DISCOVERY OF “FALSE HIV ELITE CONTROLLERS” AMONG SOUTH AFRICAN BLOOD DONORS

ISBT TTID WP

17 June 2017

Marion Vermeulen, Karin van den Berg, Genevieve Jacobs, Brian Custer, Ronel Swanevelder, Ute Jentsch, Ravi Reddy, Lubbe Wiesner, Gary Maartens and Edward Murphy for the NHLBI Recipient Epidemiology and Donor Evaluation Study-III (REDS-III)

Background

- SANBS screens all donations for HIV, HBV & HCV using ID-NAT in parallel with serology testing
- Although variable, eclipse period from infection acquisition to disseminated viraemia is estimated ~10 days using ID-NAT[1]
- Very early initiation of cART is likely to have a beneficial effect on the course of HIV disease and may facilitate HIV Cure Interventions[2]
- Use of ID HIV antibody and RNA testing of blood donors and the high incidence of HIV infection in SA enables identification of:
  - Donors with very early (Fiebig stage I & II) incident infection (HIV RNA+, Antibody-)
  - Potential Elite Controllers (EC), a group who are able to control virus replications without treatment (HIV RNA-, Antibody+)
    - Confirmed using western blot
    - Definition of an EC is a viral load <50 copies/mL
  - Prevalent HIV infections (HIV RNA+, Antibody+)

[1] Lee *et al* **Journal of theoretical Biology** 2009
[2] Barouch & Deeks **Science** 2014; Okulicz **JAMA Intern Med** 2014

Monitoring and Acute treatment of HIV study (MATHS) Objectives:

1. Determine Fiebig stage at time of blood donation. For donors identified as being Fiebig stage I or II initiate cART, and ascertain Fiebig stage at the time of therapy initiation

2. Establish the size of the peripheral blood viral reservoir at the initiation of cART and at defined time points post cART initiation for donors enrolled in the treatment study

3. Conduct a "proof of concept study" to show how blood donors identified as having "hyper-acute" HIV infection by blood testing can be successfully linked to care with the initiation of early HIV treatment

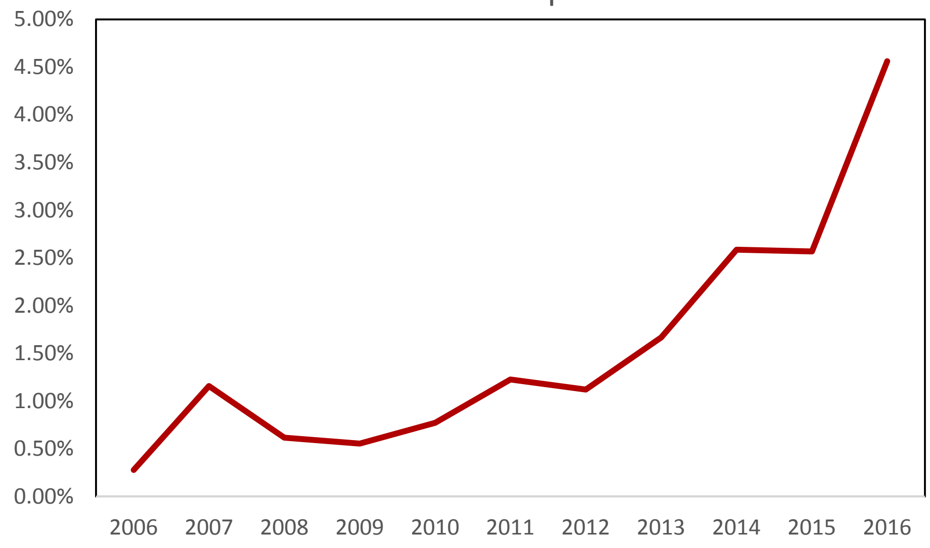
MATHS Study Design

- Enrolment commenced end-October 2015
- Acute (50 RNA+/Ab-) & Recent (25 RNA+/Ab+/Lag recent) HIV infections:
  - Open label, non-randomized treatment study
  - Rapid (<4 weeks post index donation) initiation of approved 3-drug antiretroviral therapy (cART)
- Elite Controllers (N=20)
  - Parallel prospective observational cohort of 20 elite controllers
- 2-year clinical and research follow-up
- Frequent research blood samples for HIV virology and immunology
- Less frequent, large volume leuka- and plasmapheresis for measurement of HIV reservoir

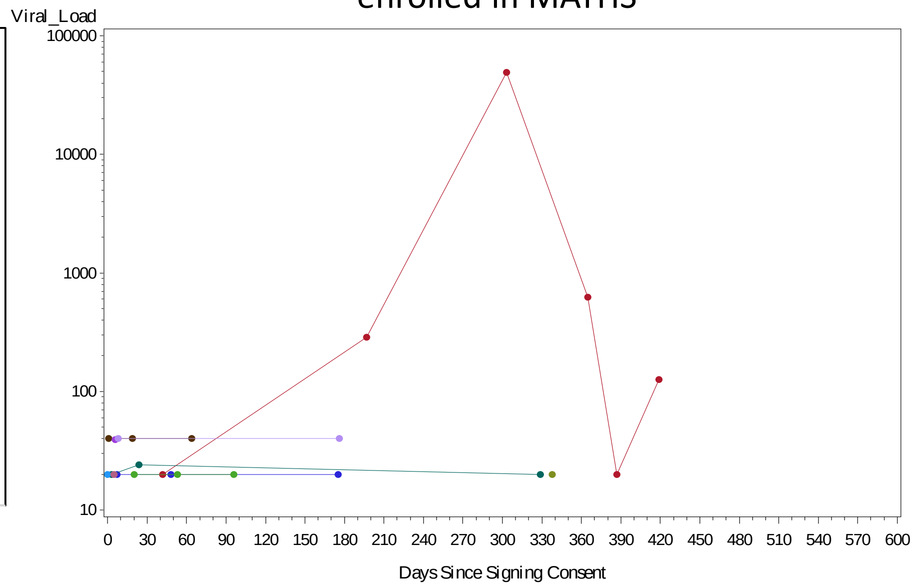
Background

- Loss of “Elite Control” by a MATHS participant
- Anecdotal evidence of Elite Controllers reporting ART and therefore “false EC” while recruiting and enrolling donors into the MATHS cohort study
- Apparent increase in EC over 1-2 years and during a winter incentive campaign

Antibody+, RNA- donations as a Proportion of all HIV positives



Loss of virologic control by an Elite controllers enrolled in MATHS*



Aim & Methods

Aim

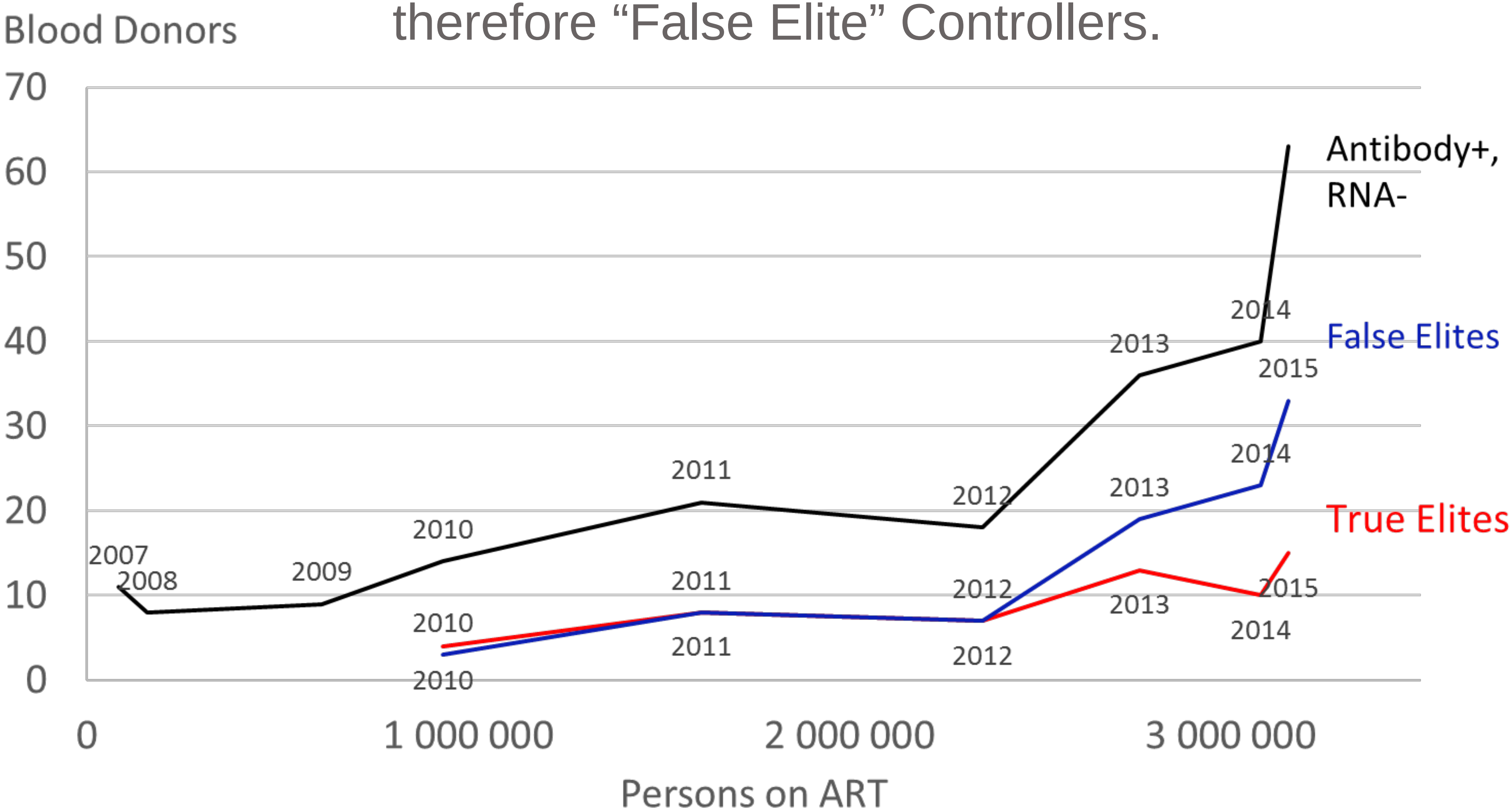
- To understand the extent of the false EC phenomenon and generate hypothesis for its genesis and prevention

Methods

- 211 Potential EC tested for five ARV drugs using qualitative liquid chromatography tandem mass spectrometry (sensitivity 0.02µg/mL)
  - Nevirapine, Efavirenz, Darunavir, Atazanavir, Lopinavir
- Compare the frequency of false EC against blood drive characteristics, donor incentives and the temporal trend of ART rollout in South Africa using chi-square, Fisher exact and trend tests

ART Rollout

Of 211 Potential Elite Controllers tested, 129 (61%) had evidence of ART and were therefore “False Elite” Controllers.



“False” Elite controllers by donation site and incentives

	False Elites n=129 (%HIV+)	True Elites n=82 (%HIV+)	Total n=211 (%HIV+)
Donation Site **(p=0.14)**			
Mobile	116 (1.6)	68 (1.0)	184 (2.6)
Fixed	13 (0.75)	14 (0.81)	27 (1.6)
Donor Incentives **(p=0.74)**			
Incentive period	26 (2.1)	15 (1.2)	41 (3.3)
Non-incentive period	103 (0.96)	67 (0.62)	170 (1.6)

False Elite controllers by gender and age

	False EC (%HIV+) N=129	True EC (%HIV+) N=82	Total (%HIV+) N=211
Gender (p=0.35)			
Female	95 (1.10)	65 (0.75)	160 (1.85)
Male	34 (0.73)	17 (0.37)	51 (1.10)
Age (p=0.03)*			
15-19	10 (0.37)	13 (0.48)	23 (0.84)
20-25	12 (0.35)	16 (0.47)	28 (0.82)
26-30	23 (0.91)	12 (0.47)	35 (1.38)
31-40	58 (2.06)	23 (0.82)	81 (2.87)
41-50	23 (1.79)	10 (0.78)	33 (2.57)
>50	3 (0.67)	8 (1.78)	11 (2.44)

* For age >30 versus <=30

False Elite controllers by race

	False EC (%HIV+) N=129	True EC (%HIV+) N=82	Total (%HIV+) N=211
Race			
Asian		1 (0.72)	1 (0.72)
Black	120 (1.00)	70 (0.59)	190 (1.59)
Coloured	1 (0.23)	1 (0.23)	2 (0.45)
Unallocated	5 (2.45)	3 (1.47)	8 (3.92)
White	3 (0.56)	7 (1.31)	10 (1.88)

Due to the small number of Potential ECs in non black race group we did not analyse for significance

Drugs detected

Drugs	Atazanavir	Darunavir	Efavirenz	Lopinavir	Nevirapine
Number (%)	0	0	111 (86)	7 (5.4)	11 (8.5)

