagnostics (Louisville, Colo.) | whole blood (fingerstick or venipuncture) | 99.7%       | 99.9%       |
|  |  | serum and plasma                          | 99.7%       | 99.9%       |
| Clearview HIV ½ STAT-PAK                     | Inverness Medical Professional Diagnostics                     | whole blood (fingerstick or venipuncture) | 99.7%       | 99.9%       |
|  |  | serum and plasma                          | 99.7%       | 99.9%       |
| Reveal G-3 Rapid HIV-1 Antibody Test         | MedMira, Inc. (Halifax, Nova Scotia)                           | serum                                     | 99.8%       | 99.1%       |
|  |  | plasma                                    | 99.8%       | 98.6%       |
| Uni-Gold Recombigen HIV                      | Trinity Biotech (Berkley Heights, N.J.)                        | whole blood (fingerstick or venipuncture) | 100%        | 99.7%       |
|  |  | serum and plasma                          | 100%        | 99.8%       |
| Multispot HIV-1/HIV-2 Rapid Test             | Bio-Rad (Redmond, Wash.)                                       | serum                                     | 100%        | 99.9%       |
|  |  | plasma                                    | 100%        | 99.9%       |

# Rapid Test Sensitivity

| Patient identifier | Initial specimen              |    |               |       |       |       |       | Viral load<br>(no. of copies/ml) |
|--------------------|-------------------------------|----|---------------|-------|-------|-------|-------|----------------------------------|
|                    | Result by the following test: |    |               |       |       |       |       |                                  |
|                    | Vironostika EIA               | WB | HIV-1/2/O EIA | OQ RT | UG RT | SP RT | MS RT |                                  |
| A                  | NR                            | I  | NR            | NR    | NR    | NR    | NR    | 5,770                            |
| B                  | NR                            | I  | NR            | NR    | NR    | NR    | NR    | ≈500,000                         |
| C                  | NR                            | I  | R             | NR    | R     | NR    | NR    | 12,183                           |
| D                  | NR                            | N  | NR            | NR    | NR    | NR    | NR    | 77                               |
| E                  | NR                            | I  | NR            | NR    | NR    | NR    | NR    | 6,373                            |
| F                  | NR                            | I  | R             | NR    | R     | NR    | R1    | ≈500,000                         |
| G                  | NR                            | N  | NR            | NR    | NR    | NR    | NR    | 12,852                           |
| H                  | NR                            | I  | NR            | NR    | NR    | NR    | NR    | 14,062                           |
| I                  | NR                            | I  | R             | R     | R     | R     | R1    | ≈500,000                         |
| J                  | NR                            | N  | NR            | NR    | NR    | NR    | NR    | 3,921                            |
| K                  | NR                            | N  | R             | NR    | R     | NR    | NR    | ≈500,000                         |
| L                  | NR                            | N  | NR            | NR    | R     | NR    | NR    | ≈500,000                         |
| M                  | NR                            | N  | R             | NR    | NR    | NR    | NR    | ≈500,000                         |
| N                  | NR                            | N  | NR            | NR    | NR    | NR    | NR    | 1,177                            |
| O                  | NR                            | N  | NR            | NR    | NR    | NR    | NR    | ≈500,000                         |
| P                  | NR                            | N  | R             | NR    | R     | NR    | R1    | ≈500,000                         |
| Q                  | NR                            | N  | NR            | NR    | NR    | NR    | NR    | 43,173                           |
| R                  | NR                            | I  | NR            | NR    | NR    | NR    | NR    | 30,734                           |
| S                  | NR                            | N  | R             | NR    | R     | NR    | R1    | ≈500,000                         |
| T                  | NR                            | N  | R             | NR    | NR    | NR    | NR    | ≈500,000                         |
| U                  | NR                            | N  | NR            | NR    | NR    | NR    | NR    | ≈500,000                         |
| V                  | NR                            | N  | NR            | NR    | NR    | NR    | NR    | ≈500,000                         |
| W                  | NR                            | I  | R             | NR    | R     | NR    | R1    | ≈500,000                         |
| X                  | NR                            | N  | NR            | NR    | NR    | NR    | NR    | ≈500,000                         |
| Y                  | NR                            | N  | R             | NR    | NR    | NR    | NR    | ≈500,000                         |
| Z                  | NR                            | N  | NR            | NR    | NR    | NR    | NR    | 102,288                          |
| AA                 | NR                            | N  | NR            | NR    | NR    | NR    | NR    | 327,333                          |
| AB                 | NR                            | N  | NR            | NR    | NR    | NR    | NR    | ≈500,000                         |
| AC                 | NR                            | I  | NR            | NR    | NR    | NR    | NR    | ≈500,000                         |
| AD                 | NR                            | N  | NR            | NR    | NR    | NR    | NR    | ≈500,000                         |
| AE                 | NR                            | N  | NR            | NR    | NR    | NR    | NR    | 389,850                          |
| AF                 | NR                            | I  | NR            | NR    | R     | NR    | R1    | 413,186                          |
| AG                 | NR                            | I  | NR            | NR    | NR    | NR    | NR    | 446,770                          |
| AH                 | NR                            | N  | NR            | NR    | NR    | NR    | NR    | 358,030                          |
| AJ                 | NR                            | N  | R             | NR    | NR    | NR    | NR    | ≈500,000                         |
| AK                 | NR                            | N  | NR            | NR    | NR    | NR    | NR    | 427,490                          |
| AL                 | NR                            | N  | R             | NR    | R     | NR    | NR    | 210,204                          |
| AM                 | NR                            | N  | NR            | NR    | NR    | NR    | NR    | ≈500,000                         |
| AN                 | NR                            | N  | NR            | NR    | NR    | NR    | NR    | ≈500,000                         |
| AO                 | NR                            | N  | R             | NR    | NR    | NR    | NR    | ≈500,000                         |
| AP                 | NR                            | N  | R             | NR    | R     | NR    | R1    | ≈500,000                         |
| AR                 | NR                            | N  | NR            | NR    | NR    | NR    | NR    | ≈500,000                         |

Multispot  
Statpak  
Unigold Recombigen  
OraQuick  
HIV-1/2/0 - 3<sup>rd</sup> gen(Genetics Systems)  
Western Blot



# OraQuick<sup>®</sup> Advance



- Synthetic gp-41 (HIV-1)
- Synthetic gp-36 (HIV-2)
- Goat anti-human IgG

# OraQuick® Advance - Fingertstick

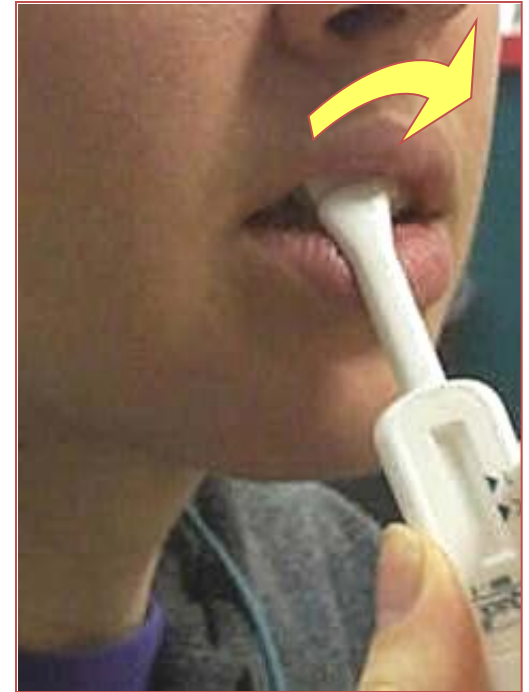
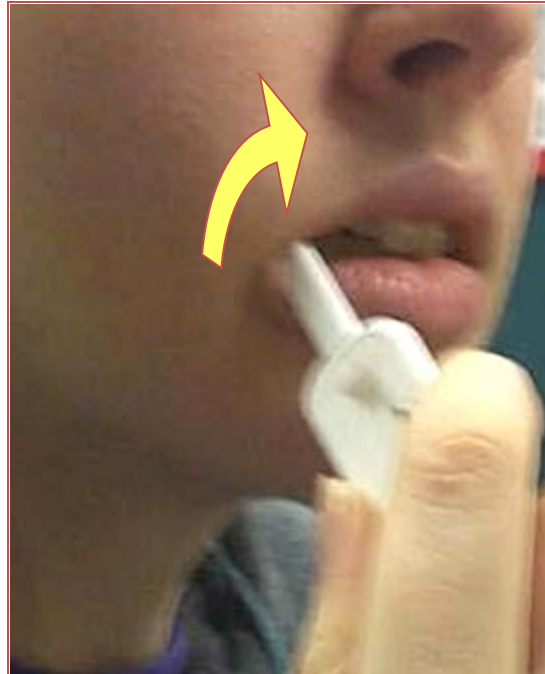
Obtain a finger stick specimen



