HIV Update in Laboratory Testing

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Objectives

- Explain the advances in HIV diagnostics, including fourth generation Ag/Ab combination HIV screening assays
- Describe the new CDC HIV diagnostic algorithm
- Explain appropriate testing algorithm and understand interpretation of laboratory results for HIV

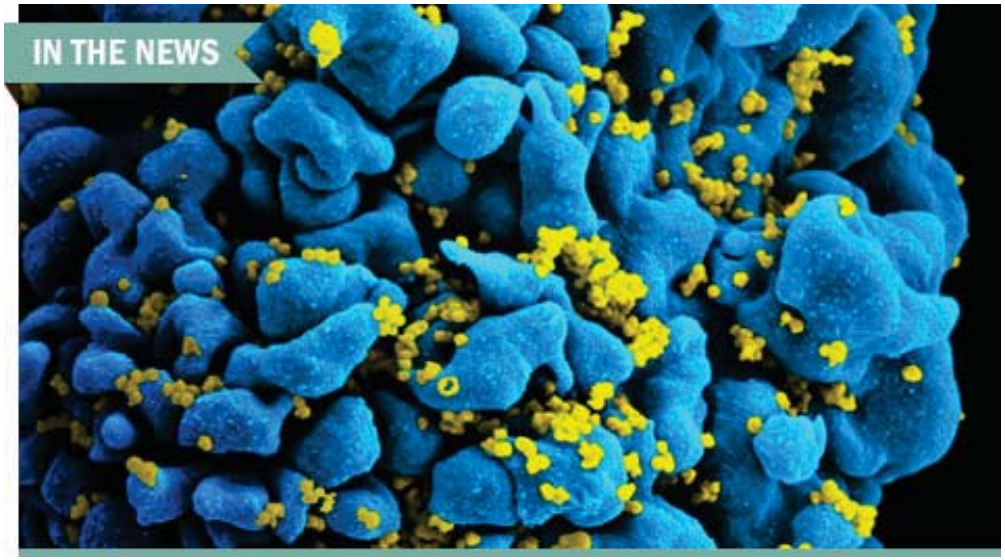
Questions

- What is a fourth generation HIV screening assay (describe)
- Is there a rapid test that detects both HIV Ag& Ab (true or false)
- Preliminary results from a rapid test must proceed to confirmation with the Western blot (true or false)

August 7, 2014

CLN Stat

NEWS AND INFORMATION FROM AACC



Updated HIV Testing Guidelines

CDC, APHL together offer recommendations for HIV testing, based on the best available scientific evidence. READ MORE

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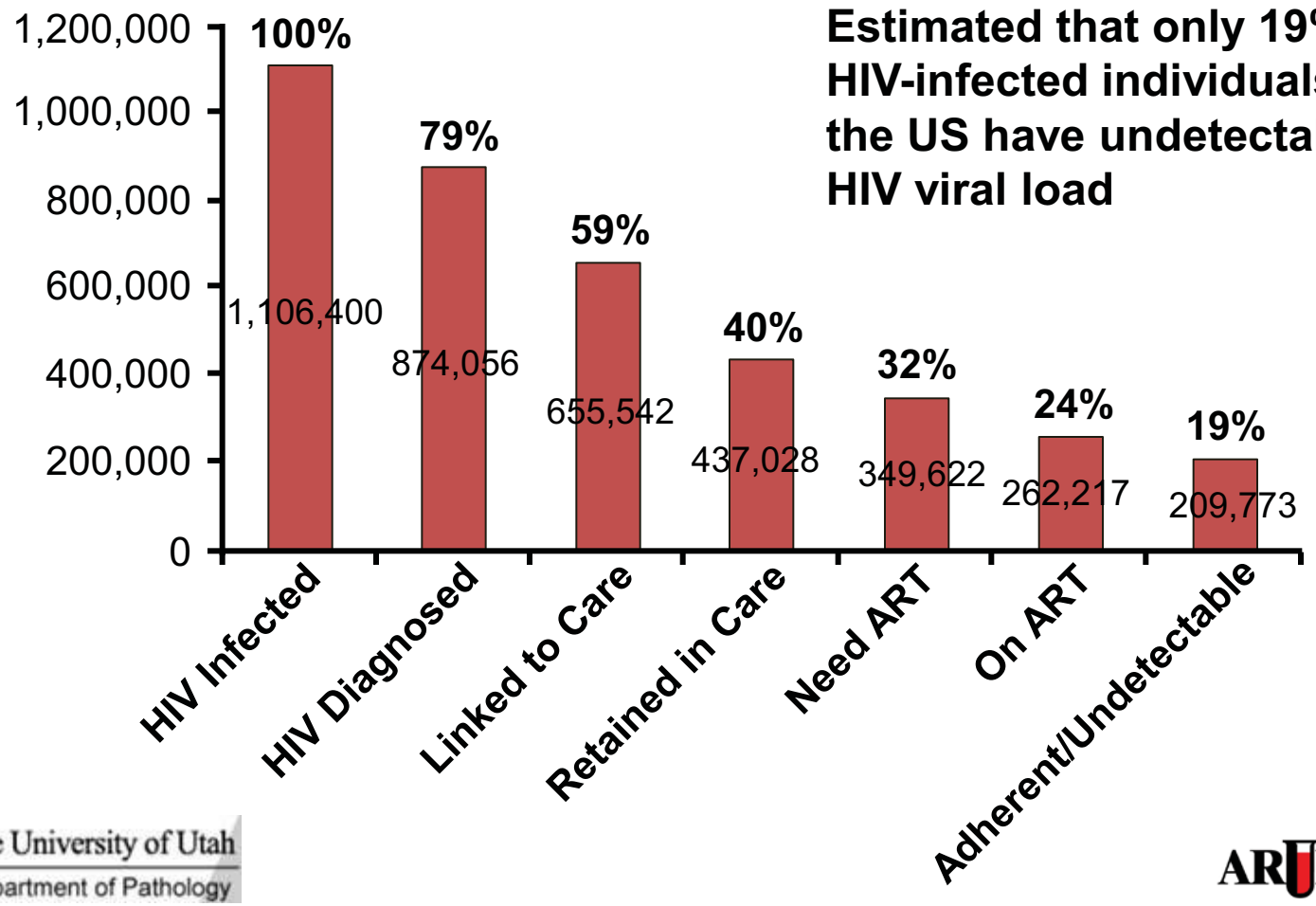
HIV in the US



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

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

USPSTF – "Universal Screening" (2013)

Grade A Recommendation for Routine HIV Testing in individuals 15-65 yrs of age

Impact - Reimbursement

Human Immunodeficiency Virus

chimpanzee

sooty mangabey

Types

HIV-1

HIV-2

Groups

Major

Non M/Non O

Outlier

Subtypes/Clades

A B C D E F G H I J K

Circulating Recombinant Forms (CRF)

