New Guidelines for HIV Diagnosis

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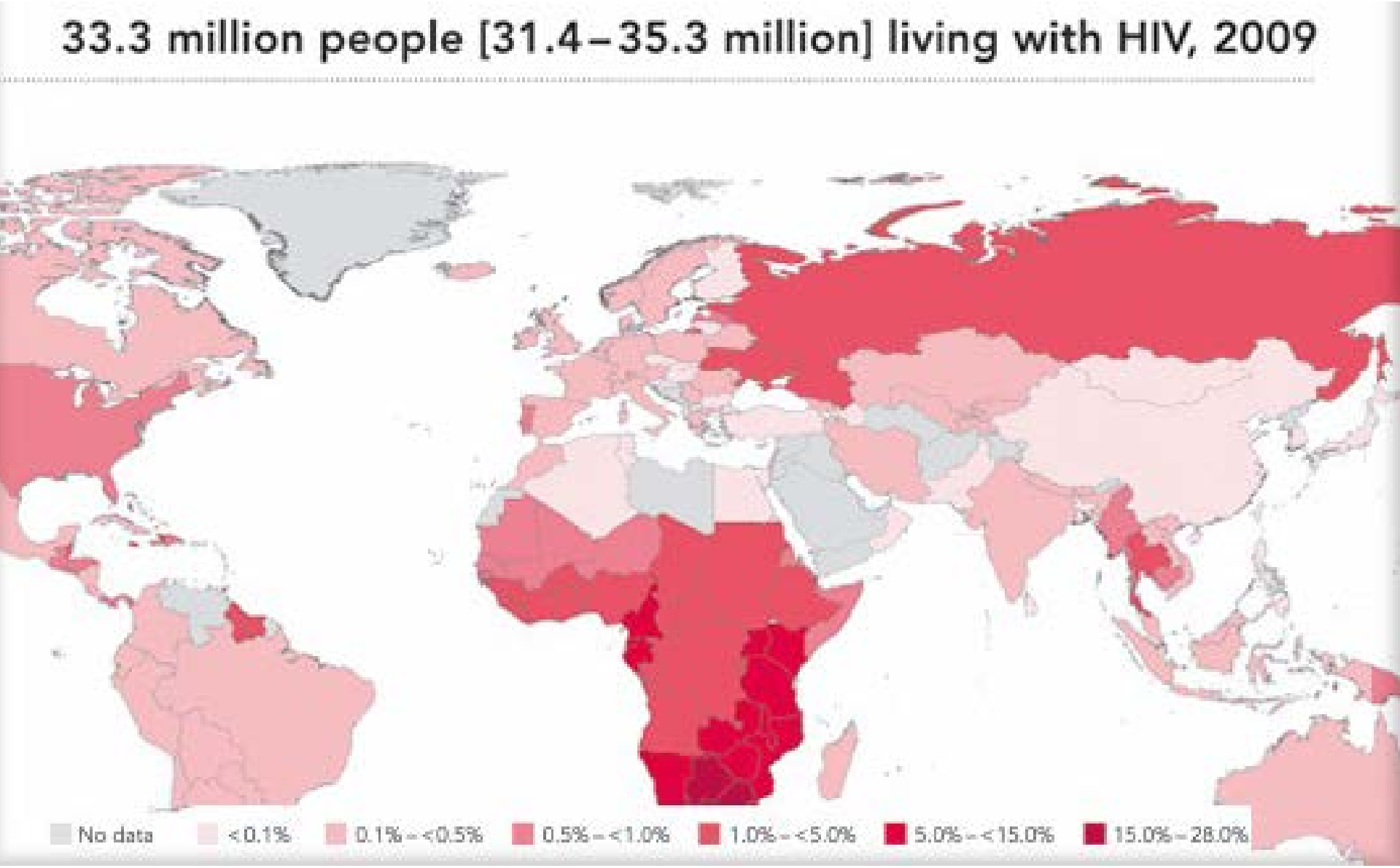
Disclosure

- None (Dr. Slev)
- Roche Diagnostics (Dr. Hillyard)

Objectives

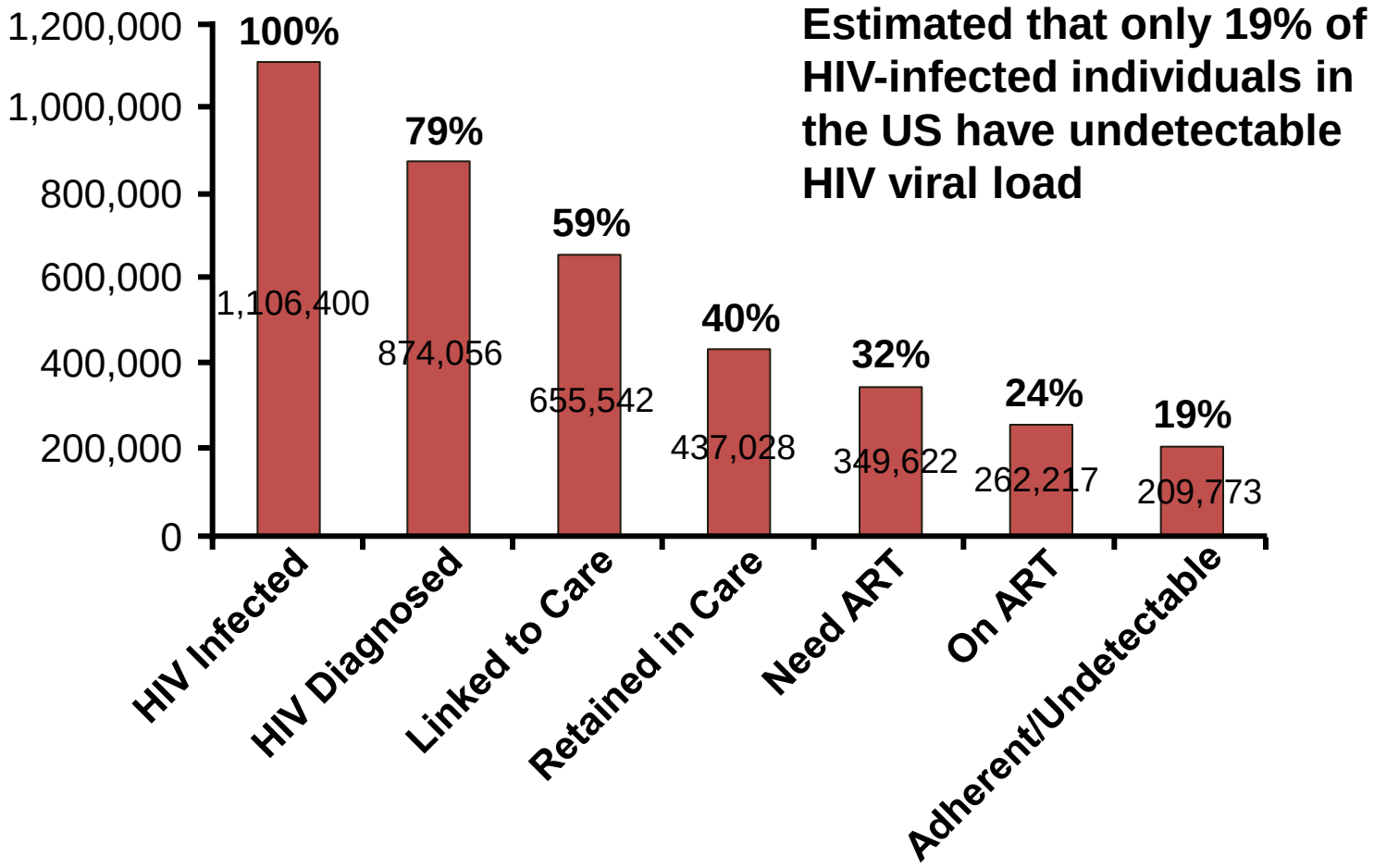
- Explain the advances in HIV diagnostics, including fourth generation Ag/Ab combination HIV screening assays.
- Describe the new CDC HIV diagnostic algorithm.
- Understand the evidence in support of the new diagnostic algorithm.
- Use screening and follow-up confirmatory tests appropriately.

HIV Globally



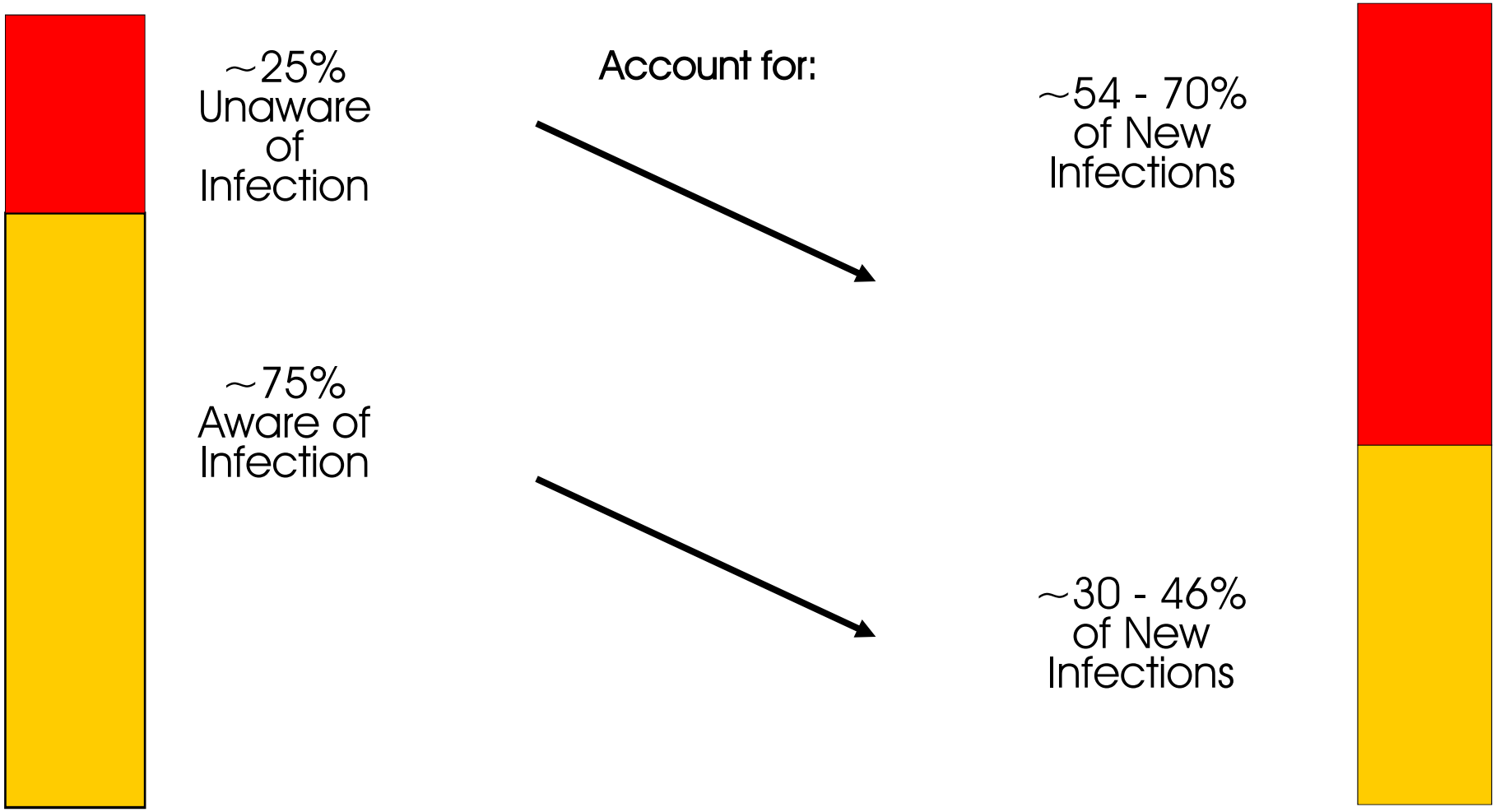
http://payvand.com/blog/files/2010/12/global-aids-2010.jpg

HIV in the US

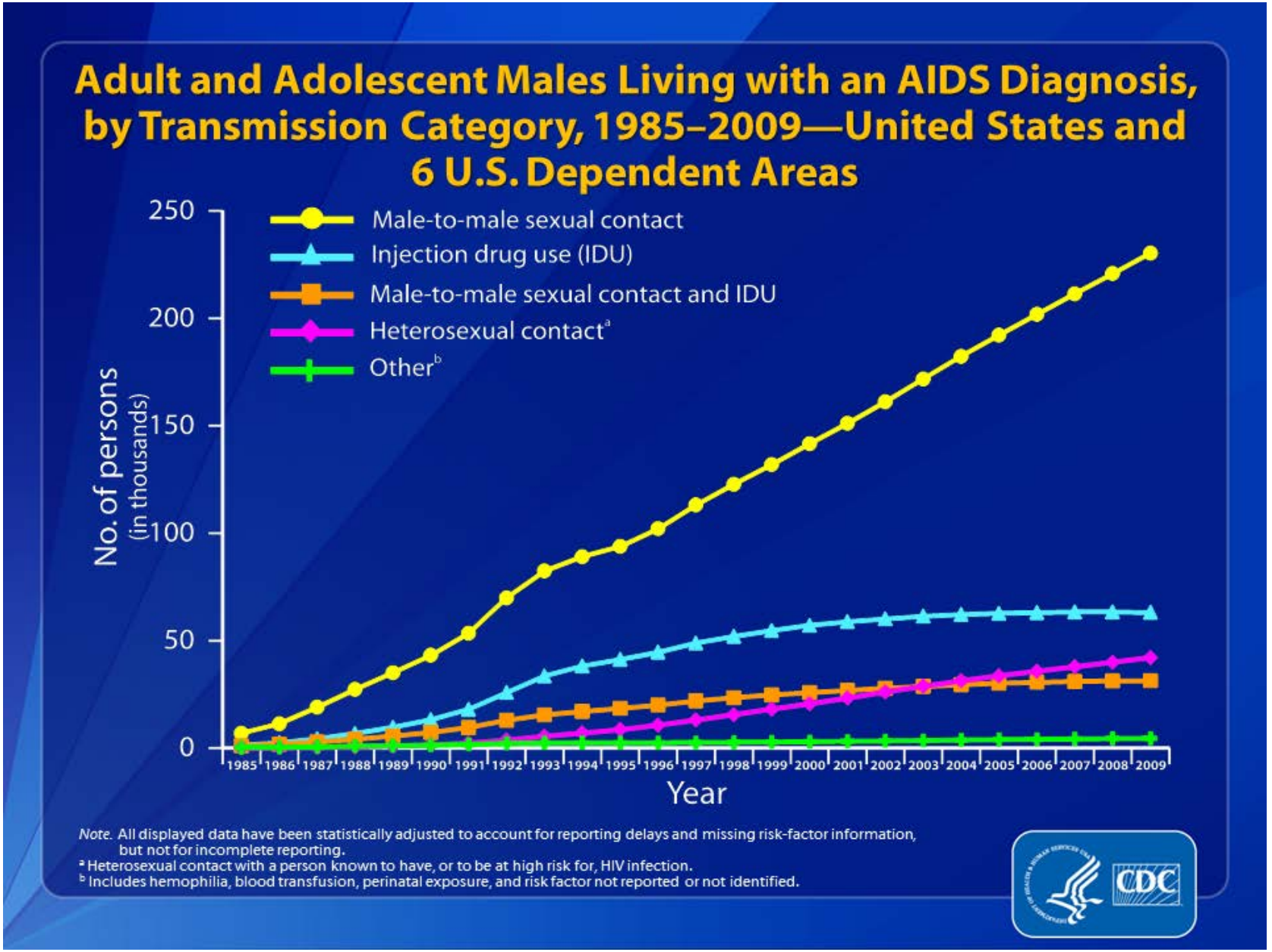


Gardner EM, et al. Clin Infect Dis. 2011;52:793-800. Clinical Care Options 2012.