Insert loop into vial and stir



# OraQuick® Advance – Oral Fluid

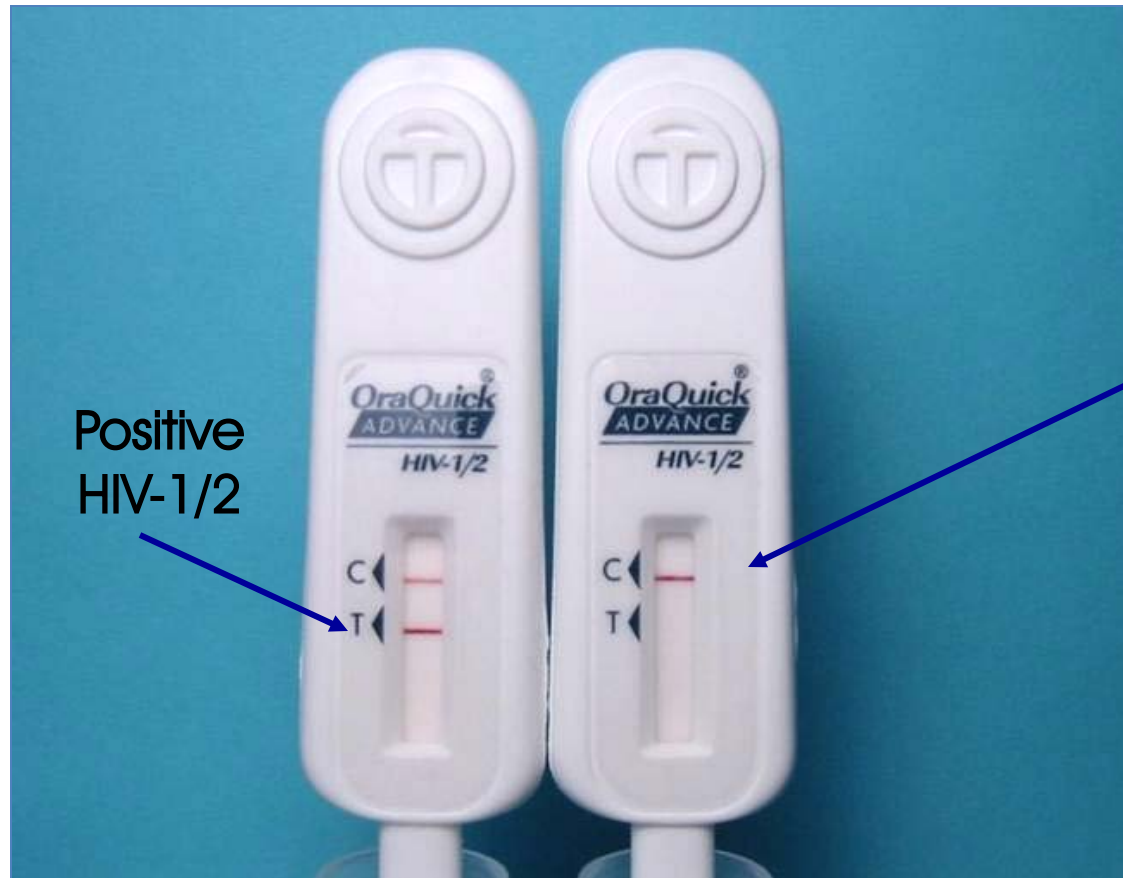


# OraQuick<sup>®</sup> Advance



Photograph courtesy of the CDC: [www.cdc.gov/hiv/rapid\\_testing](http://www.cdc.gov/hiv/rapid_testing)

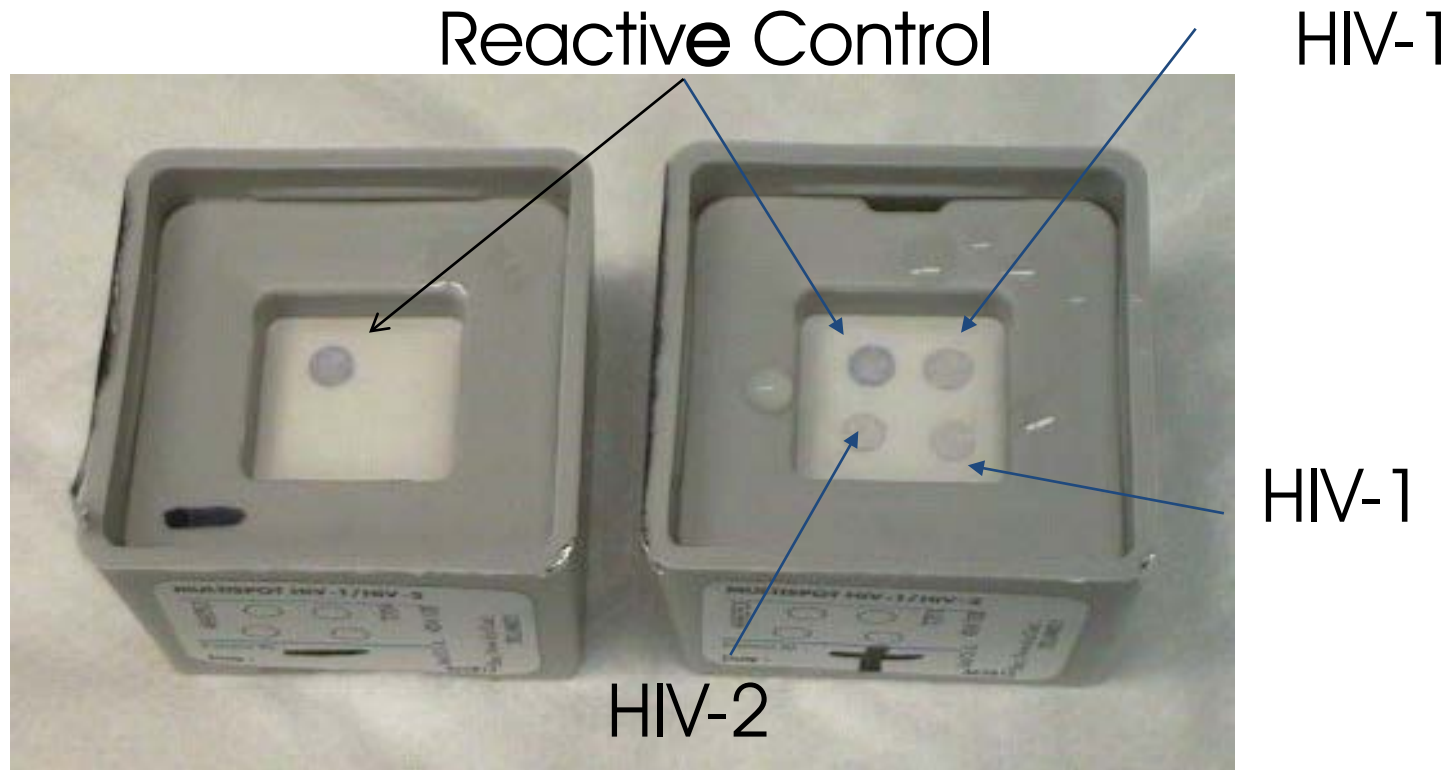
# Interpreting Results



Positive  
HIV-1/2

Reactive  
Control

# Multispot HIV-1/HIV-2 Rapid Test



Negative

Reactive  
(HIV-1 & HIV-2)

Detects and differentiates between HIV-1 and HIV-2

# 3<sup>rd</sup> Generation Anti-HIV Assays

- Platforms

  - ADVIA Centaur EHIV 1/2/0 (Siemens)

  - Ortho VITROS anti-HIV 1 +2

  - Bio-Rad GS HIV-1/HIV-2 Plus O EIA

- Characteristics

  - enzyme immunoassay (EIA) / (CIA)