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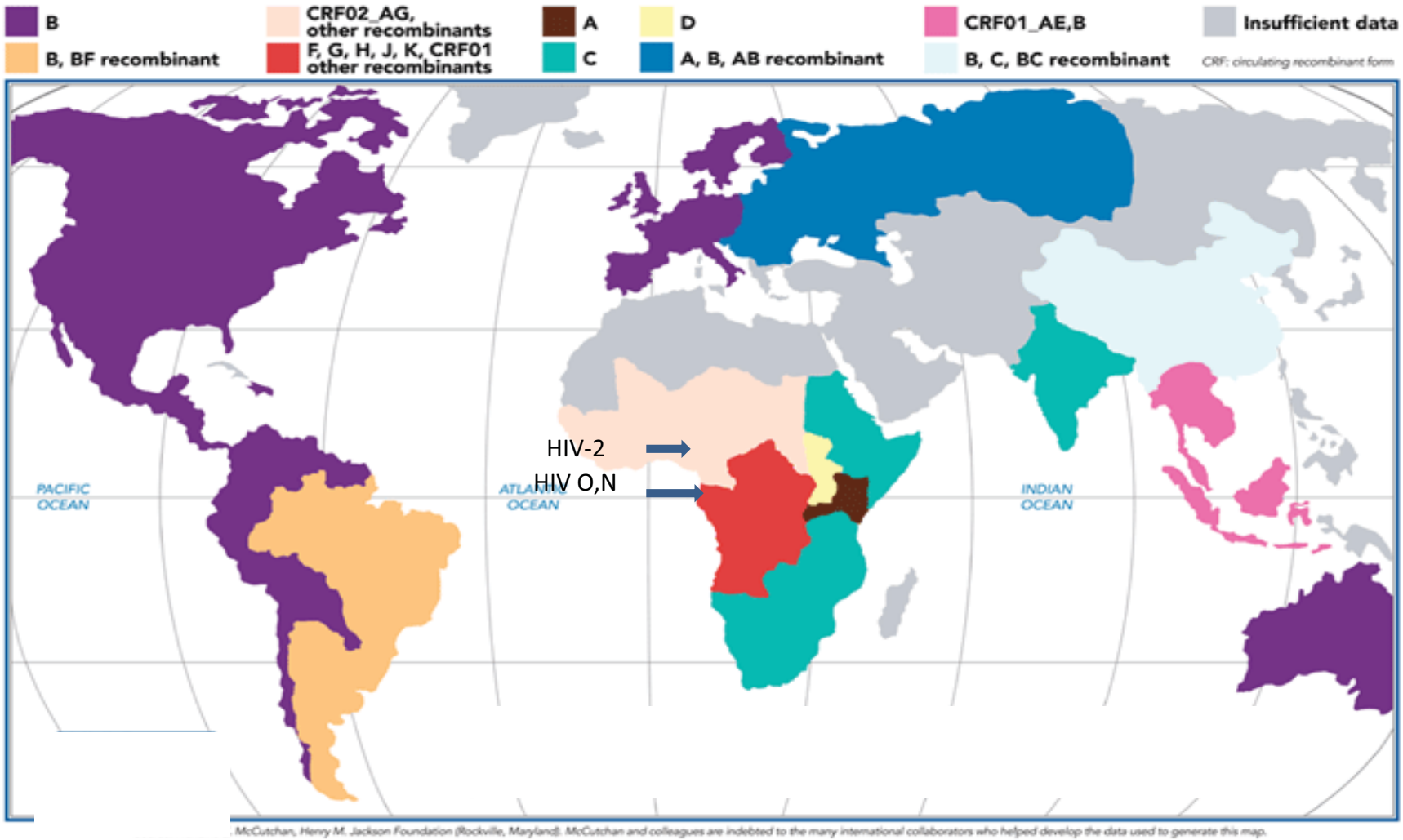
HIV Distribution



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.

HIV-2
(prior recommendations)

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

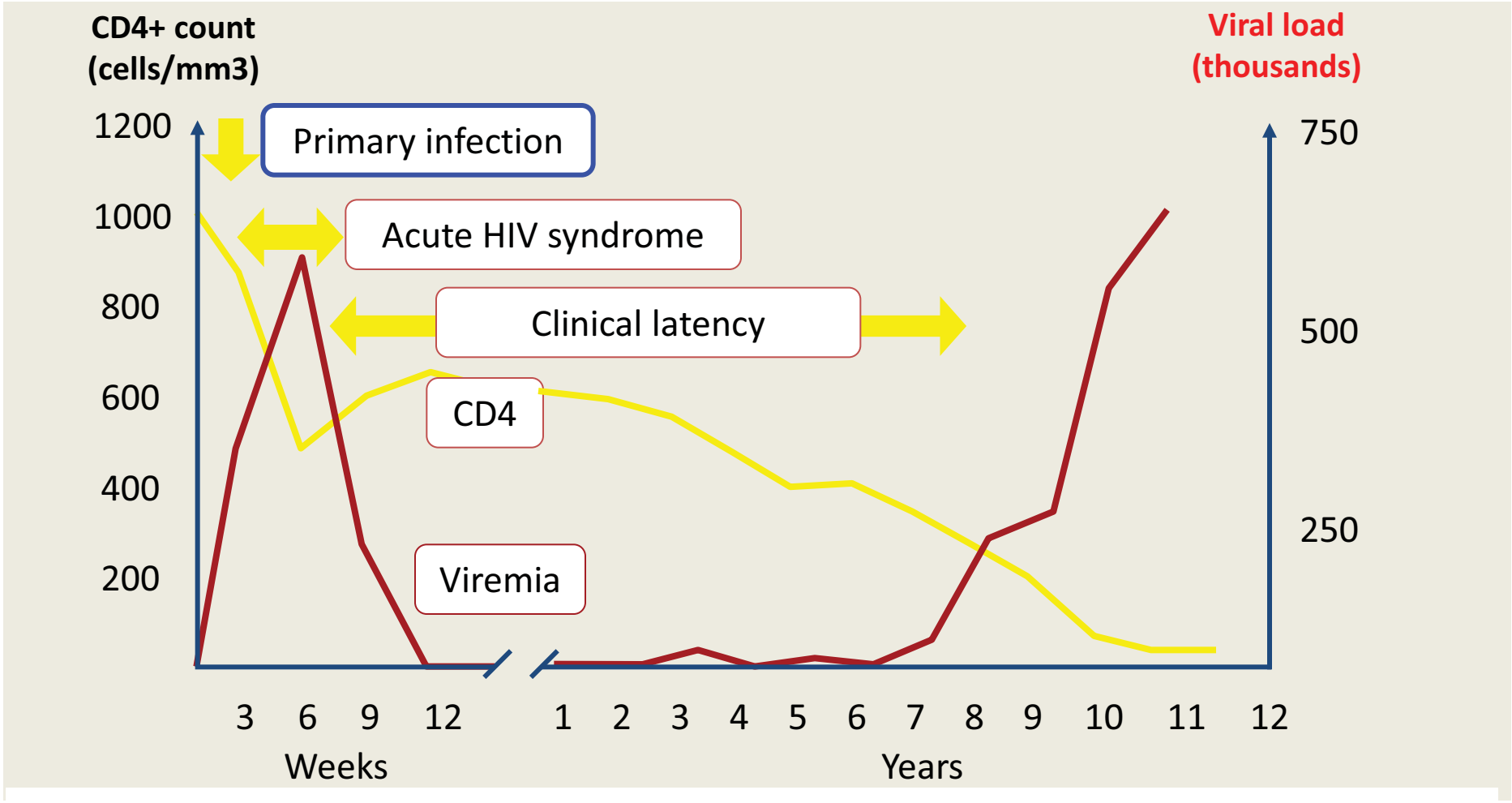
HIV-2 testing is also indicated for

- People with an illness that suggests HIV infection (such as an HIV-associated opportunistic infection) but are not HIV-1 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 Cases ?***