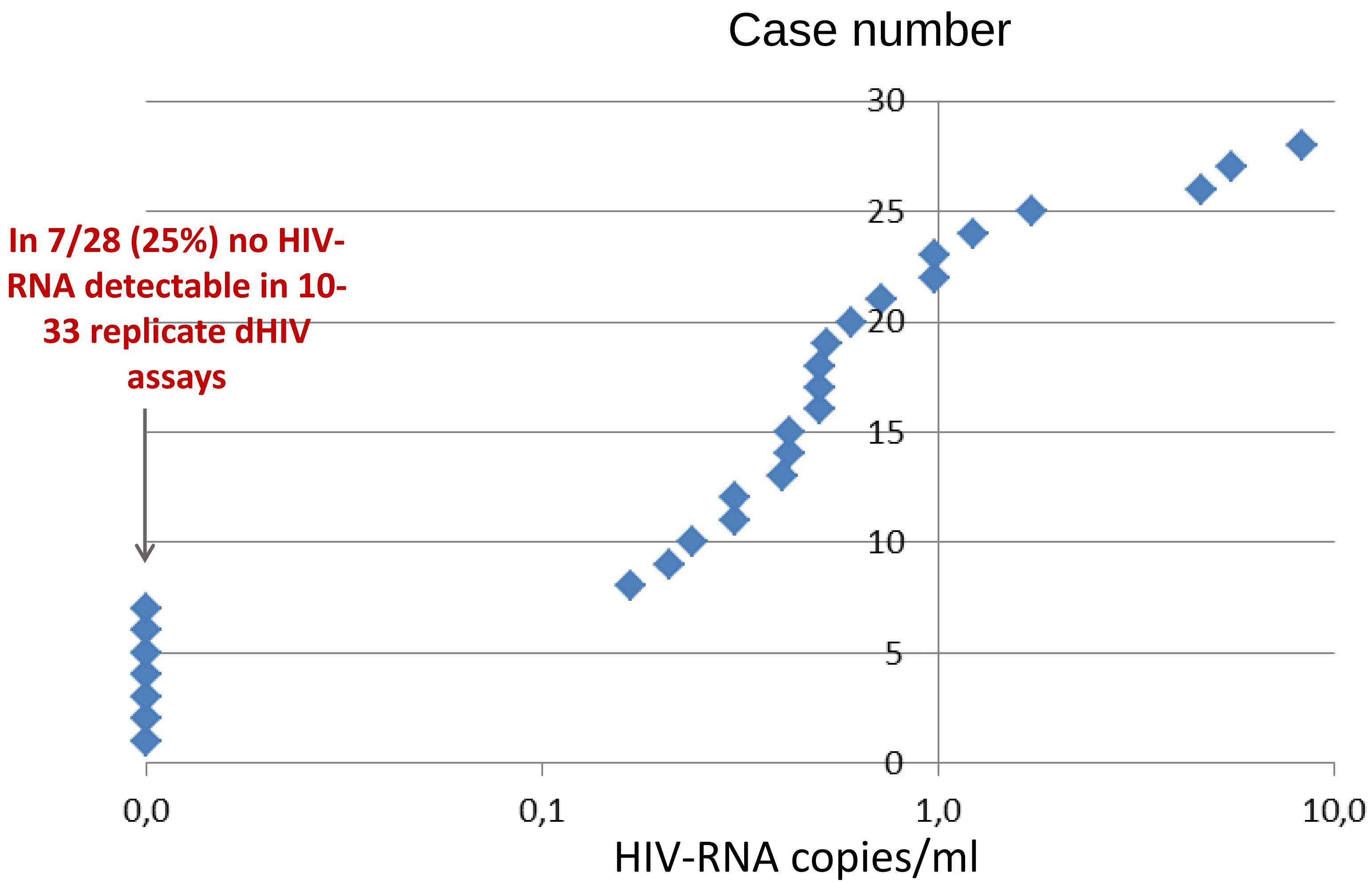
Viraemia in EC's

copies/mL

27cp/mL
9cp/mL
3cp/mL
1cp/mL
0.3cp/mL
Elite Controller
27cp/mL
9cp/mL
3cp/mL
1cp/mL
0.3cp/mL
Elite Controller
Treated "Elite Controller"
Negative Donor

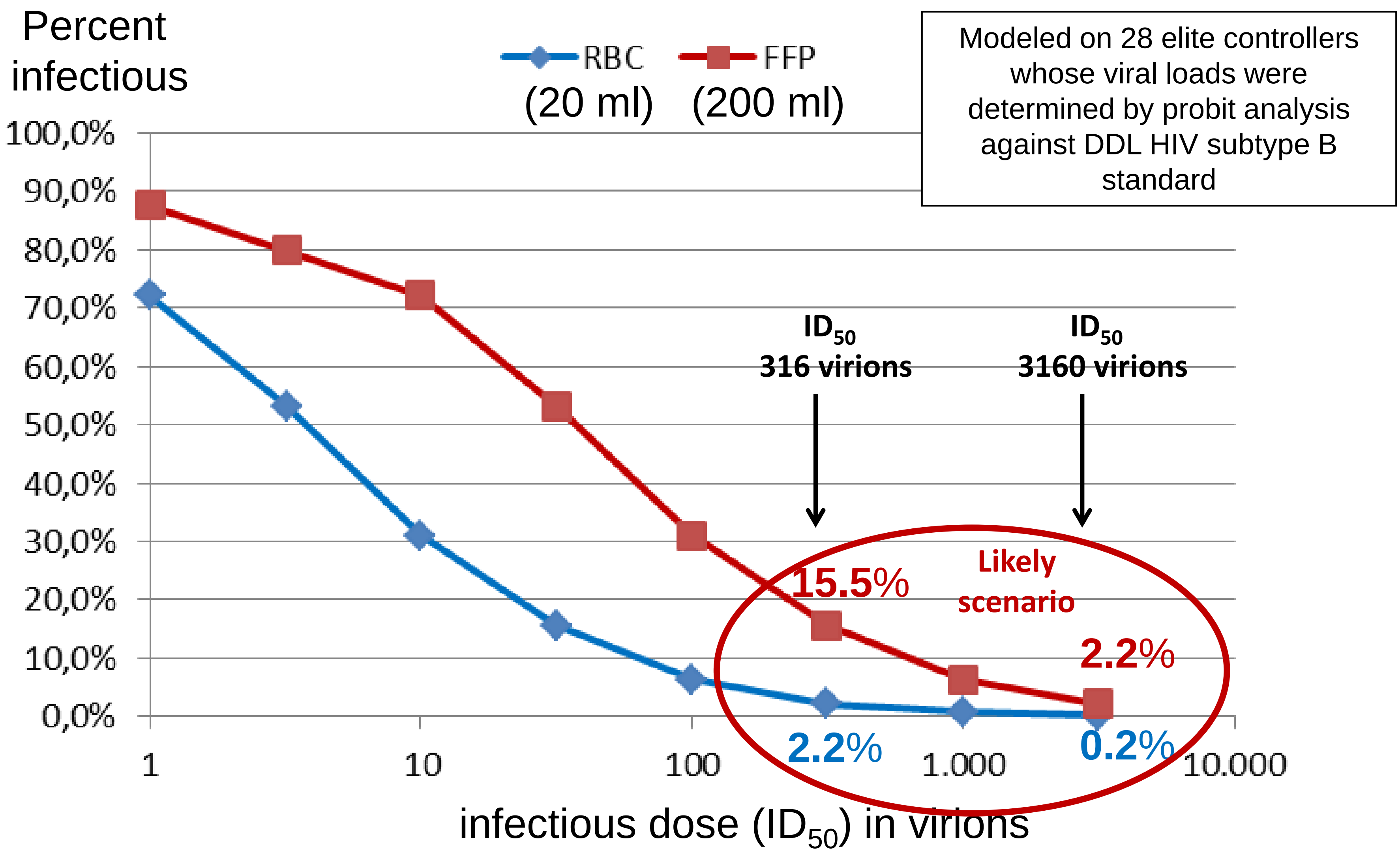
TND= 0/18 reps; <0.12 set at 0.1
(1/18 reps – poisson derived value 0.12)

Distribution of viral load in 28 elite controllers plasmas



Determined by probit alalysis from proportion of positive samples on 30 replicate Ultrio assays against DDL HIV subtype B standard dilutions

Modeling the proportion of RBC and FFP transfusions from elite controllers being infectious



Conclusions

- False EC due to undisclosed ART use, represent a large and growing proportion of potential EC in SA blood donors
- False EC status may be associated with older age but not with sex or race
- False EC status is not associated with small incentives or fixed versus mobile collection site
- False EC may seem to be increasing as ART coverage increases in South Africa
- True EC and False EC do still have viremia and therefore pose a risk to blood safety

Way forward

- Enrol False Elite controllers into a qualitative research study to determine
  - Whether donors feel peer pressure at Mobile clinics
  - Whether donors believe they are cured
  - If more education is required at the treatment clinics
  - If donors are test seeking for confirmatory purposes
  - Whether donors donate for incentives/ small gifts
- Test concordant HIV NAT+/antibody+ donors – especially those with low viral load - for ART to measure the extent of undisclosed ART usage in that group.

Acknowledgements

Karin van den Berg Genevieve Jacobs Ronel Swanevelder	Cynthia Nyoni Tinus Brits Ute Jentsch	South African National Blood Service
Coreen Barker Amisha Rama		Clinical HIV Research Unit (WITS)
Rachel Lockyear		Right to Care
Chris McClure Nathan Sikes		RTI
Brian Custer Edward Murphy	Mars Stone Mike Busch	Blood Systems Research Institute and University of California San Francisco
Jennifer Norman		University of Cape Town

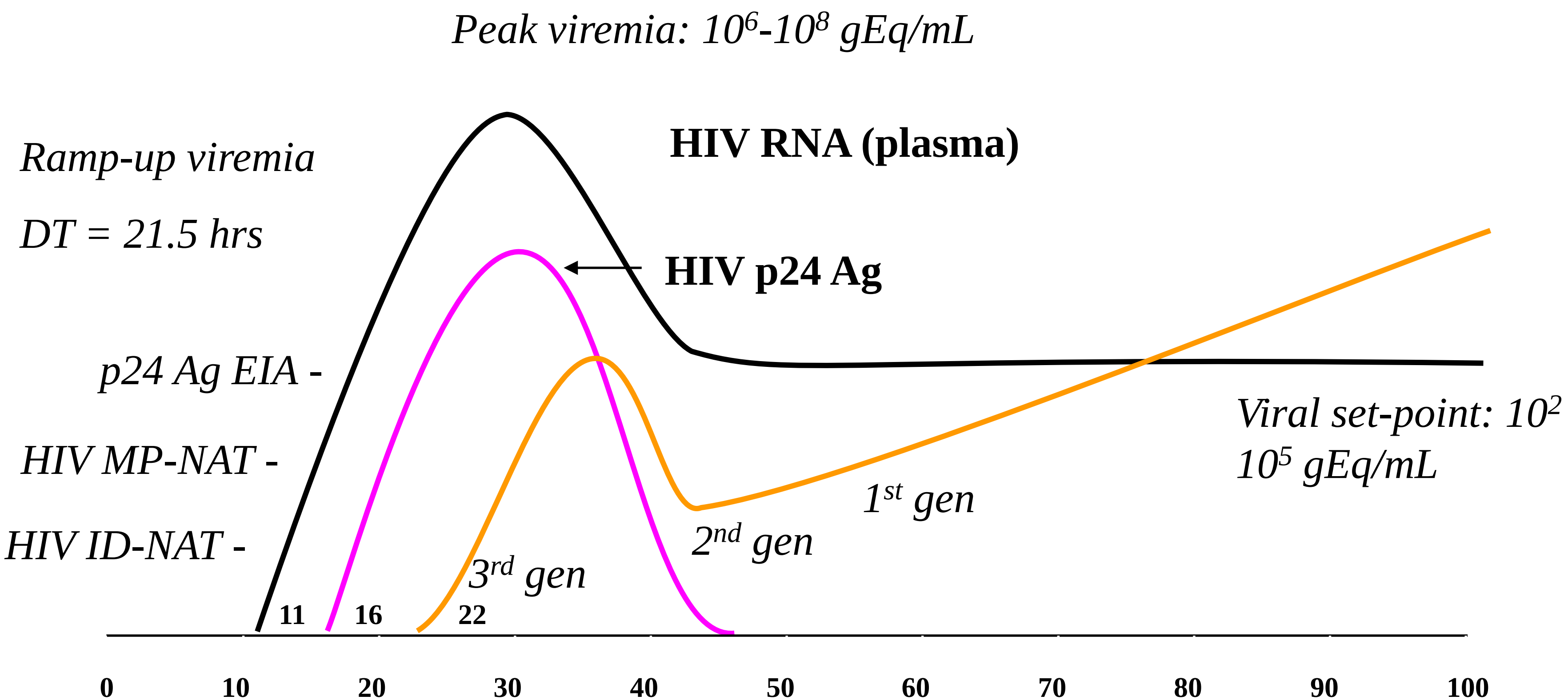
REDS-III MATHS is a NHLBI funded project

HHSN268201100009I

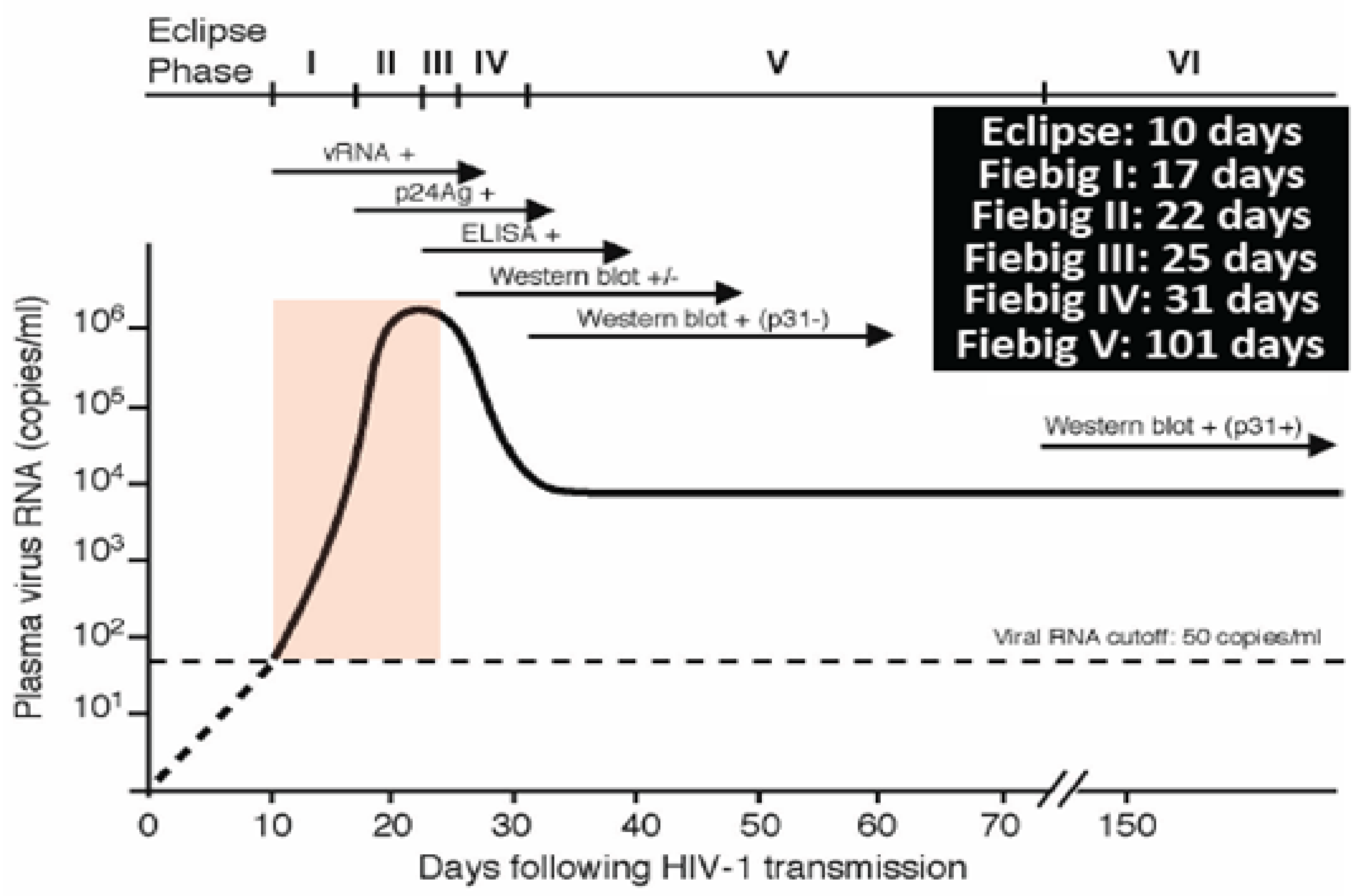
Impact of early ART treatment and pre-exposure prophylaxis on performance of assays for HIV diagnosis and donor screening