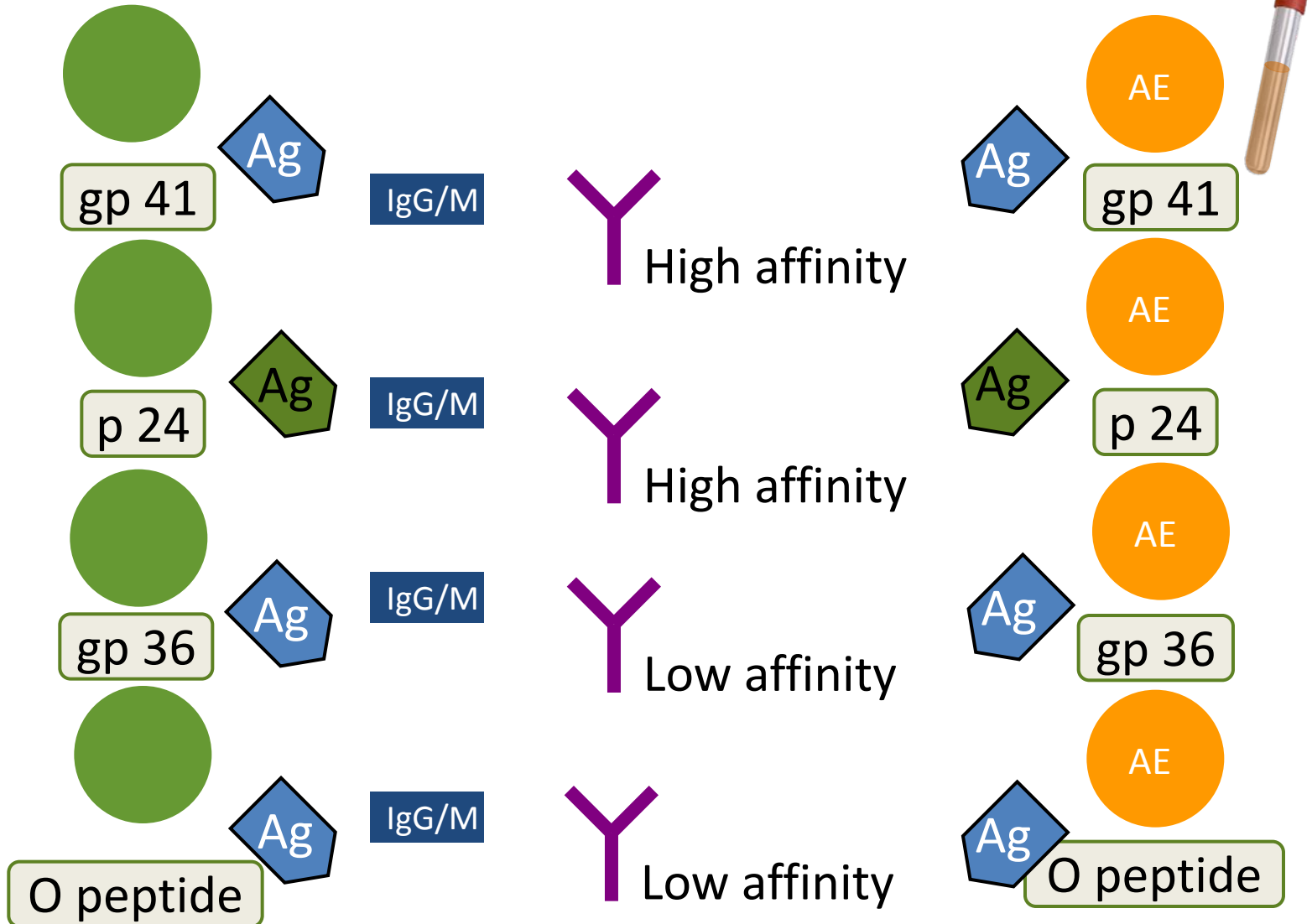
  - detect HIV infection at 22 days

  - detect HIV 1/2 and O infection (depending on assay)

  - detect HIV anti- HIV IgG and IgM

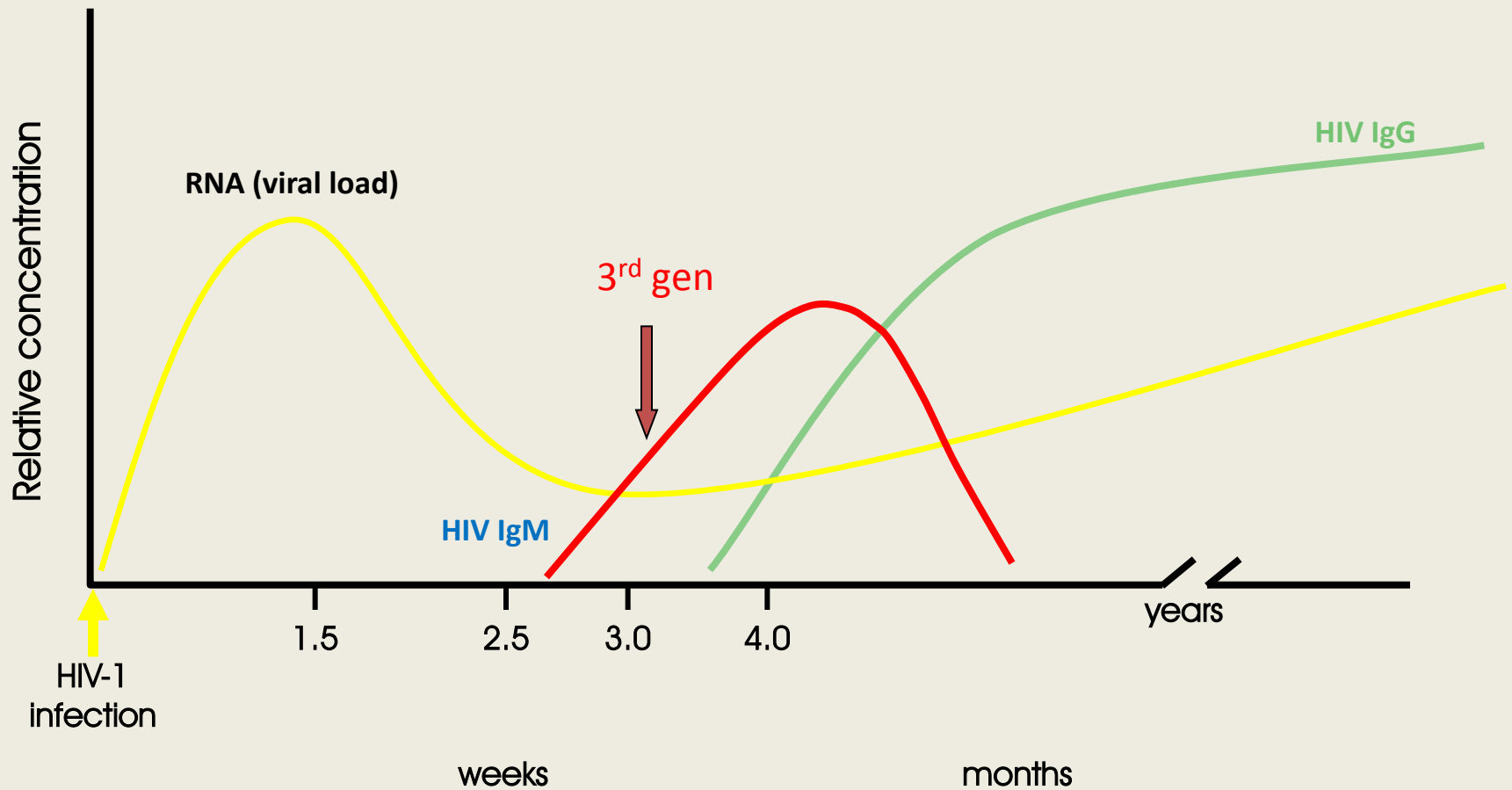
  - antigen bridging assay format

# Antigen Bridging Assay Format





# Serologic Detection of HIV Infection: 3<sup>rd</sup> gen



# Antigen/Antibody Combo (4<sup>th</sup> Generation) HIV

- Detects both anti-HIV 1 and 2 antibodies and p24 antigen
- Does not distinguish between Ab+ or Ag+
- Detects both HIV-1 and HIV-2, but does not discriminate
- 1<sup>st</sup> FDA approved June 2010 (Abbott Diagnostics – Architect Platform)
- 2<sup>nd</sup> FDA approved July 2011 (Bio-Rad, manual or semi-automated)
- Improved detection of Acute HIV
- Testing Algorithm ?

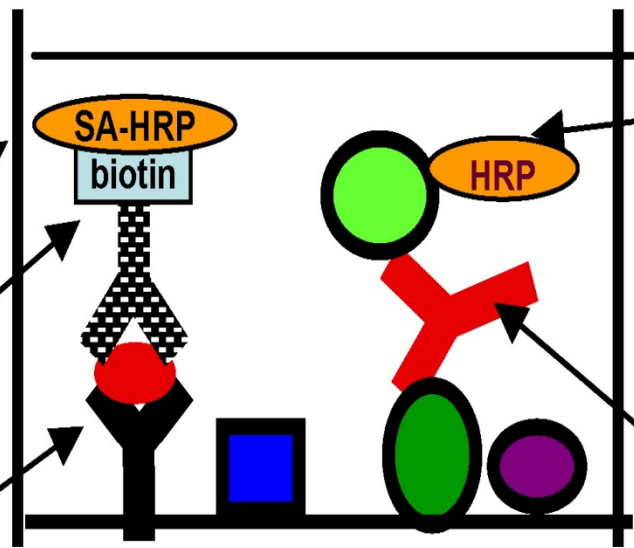
# Antigen/Antibody Combination Assay Format

**Antigen Detection Conjugates:**

**Conj 2**  
Streptavidin-  
HRP

**Conj 1**  
Sheep anti-p24  
biotin

HIV p24



MABs (3)  
anti-p24

Pep gp41  
(HIV-1 O)

Rec gp160  
(HIV-1 M)

Pep gp36  
(HIV-2)