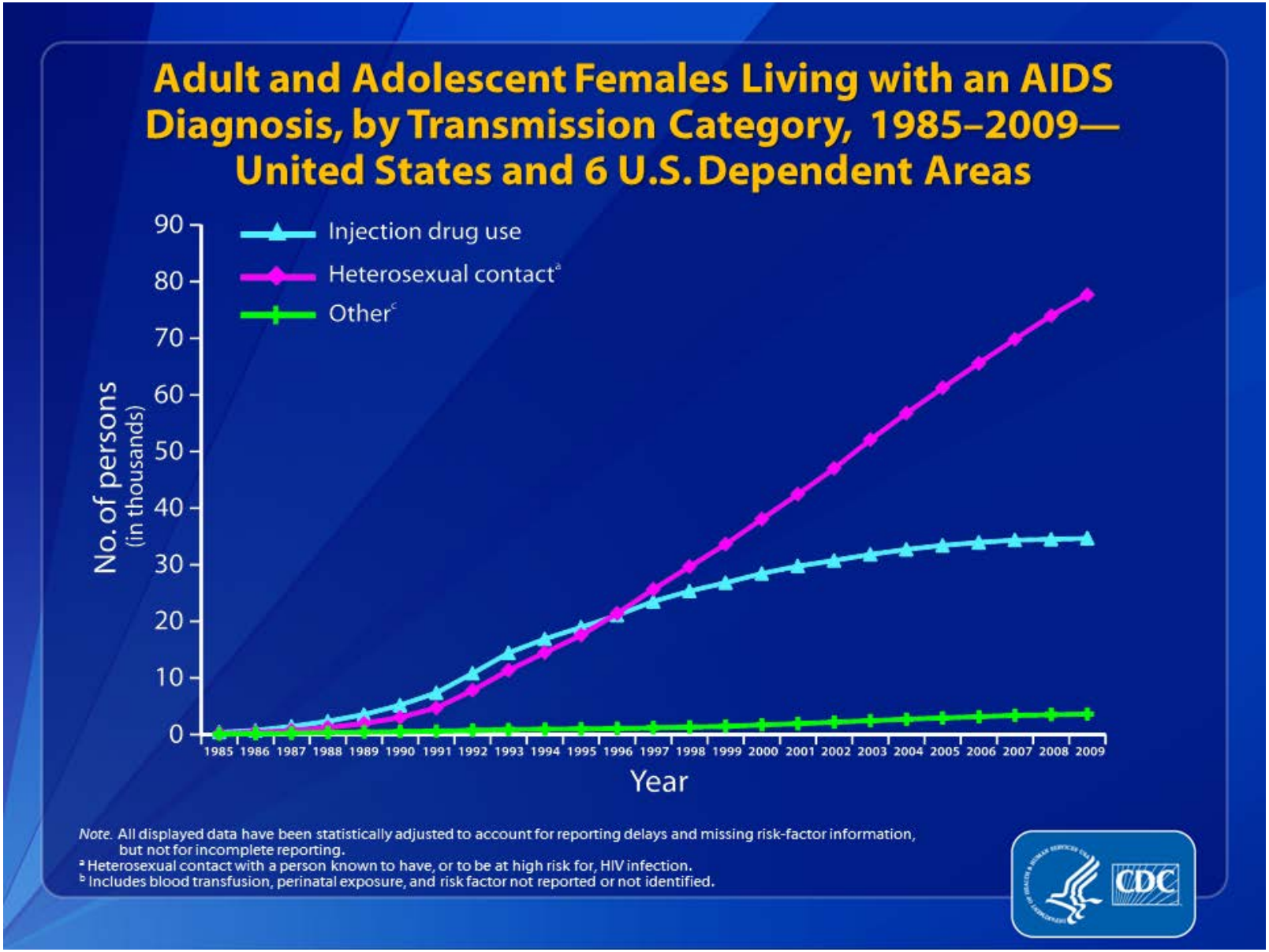
Awareness of Serostatus Among People with HIV and Estimates of STD Transmission (US)



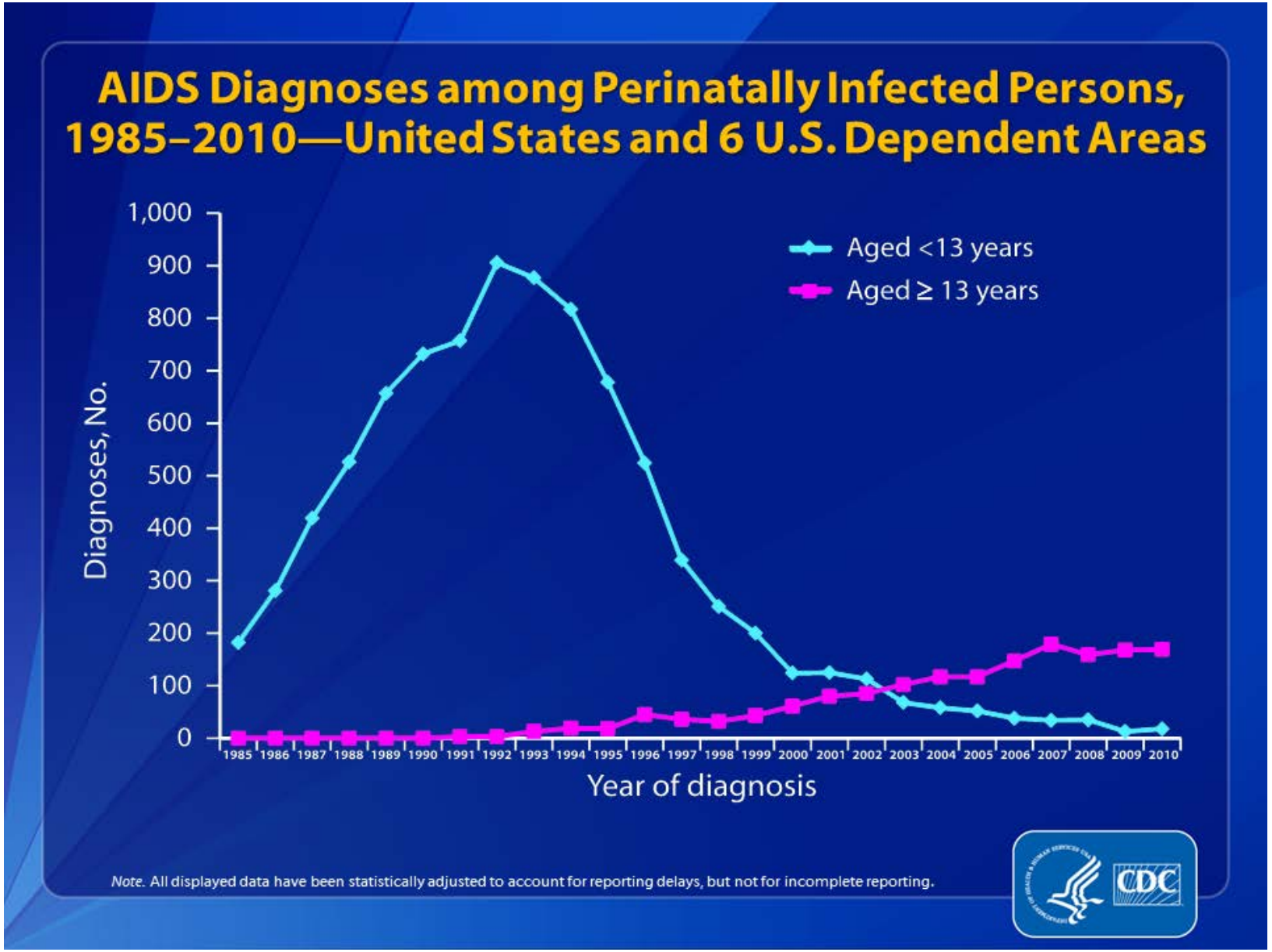
Transmission in Males



Transmission in Females



Perinatal Transmission

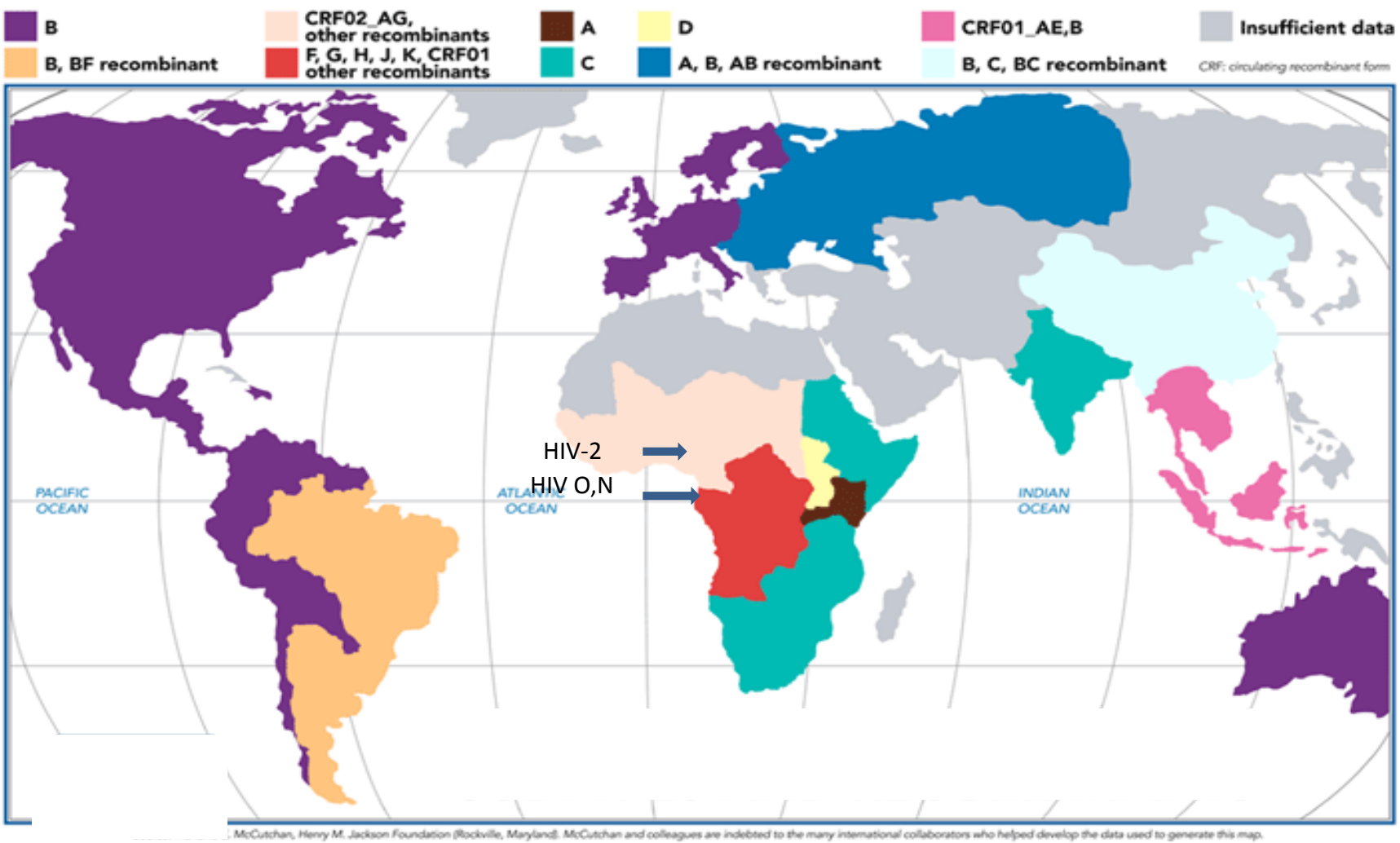


2006 CDC Guidelines
"Universal Testing"

- Routine HIV
  voluntary, not based on risk
- Opt-Out
  option to decline, general consent for care includes HIV testing
- Population
  13 -64 years old
- Venue
  inpatient services, ED, urgent care, STD clinics,
  substance abuse and correctional facilities