Michael P. Busch, MD, PhD
Blood Systems Research Institute
University of California San Francisco

HIV Viremia during early infection

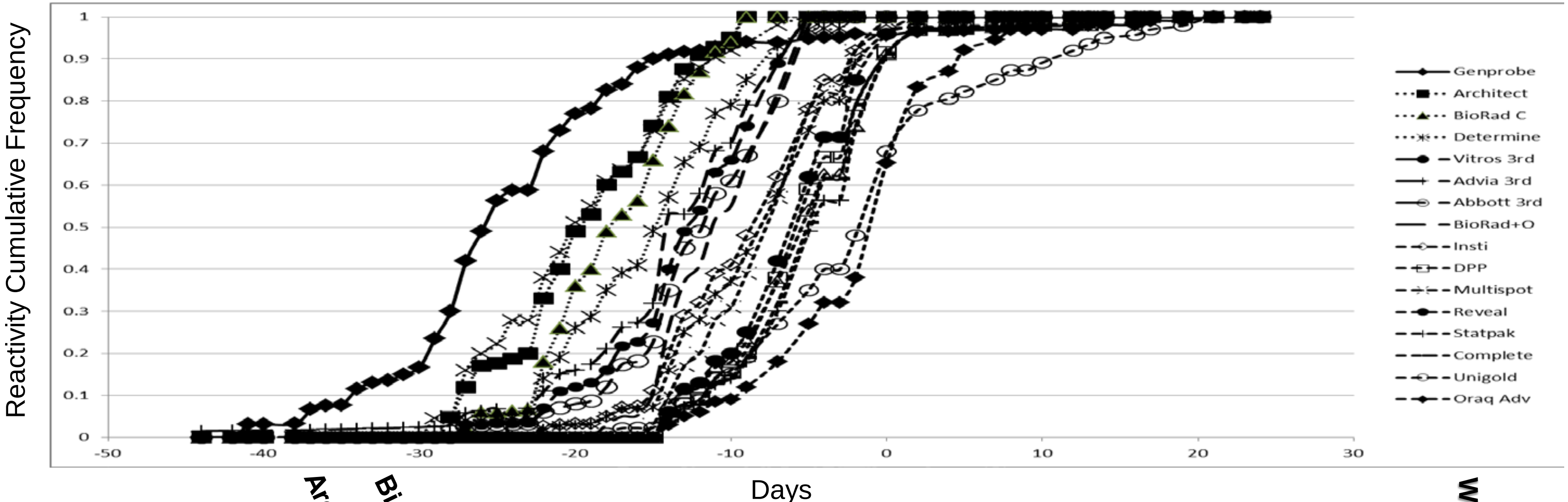


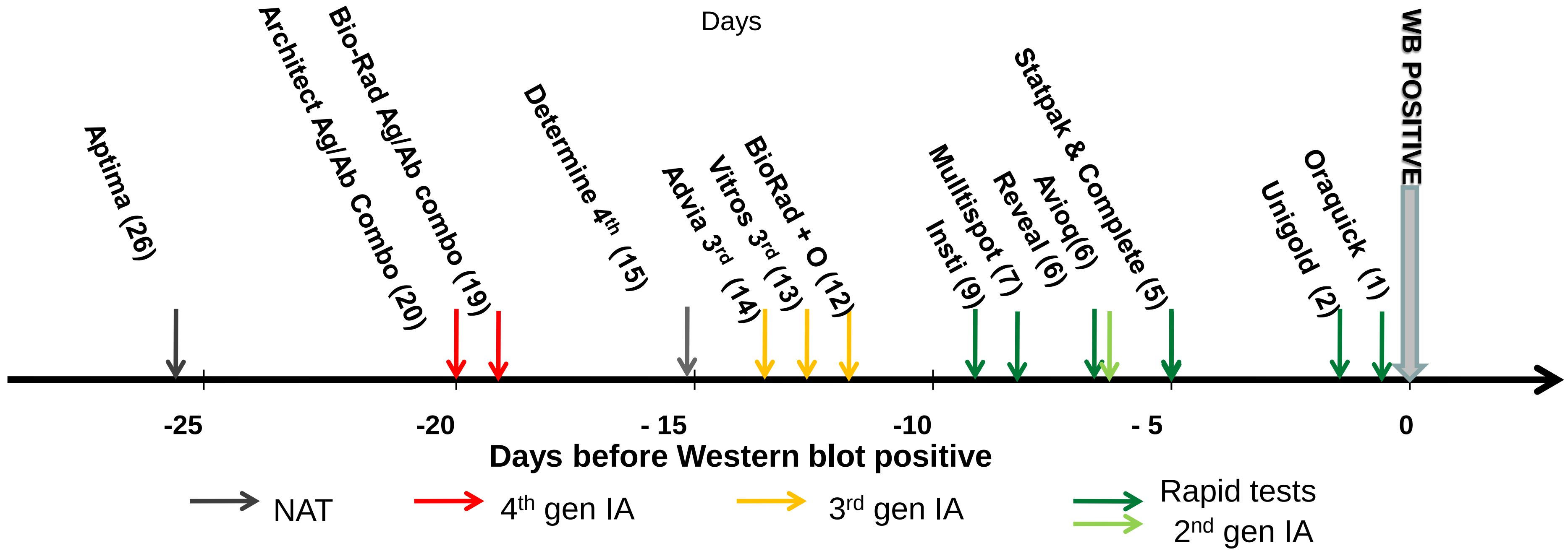
Fiebig Stages of Acute HIV Infection



Fiebig/Busch et al., AIDS 2003
Cohen/Hecht et al. JID 2010

Data from 17 plasma donors that progressed from NAT positive to WB positive used to construct a relative sequence of reactivity timeline





Adapted from Owen et al J Clin Micro 2008 and Masciotra et al J Clin Virol 2011,2013

Time Until Emergence of HIV Test Reactivity Following Infection With HIV-1: Implications for Interpreting Test Results and Retesting After Exposure

Kevin P. Delaney,[1] Debra L. Hanson,[1] Silvina Masciotra,[1] Steven F. Ethridge,[1] Laura Wesolowski,[1] and Sherry Michele Owen[2]

[1]Division of HIV/AIDS Prevention, and [2]Office of the Director, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia

Clinical Infectious Diseases®

Table 2. Inter-Test Reactivity Intervals of Plasma Specimens

HIV Test	Median (Standard Deviation)	95% Confidence Limit
Ag/Ab combo laboratory test		
ARCHITECT HIV Ag/Ab Combo	6.0 (1.14)	3.8, 8.2
BioPlex 2200 HIV Ag-Ab	5.3 (1.81)	1.7, 8.8
GS Combo Ag/Ab EIA	5.3 (2.40)	.6, 10.0
Siemens Combo HIV Ag-Ab	6.9 (1.11)	4.7, 9.1
Ag/Ab combo rapid test		
Determine HIV-1/2 Ag/Ab Combo	7.4 (1.35)	4.8, 10.1
Conjugated synthetic peptide laboratory test (IgG/IgM sensitive)		
ADVIA HIV 1/O/2 Enhanced	10.4 (2.67)	5.1, 15.6
GS HIV-1/HIV-2 PLUS O EIA	13.3 (1.58)	10.2, 16.4
VITROS Anti-HIV-1 + 2 Assay	12.0 (0.94)	10.1, 13.8
IgG/IgM-sensitive rapid test[a]		
INSTI HIV-1/HIV-2 Antibody Test	14.9 (1.66)	11.7, 18.2
Uni-Gold Recombigen HIV	20.3 (3.53)	13.4, 27.3
Synthetic or recombinant peptide rapid screening test (IgG sensitive)		
Clearview COMPLETE HIV-1/2	20.2 (2.75)	14.8, 25.6
Clearview HIV 1/2 STAT-PAK	19.5 (2.41)	14.8, 24.2
DPP HIV-1/2	18.9 (1.89)	15.2, 22.6
Multispot HIV-1/HIV-2 Rapid Test[b]	16.8 (1.53)	13.8, 19.8
Oraquick ADVANCE Rapid HIV-1/2 Antibody Assay	22.9 (4.22)	14.6, 31.2
Reveal G2 Rapid HIV-1 Antibody Test	18.6 (1.31)	16.0, 21.2
Synthetic or recombinant peptide laboratory screening test (IgG sensitive)		
Avioq HIV-1 Microelisa System[c]	19.1 (1.46)	16.3, 22.0
Synthetic or recombinant peptide supplemental HIV-1/HIV-2 differentiation test (IgG sensitive)		
Geenius HIV-1/2 Ab Supplemental Assay	21.3 (2.68)	16.0, 26.5
Multispot HIV-1/HIV-2 Rapid Test[b]	22.2 (2.67)	17.0, 27.4
Category (number of inclusive tests)		
Ag/Ab laboratory (4)	5.9 (1.01)	3.9, 7.9
IgG/IgM-sensitive laboratory (3)	11.9 (1.14)	9.6, 14.1
IgG-sensitive rapid screening (6)	19.5 (1.70)	16.2, 22.8
IgG-sensitive supplemental (2)	21.7 (2.22)	17.4, 26.1
Western blot (viral lysate) (1)	24.8 (3.38)	18.1, 31.4

Estimated median inter-test reactivity interval (ITRI), and 95% confidence limits, in days, between Aptima RNA reactivity and immunoassay reactivity. Tests are alphabetically ordered within each test category.

Table 3. Window Periods

Category (No. of Inclusive Tests)	Median (Interquartile Range; Days)	99th Percentile (Days)
Antibody/antigen laboratory (4)	17.8 (13.0, 23.6)	44.3
IgG/IgM-sensitive laboratory (3)	23.1 (18.4, 28.8)	49.5
IgG-sensitive rapid screening (6)	31.1 (26.2, 37.0)	56.7
IgG-sensitive supplemental (2)	33.4 (28.5, 39.2)	58.2
Western blot (viral lysate) (1)	36.5 (31.0, 43.2)	64.8

Estimated median, interquartile range, that is, the 25th and 75th percentiles, and 99th percentiles of the window period distribution, the duration of time between human immunodeficiency virus exposure and immunoassay reactivity, in days. Percentiles are means of respective percentiles from 4 computational methods and from all tests of a category of tests. Window period estimates were sums of 10 000 simulated days inter-test reactivity interval, using parameters from the observed data (testing of plasma specimens), and 10 000 simulated eclipse period days as graphed in Figure 1.

Abbreviation: Ig, immunoglobulin.

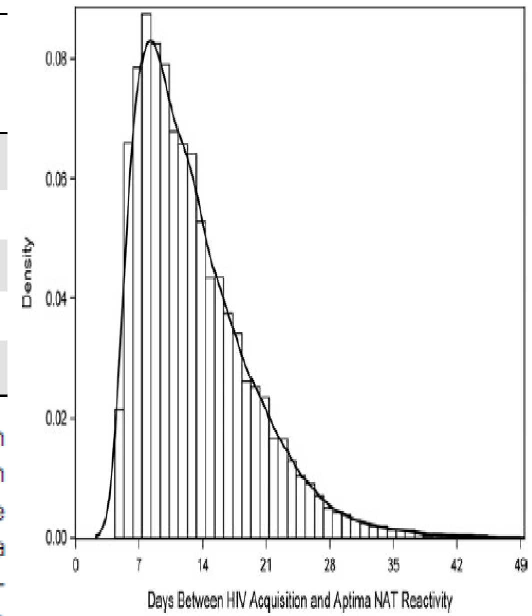


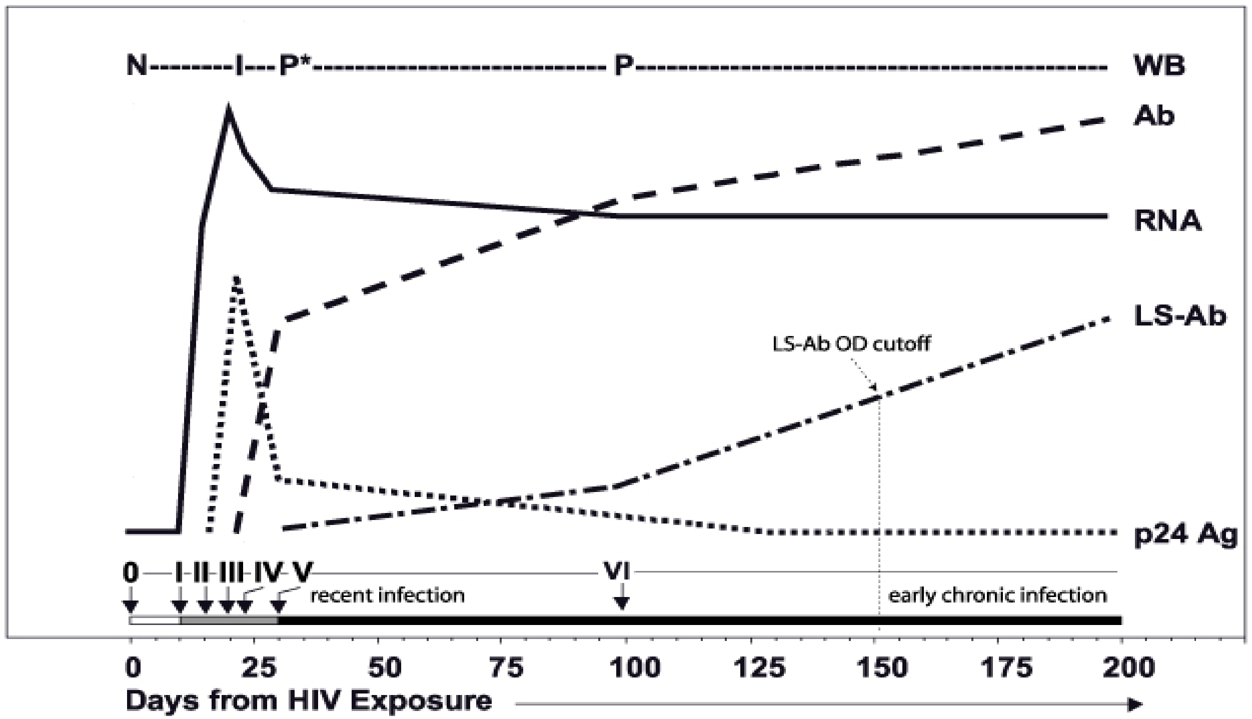
Figure 1. Simulated eclipse period probability density function (PDF) translated from a 3-parameter Weibull prior distribution. Parameters for location, shape, and