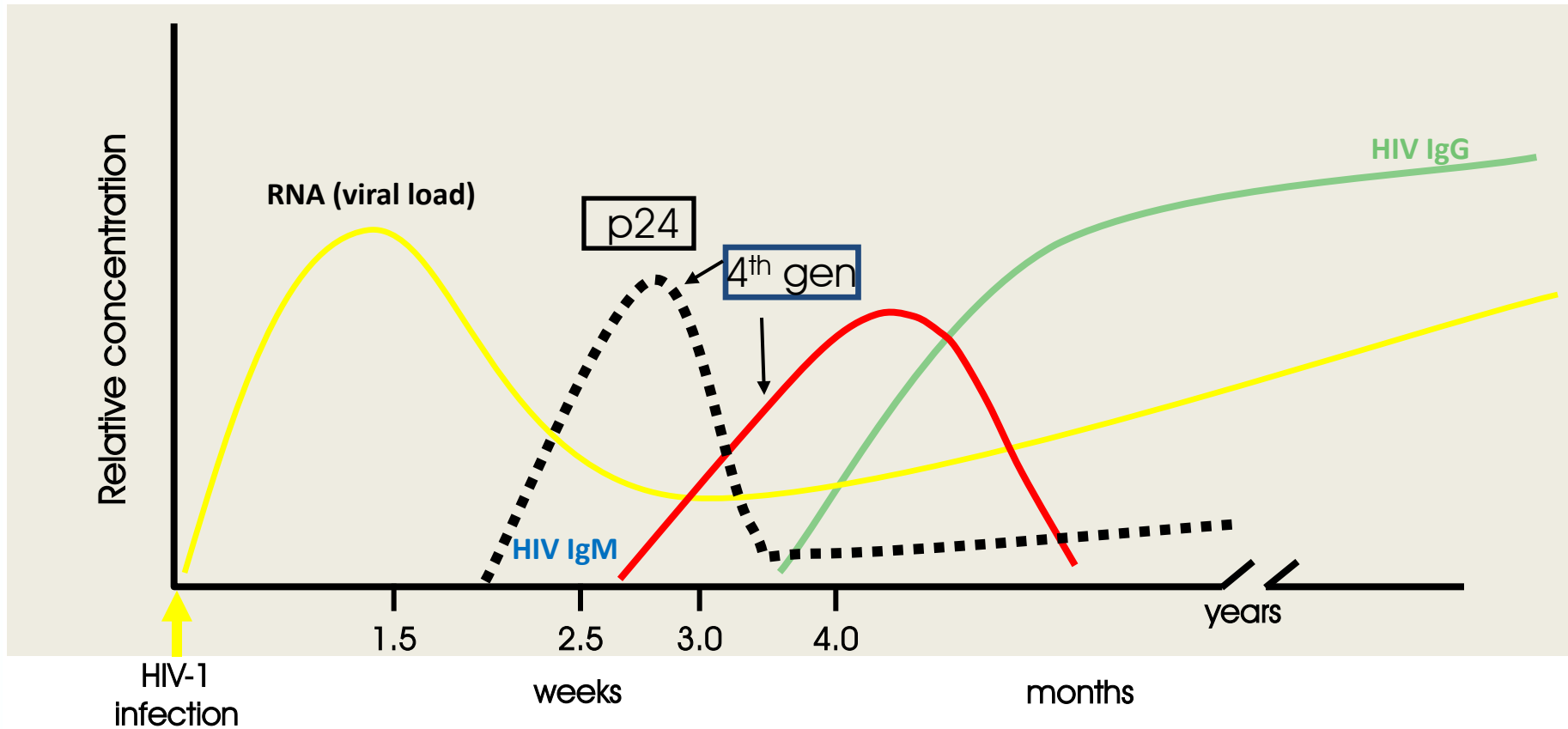
**Antibody Detection Conjugates:**

Pep gp41 (HIV-1 M)-HRP  
Pep gp41 (HIV-1 O)-HRP  
Pep gp36 (HIV-2)-HRP

HIV-1 Antibody

Bentsen et al. Journal of Clinical Virology. 2011

# Serologic Detection of HIV Infection: 4<sup>th</sup> gen

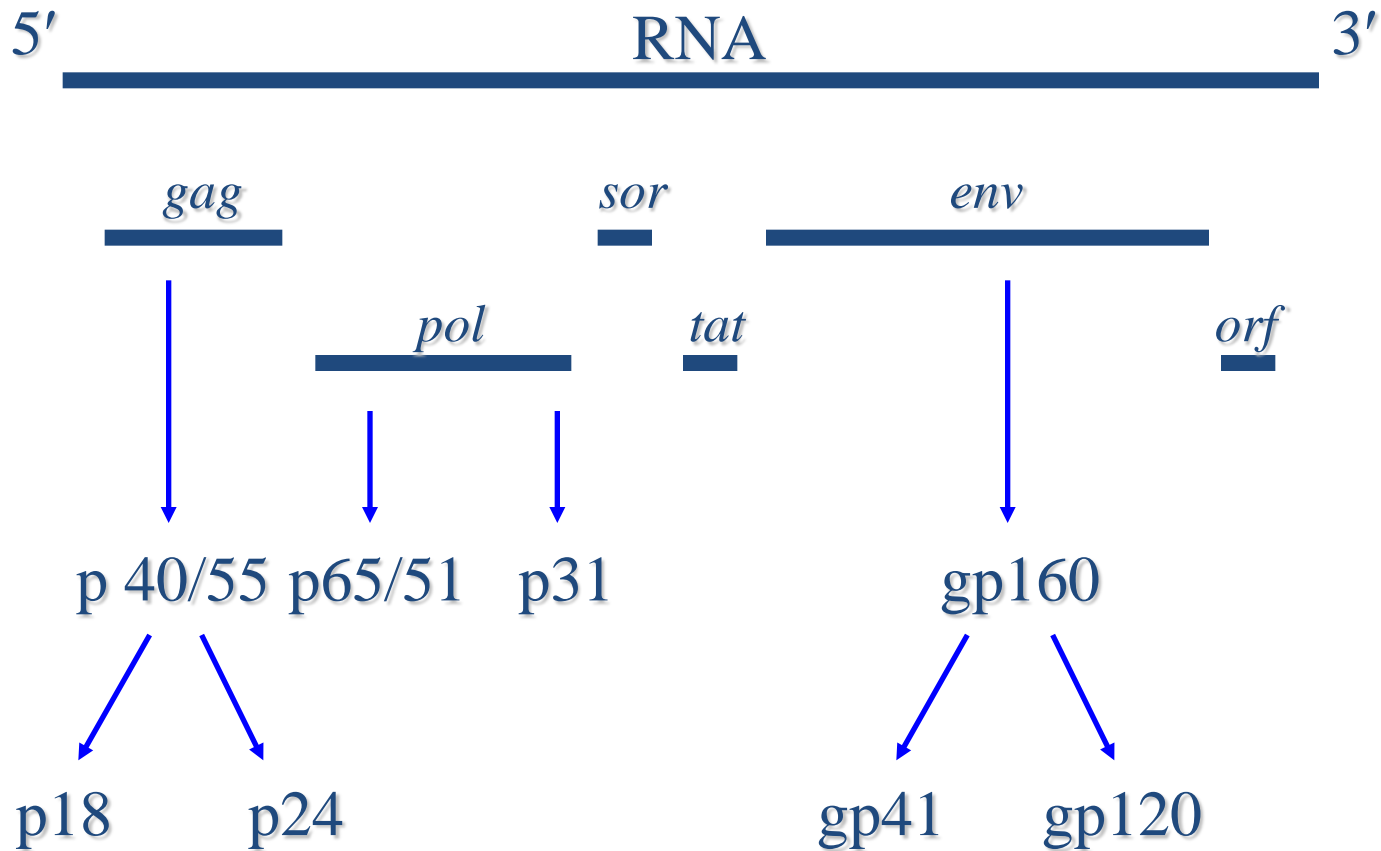


Detects infection at 2.5 -3.0 weeks, 5 days earlier than 3<sup>rd</sup> gen

# Confirmation for HIV-1 Infection

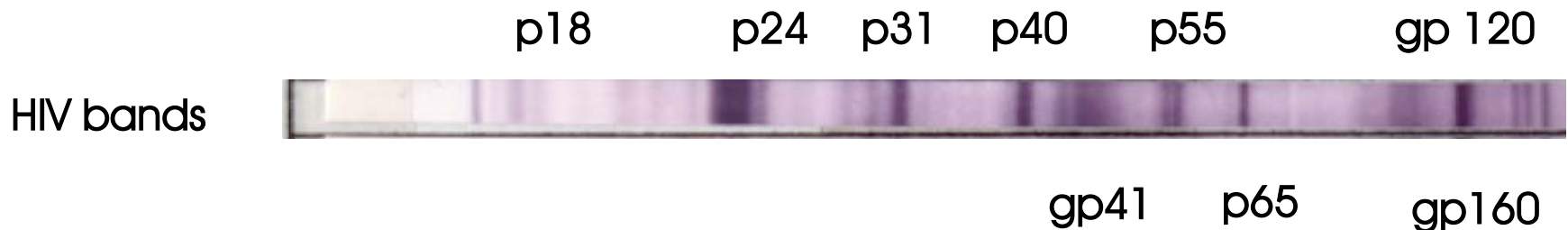
- All repeatedly reactive EIA/CIA screening assay results must be confirmed
- POC results are considered “preliminary positive” results and must also be confirmed
- Confirmation for HIV-1 Infection
  - Indirect Immunofluorescence (IFA)
  - Western Blot