Human Immunodeficiency Virus

HIV Distribution



Francine E. McCutcham, Henry M. Jackson Foundation (Rockville, Maryland). IAVI Report , August 2003

HIV-2

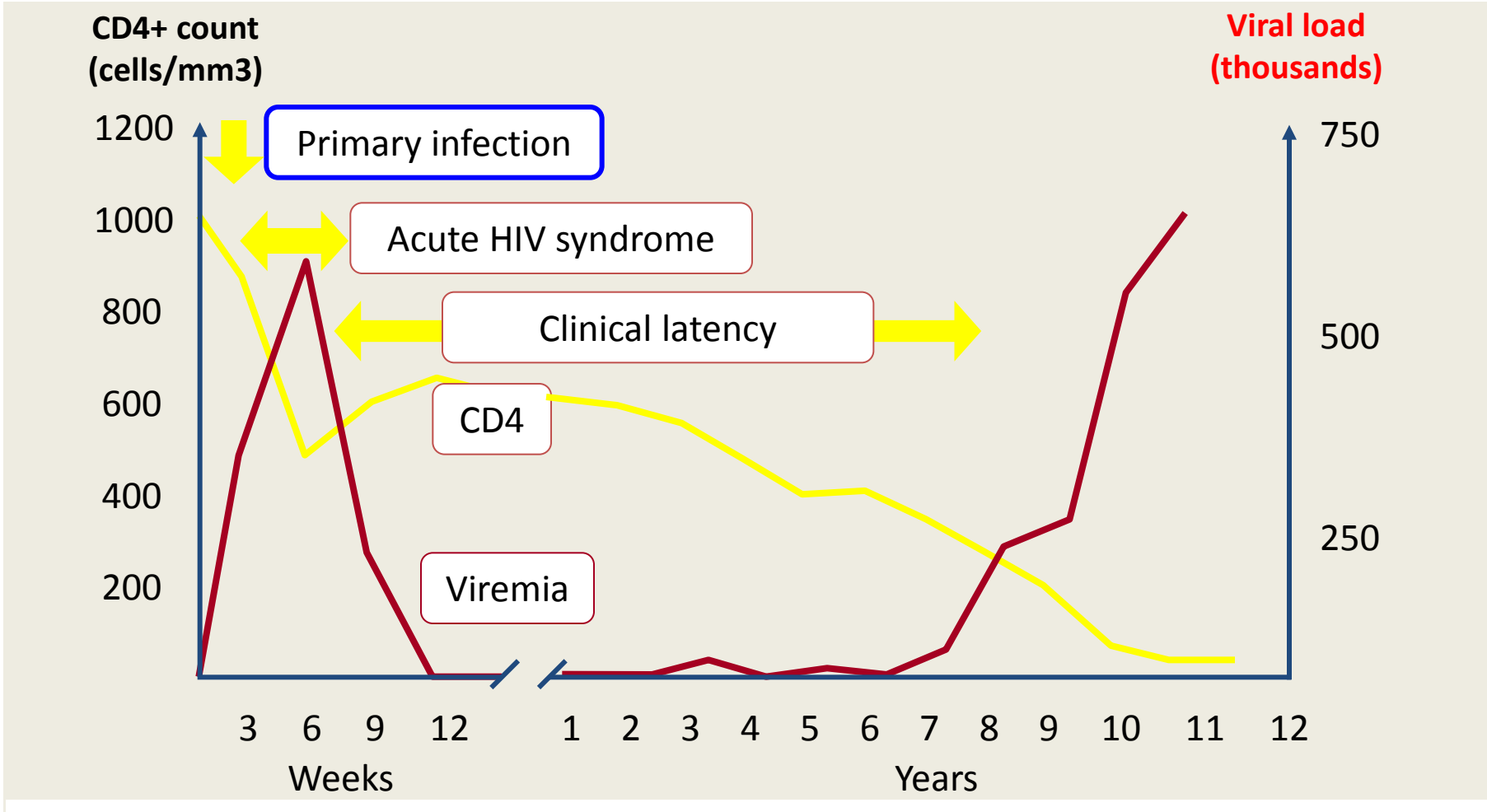
Persons at risk for HIV-2 infection include

- 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)**

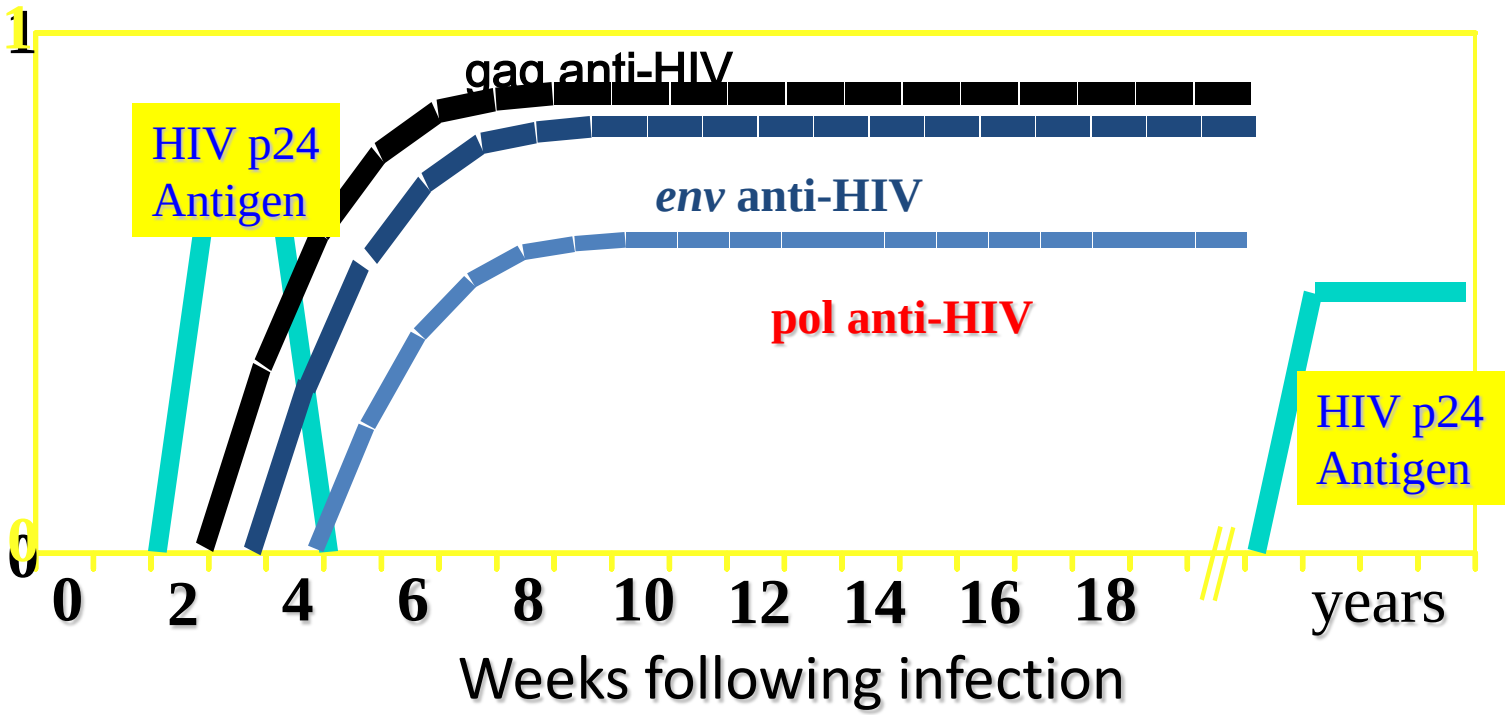
HIV Infection Course



Adapted from Roche and Siemens slides

HIV Serological Response

Typical response following infection

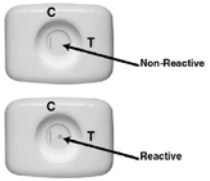


Current HIV Diagnostic Algorithm

1 Screen

immunoassay (EIA/CIA)

rapid tests

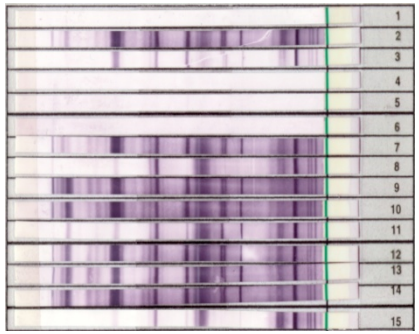


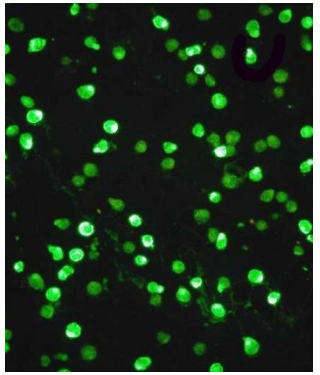
2 Confirmation

Western blot (98%)

IFA

APTIMA*



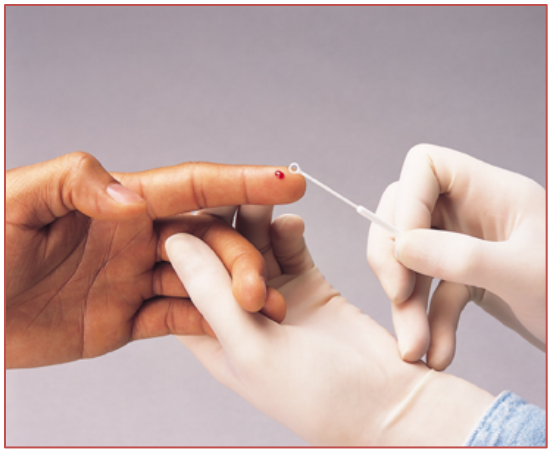


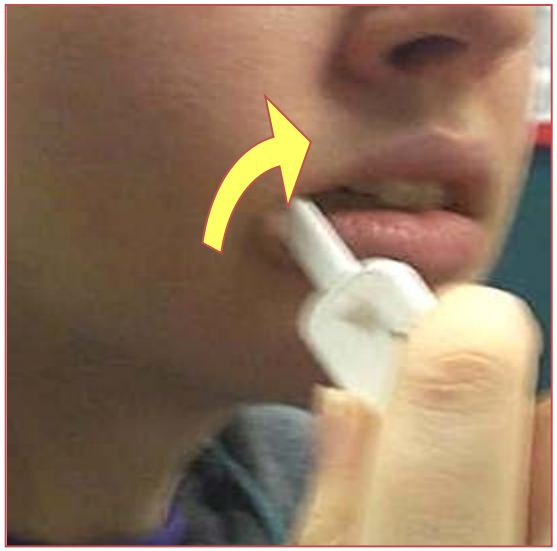
*Note: APTIMA, Genprobe (TMA format) qualitative assay only FDA approved nucleic acid amplification test (NAAT) for diagnosis and confirmation

Screening Assays - Rapid Antibody Tests (2nd gen - IgG)

Assay	Sample Type	Sensitivity	Specificity
OraQuick ADVANCE Rapid HIV-1/2 Antibody Test	Oral fluid	99.3%	99.8%
	Whole Blood	99.6%	100%
	Plasma	99.6%	99.9%
Clearview COMPLETE HIV 1/2	Whole Blood	99.7%	99.9%
	Serum & Plasma	99.7%	99.9%
Clearview HIV ½ STAT-PAK	Whole Blood	99.7%	99.9%
	Serum & Plasma	99.7%	99.9%
Reveal G-3 Rapid HIV-1 Antibody Test	Serum	99.8%	99.1%
	Plasma	99.8%	98.6%
Uni-Gold Recombigen HIV	Whole Blood	100%	99.7%
	Serum & Plasma	100%	99.8%
Multispot HIV-1/HIV-2 Rapid Test	Serum	100%	99.9%
	Plasma	100%	99.9%
INSTI HIV-1Antibody Test*	Plasma	99.9%	100.0%
	Whole blood (venipuncture)	99.9%	100.0%
	Whole blood (fingerstick)	99.8%	99.5%