JAMA®
The Journal of the American Medical Association

July 1998

New Testing Strategy to Detect Early HIV-1 Infection for Use in Incidence Estimates and for Clinical and Prevention Purposes

Robert S. Janssen, MD; Glen A. Satten, PhD; Susan L. Stramer, PhD; Bhupat D. Rawal, PhD; Thomas R. O'Brien, MD, MPH; Barbara J. Weiblen, MS; Frederick M. Hecht, MD; Noreen Jack, MBBS, MPH; Farley R. Cleghorn, MD, MPH; James O. Kahn, MD; Margaret A. Chesney, PhD; Michael P. Busch, MD, PhD



Beyond detuning: 10 years of progress and new challenges in the development and application of assays for HIV incidence estimation

Michael P. Busch[a,b], Christopher D. Pilcher[c], Timothy D. Mastro[d], John Kaldor[e], Gaby Vercauteren[f], William Rodriguez[g], Christine Rousseau[g], Thomas M. Rehle[h], Alex Welte[i], Megan D. Averill[d], Jesus M. Garcia Calleja[f], for the WHO Working Group on HIV Incidence Assays

AIDS 2010, **24**:2763–2771

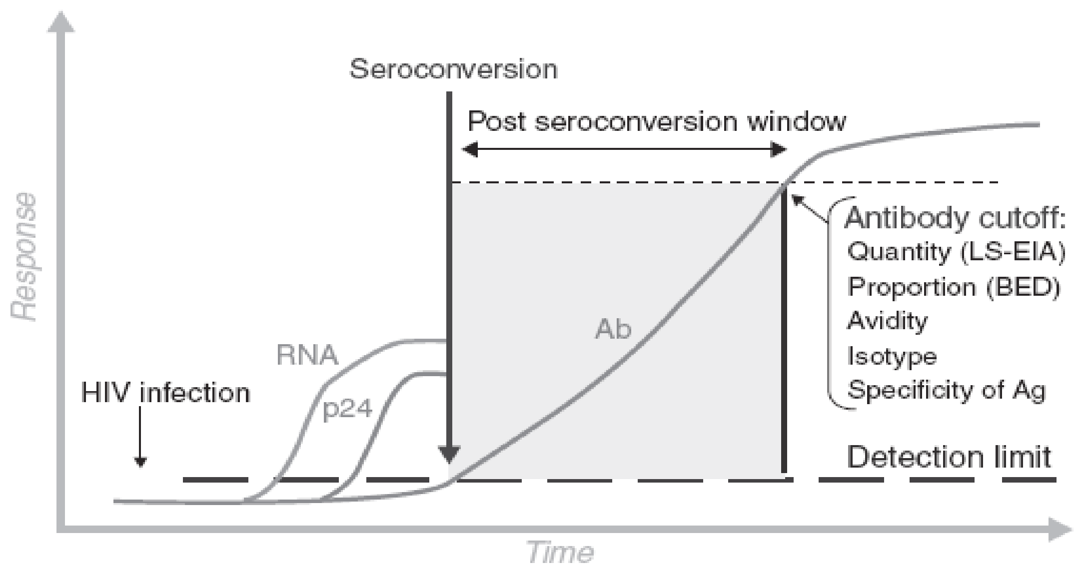


Fig. 1. Principle of assays that discriminate recent from long-standing HIV infections, based on maturation of HIV-specific antibody responses, for use in cross-sectional incidence estimation. LS-EIA, less-sensitive-enzyme immunoassay.

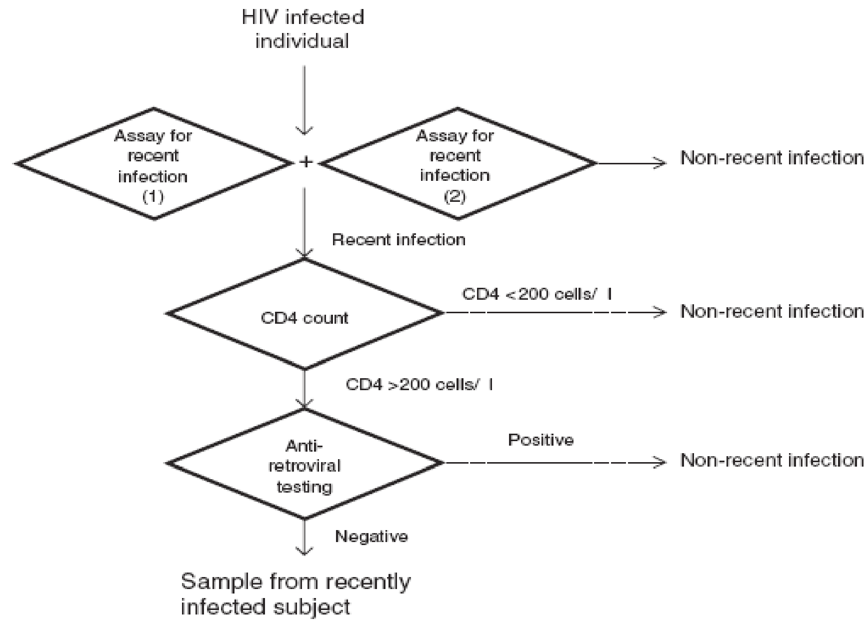


Fig. 2. Representative testing algorithm incorporating two incidence assays and available clinical data (CD4 count and antiretroviral treatment history) for determination of recent HIV infection status of specimens evaluated for HIV incidence estimation. Assay 1 and assay 2 represent two assays

Epidemiol. Infect., Page 1 of 17.
doi:10.1017/S0950268816002910

REVIEW ARTICLE
Moving towards a reliable HIV incidence test – current status, resources available, future directions and challenges ahead

G. MURPHY[1]*, C. D. PILCHER[2], S. M. KEATING[3], R. KASSANJEE[4,5], S. N. FACENTE[2], A. WELTE[4], E. GREBE[4], K. MARSON[2], M. P. BUSCH[3], P. DAILEY[6], N. PARKIN[6,7], J. OSBORN[6], S. ONGARELLO[6], K. MARSH[8] AND J. M. GARCIA-CALLEJA [9] on behalf of the Consortium for the Evaluation and Performance of HIV Incidence Assays (CEPHIA)† Foundation for Innovative New Diagnostics, UNAIDS and the WHO Technical Working Group

Fig. 2. CEPHIA incidence assay critical path.

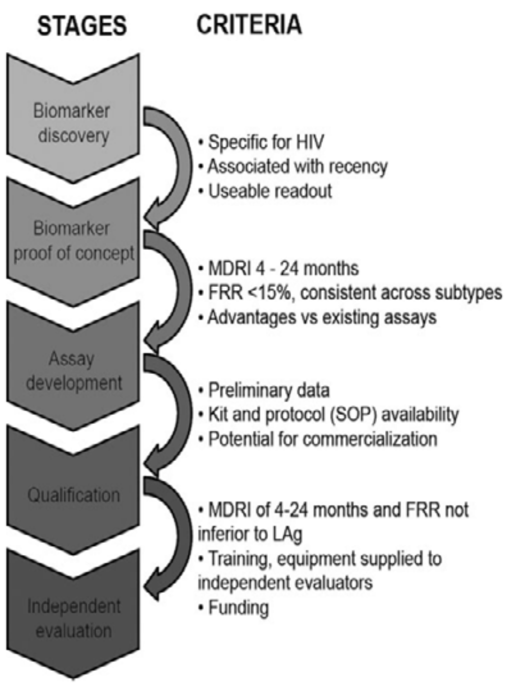
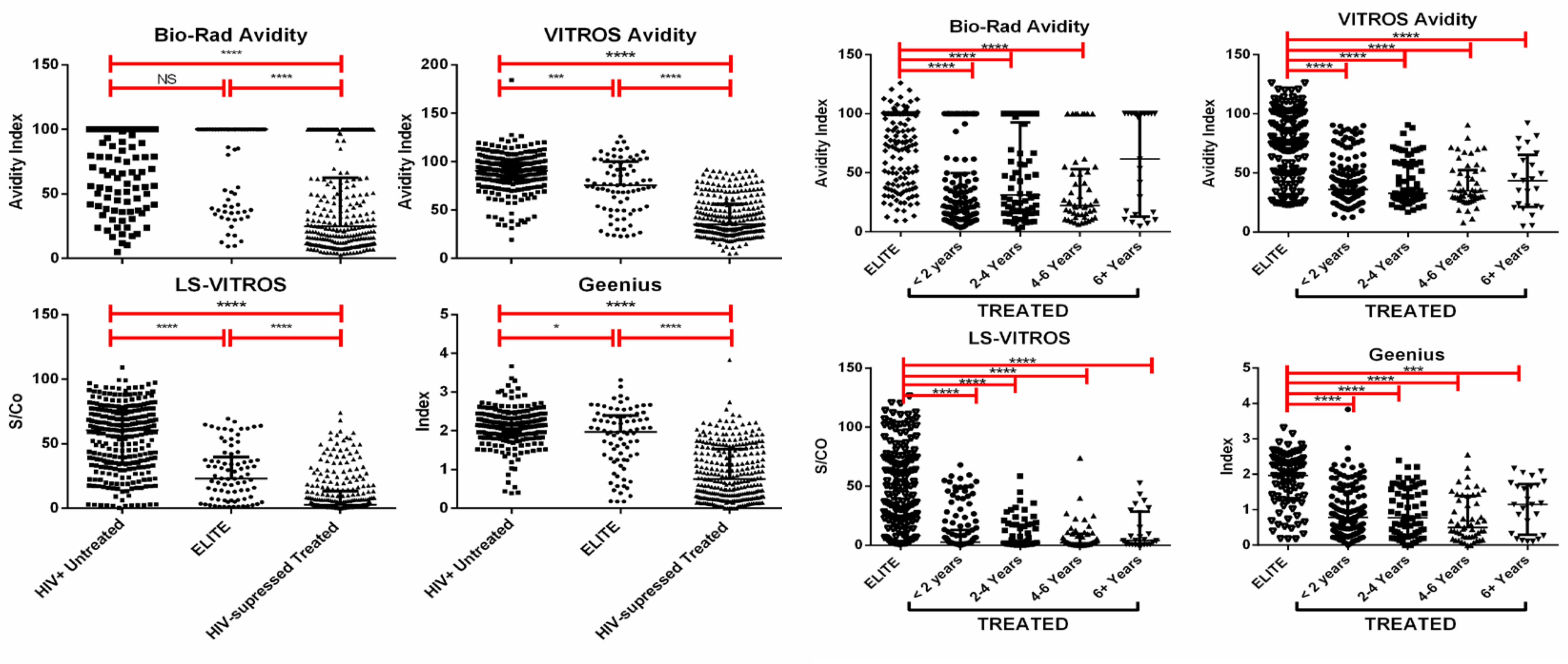


Table 2. *Potential uses for HIV incidence assays*

Use	Description of use
1. Incidence estimate for national surveillance	To provide national estimate of incidence; may be part of a broader demographic study
2. Incidence estimation for programme, prevention or trial planning	To provide incidence estimate in sub-populations for planning, prioritizing, or other instances when an estimate of incidence is required. May often be for only a city or region (e.g. prioritize programmes or investments, or identify sites for intervention trials)
3. Incidence estimate in key or sentinel populations	To provide incidence estimates in special sub-population using targeted sampling methods
4. Incidence estimation to assess the impact of population-level interventions	To assess the impact of a population-level intervention (e.g. community-level intervention) by comparing incidence before and after the intervention
5. Incidence estimate from case-based surveillance	To provide national or regional incidence estimates via case-based reporting of newly identified HIV+ individuals
6. Identification of individuals with 'recent' infections for research purposes	Identification of individuals with 'recent' infections for multiple potential applications (e.g. recruitment of recently infected individuals into longitudinal cohort studies)
7. Identification of patients with 'recent' infections for individual patient management	Identification of patients with 'recent' infections for to guide clinical management and/or public health programmes (e.g. selecting therapy, and/or prioritizing contact tracing)
8. Targeted prevention planning	To provide population-level data on recent infections to enable risk factors analysis or identify hotspots to inform targeted prevention planning (no incidence estimate is obtained)

Reduction in HIV Ab reactivity in EC and Following ART in CEPHIA



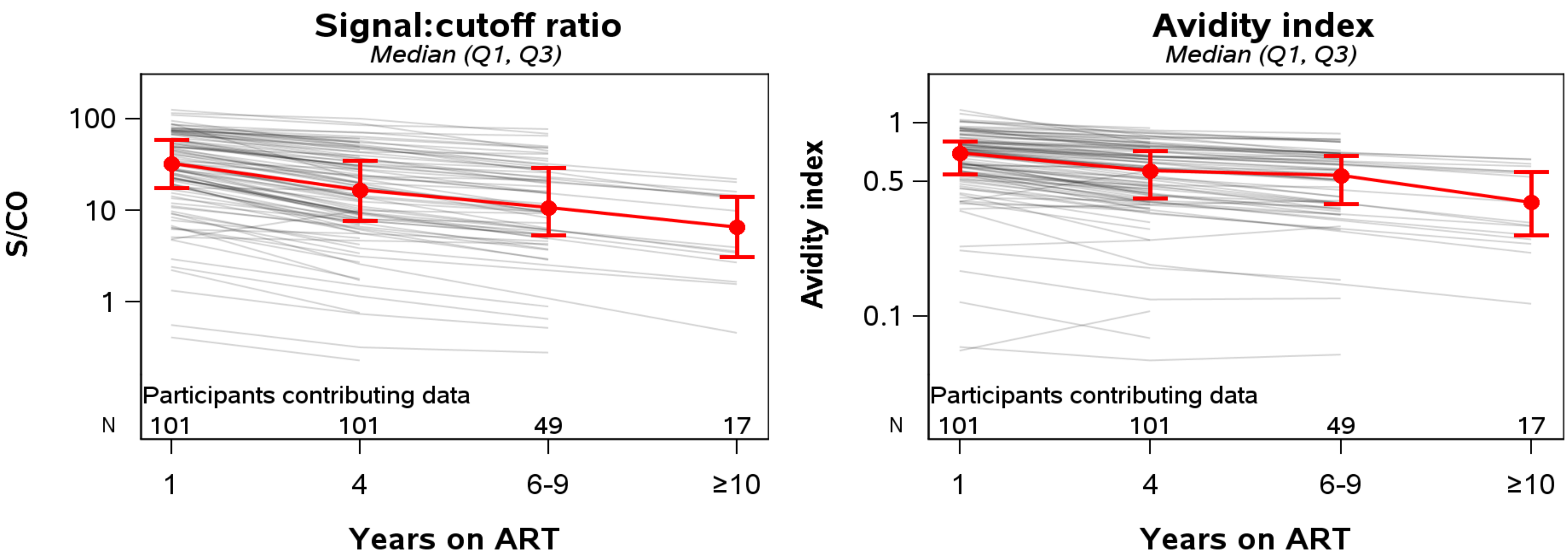
Keating et al. JID, in press

HIV Abs in Early/Late Treatment Cohort

L S - V I T R O S -

Keating et al, JID, in press

HIV Antibody Levels and Avidity Slowly but Steadily Decrease during ART in A5321 Cohort



Sheila et al, in preparation

When is HIV latency established?