# Western Blot HIV Antigens



# Western Blot Pattern Interpretations

- Nine characteristic HIV antibody bands
- Cardinal: p24, gp41, and gp120/160 (combined)
- Noncardinals: p18, p31, p51, p55, p65
- Positive = at least 2 of the 3 cardinals
- Negative = no bands present (FDA criteria)
- Indeterminate = anything that does not meet + or – interpretation



# False Positive Immunoassay Results

- Vaccinations  
    flu, rabies
- HIV vaccine trials
- Autoimmune disease
- Liver disease
- Undefined cross reactivity



# Western Blot “Indeterminate”

- Indeterminate results may be due to
  - infected but in the “window”
  - advanced disease, AIDS
  - HIV vaccinated
  - infected with HIV-2
  - uninfected, cross reactivity
    - viral or non-viral bands, recent flu and rabies vaccinations, multiple pregnancies, recipients of multiple transfusions, autoimmune disease
    - study followed 99 blood donors – 91 stable indeterminate Western blot patterns over 30 months
- Indeterminate results require follow-up
  - repeat Western blot
  - NAAT

# HIV-2 Testing

## *Persons at risk for HIV-2 infection include*

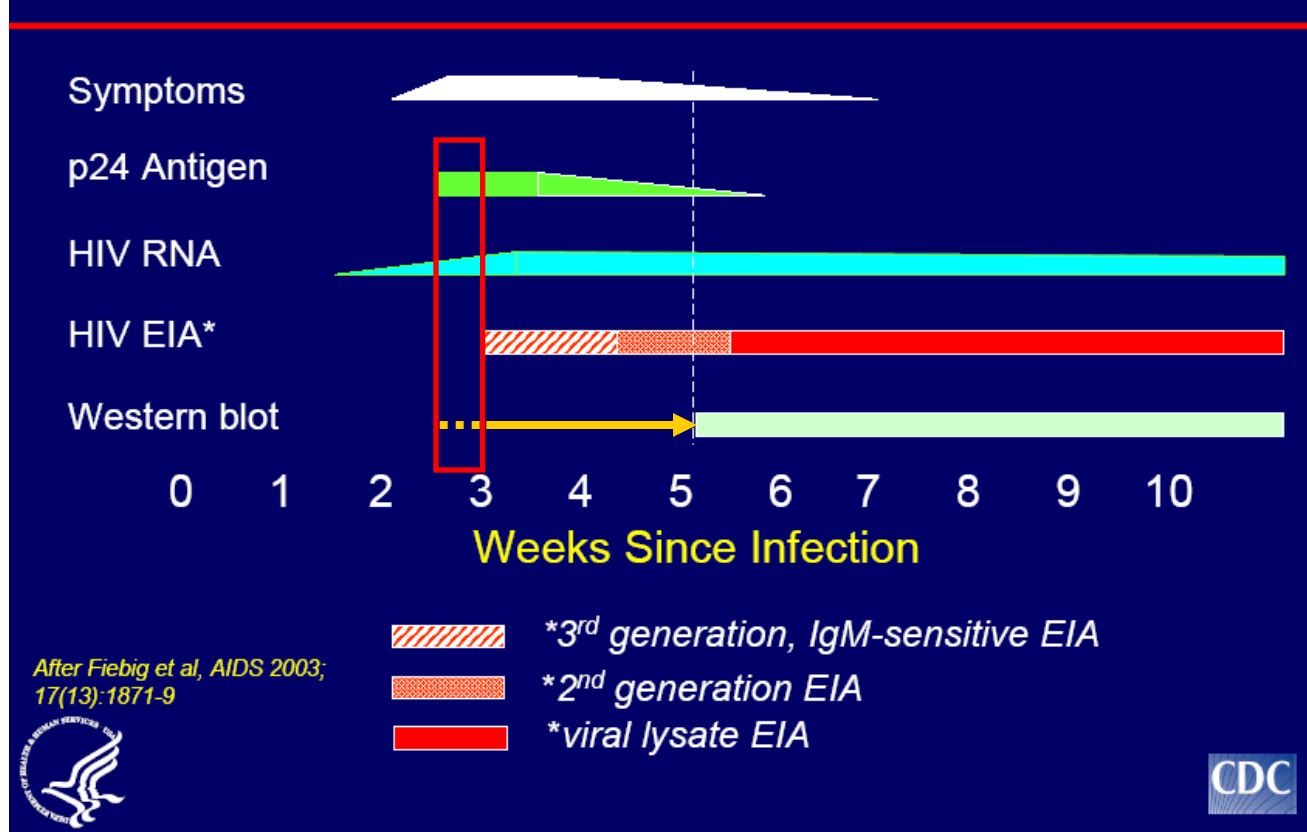
- Sex partners of a person from a country where HIV-2 is endemic
- Sex partners of a person known to be infected with HIV-2
- People who received a blood transfusion or a nonsterile injection in a country where HIV-2 is endemic
- People who shared needles with a person from a country where HIV-2 is endemic or with a person known to be infected with HIV-2
- Children of women who have risk factors for HIV-2 infection or are known to be infected with HIV-2

## *HIV-2 testing also is indicated for*

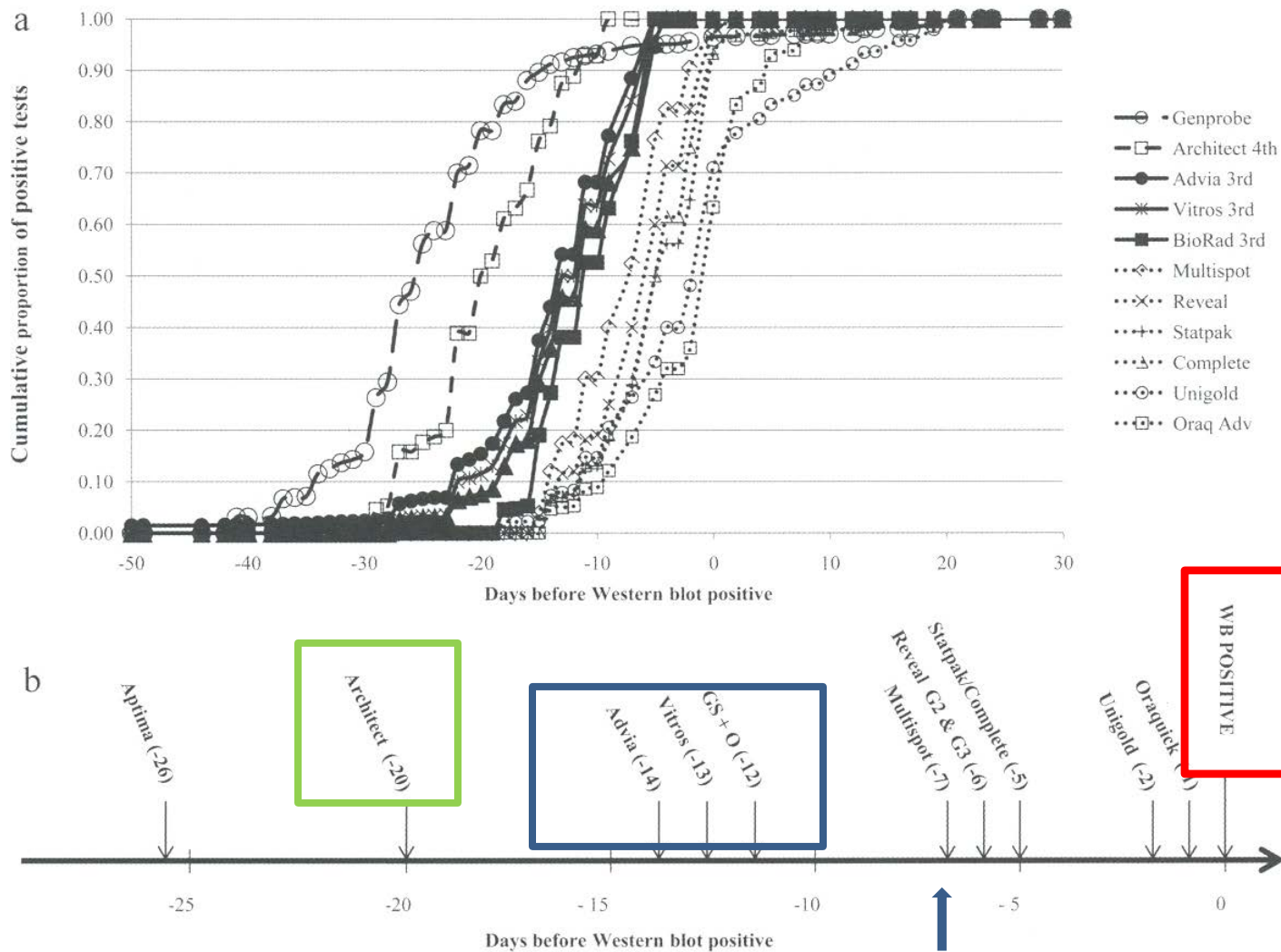
- People with an illness that suggests HIV infection (such as an HIV-associated opportunistic infection) but whose HIV-1 test result is not positive
- People for whom HIV-1 Western blot exhibits the unusual indeterminate test band pattern of gag (p55, p24, or p17) plus pol (p66, p51, or p32) in the absence of env (gp160, gp120, or gp41)