OraQuick® Advance

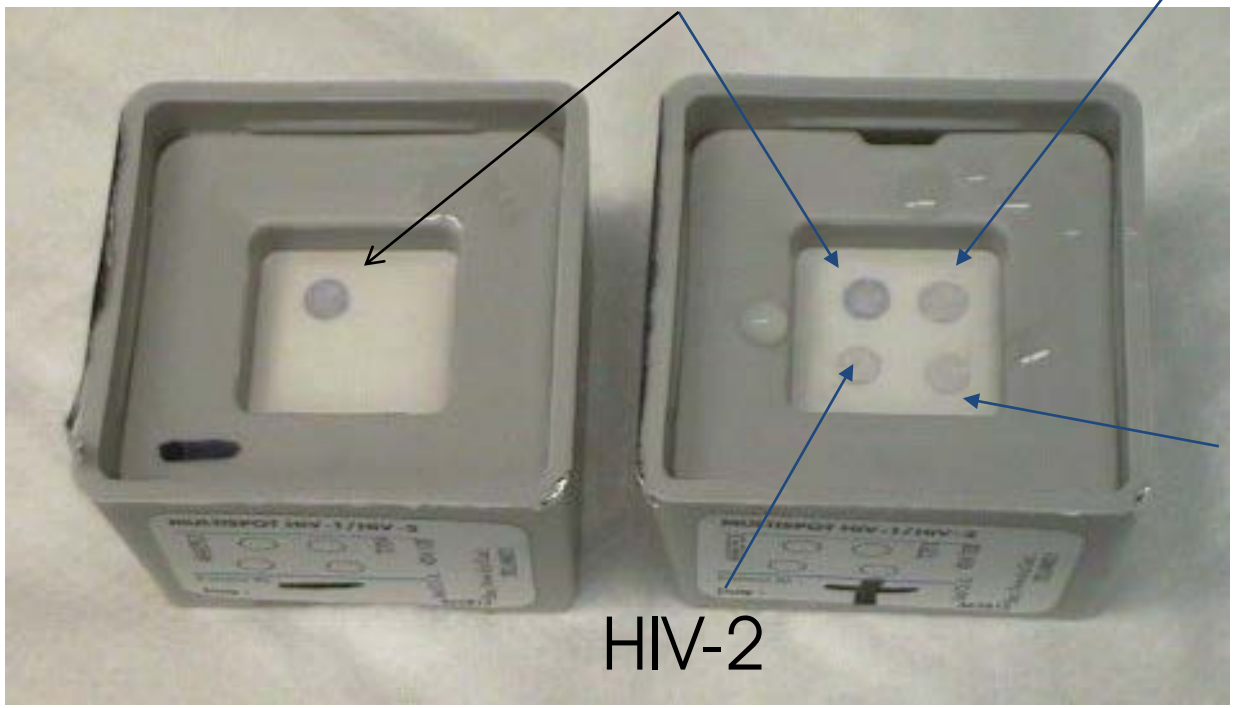




- Synthetic gp-41 (HIV-1)
- Synthetic gp-36 (HIV-2)
- Goat anti-human IgG

Photograph from CDC: www.cdc.gov/hiv/rapid_testing

Multispot HIV-1/HIV-2



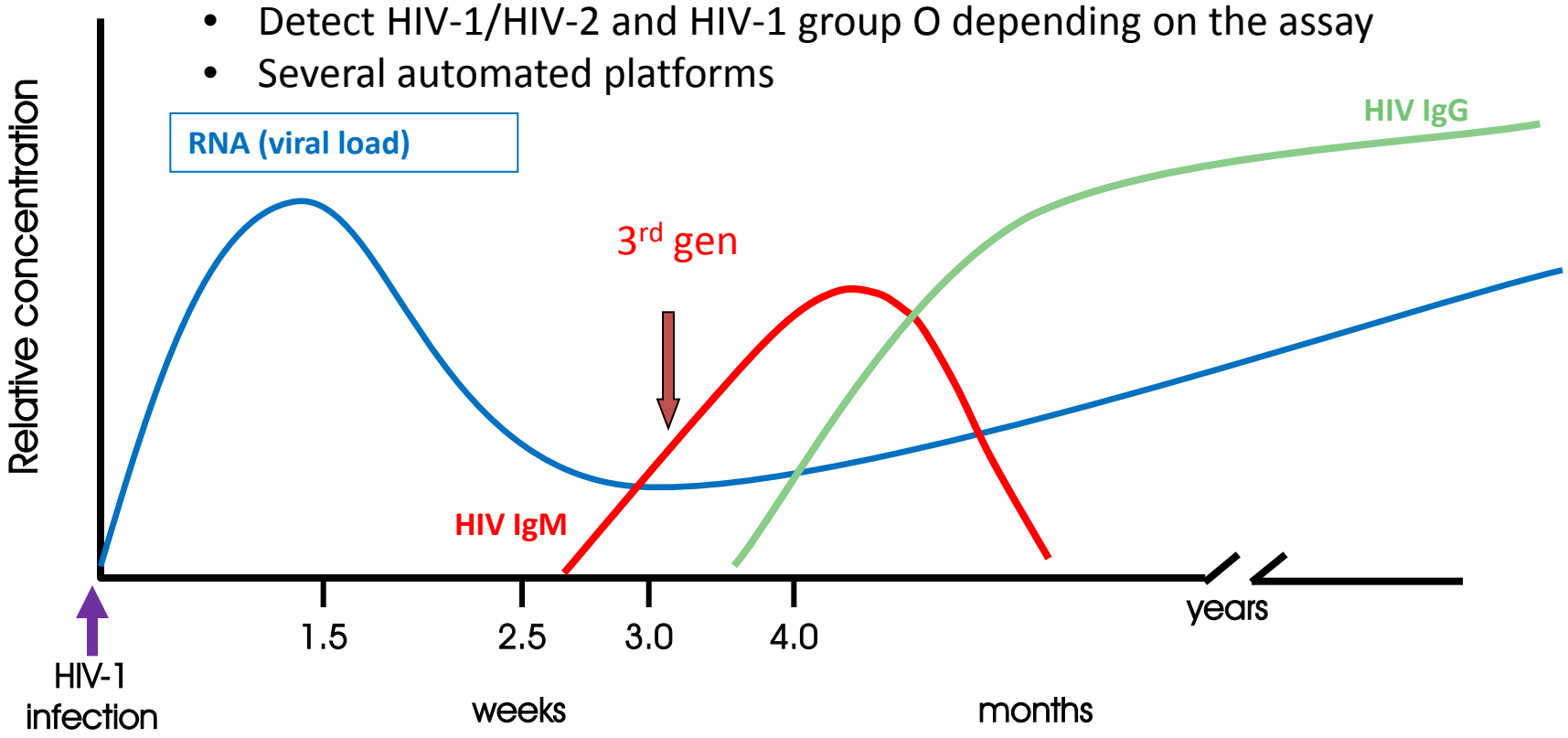
Negative

Reactive
(HIV-1 & HIV-2)

Detects and differentiates between HIV-1 and HIV-2

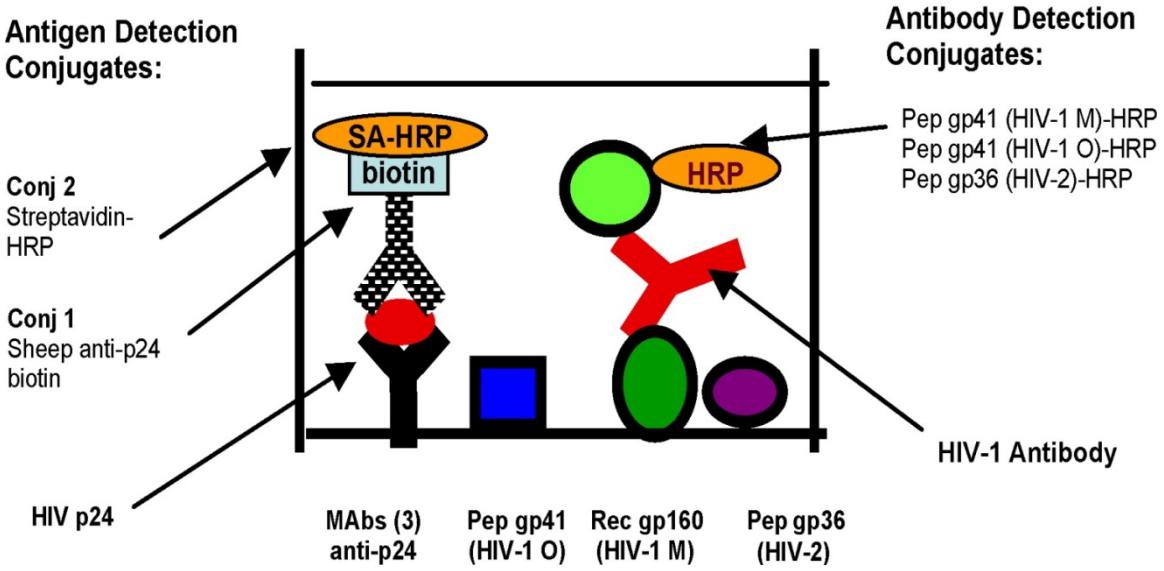
HIV Ab Screening Assays
(3rd gen – IgM and IgG)

- Third generation assays (IgG/IgM)
- Detect HIV infection on day 22
- Detect HIV-1/HIV-2 and HIV-1 group O depending on the assay
- Several automated platforms



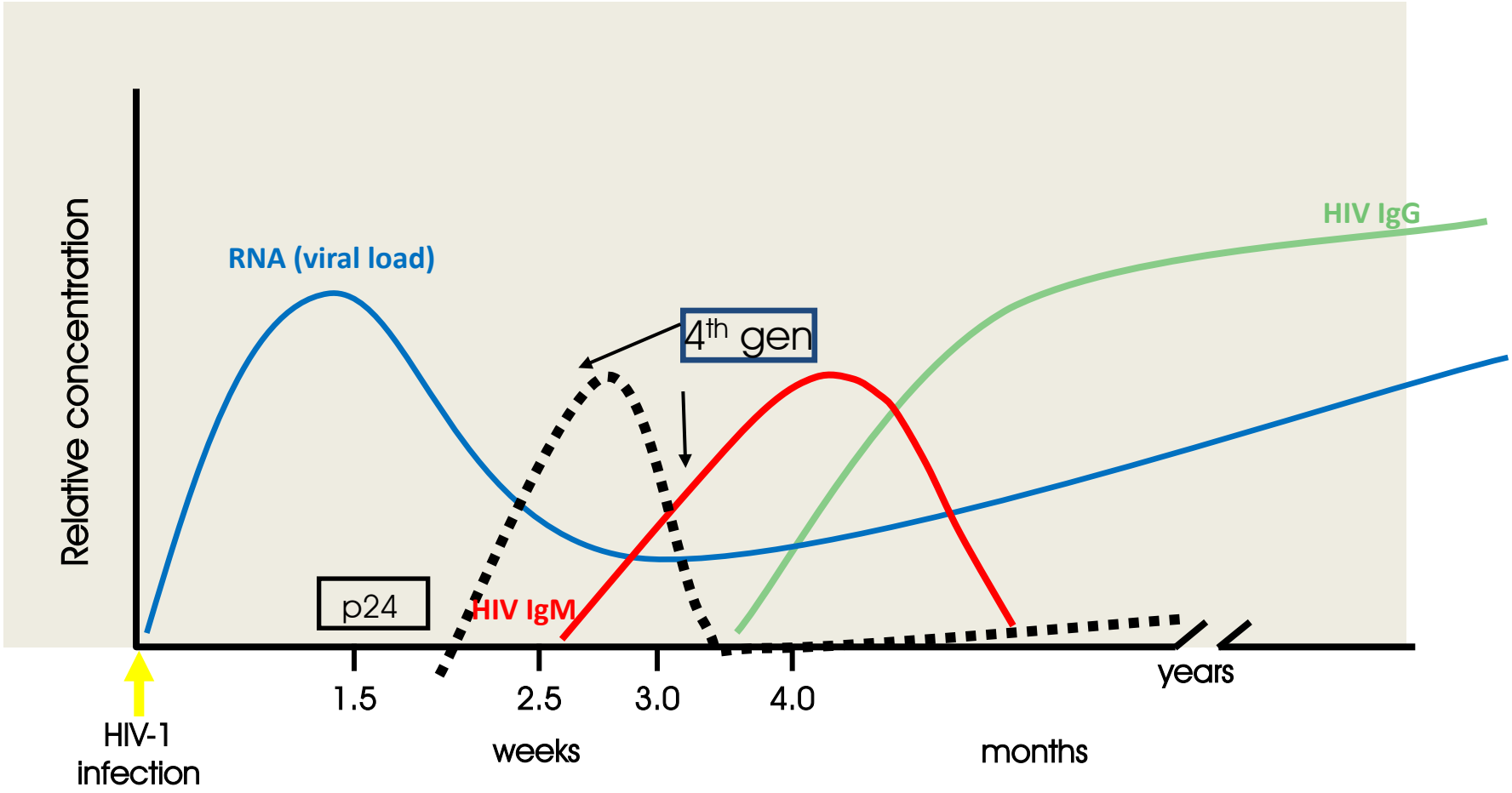
HIV Antigen/Antibody Combination Assays (4th gen – p24 Ag/IgM/IgG)

- Detects both HIV -1 (group O) and HIV-2 antibodies and p24 antigen
- Does not distinguish between Ab+ or Ag+
- Only two FDA – cleared assays



Bentsen et al. Journal of Clinical Virology. 2011

Earlier Detection of HIV Infection: (4th generation)

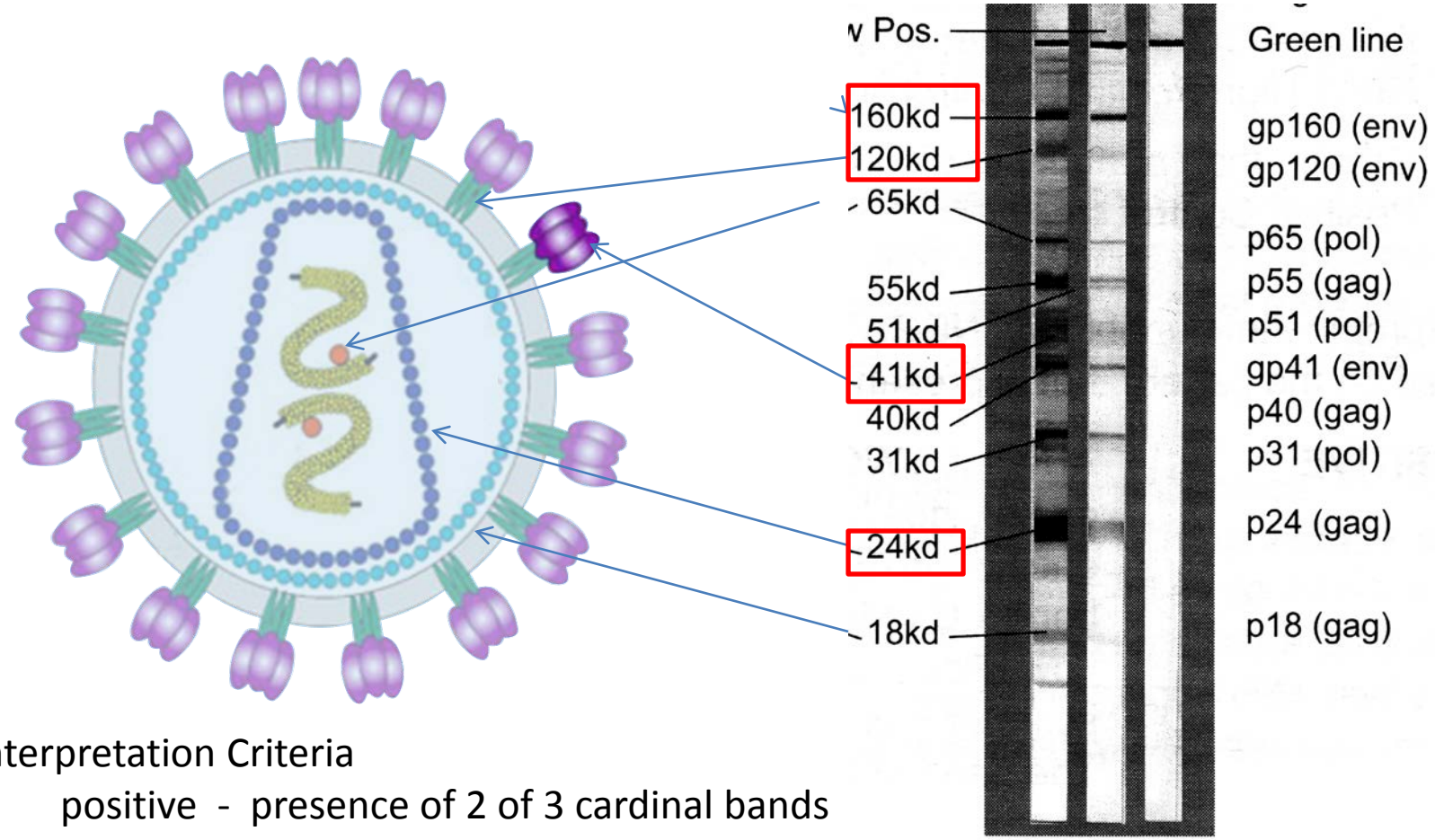


Detects infection at 2.5 -3.0 weeks, 5 days earlier than 3rd gen

False Positive Immunoassay Results

- Vaccinations
  flu, rabies
- HIV vaccine trials
- Autoimmune disease
- Heterophile Antibodies
- Other viral infections