Immediate antiviral therapy appears to restrict resting CD4^{+} cell HIV-1 infection without accelerating the decay of latent infection

Nancie M. Archin[a,1], Naveen K. Vaidya[b,c,1], JoAnn D. Kuruc[a], Abigail L. Liberty[a], Ann Wiegand[d], Mary F. Kearney[d], Myron S. Cohen[a], John M. Coffin[e], Ronald J. Bosch[f], Cynthia L. Gay[a], Joseph J. Eron[a], David M. Margolis[a,2], and Alan S. Perelson[b,2]

[a]Department of Medicine, University of North Carolina, Chapel Hill, NC 27599; [b]Theoretical Biology and Biophysics Group, MS K710, Los Alamos National Laboratory, Los Alamos, NM 87545; [c]Department of Applied Mathematics, University of Western Ontario, London, ON, Canada N6A 5B7; [d]HIV Drug Resistance Program, National Cancer Institute, National Institutes of Health, Frederick, MD 21702; [e]Department of Molecular Biology and Microbiology, Tufts University, Boston, MA 02111; and [f]Harvard School of Public Health, Harvard University, Boston, MA 02115

Edited by Stephen P. Goff, Columbia University College of Physicians and Surgeons, New York, NY, and approved April 27, 2012 (received for review December 9, 2011)

- ❖ HIV latency is established in acute HIV infection
- ❖ Latency persists despite early and long-term ART
- ❖ Pool of latently infected cells is stable with little to no decay in the presence of long-term ART

Archin N *et al*. *PNAS*, 2011

HIV DNA Set Point is Rapidly Established in Acute HIV Infection and Dramatically Reduced by Early ART☆,☆☆

Jintanat Ananworanich [a,b,c,*], Nicolas Chomont [d,e,1], Leigh Ann Eller [a,b], Eugene Kroon [c,f], Sodsai Tovanabutra [a,b], Meera Bose [a,b], Martin Nau [a,b], James L.K. Fletcher [c], Somporn Tipsuk [c], Claire Vandergeeten [e,1], Robert J. O'Connell [d], Suteeraporn Pinyakorn [a,b], Nelson Michael [a], Nittaya Phanuphak [c], Merlin L. Robb [a,b], on behalf of the, RV217 and RV254/SEARCH010 study groups:

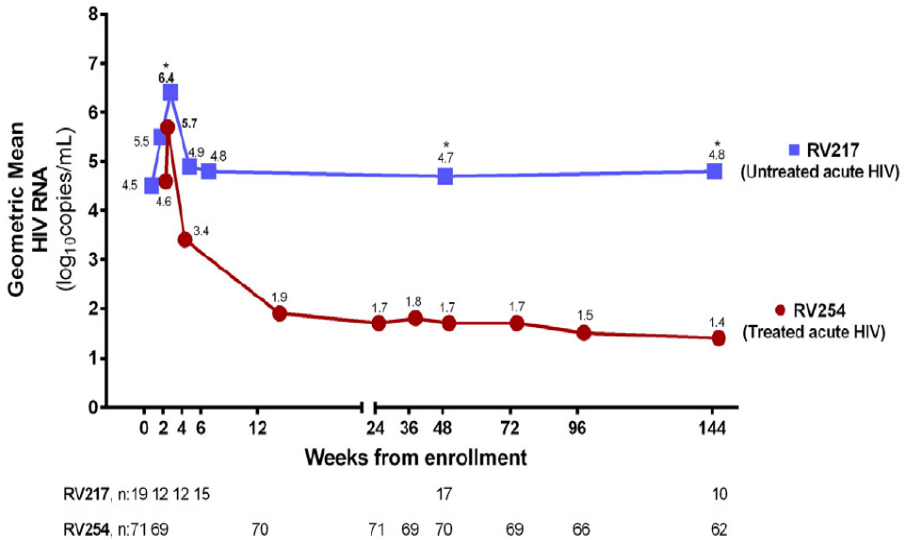


Fig. 1. Plasma HIV RNA of RV217 untreated and RV254 treated acute HIV infection participants. Footnote: The detection limit of HIV RNA was either 1.7 or 1.3 log_{10}copies/ml.

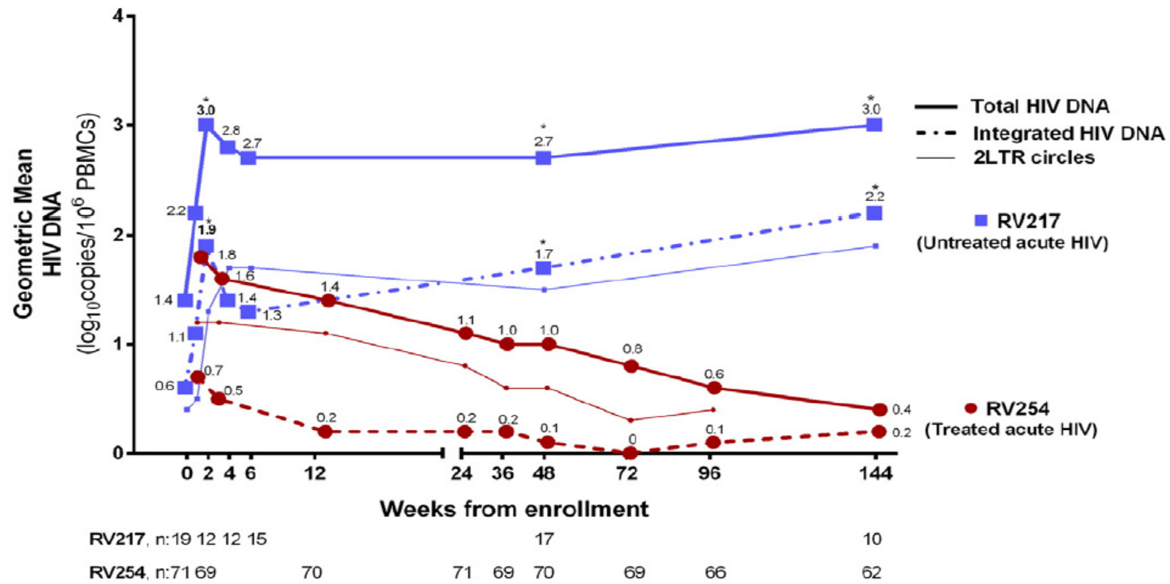


Fig. 2. Total and integrated HIV DNA and 2-LTR circles in peripheral blood mononuclear cells of Fiebig I to IV RV217 untreated and RV254 treated acute HIV infection participants. Footnote: PBMCs: peripheral blood mononuclear cells.

Commentary

The Benefits of Early Antiretroviral Therapy for HIV Infection: How Early is Early Enough?

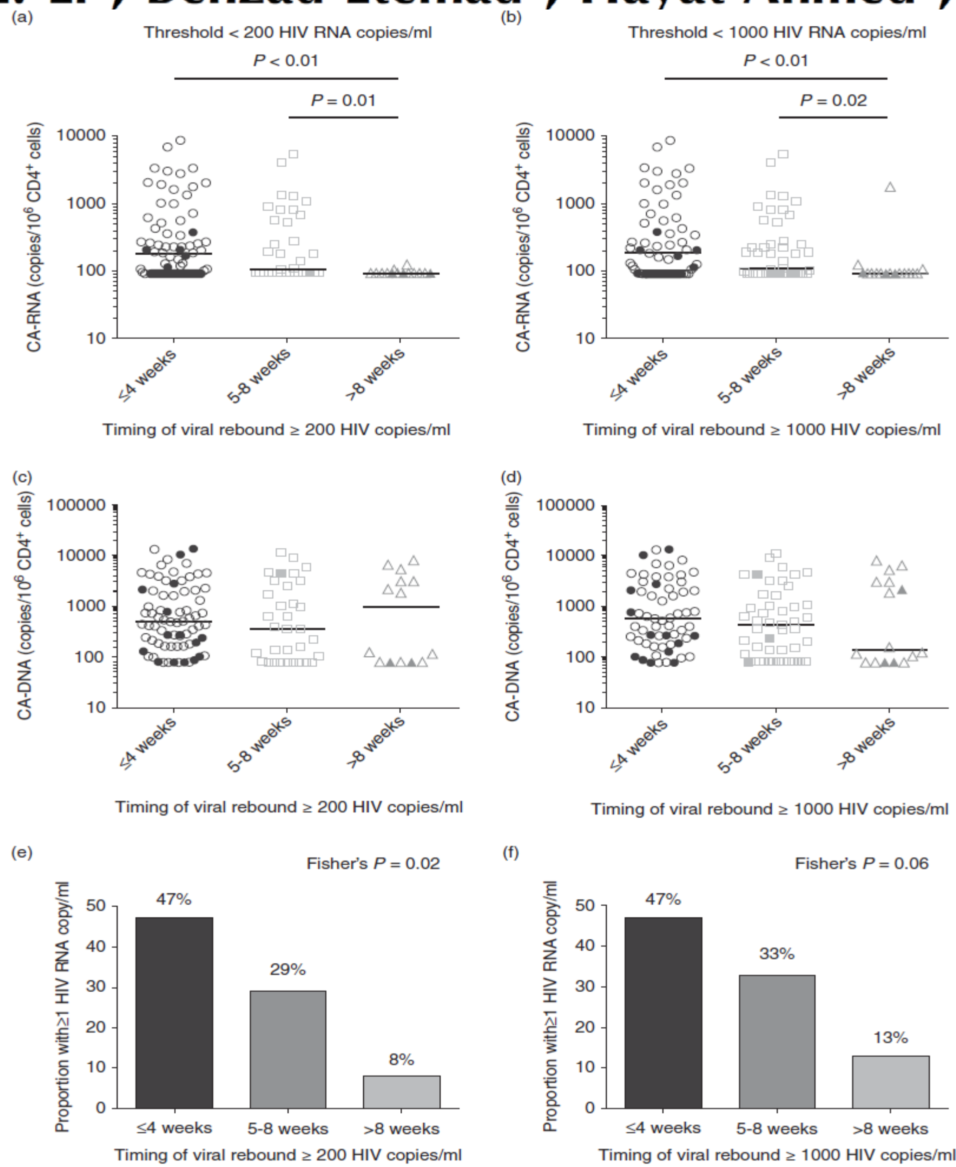
Sulggi A. Lee, Steven G. Deeks *

University of California San Francisco, CA, USA

http://dx.doi.org/10.1016/j.ebiom.2016.07.024.