# Sensitivity of HIV Assays

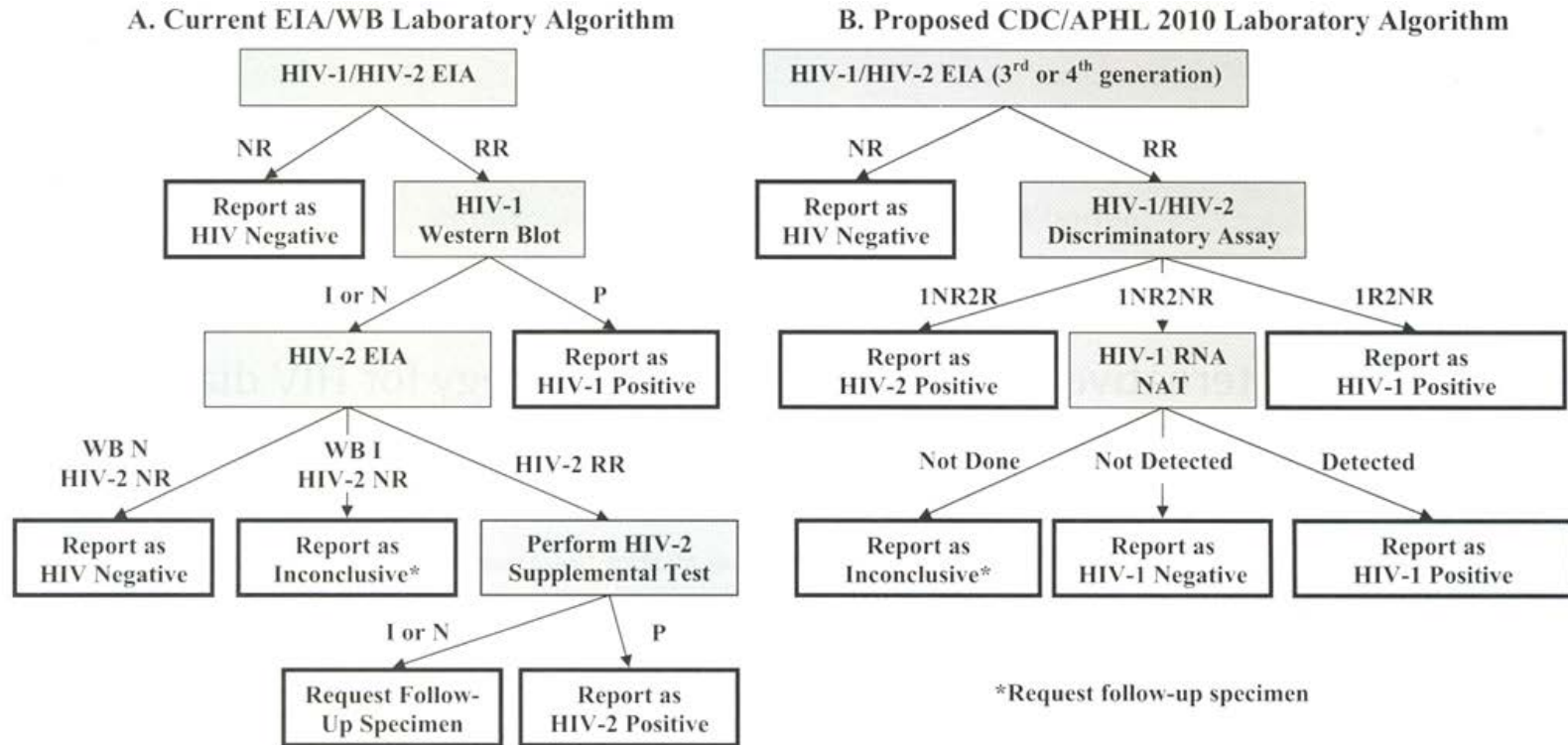
## Detection of HIV by Diagnostic Tests



# Detecting HIV Infection and Current Assays



# HIV Algorithms

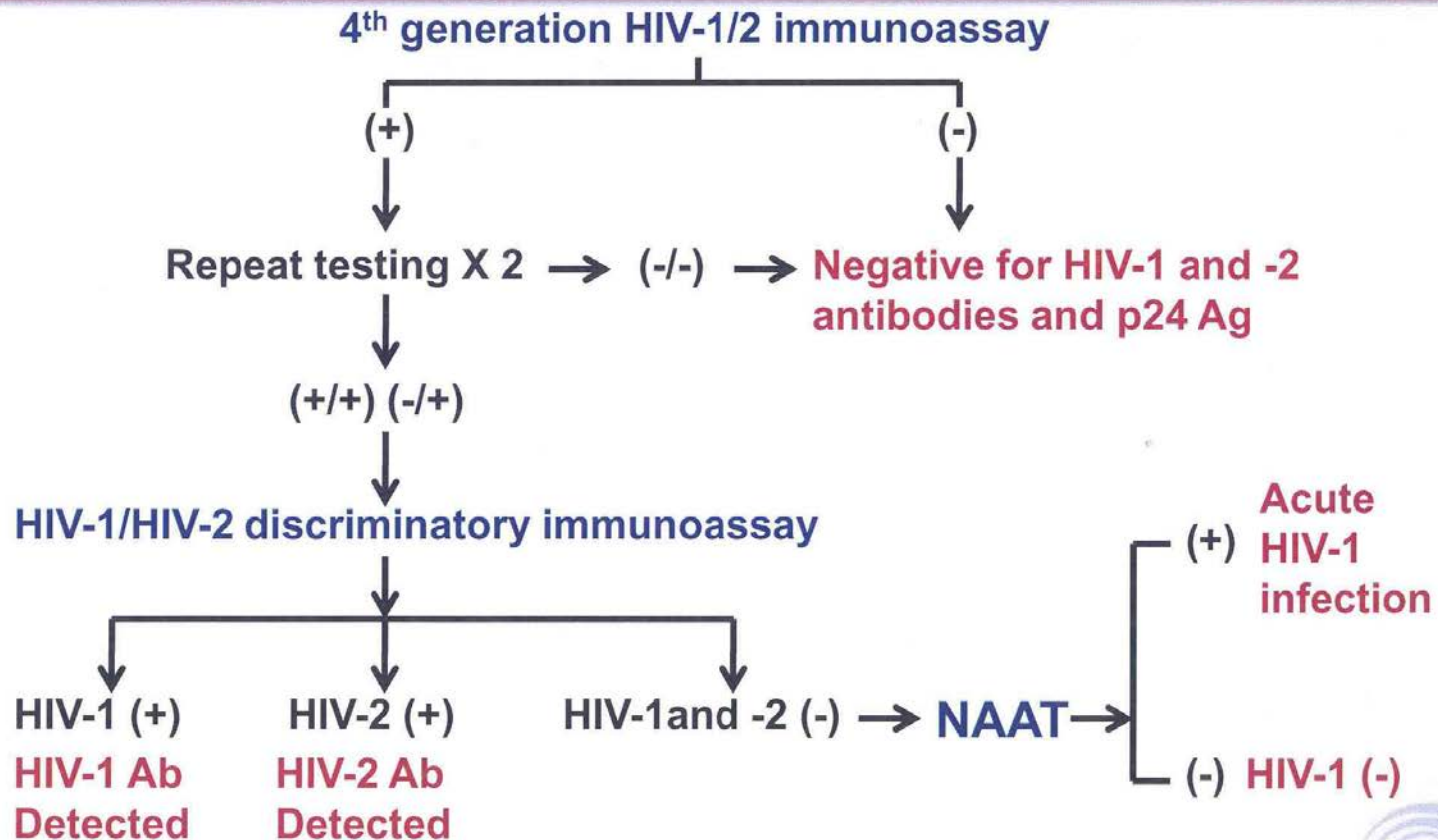


**Fig. 1.** Components and interpretations of (A) the current EIA/WB algorithm and (B) the proposed CDC/APHL 2010 algorithm. Shaded boxes, tests performed; unshaded boxes, interpretation of test results; EIA, enzyme immunoassay; WB, Western blot; NAT, nucleic acid test; NR, non-reactive; RR, repeatedly reactive; I, indeterminate; N, negative; P, positive.