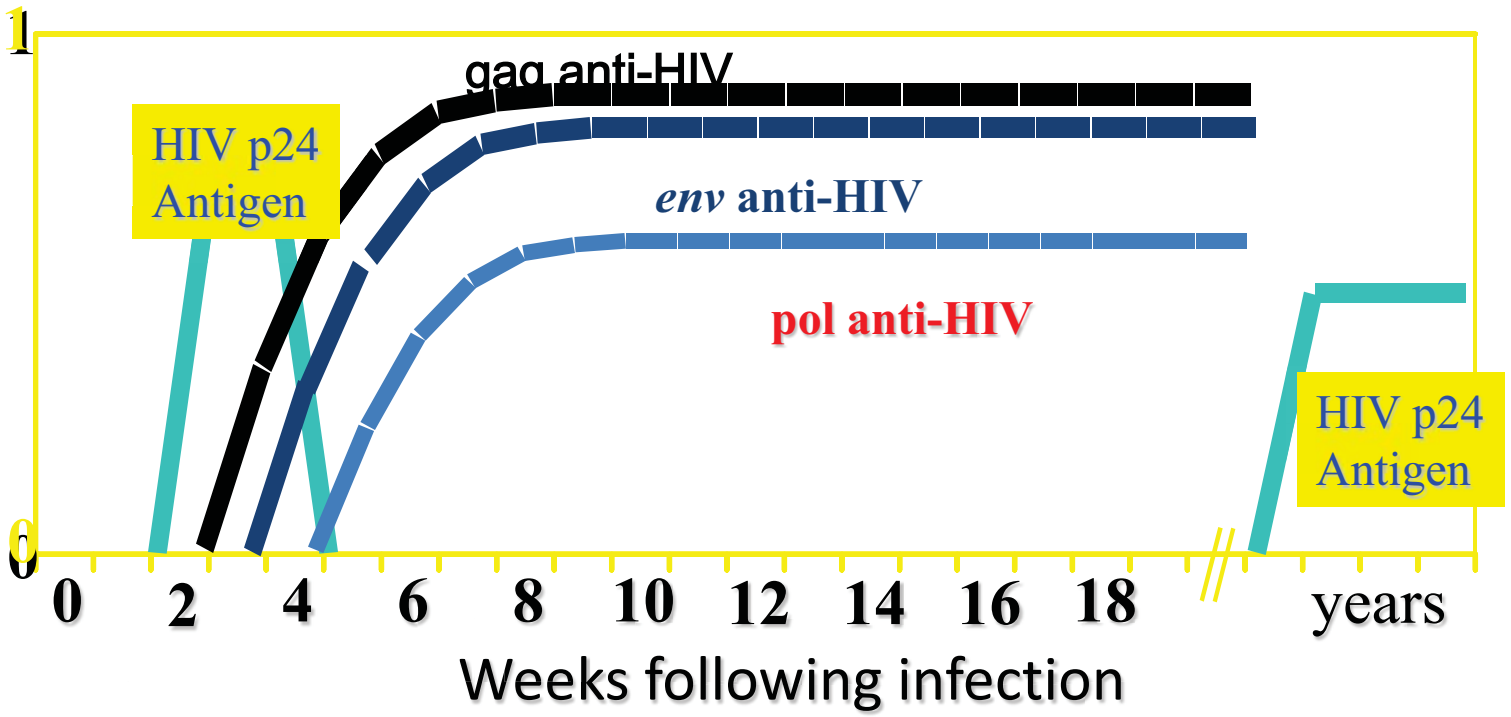
  166 confirmed cases between 1988-2010; 0.01% of all HIV cases in the US
  81% people born in West Africa; most positive on HIV-1 Western blot

HIV Infection Course



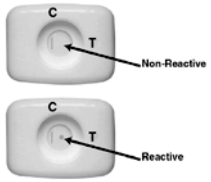
HIV Serological Response

Typical response following infection

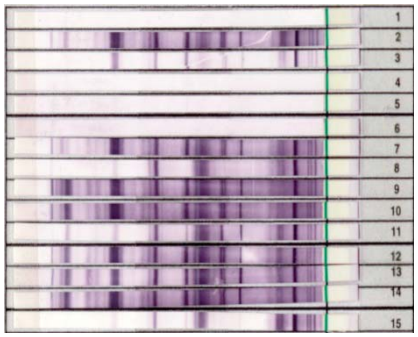


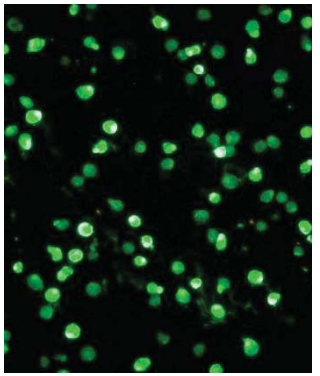
“Traditional” HIV Diagnostic Algorithm

1 Screen
- immunoassay (EIA/CIA)
- rapid tests



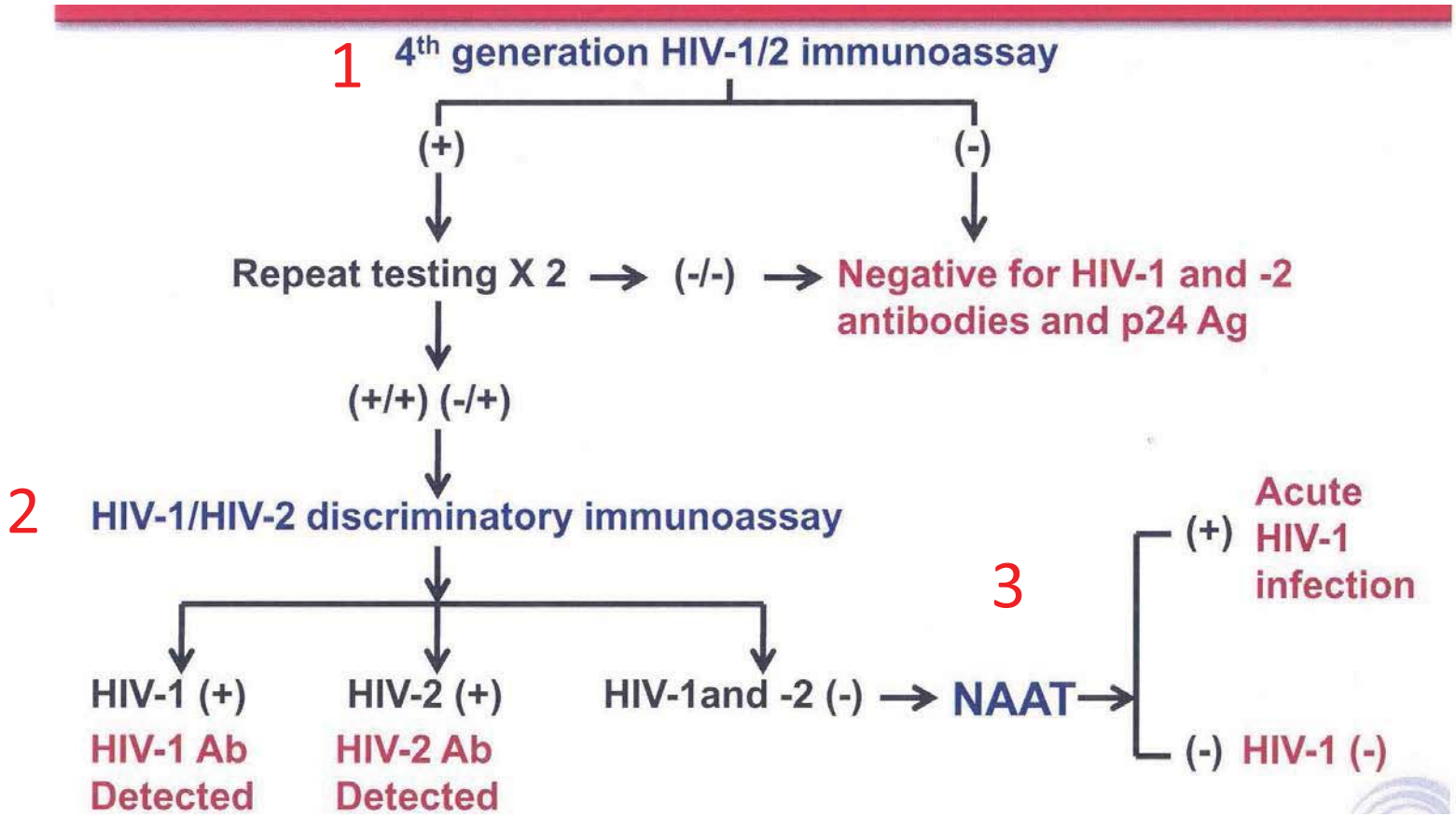
2 Confirmation for HIV-1
- Western blot (98%)
- IFA
- Nucleic Acid Amplification Test *