The size of the expressed HIV reservoir predicts timing of viral rebound after treatment interruption

Jonathan Z. Li[a], Behzad Etemad[a], Hayat Ahmed[a], Evgenia Aga[b],
Ronalc ... itzkes[a],
Michael ... . Gandhi[f]

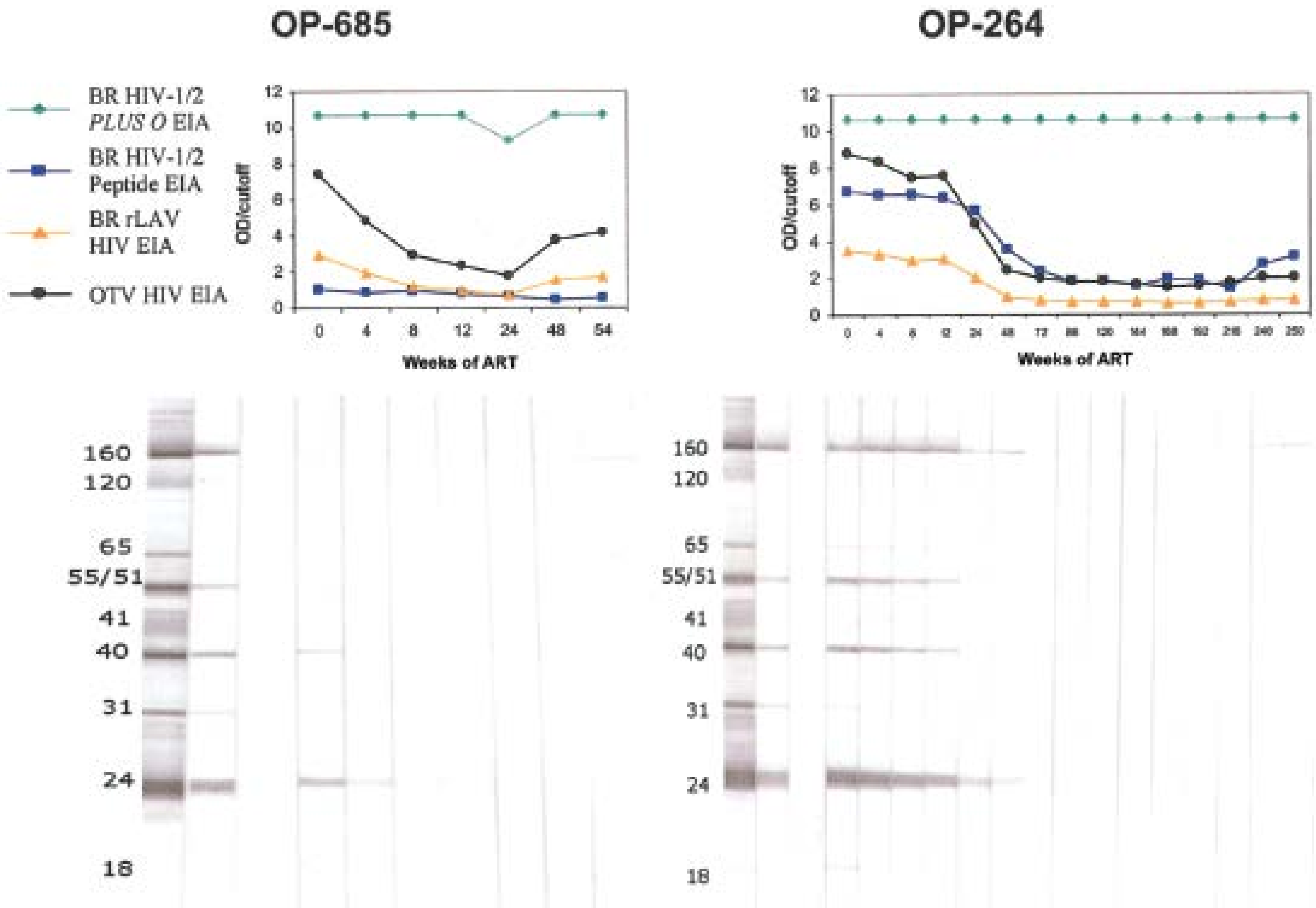


. **Association of pre-ATI levels of CA-RNA, CA-DNA, and residual viremia with timing of viral rebound.** Levels of pre-ATI

Seroreversion in Subjects Receiving Antiretroviral Therapy during Acute/Early HIV Infection

C. Bradley Hare,[1] Brandee L. Pappalardo,[4,a] Michael P. Busch,[4] Annika C. Karlsson,[2,a] Bruce H. Phelps,[5] Steven S. Alexander,[6] Christopher Bentsen,[7] Clarissa A. Ramstead,[1] Douglas F. Nixon,[2] Jay A. Levy,[3] and Frederick M. Hecht[1]

[1]Positive Health Program, [2]Gladstone Institute of Virology and Immunology, and [3]Department of Medicine, University of California, San Francisco, and [4]Blood Systems Research Institute, San Francisco, and [5]Chiron Corporation, Emeryville, California; [6]Ortho Clinical Diagnostics, Raritan, New Jersey; and [7]Bio-Rad Laboratories, Redmond, Washington



Timing is Everything – Shortcomings of Current HIV Diagnostics in the Early Treatment Era

Keating SM, Pilcher CD, Busch MP: Clin Infect Dis. 2016.

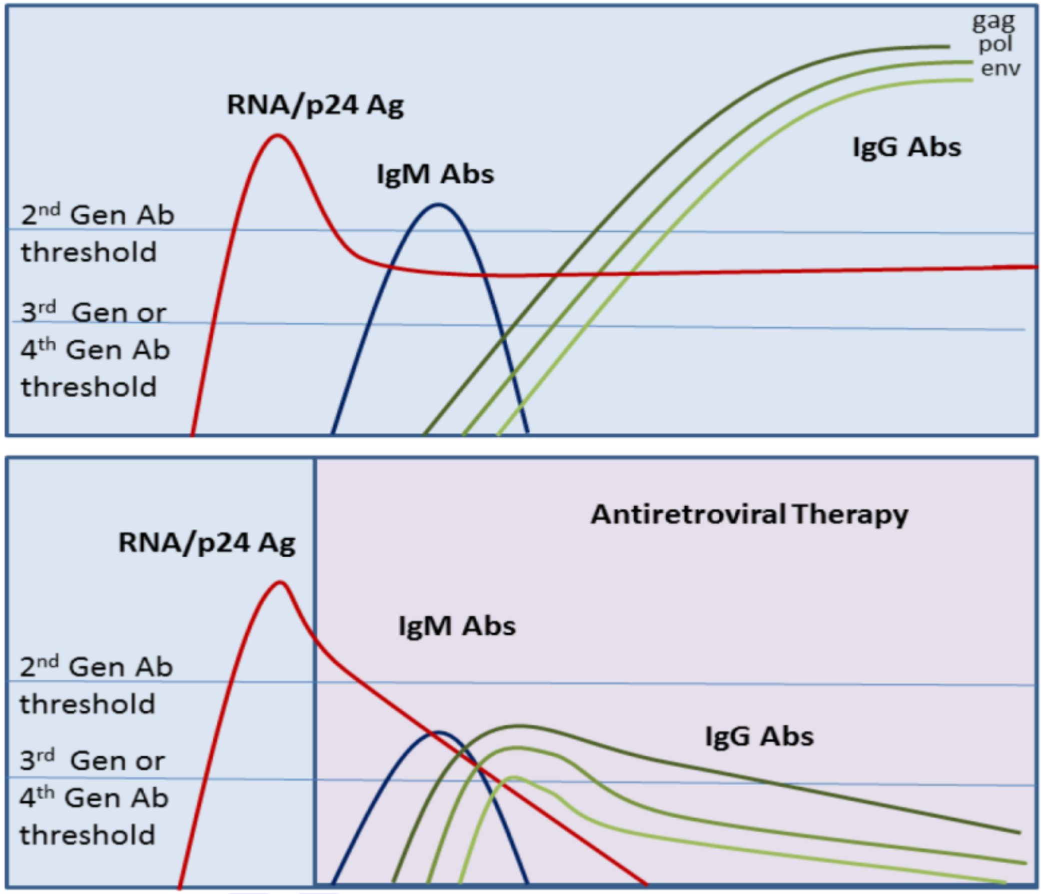


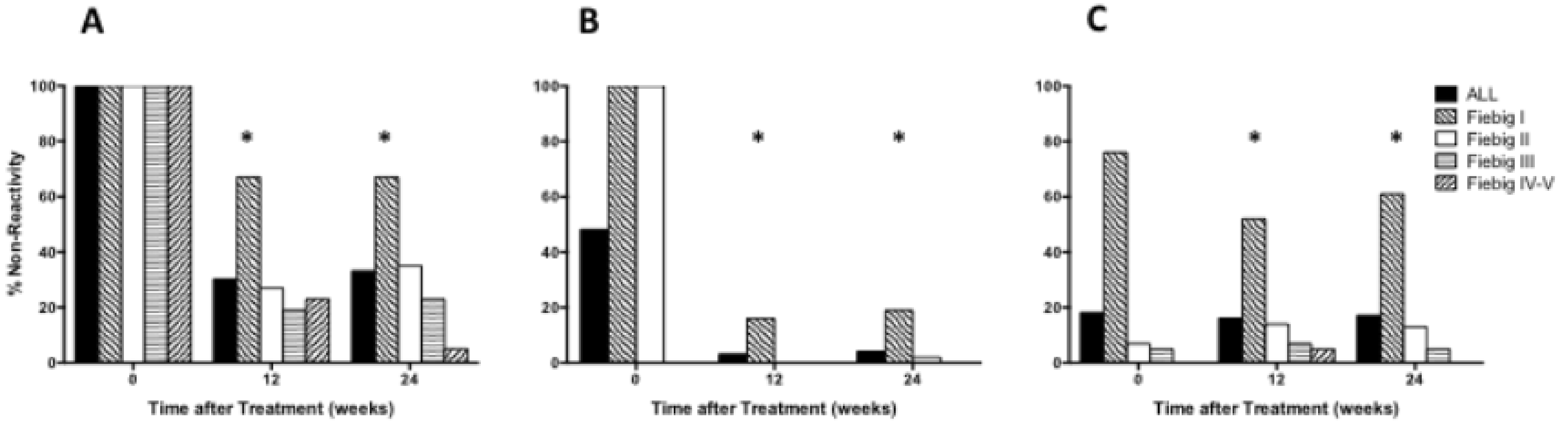
Figure 1. After HIV infection, p24 antigen and IgM and IgG antibody seroconversion occur which are progressively detected by 4th, 3rd or 2nd generation assays over the weeks post-infection. Diverse antigen-specific responses can be differentially detected during this period by western blot (WB) and other confirmatory assays. Sustained antigenic stimulation is required for maturation and maintenance of these antibody responses. Early treatment with ART aborts the development of antibodies if treatment is initiated very early and subsequent seroreversion may occur if treatment is initiated shortly following seroconversion, making it difficult to detect or confirm HIV infection by standard diagnostic tests.

Initiation of Antiretroviral Therapy During Acute HIV-1 Infection Leads to a High Rate of Nonreactive HIV Serology

Clinical Infectious Diseases®

Mark S. de Souza,[1,2,3] Suteeraporn Pinyakorn,[4,5] Siriwat Akapirat,[6] Supanit Pattanachaiwit,[2] James L. K. Fletcher,[1] Nitiya Chomchey,[1] Eugene D. Kroon,[1,2] Sasiwimol Ubolyam,[7] Nelson L. Michael,[5,8] Merlin L. Robb,[4,5] Praphan Phanuphak,[1,2] Jerome H. Kim,[9] Nittaya Phanuphak,[1,2] and Jintanat Ananworanich[1,4,5]; for the RV254/SEARCH010 Study Group

[1]South East Asia Research Collaboration with Hawaii (SEARCH), and [2]Thai Red Cross AIDS Research Centre, Bangkok, Thailand; [3]Cooper Human Systems, Cambridge, Massachusetts; [4]Henry M. Jackson Foundation for the Advancement of Military Medicine, and [5]United States Military HIV Research Program, Bethesda, Maryland; [6]Department of Retrovirology, Armed Forces Research Institute of Medical Sciences, United States Component, and [7]HIV Netherlands Australia Thailand Research Collaboration, Bangkok; [8]Walter Reed Army Institute of Research, Silver Spring, Maryland; and [9]International Vaccine Institute, Seoul, South Korea



Results. Participants (N = 234) initiating ART at a median of 19 days (range, 1–62 days) from HIV exposure demonstrated different frequencies of reactivity prior to and following 24 weeks of ART depending on the IA. Third-generation IA nonreactivity prior to ART was 48%, which decreased to 4% following ART ($P < .001$). Fourth-generation IA nonreactivity was 18% prior to ART and 17% following ART ($P = .720$). Negative WB results were observed in 89% and 12% of participants prior to and following 24 weeks of ART, respectively ($P < .001$). Seroreversion to nonreactivity during ART was observed to at least one of the tests in 20% of participants, with fourth-generation IA demonstrating the highest frequency (11%) of seroreversion.

Absence of Serological Response Following Early Treatment of Acute HIV Infection

Manak M[1], J Malia[2], A Shutt[1], S Akapirat[3], D Colby[4], N Chomchey[4], LA Eller[1], JH Kim[2], J Ananworanich[1], and S Peel[2]

[1]MHRP HJF, Silver Spring, MD; [2]MHRP WRAIR, Silver Spring, MD; [3]AFRIMS, Bangkok, Thailand; [4]SEARCH, TRCARC, Bangkok, Thailand

HIV Diagnostics Conference
Mar 22, 2016
Atlanta, GA

In press, Clinical Infectious Diseases

WRAIR
Walter Reed Army Institute of Research
Soldier Health • World Health

MHRP
U.S. MILITARY HIV RESEARCH PROGRAM

Background

Initiation of HAART at very early times in acute HIV infection (AHI) can reduce viral load to below detectable levels.

The reduction of plasma viremia may reduce HIV-1 immune response and emergence of HIV diagnostic markers in blood.

HIV infected infants who initiated treatment before 12 weeks of age frequently become HIV seronegative by 2 years of age, but have not cleared virus from latent reservoirs.

Examine evolution of HIV serological markers following early HAART Therapy

Study Populations and Assays

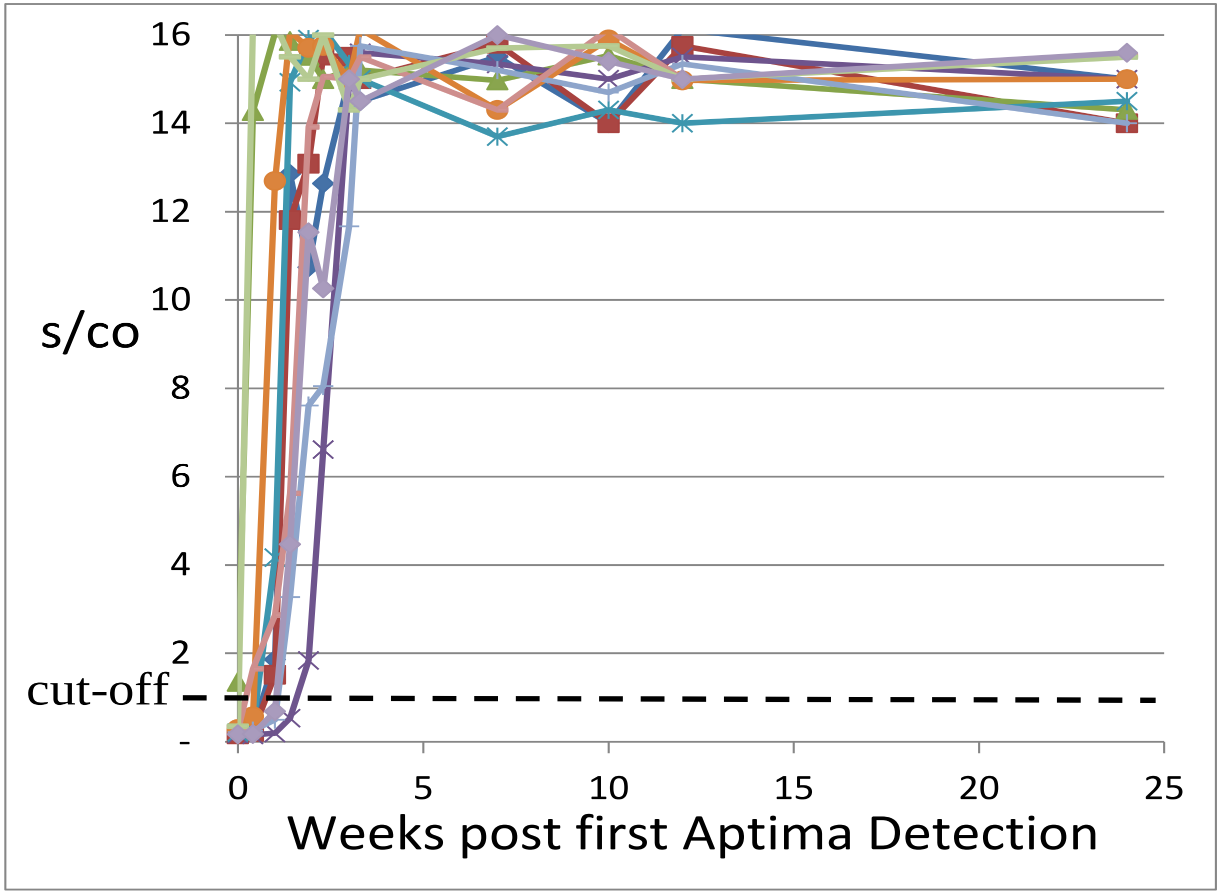
Evolution of HIV Markers in two Acute HIV Infection Cohorts