Confirmation by Western Blot



CDC Interpretation Criteria

positive - presence of 2 of 3 cardinal bands

negative - absence of all bands

indeterminate – does not meet + or - criteria

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
  - nucleic acid amplification test (NAAT)

HIV-1 vs HIV-2 and Western Blot

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-1by Western blot and Multispot HIV-1/HIV2 assay.

	p17	p24	p31	p40	gp41	p51	p55	p66	gp120	gp160
HIV-2 (n=114)										
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
HIV-1 (n=1761)										
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

Adapted from Nasrullah et al. Journal of Clinical Virology 2011.

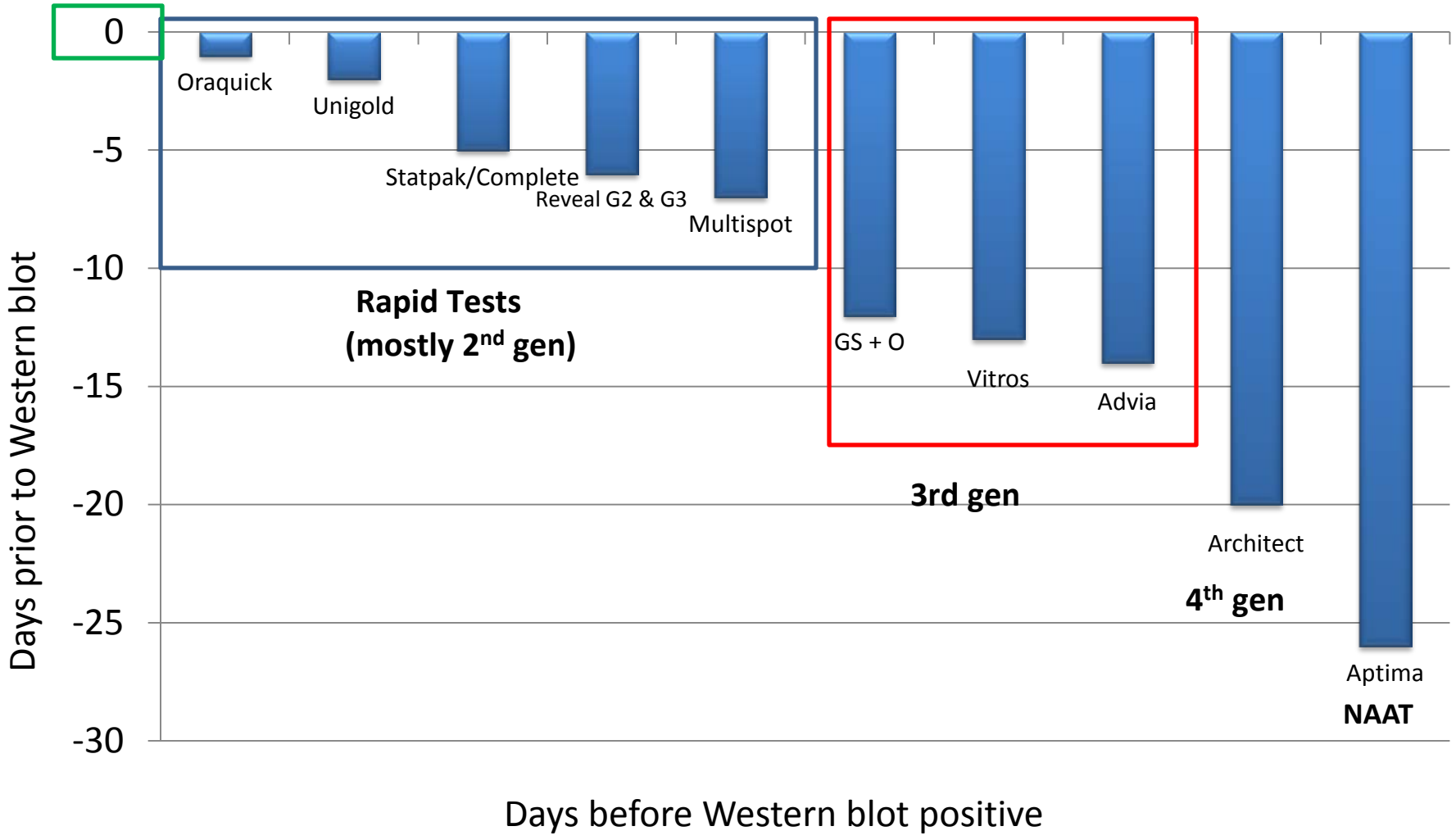
HIV-2 Infection Classification by Western Blot

Comparison of two HIV-1 Western blot interpretive criteria applied to specimens collected from 114 persons known to be infected with HIV-2,[a]

Current CDC HIV-1 WB criteria[a]	Alternative HIV-1 WB criteria Þ , ŋ (%)			
	Negative	Indeterminate	Positive	Total
Negative	1 (0.9)	0 (0.00)	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)

Adapted from Nasrullah et al. Journal of Clinical Virology 2011 .

Sensitivity of Current Assays



Adapted from Masciotra et al. Journal of Clinical Virology 2011.

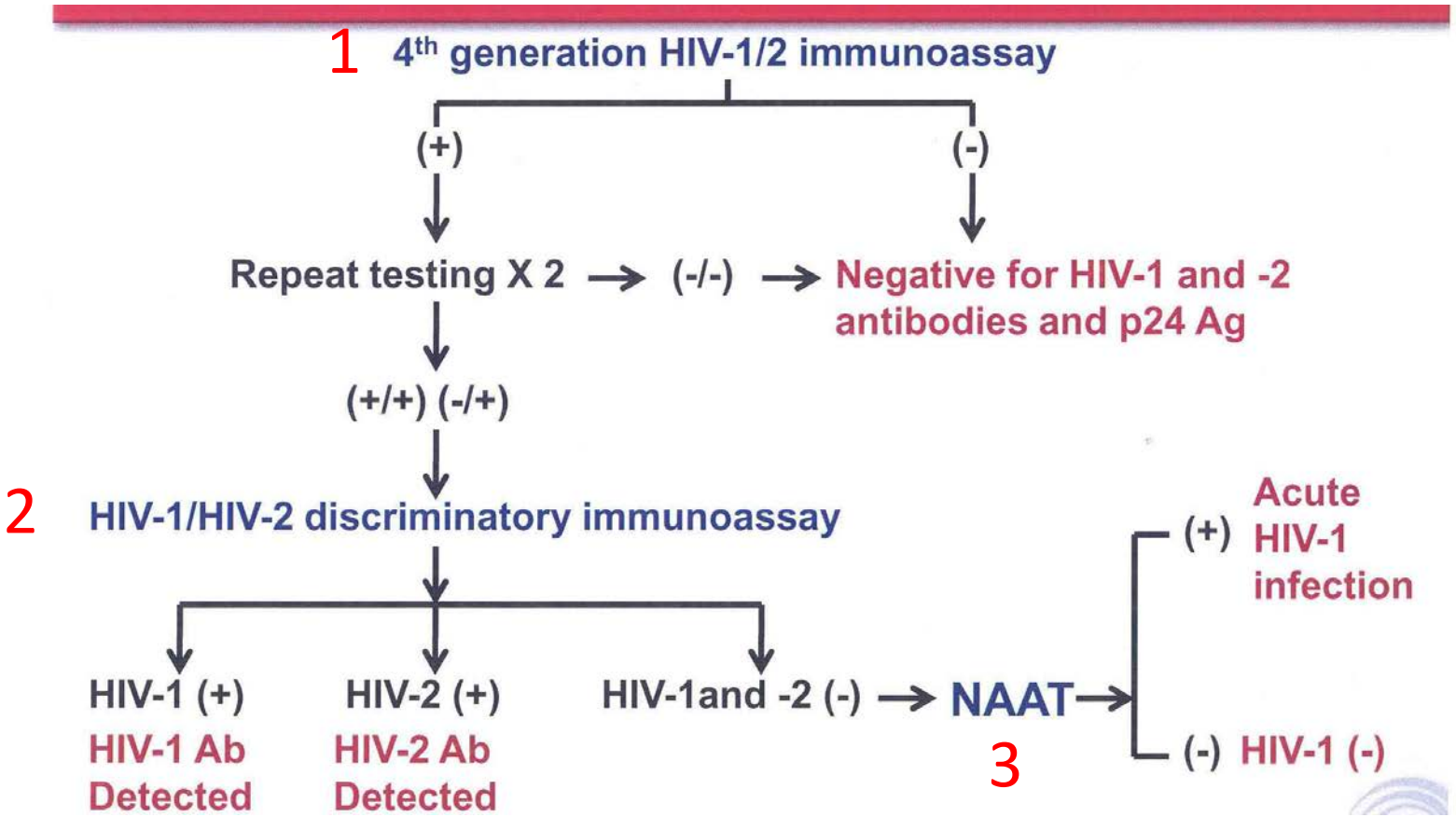
Slide - Anna Gardiner

Western Blot Disadvantages

- Diagnostic Considerations
  - insensitive compared to current screening assays
  - indeterminate/inconclusive results - follow-up
  - HIV-2 misdiagnosis

- Practical Considerations
  - access
  - expense
  - turn around time

CDC Proposed Diagnostic Algorithm



***Could be an IgM sensitive Ab immunoassay if Ag/Ab combination assay is unavailable**

AACC. Clinical Laboratory News. 2010

GS Combo Ag/Ab and Long Standing HIV Infection Sensitivity (4th generation)

Population	N	GS HIV Combo Ag/Ab	Licensed HIV-1/HIV-2
		EIA Repeatedly Reactive	EIA Repeatedly Reactive
Known HIV-1 Ab positive US	100	1000 (100%)	1000 (100%)
Known HIV Ab positive, Non-US	200	200 (100%)	200 (100%)
AIDS	100	100 (100%)	100 (100%)
Known HIV-1 Ab positive, pediatric	40	40 (100%)	40 (100%)
Total	1340	1340	1340 (100%)

Adapted from Bentsen et al. Journal of Clinical Virology 2011.

GS HIV Combo Ag/Ab Specificity (4th generation)

Low Risk Population	Number tested	GS HIV Ag/Ab Combo	Repeatedly reactive Samples		Specificity (#negative/total)
		Repeatedly Reactive (% Reactive)	WB positive (%positive)	HIV-2 positive (%positive)	
Health insurance applicants	2000	6 (0.30%)	2	0 (0.00%)	99.8%
Normal blood donors	2000	0 (0.0%)	NT	NT	100%
Pregnant women	1000	2 (0.20%)	1	0 (0.00%)	99.9%
Military recruits	1000	3 (0.30%)	1	0 (0.0%)	99.8%
Healthy pediatric subjects	100	0(0.0%)	NT	NT	100%
Total	6100	11 (0.18%)	4	0 (0.0%)	99.89%

Adapted from Bentsen et al. Journal of Clinical Virology 2011.

Architect Ag/Ab Combo Performance Data (4th generation)

Result	No of samples		Sensitivity	Specificity
	HIV-1 infected (n-3386)	HIV-1 Uninfected (N=7551)		
Initial screening				
Positive	3384	92		
Negative	2	7459		
Performance			99.94%	98.78%
Retest Screening				
Positive	3384	38		
Negative	2	7513		
Performance			99.94%	99.50%

Result	Acute Infection	Sensitivity
Positive	48	
Negative	10	
Performance		82.76%

Adapted from Chavez et al. Journal of Clinical Virology 2011

Multispot vs. Western Blot

	Multispot Positive		Multispot Negative		Total
	N	Row %	N	Row%	N
WB positive	8670	99.9%	8	0.1%	8678
WB negative	3	15.8%	16	84.2%	19
WB indeterminate	23	36.5%	40	63.5%	63
Total	8696	99.3%	64	0.7%	8760

Adapted from Torian et al. Journal of Clinical Virology 2011.