*Note: TMA format, qualitative assay only FDA approved nucleic acid amplification test (NAAT) for diagnosis and confirmation. There are no viral load tests approved for diagnosis

CDC Diagnostic Algorithm (2014)



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

AACC. Clinical Laboratory News. 2010

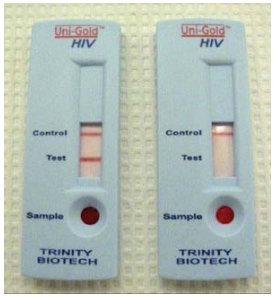
Rapid Test – Point of Care

- Most are equivalent to 2nd gen assays
- One kit Ag/Ab combo (not incorporated in the algorithm)
- One kit approved for in-home testing

- Sample types

  plasma, serum, whole blood, oral fluid

  unprocessed sample types (oral fluid & whole blood) are CLIA waived, all others are moderately complex



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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

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HIV-1/HIV-2 Differentiation Assay

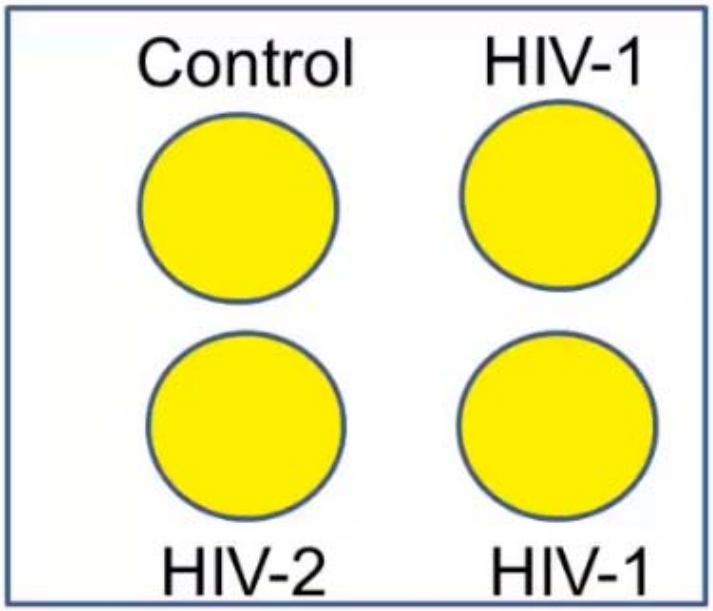
- Rapid test

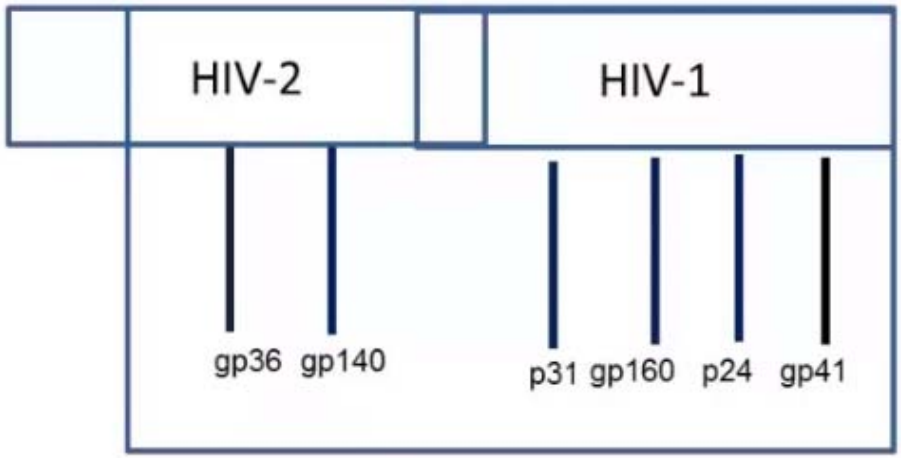
  2 Ags for HIV-1 (gp41); 1 Ag for HIV-2(gp36)