- RV217 - Untreated population
- RV254 – Initiated HAART Treatment during AHI

Viral markers were followed at various times post first infection

RNA	Screen – Aptima (Hologic)
	Viral Load – (Abbott m200)
p24 Ag	Bio-Rad p24 Ag (RUO)
4th Gen EIA	Bio-Rad Ab/Ag Combo
3rd Gen EIA	Bio-Rad EIA 1/2/O
Supplementary	Bio-Rad Western blot
	Bio-Rad MultiSpot

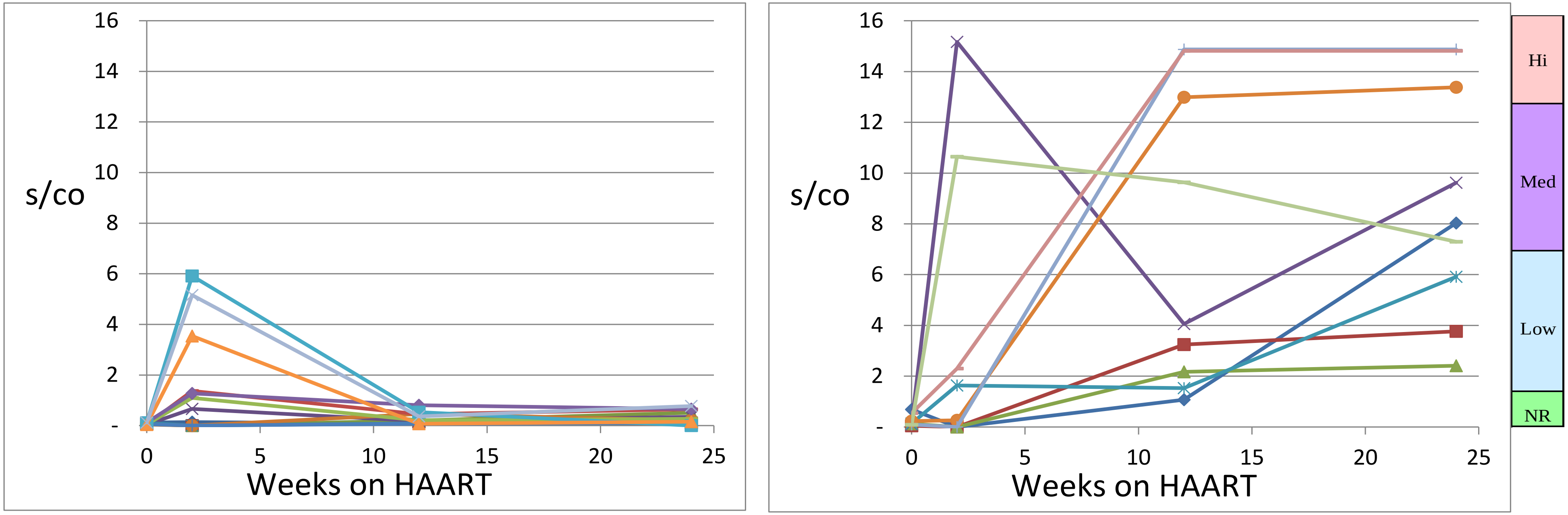
3rd Gen EIA signal in Untreated HIV Volunteers



Untreated Volunteers were Reactive by 3rd Gen EIA by 2 weeks with s/co >14.

Once seroconversion took place, all samples remained EIA Reactive throughout subsequent testing periods

3rd Gen EIA signal in Individuals Treated at Fiebig I



Treated at Fiebig I N = 20

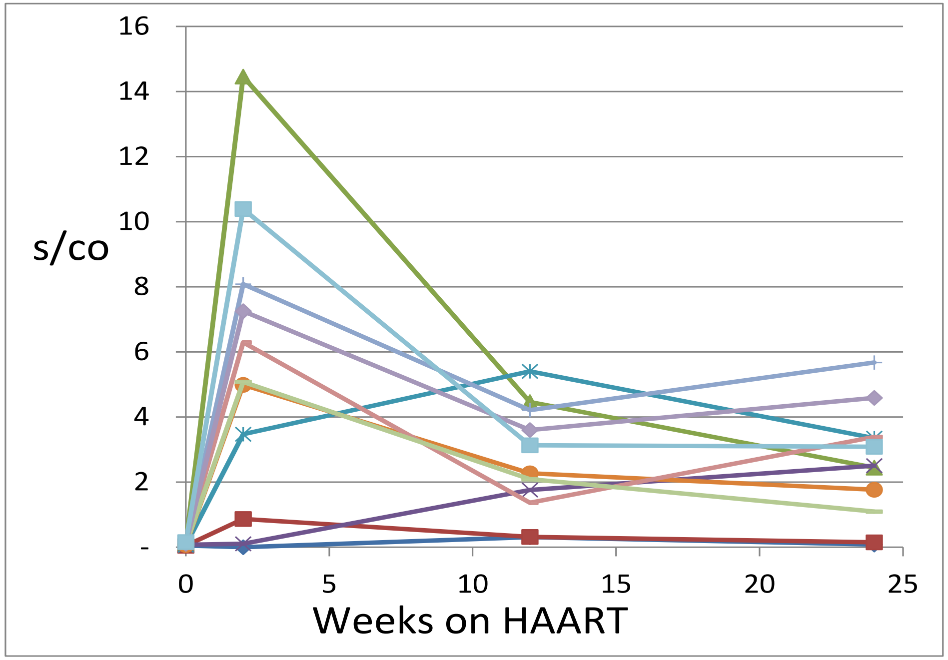
11 (55%) Non Reactive (NR) [6 (30%) Transient]

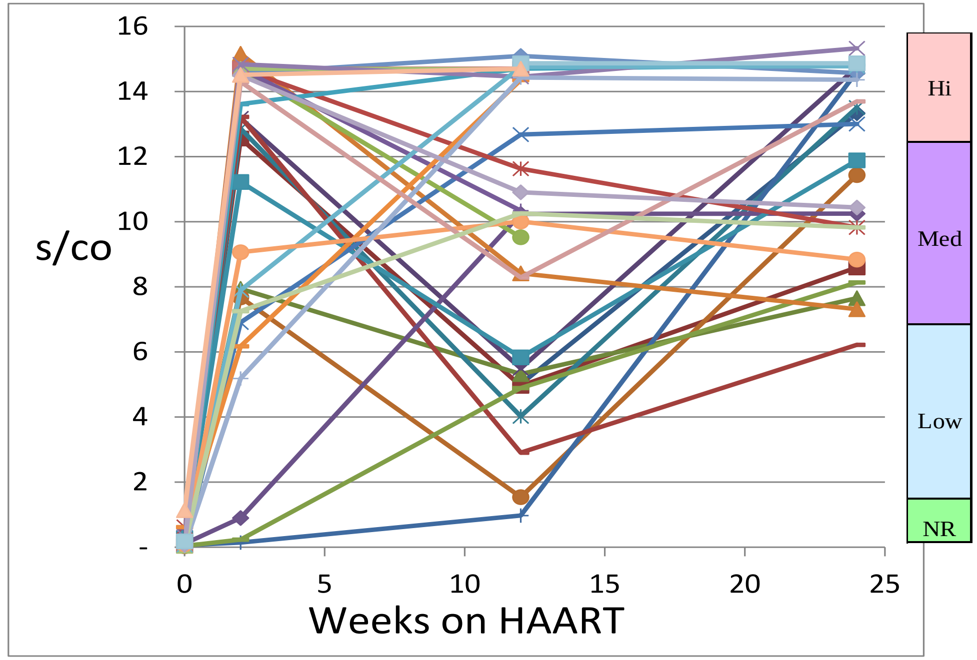
3 (15%), Low

3 (15%) Medium

3 (15%) High.

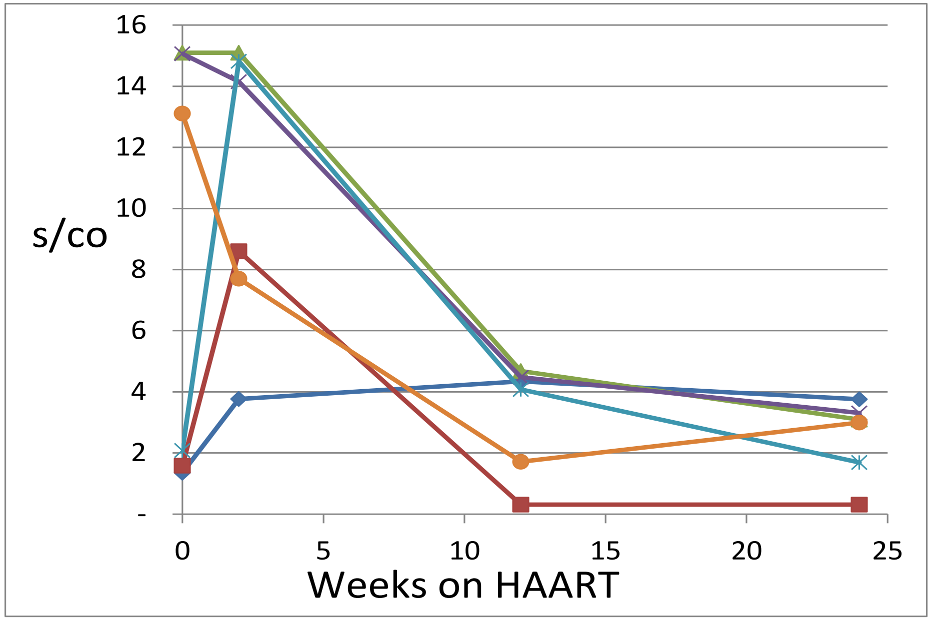
3rd Gen EIA signal in Individuals Treated at Fiebig II

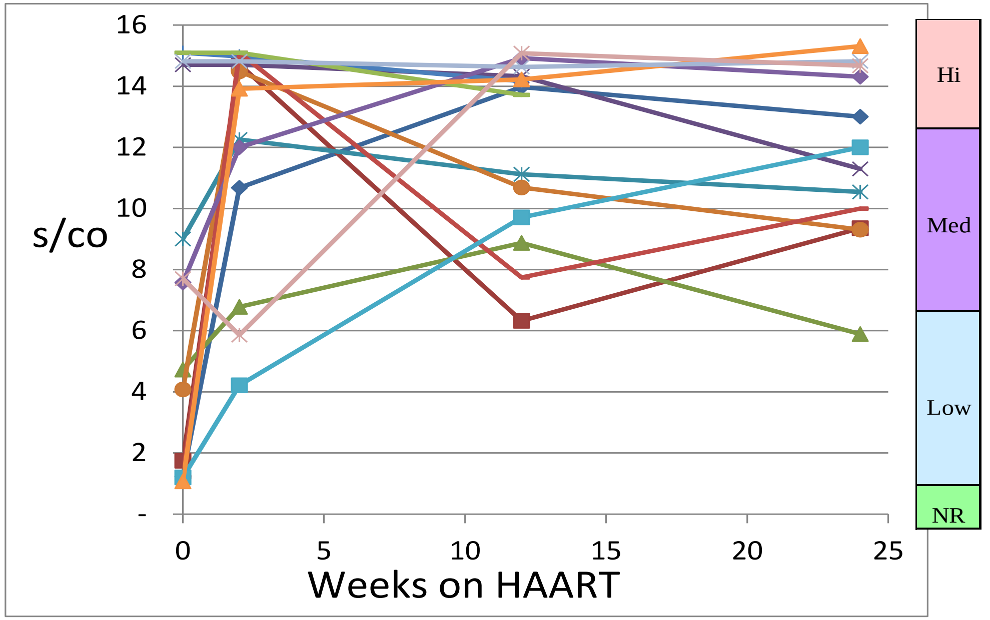




Treated at Fiebig II	N = 30
2 (6.7%)	NR
8 (26.7%)	Low
14 (46.7%)	Medium
6 (20%)	High