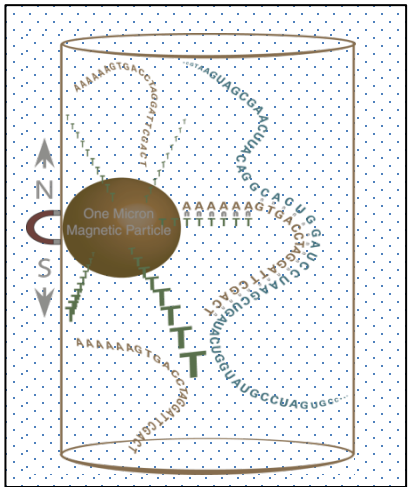
NAAT for HIV Diagnosis

- APTIMA® HIV-1 Qualitative Assay (FDA approved 2006)
  - TMA, Hybrid capture for RNA purification
- Screening of high-risk populations
- Known exposure such as needle-stick
- Testing patients with acute HIV-1 symptoms and known exposure
- Screening of newborn babies born to infected mothers
- HIV vaccine studies
- **Resolution arm for new screening algorithms**

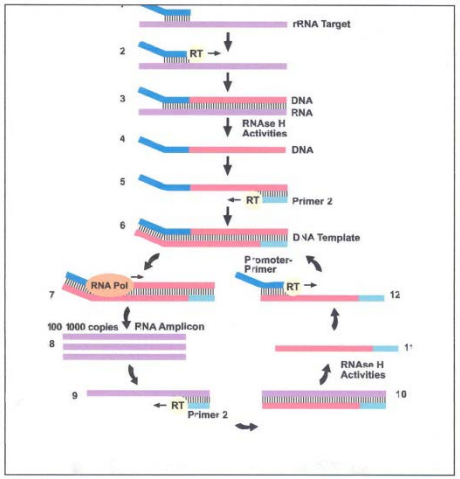
APTIMA® HIV-1 Qualitative Assay

- Poses dilemma for diagnostic algorithm:
  - Only approved test with diagnostic claim few installations, limited test availability
  - Manual test format
  - 1st generation chemistry design, ability to see new strains?

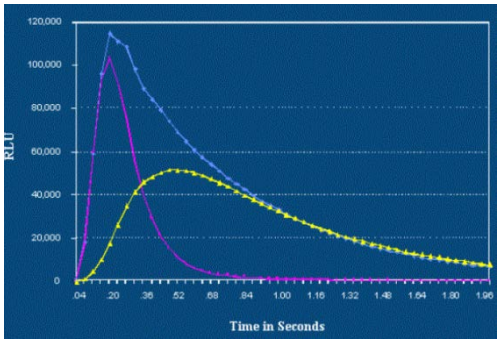
Hybrid Capture Purification



Transcription Mediated Amplification (TMA)



Hybridization Protection Dual Kinetic Detection



Aptima vs Real-time PCR Tests

	Aptima Gen-Probe	Real Time Abbott	TaqMan 1.0 Roche	TaqMan 2.0 Roche
Sensitivity	30 copies/ml	40 copies/ml	43 copies/ml	20 copies/ml
Genotypes	A-O	A-O	A-G	A-G
Amplicon control	Strand Capture	closed	UTP/UNG, closed	UTP/UNG closed
Automation	No (U.S.)	yes	yes	yes
FDA approval	Diagnosis	Monitor	Monitor	Monitor

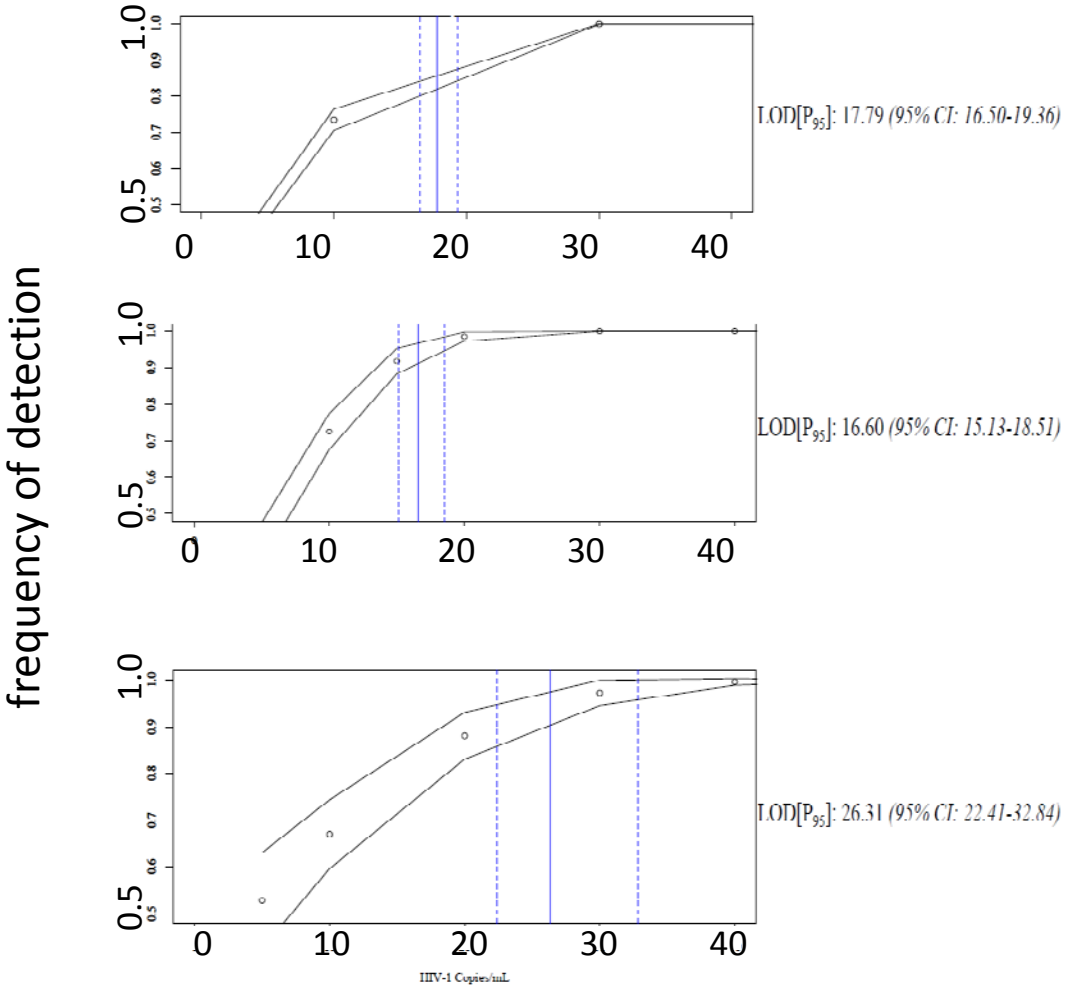
Note: bDNA (signal amplification assay) has ~1% low positivity rate
In negative samples …. not suitable for resolution testing

Comparative Assay Sensitivities (probit modeling of PI data)

Aptima

Roche 2.0

Abbott

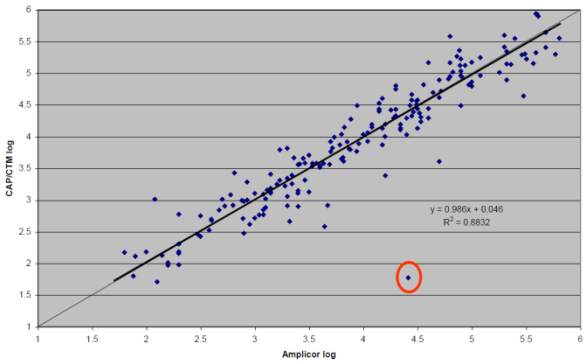


Andrew Wilson

Targets, Chemistry, Issues

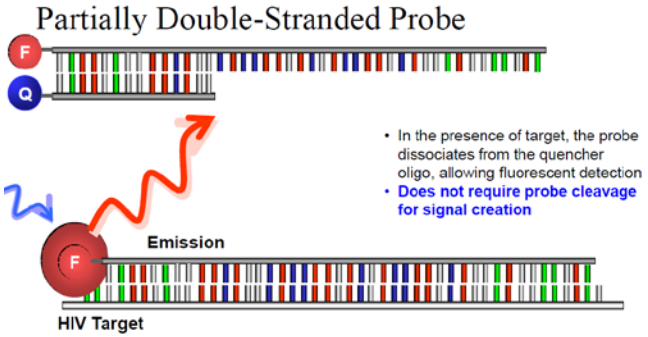
Cobas 1.0, 1.5
end-point PCR Roche

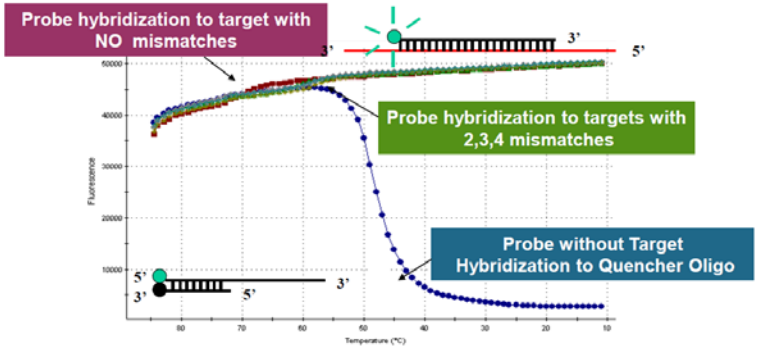
CapTaq 2.0 CapTaq 1.0 real-time PCR Roche



Aptima

Real Time Abbott





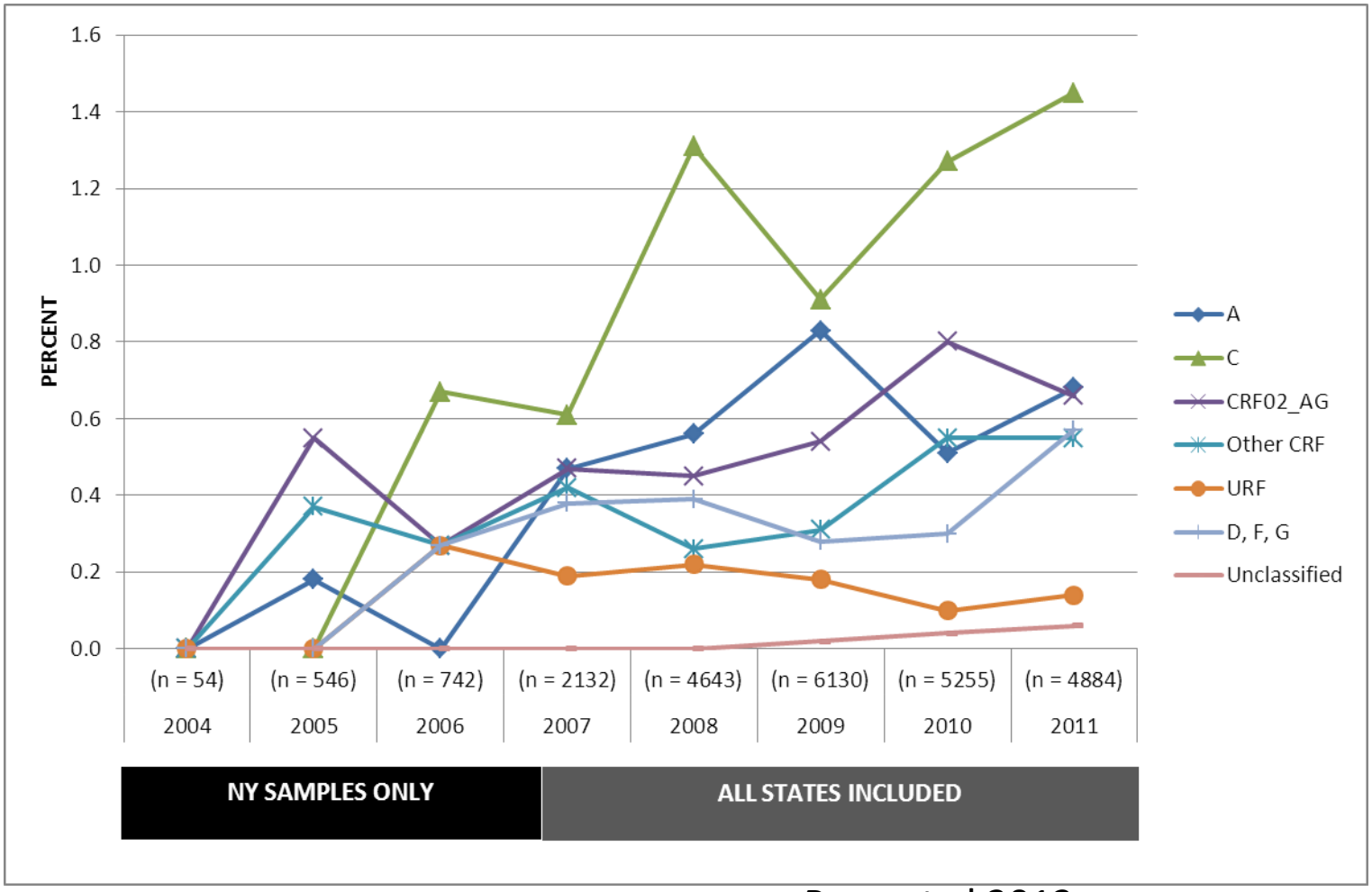
Pyne, M. J Clin Microbiol, *48*(8), 2852-2858.