- Geenius

  2 Ags for HIV-2 (gp36, gp140)

  4 Ags for (p24, gp41-O&M, gp160, p31)





HIV-1/HIV-2 Differentiation Assays

Multispot

- No bar code
- Interpretation- visual
- 30 minutes
- No result storage

Geenius

- Bar code
- Interpretation – automated
- 30 minutes
- Result is stored

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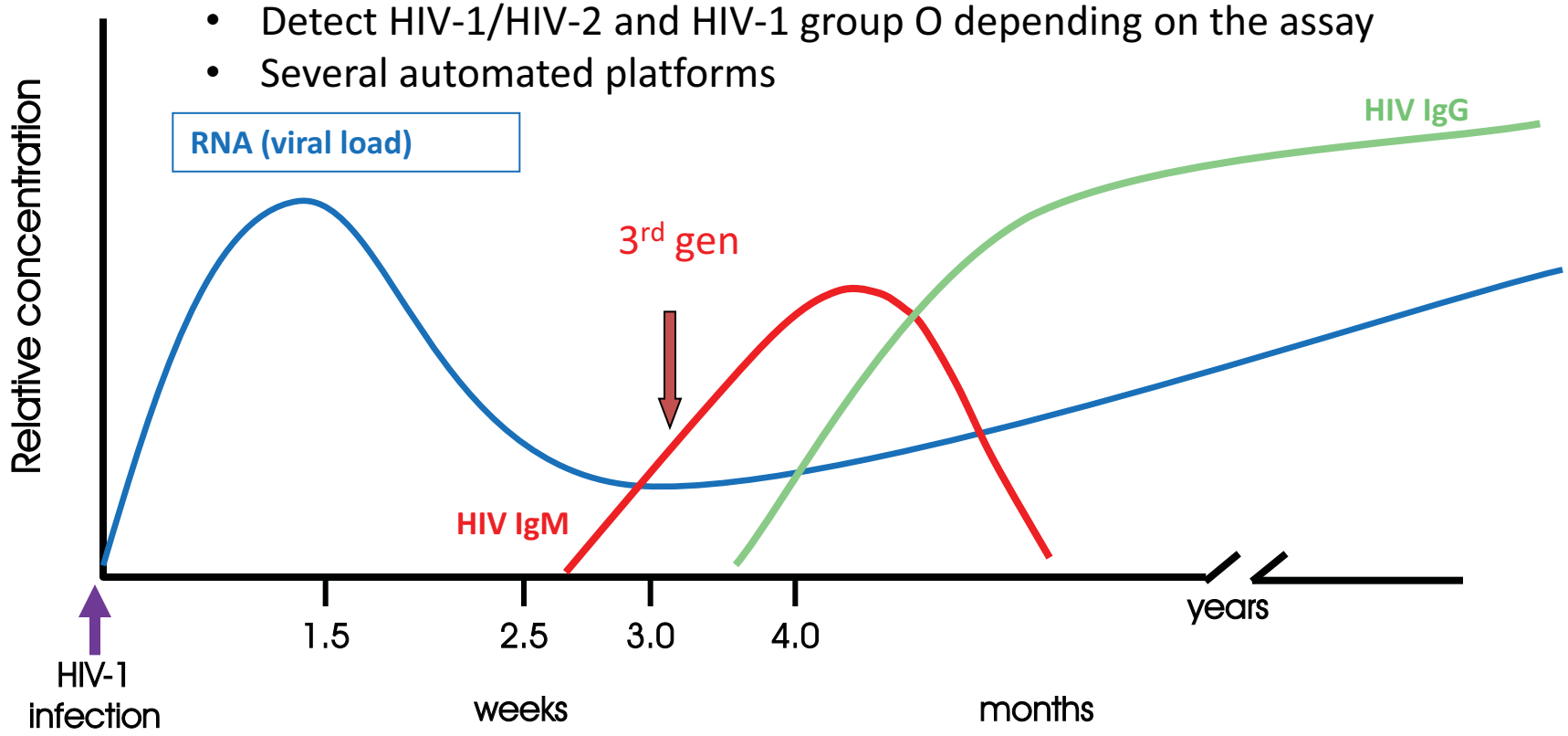
HIV Ab Screening Assays
(3rd gen – IgM and IgG)

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



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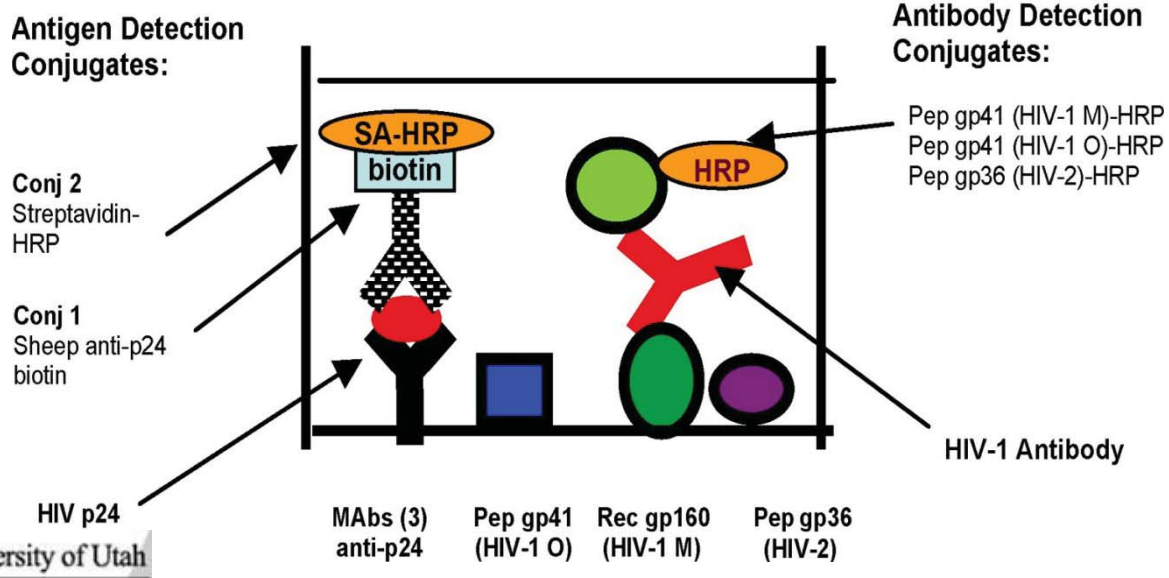
HIV Antigen/Antibody Combination Assays (4th gen – p24 Ag/IgM/IgG)

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



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Bentsen et al. Journal of Clinical Virology. 2011

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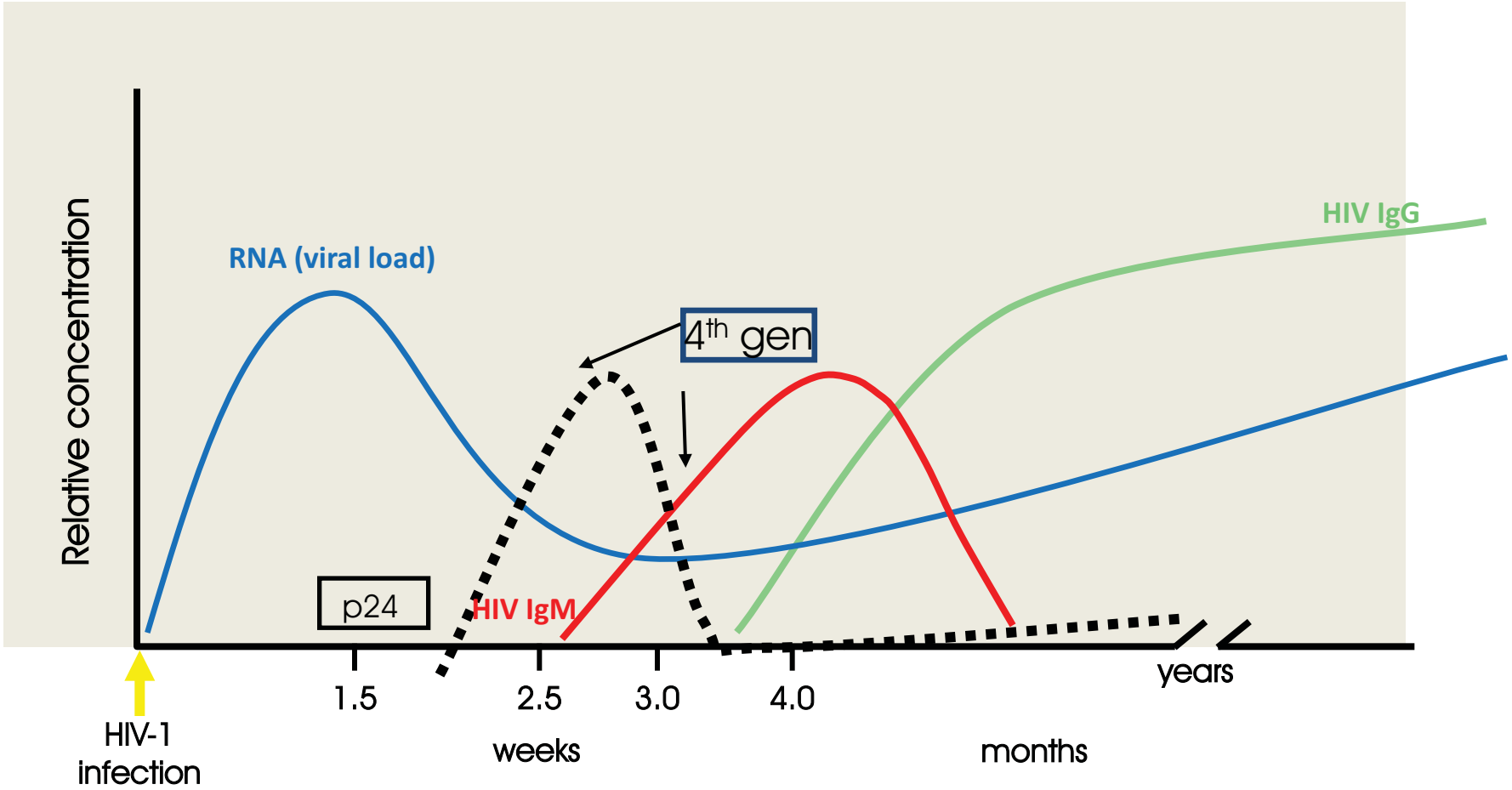
Earlier Detection of HIV Infection: (4th generation)