# Current Algorithm Issues

- Detecting Acute HIV infection
  - increased sensitivity with 4<sup>th</sup> gen, Ag/Ab Combo
- Western Blots
  - insensitive compared to current screening assays
  - indeterminate/inconclusive
- Diagnosing HIV-2 infection

# CDC Proposed Algorithm



# Acute HIV Detection

**Table 2**

Analysis of the current two-test algorithm in acute HIV-1 infections (seroconversion panels).

| Screening test                          | GS+O | Vitros | Advia | Architect |
|---|------|--------|-------|-----------|
| Number of first positive results        | 108  | 110    | 111   | 135       |
| WB positive ( <i>n</i> )                | 56   | 56     | 56    | 56        |
| WB indeterminate ( <i>n</i> )           | 38   | 39     | 39    | 43        |
| +NAAT positive ( <i>n</i> )             | 36   | 37     | 37    | 41        |
| +NAAT negative ( <i>n</i> )             | 1    | 1      | 1     | 1         |
| +NAAT not available ( <i>n</i> )        | 1    | 1      | 1     | 1         |
| WB negative ( <i>n</i> )                | 14   | 15     | 16    | 36        |
| Current algorithm positive ( <i>n</i> ) | 56   | 56     | 56    | 56        |

Masciotra et al. Journal of Clinical Virology 2011.



# HIV-1 vs HIV-2 and Western Blot

**Table 1**

Percentage of specimens with each HIV-1 Western blot band in 114 specimens collected from persons infected with HIV-2 and 1761 specimens positive for HIV-1 by Western blot and Multispot HIV-1/HIV-2 differentiation assay.

|                         | p17  | p24  | p31  | p40  | gp41 | p51  | p55  | p66  | gp120 | gp160 |
|-------------------------|------|------|------|------|------|------|------|------|-------|-------|
| <b>HIV-2 (n = 114)</b>  |      |      |      |      |      |      |      |      |       |       |
| Present                 | 18.4 | 93.9 | 83.3 | 88.6 | 1.8  | 74.6 | 73.7 | 29.8 | 10.5  | 48.3  |
| Present but weak        | 14.9 | 4.4  | 7.0  | 9.7  | 0.9  | 17.5 | 17.5 | 10.5 | 10.5  | 22.8  |
| Absent                  | 66.7 | 1.8  | 9.7  | 1.8  | 97.4 | 7.9  | 8.8  | 59.7 | 79.0  | 29.0  |
| <b>HIV-1 (n = 1761)</b> |      |      |      |      |      |      |      |      |       |       |
| Present                 | 78.8 | 91.4 | 95.2 | -    | 97.4 | 97.2 | 93.3 | 95.0 | 98.6  | 99.9  |
| Present but weak        | 6.3  | 7.3  | 2.0  | -    | 1.7  | 1.4  | 1.3  | 2.8  | 0.6   | 0.1   |
| Absent                  | 14.9 | 1.4  | 2.8  | -    | 0.9  | 1.4  | 5.4  | 2.2  | 0.8   | 0.0   |

Nasrullah et al. Journal of Clinical Virology 2011.

# HIV-2 Infection Misclassification by Western Blot

Table 2

Comparison of two HIV-1 Western blot interpretive criteria applied to specimens collected from 114 persons known to be infected with HIV-2.<sup>a</sup>

| Current CDC HIV-1 WB criteria <sup>a</sup> | Alternative HIV-1 WB criteria <sup>b</sup> , n (%) |               |          | Total      |
|--|--|---------------|----------|------------|
|  | Negative   | Indeterminate | Positive |            |
| Negative                                   | 1(0.9)   | 0(0.0)        | 0(0.0)   | 1(0.9)     |
| Indeterminate                              | 0(0.0)   | 60(52.6)      | 0(0.0)   | 60(52.6)   |
| Positive                                   | 0(0.0)   | 40(35.1)      | 13(11.4) | 53(46.5)   |
| Total                                      | 1(0.9)   | 100(87.7)     | 13(11.4) | 114(100.0) |

Nasrullah et al. Journal of Clinical Virology 2011 .

# GS Ag/Ab Combo and Long Standing HIV Infection

Reactivity in known HIV-1 antibody positive samples.

| Population                         | N                | GS HIV Combo Ag/Ab EIA repeatedly reactive | Licensed HIV-1/HIV-2 EIA repeatedly reactive |
|------------------------------------|------------------|--|--|
| Known HIV-1 Ab positive U.S.       | 1000             | 1000 (100%)                                | 1000 (100%)                                  |
| Known HIV-1 Ab positive, Non-U.S.  | 200 <sup>a</sup> | 200 (100%)                                 | 200 (100%)                                   |
| AIDS                               | 100              | 100 (100%)                                 | 100 (100%)                                   |
| Known HIV-1 Ab positive, pediatric | 40               | 40 (100%)                                  | 40 (100%)                                    |
| Total                              | 1340             | 1340 (100%)                                | 1340 (100%)                                  |

Ab: antibody.

<sup>a</sup> Columbia (9), Thailand (31), Australia (100), Nigeria (10), Central African Republic (10), Sierra Leone (10), Ghana (10), Senegal (10), and Zimbabwe (10).

Bentsen et al. Journal of Clinical Virology 2011.

# Acute HIV and GS HIV Combo Ag/Ab

**Table 1**  
Individuals with acute HIV infections and follow-up sample testing.

| Acute HIV patient   | Days from 1st bleed | HIV-1 RNA copies (mL) | GS HIV Combo Ag/Ab EIA<br>Result | Historical results <sup>a</sup> |                     |                           |
|---|---------------------|-----------------------|----------------------------------|---------------------------------|---------------------|---------------------------|
|   |                     |                       |                                  | HIV-1/HIV-2 EIA<br>Result       | HIV-1 EIA<br>Result | HIV-1 Western Blot Result |
| 1   | 0                   | >500,000              | RR                               | NR                              | NR                  | NEG                       |
|   | 56                  | NA                    | RR                               | R                               | R                   | POS                       |
| 2   | 0                   | 183,850               | RR                               | NR                              | NR                  | NEG                       |
|   | 16                  | 10,479                | RR                               | R                               | R                   | POS                       |
|   | 42                  | NA                    | RR                               | R                               | R                   | POS                       |
| 3   | 0                   | >500,000              | RR                               | R                               | NA                  | NEG                       |
|   | 141                 | NA                    | RR                               | R                               | R                   | POS                       |
| 4   | 0                   | >500,000              | RR                               | NR                              | NR                  | NEG                       |
|   | 19                  | NA                    | RR                               | R                               | R                   | POS                       |
| 5   | 0                   | >500,000              | RR                               | R                               | R                   | NEG                       |
|   | 21                  | NA                    | RR                               | R                               | R                   | IND                       |
|   | 64                  | NA                    | RR                               | R                               | R                   | POS                       |
| 6   | 0                   | 795,520               | RR                               | NR                              | NR                  | NEG                       |
|   | 25                  | NA                    | RR                               | R                               | R                   | POS                       |
|   | 32                  | NA                    | RR                               | R                               | R                   | POS                       |
| 7   | 0                   | 72,000                | RR                               | NR                              | NR                  | NEG                       |
|   | 34                  | NA                    | RR                               | R                               | R                   | POS                       |
| 8   | 0                   | 460,790               | RR                               | R                               | NR                  | NEG                       |
|   | 15                  | NA                    | RR                               | R                               | R                   | POS                       |
|   | 29                  | NA                    | RR                               | R                               | R                   | POS                       |
| 9   | 0                   | 20,420                | NR                               | NR                              | NR                  | NEG                       |
| Number EIA reactive or WB positive/number tested<br>(% EIA reactive or WB positive) |                     |                       | 20/21 (95.24%)                   | 15/21 (71.43%)                  | 13/20 (65.00%)      | 11/21 (52.38%)            |

# GS HIV Combo Ag/Ab Specificity

Specificity in samples from low risk populations.

| Low risk population         | Number tested | GS HIV Combo Ag/Ab EIA<br>Repeatedly reactive<br>(% reactive) | Repeatedly reactive specimens               |                                | Specificity<br>(# negative/total) <sup>a</sup> |
|-----------------------------|---------------|---|---|--------------------------------|--|
|                             |               |   | HIV-1 Western blot<br>positive (% positive) | HIV-2 positive<br>(% positive) |  |
| Health insurance applicants | 2000          | 6 (0.30%)   | 2 (0.10%)                                   | 0 (0.00%)                      | 99.80% (1994/1998)                             |
| Normal blood donors         | 2000          | 0 (0.00%)   | NT  | NT                             | 100% (2000/2000)                               |
| Pregnant women              | 1000          | 2 (0.20%)   | 1 (0.10%)                                   | 0 (0.00%)                      | 99.90% (998/999)                               |
| Military recruits           | 1000          | 3 (0.30%)   | 1 (0.10%)                                   | 0 (0.00%)                      | 99.80% (997/999)                               |
| Healthy pediatric subjects  | 100           | 0 (0.00%)   | NT  | NT                             | 100% (100/100)                                 |
| Total                       | 6100          | 11 (0.18%)  | 4 (0.07%)                                   | 0 (0.00%)                      | 99.89% (6089/6096)                             |

NT: not tested and #: number.

<sup>a</sup> Four HIV-1 Western blot positive samples were removed from the specificity calculation.

Bentsen et al. Journal of Clinical Virology 2011.