Pyne, M. JOURNAL OF CLINICAL MICROBIOLOG
Aug. 2010, p. 2852–2858

HIV-1 Proviral DNA Testing

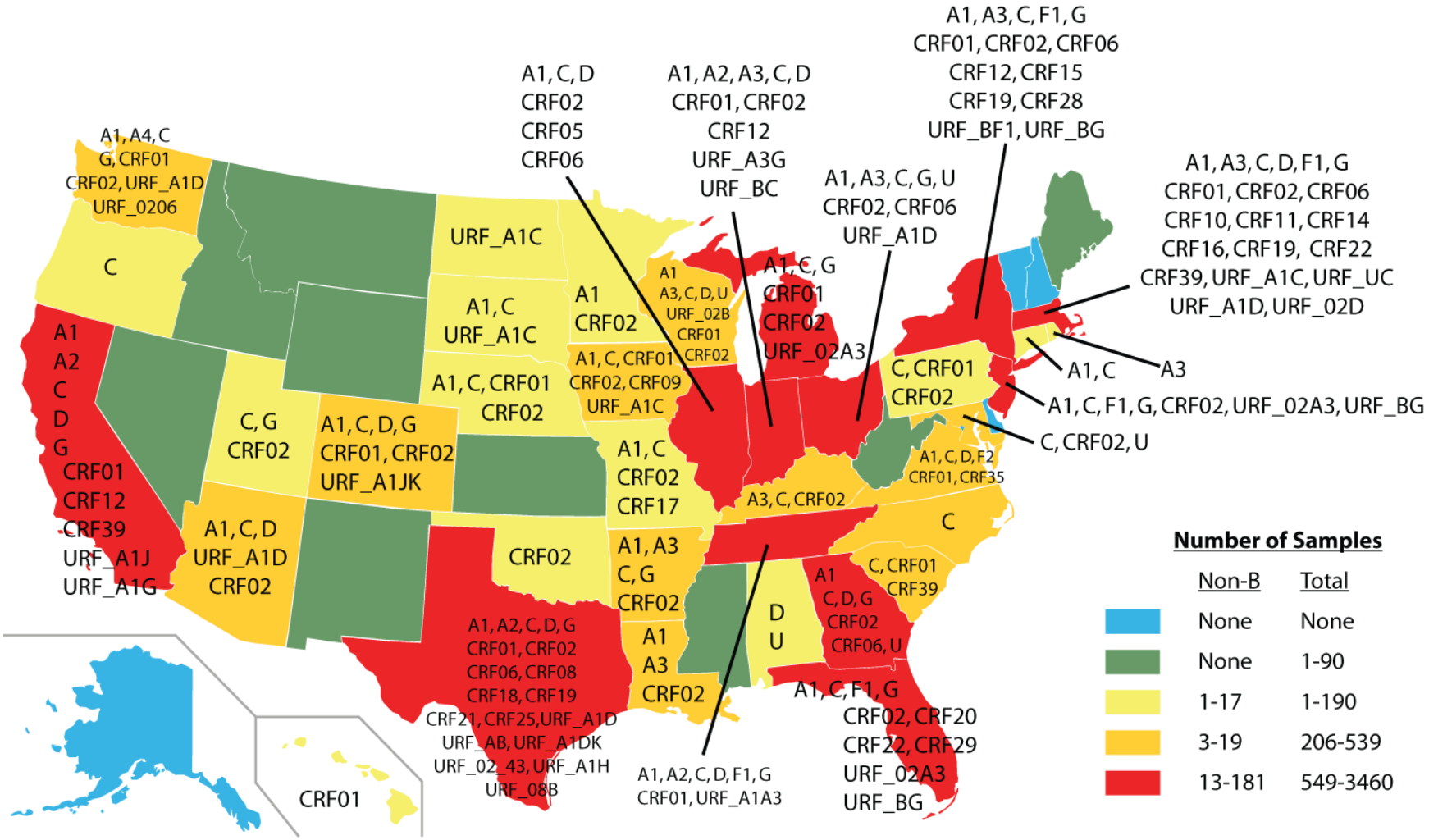
- Whole blood assay, detects RNA and DNA
- Uses
  - Infants born to HIV-1 infected mothers
  - 2 serially positive RNA or DNA tests necessary for diagnosis in infants < 2 years of age
  - Early diagnosis of infants on prophylactic therapy (RNA suppressed)
- Whole blood assay (Roche)
  - 1.0 chemistry
  - CAPTAQ robot

Expanding HIV Diversity in U.S.



Pyne et al 2012

Expanding HIV Diversity in U.S.



Pyne et al 2012

Molecular Take-Home Points

- Only APTIMA® is approved for HIV diagnosis
  - Automation may eventually occur on Panther platform
- Viral Load tests have equivalent "analytic performance" compared to APTIMA®
  - Guidelines stirred interest in claims for diagnosis
  - Process will be slow
- Precedent for off-label use molecular tests for confirmation/resolution (HCV)
- Proviral HIV-1 DNA testing available, not approved
- Very few LDT HIV-2 RNA assays available

Comparing Algorithms

Algorithm	Sensitivity		Specificity	
	%	95% CI	%	95% CI
Two-test current algorithm				
Architect/WB	99.76	98.65 - 99.06	100.00	99.08 - 100.00
GS+O /WB	100.00	99.09 – 100.00	100.00	99.08 – 100.00
Three-test proposed algorithm				
Architect or GS+O/Multispot/NAAT	99.76	98.65 – 99.96	100.00	99.08 – 100.00

Adapted from Masciotra et al. Journal of Clinical Virology 2011.

ARUP

Offers both third and fourth generation screening assays
Acute HIV Case
Validating Multispot

	Multispot negative	Multispot HIV-1 positive	Multispot HIV-2 positive	Total
WB negative	12			12
WB positive		9		9
WB indeterminate	2	5		7
HIV-2 confirmed positive			5	5

New Algorithm Benefits

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

New Algorithm Challenges

- Only two platforms currently available for Ag/Ab Combo assays
- Multispot is a rapid test, not approved for confirmation of HIV infection
- There is only one qualitative molecular assay approved for HIV diagnosis (Aptima) that is not automated and therefore not routinely available
- High-throughput quantitative or viral load HIV assays are widely utilized but none is approved for diagnosis

Clinical Considerations

- Ag/Ab combination or 4th assays are the most sensitive screening assays and should be used if acute HIV infection is suspected
- Multispot discriminates between HIV-1 and HIV-2 infection
- Both the Multispot and Western blot can detect established HIV infection, majority of HIV diagnoses
- If a result is positive by 4th gen screening assay but negative by either the Western blot or Multispot (Ab detection only), further testing by molecular assays (NAAT) is necessary
- NAAT testing cannot be used as the second step because it can be negative in:

    HIV-2 infection

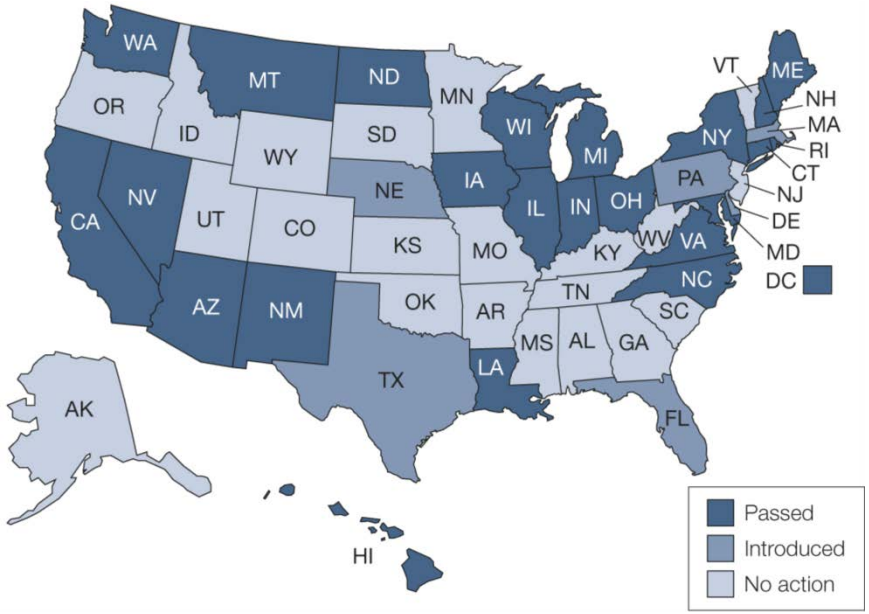
    HIV infected individuals that are elite suppressors/controllers - 0.5%

Thanks

- Orly Ardon
- Jennifer Blackley
- Scott Griffiths
- Michael Pyne
- Melanie Mallory
- Malissa Jones
- Jason Metz

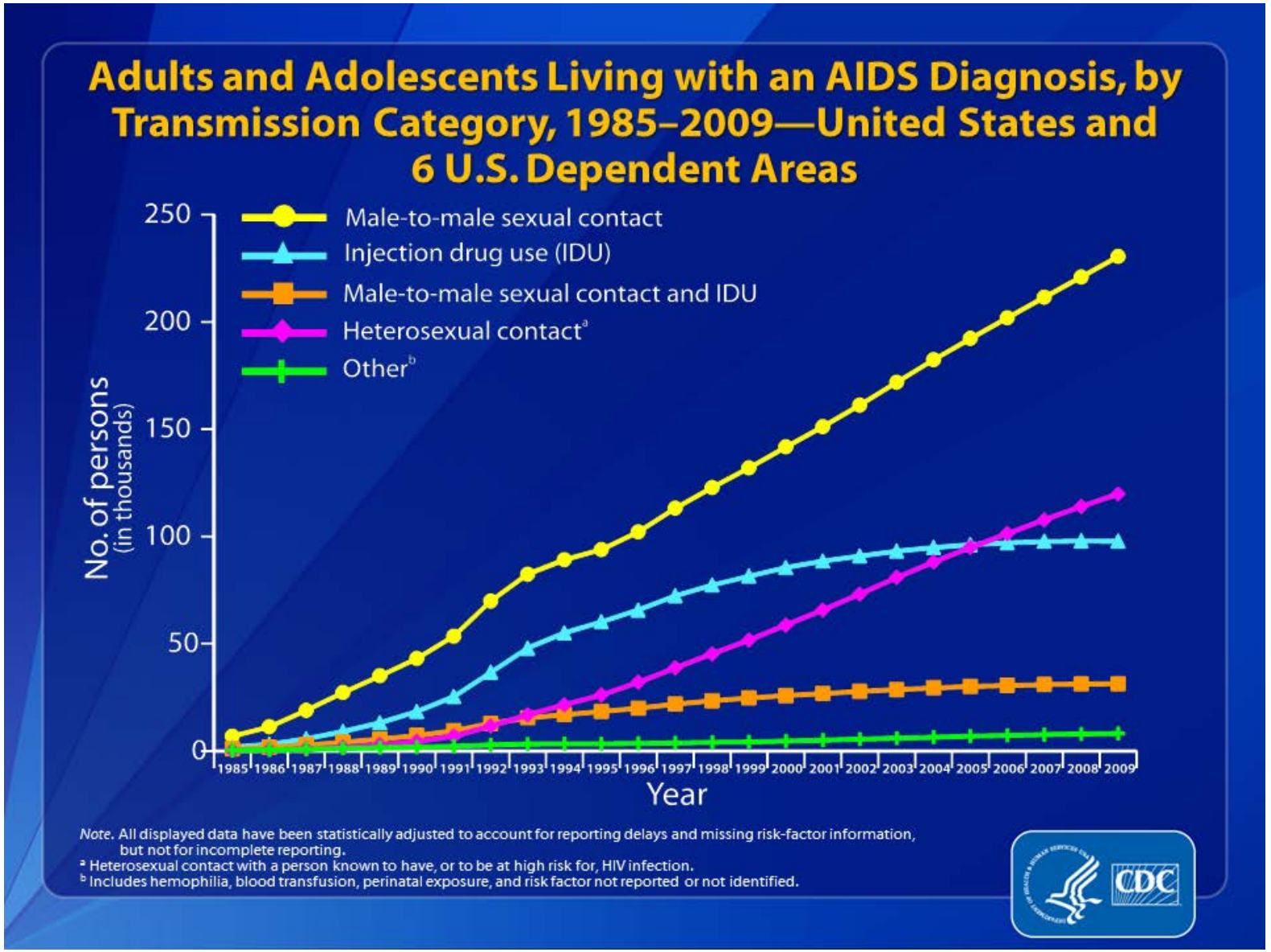
Universal Screening ?