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

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.

HIV Combo Ag/Ab Specificity (4th generation)

Low Risk Population	Number tested	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.

False Positive Immunoassay Results

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

Screening Test

- Sensitivity = 96% and Specificity = 99.8%

$$Sensitivity = \frac{TP}{TP + FN}$$

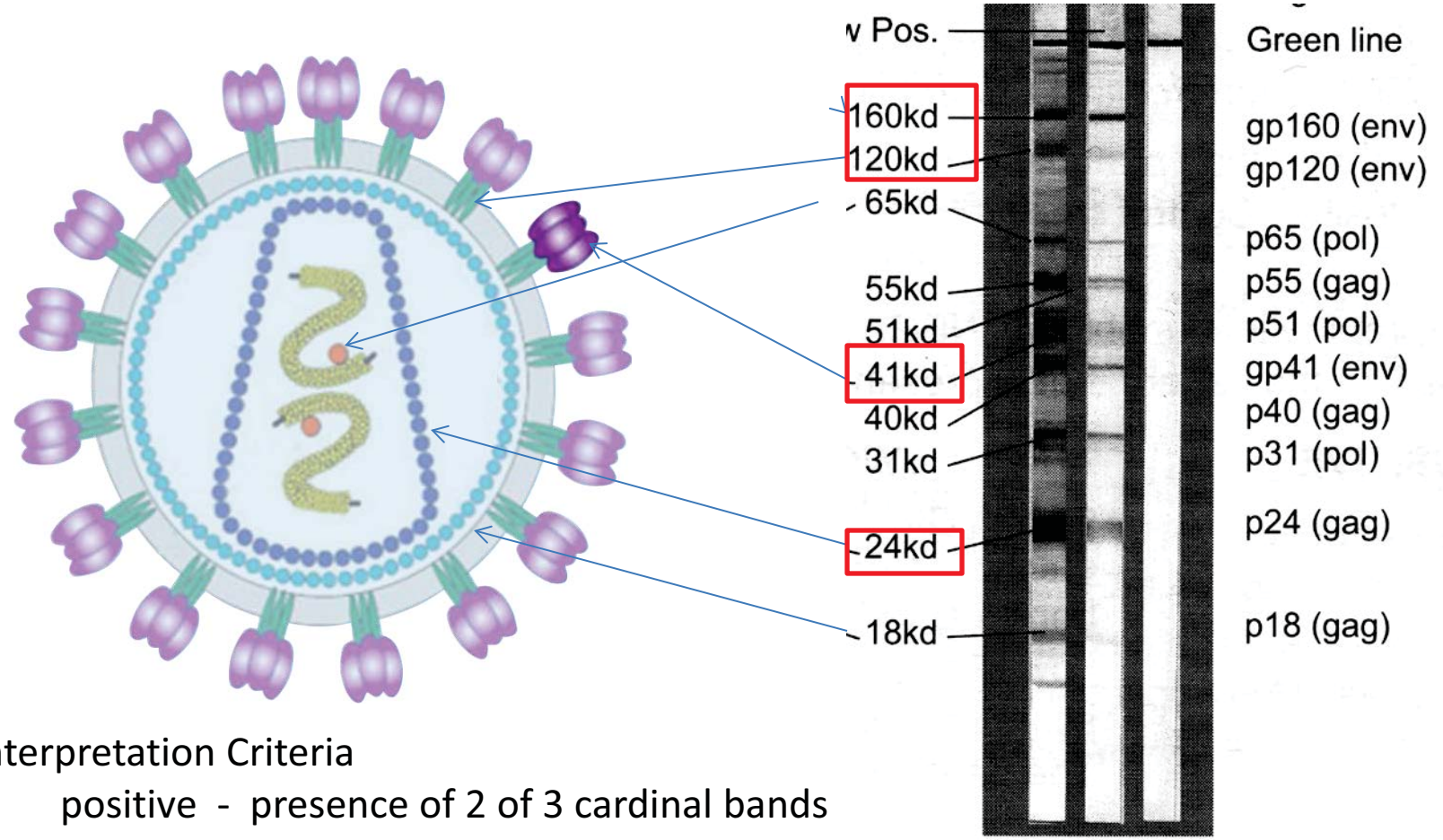
$$Specificity = \frac{TN}{TN + FP}$$

- But we need to know the predictive value

Supplemental/Confirmatory Testing

- Assume the infection rate is 1 per 500
- Testing 10,000 random subjects will yield
  - 20 false repeatedly reactive
  - 19 true repeatedly reactive
  - 9,960 true nonreactives
  - 1 false nonreactives
- Therefore, PV^{+} = 49%, PV^{-} = 99.99%
- Testing needed to separate repeat reactives

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

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Why Not the Western Blot ?