# Architect Ag/Ab Combo

**Table 2**  
Sensitivity and specificity of the ARCHITECT Ag/Ab Combo assay.<sup>a</sup>

| Result                              | No. of samples            |                             | Sensitivity (95% CI)  | Specificity (95% CI)  |
|-------------------------------------|---------------------------|-----------------------------|-----------------------|-----------------------|
|                                     | HIV-1-infected (n = 3386) | HIV-1-uninfected (n = 7551) |                       |                       |
| <b>Initial screening</b>            |                           |                             |                       |                       |
| Positive                            | 3384                      | 92                          |                       |                       |
| Negative                            | 2                         | 7459                        |                       |                       |
| Performance                         |                           |                             | 99.94% (99.79, 99.99) | 98.78% (98.51, 99.02) |
| <b>Retest screening<sup>b</sup></b> |                           |                             |                       |                       |
| Positive                            | 3384                      | 38                          |                       |                       |
| Negative                            | 2                         | 7513                        |                       |                       |
| Performance                         |                           |                             | 99.94% (99.79, 99.99) | 99.50% (99.31, 99.64) |
| <b>Acute infections (n = 58)</b>    |                           |                             |                       |                       |
| Result                              | Acute infections (n = 58) |                             | Sensitivity (95% CI)  |                       |
| Positive                            | 48                        |                             |                       |                       |
| Negative                            | 10                        |                             |                       |                       |
| Performance                         |                           |                             | 82.76% (70.57, 91.41) |                       |

Chavez et al. Journal of Clinical Virology 2011

# Multispot

L.V. Torian et al. / Journal of C

|                                   | <b>Multispot Positive</b> |              | <b>Multispot Negative</b> |              | <b>Total</b> |
|-----------------------------------|---------------------------|--------------|---------------------------|--------------|--------------|
|                                   | <i>N</i>                  | <i>Row %</i> | <i>N</i>                  | <i>Row %</i> |              |
| <b>Western Blot Positive</b>      | 8670                      | 99.9%        | 8                         | 0.1%         | <b>8678</b>  |
| <b>Western Blot Negative</b>      | 3                         | 15.8%        | 16                        | 84.2%        | <b>19</b>    |
| <b>Western Blot Indeterminate</b> | 23                        | 36.5%        | 40                        | 63.5%        | <b>63</b>    |
| <b>Total</b>                      | <b>8696</b>               | <b>99.3%</b> | <b>64</b>                 | <b>0.7%</b>  | <b>8760</b>  |

Fig. 2. Western blot vs. Multispot sensitivity.

Torian et al. Journal of Clinical Virology 2011.

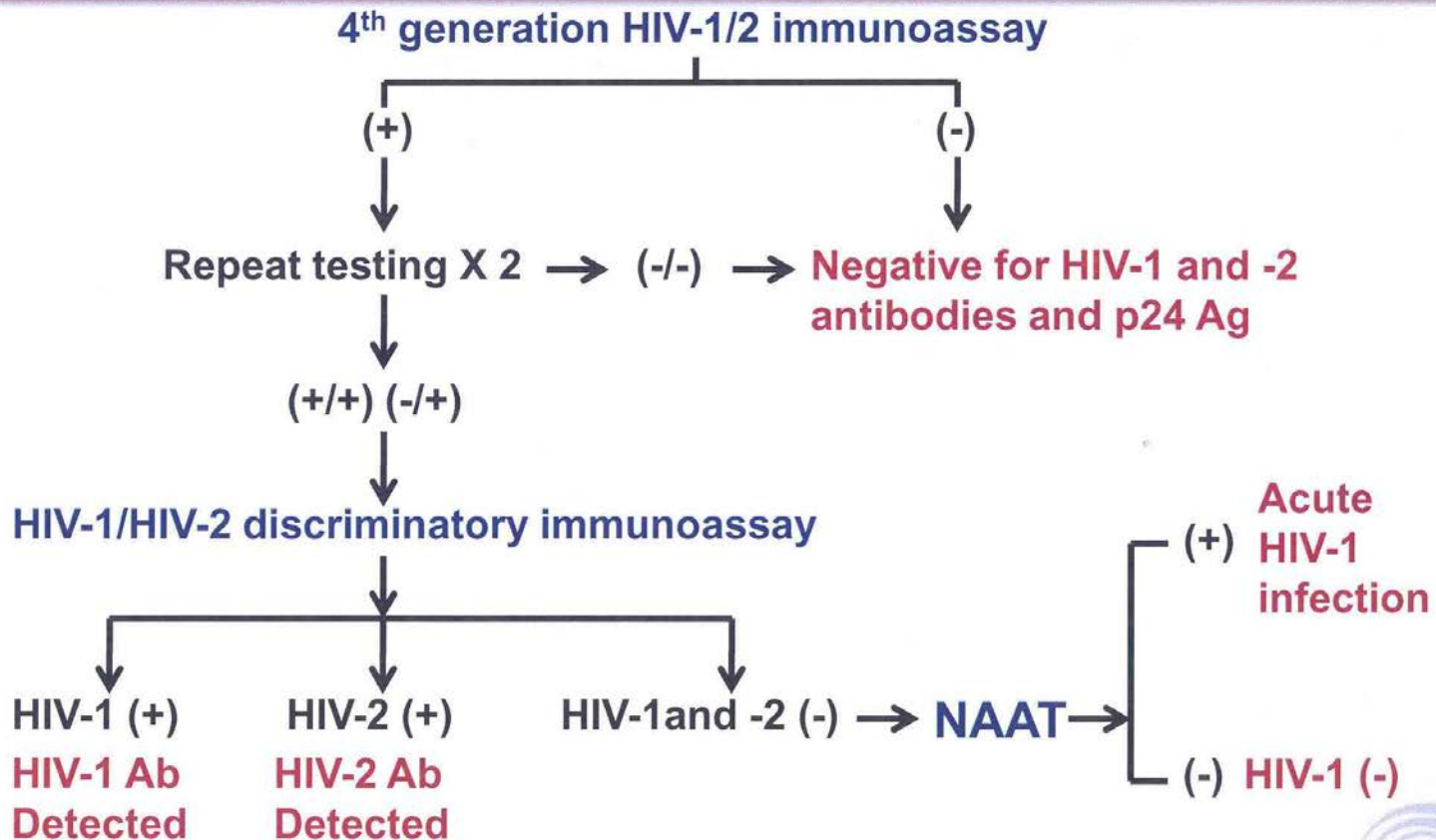
# Comparison of Algorithms

|   | Sensitivity |              | Specificity |              |
|---|-------------|--------------|-------------|--------------|
|   | %           | 95% CI       | %           | 95% CI       |
| <b>Single test</b>                            |             |              |             |              |
| Architect                                     | 99.76       | 98.65-99.96  | 100.00      | 99.08-100.00 |
| GS+O  | 100.00      | 99.09-100.00 | 99.52       | 98.26-99.87  |
| Multispot                                     | 99.52       | 98.26-99.62  | 99.03       | 97.54-99.61  |
| Oraquick                                      | 98.80       | 97.22-99.49  | 99.76       | 98.64-99.96  |
| Reveal G2                                     | 99.28       | 97.90-99.75  | 99.76       | 98.64-99.96  |
| Unigold                                       | 98.80       | 97.22-99.49  | 99.52       | 98.26-99.87  |
| <b>Two-test current algorithm</b>             |             |              |             |              |
| Architect/WB                                  | 99.76       | 98.65-99.96  | 100.00      | 99.08-100.00 |
| GS+O/WB                                       | 100.00      | 99.09-100.00 | 100.00      | 99.08-100.00 |
| <b>Three-test proposed algorithm</b>          |             |              |             |              |
| Architect or GS+O/Multispot/NAAT <sup>a</sup> | 99.76       | 98.65-99.96  | 100.00      | 99.08-100.00 |
| Architect or GS+O/Oraquick/NAAT               | 99.28       | 97.96-99.75  | 100.00      | 99.08-100.00 |
| Architect or GS+O/Reveal G2/NAAT              | 99.28       | 97.96-99.75  | 100.00      | 99.08-100.00 |
| Architect or GS+O/Unigold/NAAT                | 99.52       | 98.26-99.87  | 100.00      | 99.08-100.00 |

Masciotra et al. Journal of Clinical Virology 2011.



# CDC Proposed Algorithm



# New Algorithm Benefits

- Increased detection of acute HIV infection
  - Ag/Ab Combo Assay
  - NAAT confirmation of acute HIV infection
- Eliminate inconclusive/indeterminate results
  - eliminating the Western Blot
- Decrease turn around time & linkage to care
  - replacing Western blot with Multispot
- Increased detection of HIV-2 infection
  - replacing Western blot with Multispot

# New Algorithm Challenges

- Only two platforms currently available for Ag/Ab Combo assays
- Multispot is a rapid test, originally approved as a screening assay
- There is only one qualitative molecular assay approved for HIV diagnosis (Aptima) that is not widely available and not automated
- Quantitative or viral load HIV assays are widely utilized but none is approved for diagnosis
- If a laboratory is using an antigen/antibody combo assay for screening then confirmation by Western blot is insufficient because it only detects anti-HIV antibody

# Thank you!