- Laws
- Reimbursement

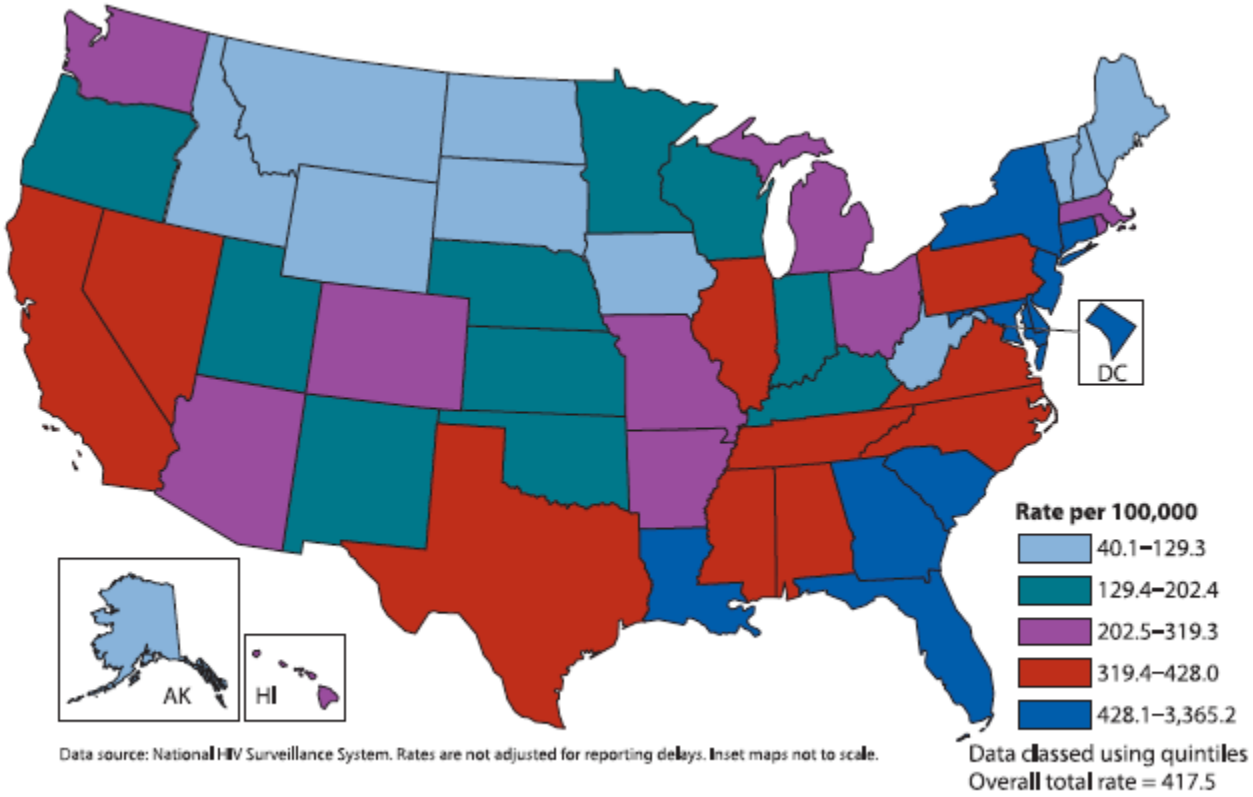


Adapted from JAMA 2011

Transmission in Adults

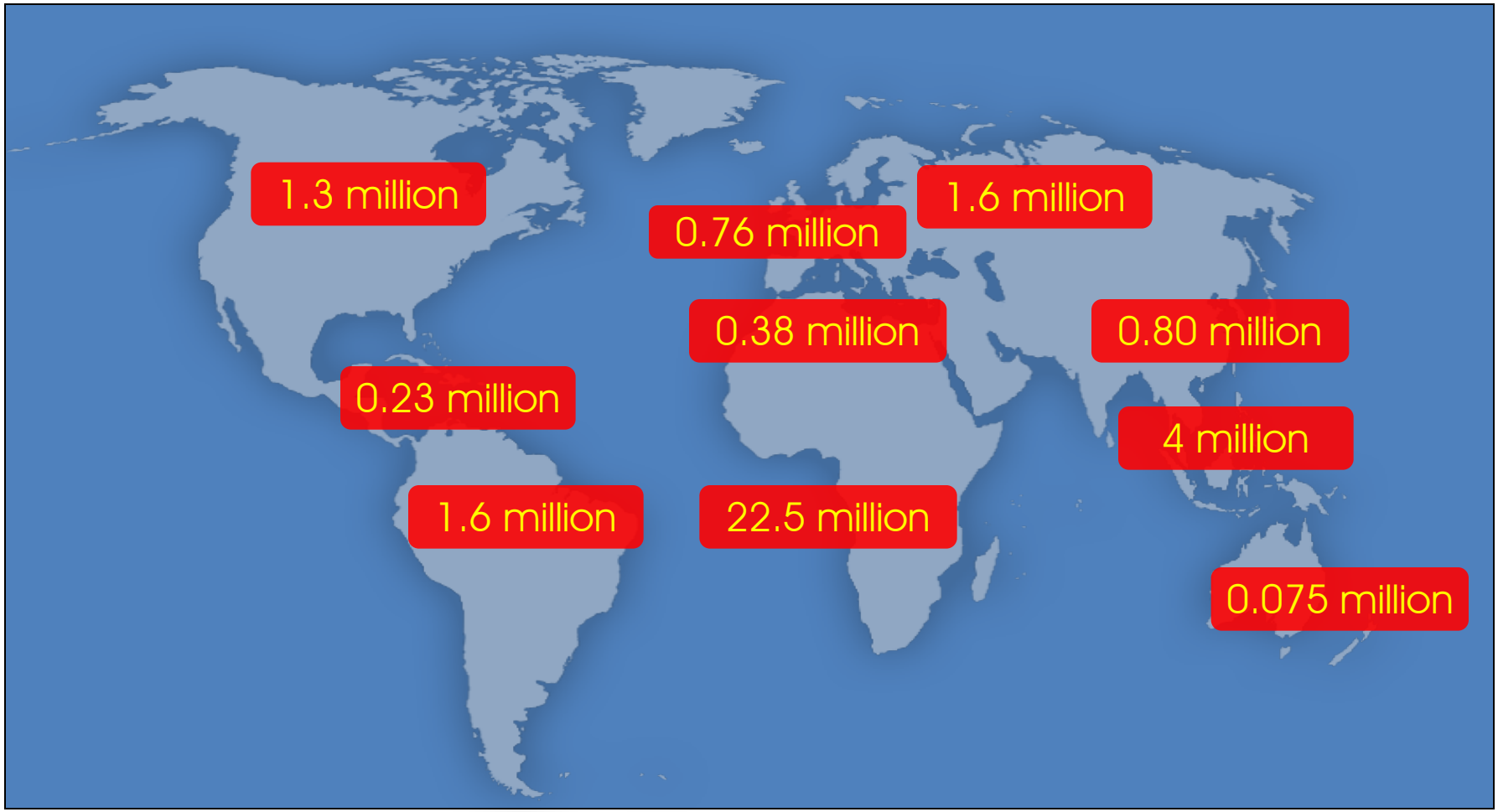


US HIV Prevalence



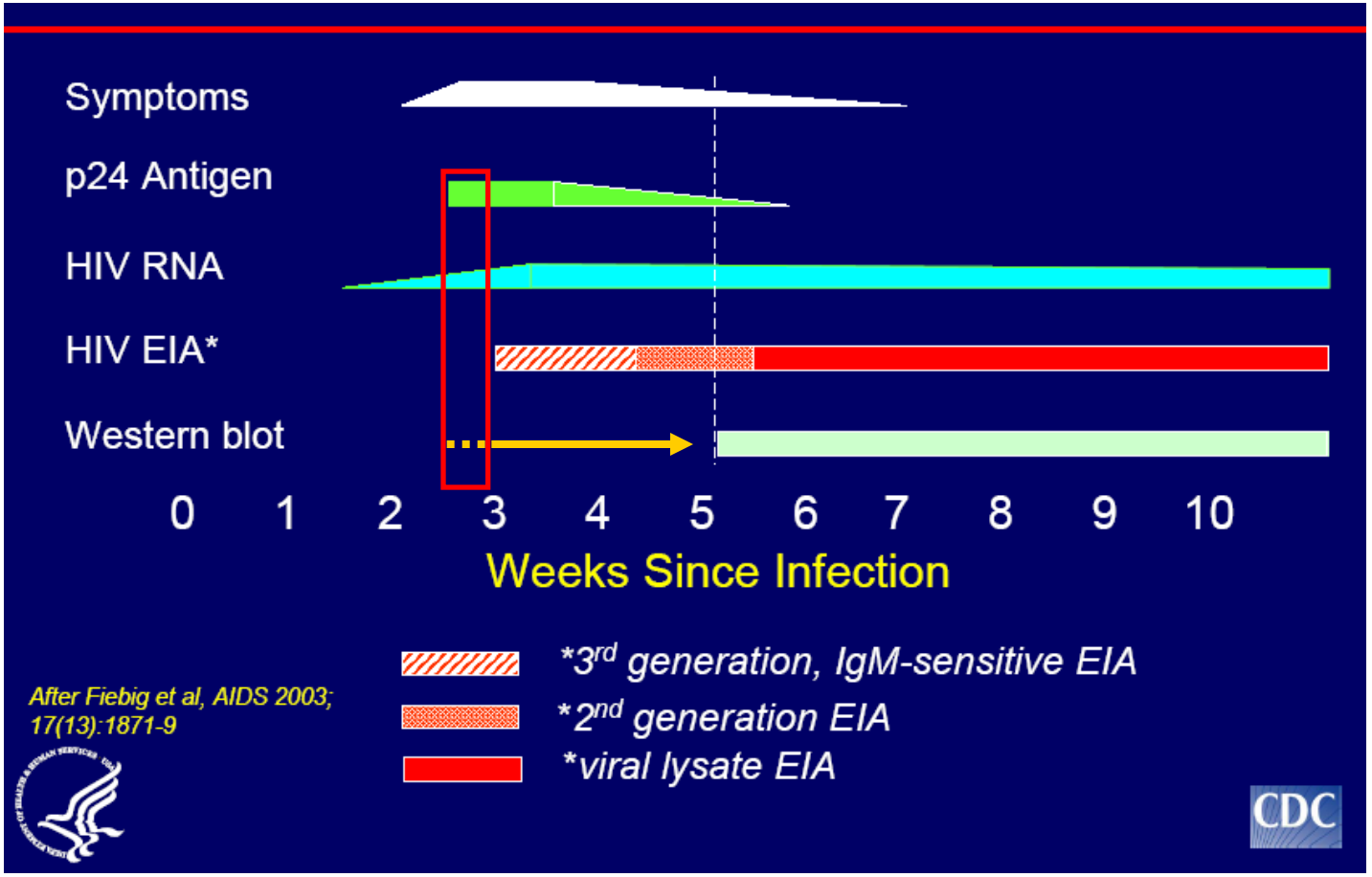
CDC Surveillance Data 2012

Global HIV Epidemiology



WHO, UNAIDS. 07 AIDS epidemic update.http://data.unaids.org/pub/EPISlides/2007/2007_epiupdate_en.pdf.

Detection of HIV by Diagnostic Tests



Branson et al. AIDS Therapy 2008.

Confirmation for HIV-1 Infection

- All repeatedly reactive EIA/CIA screening assay results must be confirmed

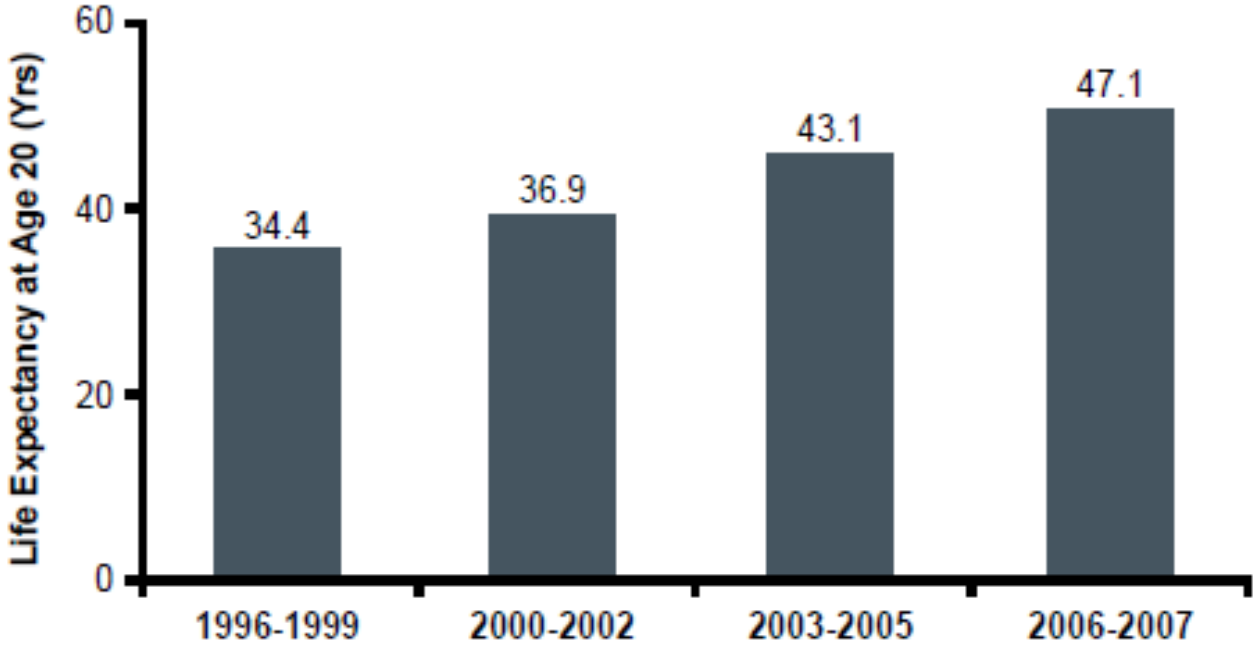
- POC results are considered “preliminary positive” results and must also be confirmed

- Confirmation for HIV-1 Infection

  Indirect Immunofluorescence (IFA)

  Western Blot

NA-ACCORD: Increasing Life Expectancy in HIV+ Adults Receiving ART



Hogg R, et al. CROI 2012. Abstract 137. Clinical Care Options 2012

GS Combo Ag/Ab & Acute HIV Infection (4th generation)

Acute HIV patient	Days from 1st bleed	HIV-1 RNA copies (mL)	GS HIV Combo Ag/Ab	Historical results		
				HIV-1/HIV-2 EIA	HIV-1 EIA	WB
1	0	>500,000	R	NR	NR	Neg
	56		R	R	R	Pos
2	0	183,850	R	NR	NR	Neg
	16	10,479	R	R	R	Pos
	42		R	R	R	Pos
3	0	>500,000	R	R		Neg
	141		R	R	R	Pos
4	0	>500,000	R	NR	NR	Neg
	19		R	R	R	Pos
5	0	>500,000	R	R	R	Neg
	21		R	R	R	Ind
	64		RR	R	R	Pos

Adapted from Bentsen et al. Journal of Clinical Virology 2011.

Detection of Rare HIV Genotypes

HIV Non-B Infections			
Assay Type	HIV-1 group M, non-B	HIV-1 group O	HIV-2
HIV-1/HIV-2/O Ab (3rd gen)	detected	detected	detected
HIV-1/HIV-2/O Ag/Ab Combination (4th gen)	Ab detected Ag sensitivity is assay dependent	Ab detected Ag sensitivity is assay dependent	Ab detected Ag sensitivity is assay dependent and HIV-2 detection is dependent on cross-reactivity
HIV-1 WB	detected	negative indeterminate	negative indeterminate
HIV-1 RNA NAAT (qualitative)	detected	detected	not detected
HIV-1 RNA NAAT (quantitative)	detected but quantification is assay dependent	detection and quantification assay dependent	not detected
HIV-1 RNA NAAT (genotyping)	detected	not detected	not detected

Acute HIV Infection
(3rd gen, 4th gen, Western blot and NAAT)

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 (*n*)	56	56	56	56
WB indeterminate (*n*)	38	39	39	43
+NAAT positive (n)	*36*	*37*	*37*	*41*
+NAAT negative (n)	*1*	*1*	*1*	*1*
+NAAT not available (n)	*1*	*1*	*1*	*1*

Adapted from Masciotra et al. Journal of Clinical Virology 2011.