- Diagnostic Limitations
  - indeterminate/inconclusive results, require follow-up
  - insensitive compared to current screening assays
  - HIV-2 misclassification

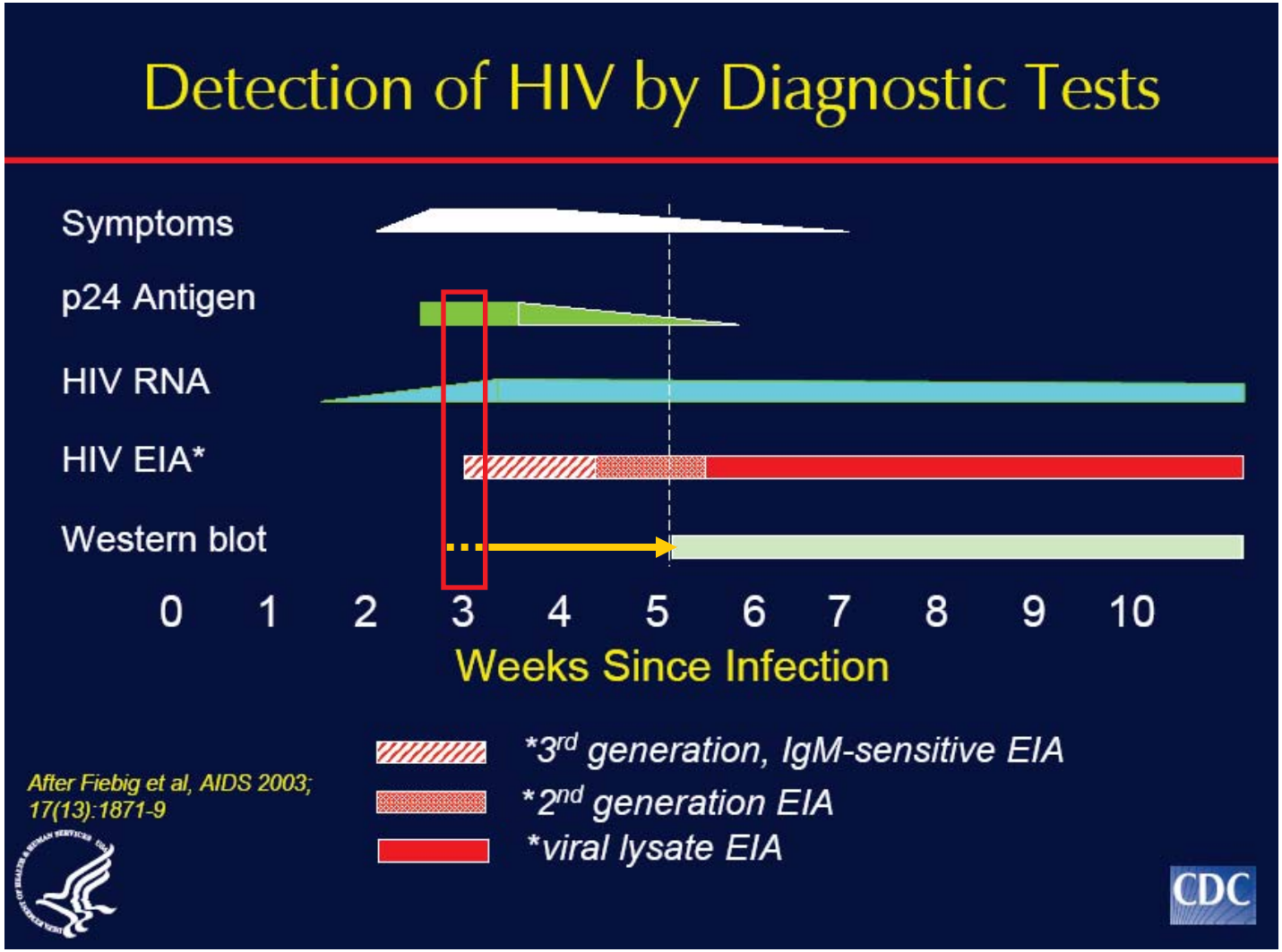
- Practical Limitations
  - access
  - expense
  - turn around time

- High Specificity for HIV Infection

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 – 3 indeterminate results spanning 6 months = negative
  - nucleic acid amplification test (NAAT)

Sensitivity of HIV Assays



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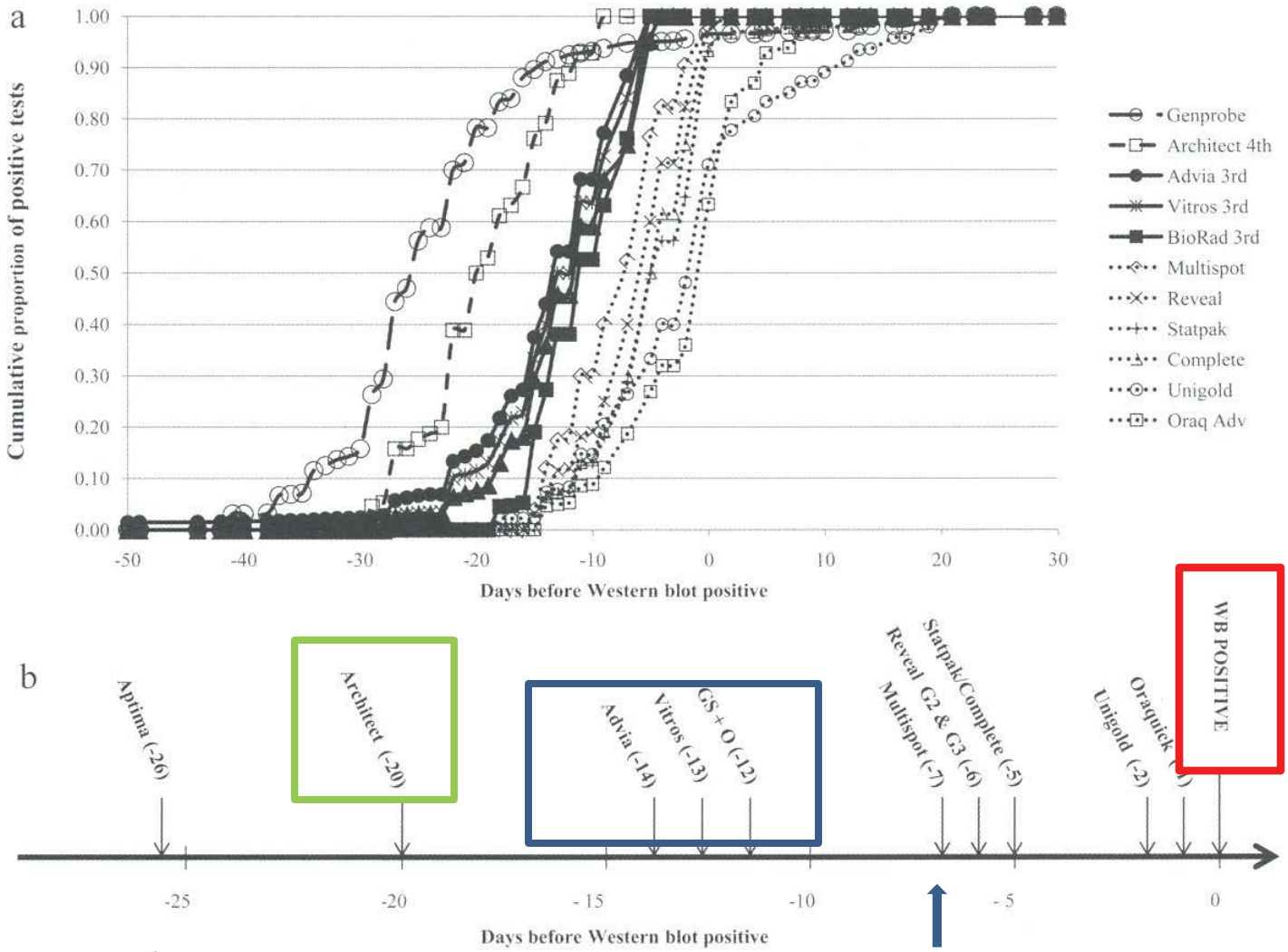
Detecting HIV Infection and Current Assays



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Masciotra et al. Journal of Clinical Virology 2011.

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.

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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 + , η (%)			
	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 .

HIV-1 /HIV-2 Differentiation Assay

vs

Western Blot

	HIV 1/2 Diff Assay Positive		HIV1/2 Diff Assay 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.

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NAAT for HIV Diagnosis

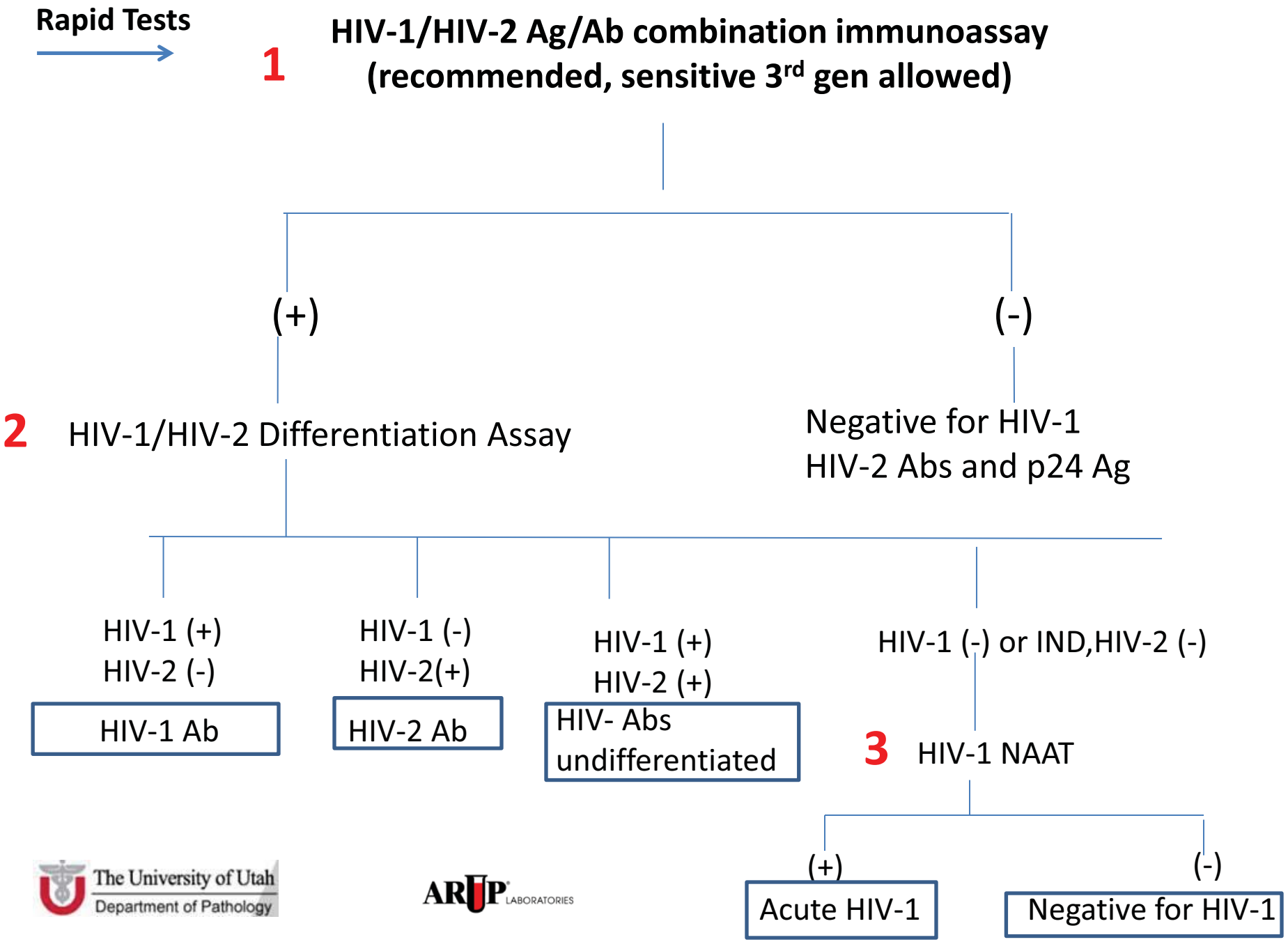
- Transcription Mediated Amplification (TMA)
- Screening of high-risk populations
- Known exposure such as needle-stick
- Testing patients with acute HIV-1 symptoms and known exposure
- Screening of newborn babies born to infected mothers
- HIV vaccine studies
- **Resolution arm for new screening algorithms**

TMA vs Real-time PCR Tests

	TMA	Real Time (1)	Real Time (2)
Sensitivity	30 copies/ml	40 copies/ml	20 copies/ml
Genotypes	A-O	A-O	A-G
Amplicon control	Strand Capture	Closed	UTP/UNG, closed
Automation	No (U.S.)	Yes	Yes
FDA approval	Diagnosis	Monitor	Monitor

Molecular Take-Home Points

- Only TMA format is approved for HIV diagnosis
  - Automation may eventually occur
- Viral Load tests may have equivalent "analytic performance" compared to TMA
  - Guidelines stirred interest in claims for diagnosis
  - Process will be slow
- Very few LDT HIV-2 RNA assays available


Rapid Tests
1
HIV-1/HIV-2 Ag/Ab combination immunoassay
(recommended, sensitive 3rd gen allowed)
(+)
(-)
2
HIV-1/HIV-2 Differentiation Assay
Negative for HIV-1
HIV-2 Abs and p24 Ag
HIV-1 (+)
HIV-2 (-)
HIV-1 Ab
HIV-1 (-)
HIV-2(+)
HIV-2 Ab
HIV-1 (+)
HIV-2 (+)
HIV- Abs
undifferentiated
HIV-1 (-) or IND,HIV-2 (-)
3
HIV-1 NAAT
(+)
Acute HIV-1
(-)
Negative for HIV-1
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HIV Summary

- New algorithm encourages use of HIV Ag/Ab combo assay to improve detection of acute HIV infection
  - Only two lab platforms currently available for Ag/Ab Combo assays
  - Sensitive 3rd gen allowed
- New algorithm replaces the Western blot supplemental testing with HIV-1/HIV-2 discriminatory assay to improve detection of HIV-2 infection
  - Only one rapid test platform can discriminate between HIV-1 and HIV-2 infection
  - Interpretation for the differentiation assay depends on use (screen vs supplemental)
  - Indeterminate results are possible

HIV Summary

- NAAT is formally incorporated into the algorithm
  - There is only one qualitative molecular assay approved for HIV diagnosis, TMA format, that is not automated and therefore not readily available
  - NAAT are designed to detect HIV-1
  - NAAT for HIV-2 are not FDA –cleared
- Rapid tests must proceed to 4th gen lab test, the starting point in the algorithm
  - **including preliminary positive samples with Ag/Ab 4th gen rapid test**
  - rapid tests are no longer confirmed with Western blot

Thank you!