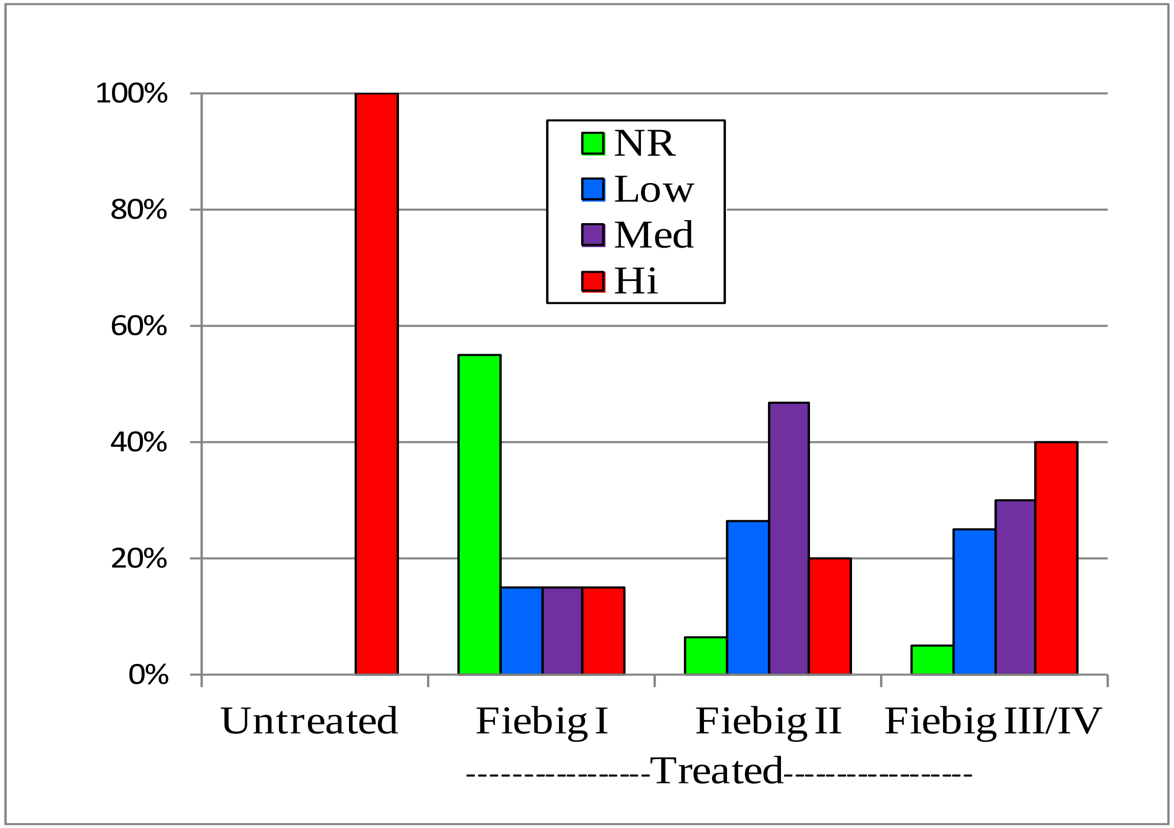
3rd Gen EIA signal in Individuals Treated at Fiebig III/IV





Treated at Fiebig III/IV	N = 20
1 (5.0%)	NR
5. (25.0%)	Low
6. (30.0%)	Medium
8 (40%)	High.

Delay/Reversion of 3rd Gen EIA following early HAART



EIA Reactivity by Wk 24

HAART treatment at Fiebig I - IV results in decreased seroreactivity at later times.

.

			3rd Gen EIA Reactivity (s/co)							
		N	NR (<1.0)		Low (1 - 50		Med (6 - 12)		High (>12)	
Untreated		24	0	0%	0	0%	0	0%	24	100%
Treated	Fiebig I	20	11	55.0%	3	15.0%	3	15.0%	3	15.0%
	Fiebig II	30	2	6.7%	8	26.7%	14	46.7%	6	20.0%
	Fiebig III/IV	20	1	5.0%	5	25.0%	6	30.0%	8	40.0%

Conclusions

In untreated individuals, serological markers evolve with time post infection:

- 4th Gen EIA (Ag/Ab Combo) reactive within 7 days,
- 3rd Gen (Ab) within 14 days, and
- WB Pos within 24 days post infection.

Treatment at Fiebig I blocks subsequent emergence of anti-HIV

- 72.7% of individuals remained serologically HIV negative by week 24

Treatment at Fiebig II caused delay or decrease of serological markers,

- 68.6% of individuals at Wk 12, and 70.7% by Wk 24.

Treatment at Fiebig III and IV results in delay/decrease in seroreactivity

- in 60% participants

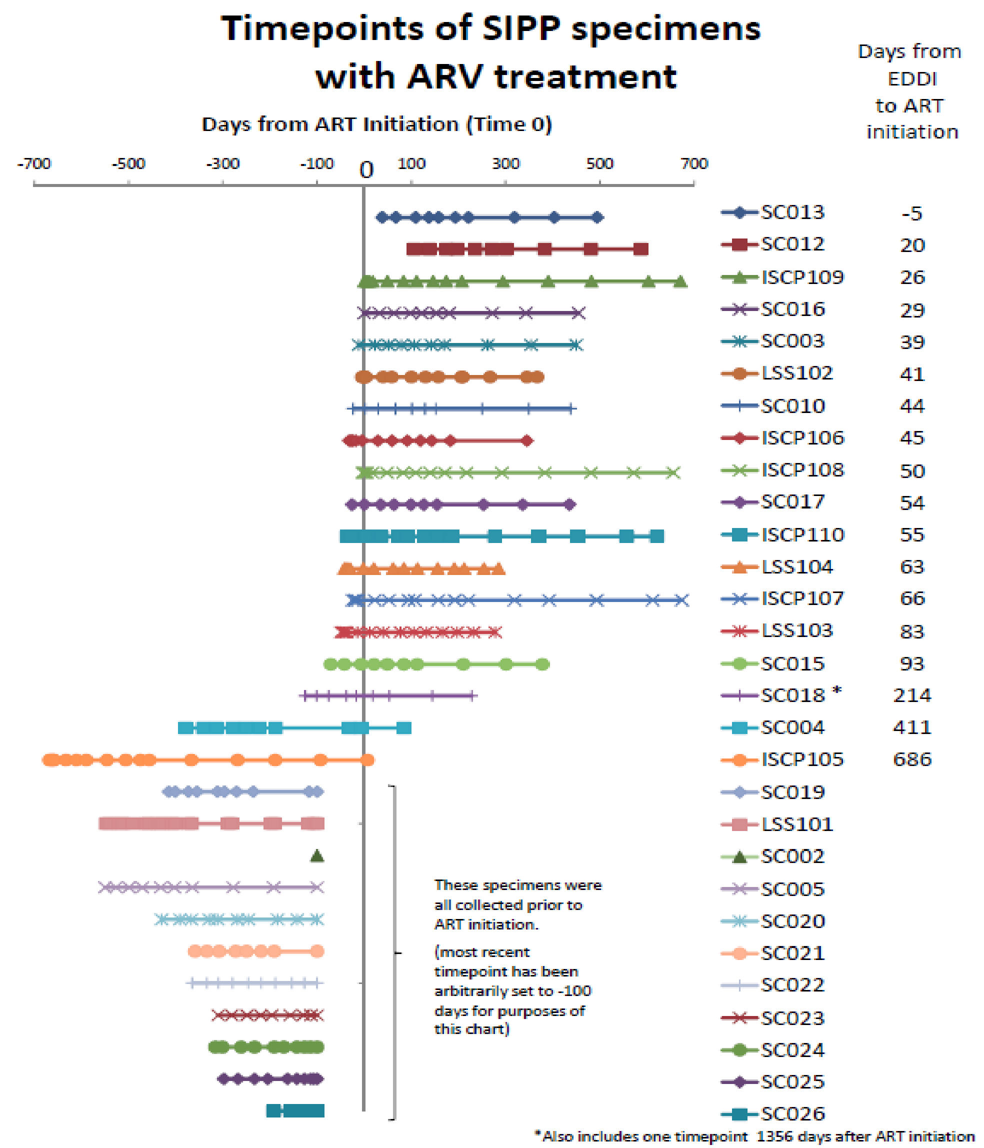
Western Blot reactivity is significantly delayed/reduced

- 45% of individuals had IND or Neg WB even after Wk 12 and 24.

Caution is urged in interpretation of negative serological signal in individuals on early HAART as absence of infection.

HIV Seroincidence Panel Project

(SIPP)



The Effect of Oral Pre-Exposure Prophylaxis (PrEP) on the Progression of HIV-1 Seroconversion: The Known Unknowns

Deborah Donnell PhD, Eric M Ramos MD MSc, Connie Celum MD MPH, Jared M Baeten MD PhD, Joan Dragavon MLS, Jordan Tappero MD MPH, Jairam R Lingappa MD PhD, Allan Ronald MD, Kenneth Fife MD PhD and
Robert Coombs MD PhD FRCPC for the Partners PrEP Study Team

Centers for Disease Control, Indiana University
University of Manitoba and University of Washington

Background

- Six randomized clinical trials of tenofovir disoproxil fumarate (TDF) alone or in combination with emtricitabine (TDF/FTC) [TRUVADA®] used as pre-exposure prophylaxis (PrEP) for HIV-1 infection have shown a reduced risk of HIV-1 acquisition between 44% and 87%
- Success of PrEP is associated with antiretroviral drug adherence
- Primate model suggests that PrEP reduces viral load associated with break-through of $SHIV_{SF162P3}$ infection but has little impact on the timing of seroconversion or neutralizing antibodies; however, maturation of antibody avidity is delayed

(Curtis et al., *J Acquir Immune Defic Syndrome* 2011;57:355-362)

Background (cont'd)

- It is biologically plausible that PrEP could affect the appearance and concentration of biomarkers of acute HIV-1 infection (AHI) – HIV-1 RNA, p24 antigen, HIV-1 antibodies – i.e., Fiebig Stage
- PrEP could result in the delay or attenuation or perhaps even skipping the detection of expected biomarkers of AHI
- <u>These potential effects of PrEP would have implications for the routine laboratory diagnosis of acute/early HIV-1 infection</u>

Partners PrEP study

- Enrolled 4,747 HIV serodiscordant couples in Kenya and Uganda between July 2008 and November 2010 (Baeten et al., *Lancet Infect Dis 2014; 14:1055-64*)
- Randomized 1:1:1 to FTC/TDF:TDF:Placebo
- Stopped early for efficacy
  - re-randomized Placebo 1:1 to FTC/TDF:TDF after DSMB meeting July 2013
- N = 138 seroconversions observed

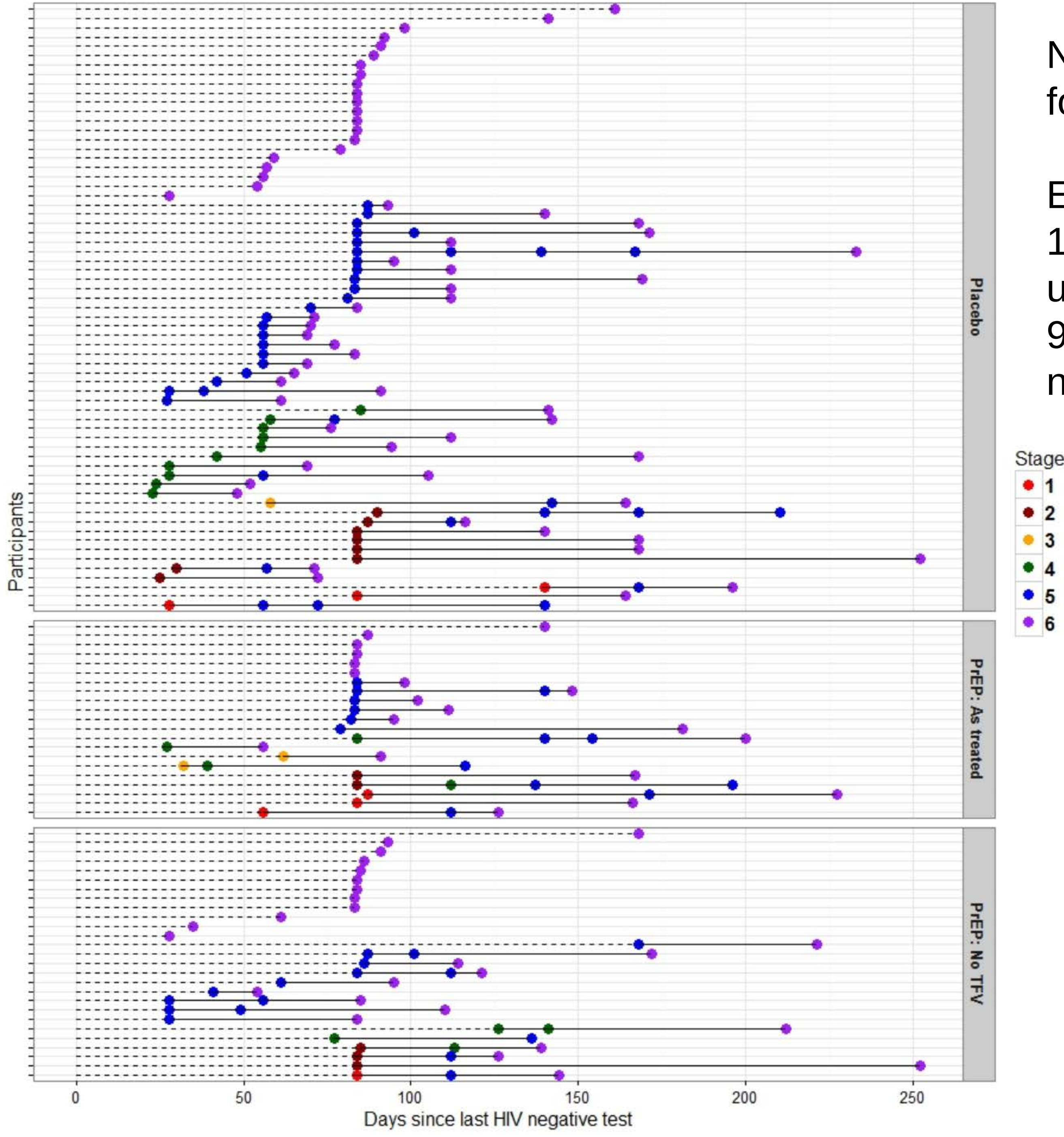
Specific Aims

- ***Hypothesis****: PrEP will delay the expected biomarker-defined Fiebig stages of acute/recent/early HIV-1 infection for human clinical trial subjects infected with non-clade B virus*
  - Assess whether time to progress through Fiebig stages was affected by PrEP
  - Assess whether virologic or antibody response was affected by PrEP
  - Assess an association of PrEP with a delay in detection of seroconversion by the site versus the central laboratory

Seroconverter characteristics (N=138)

	PrEP (N = 67)	Placebo (N = 71)
Male	27 (40%)	37 (52%)
Viral load of partner (median RNA log10 copies/mL)	4.33	4.43
Age (median, IQR)	31	30
Infected at Randomization	9 (14%)	6 (8%)
No site HIV test for >100 days prior to first HIV-infected visit	9	0
Time to detect seroconversion at site*	N = 58	N = 71
0 days	21 (36%)	36 (51%)
Within 100 days	27 (47%)	31 (44%)
>100 days	10 (17%)	4 (6%)

*Time from first HIV-infected sample to site detection of seroconversion



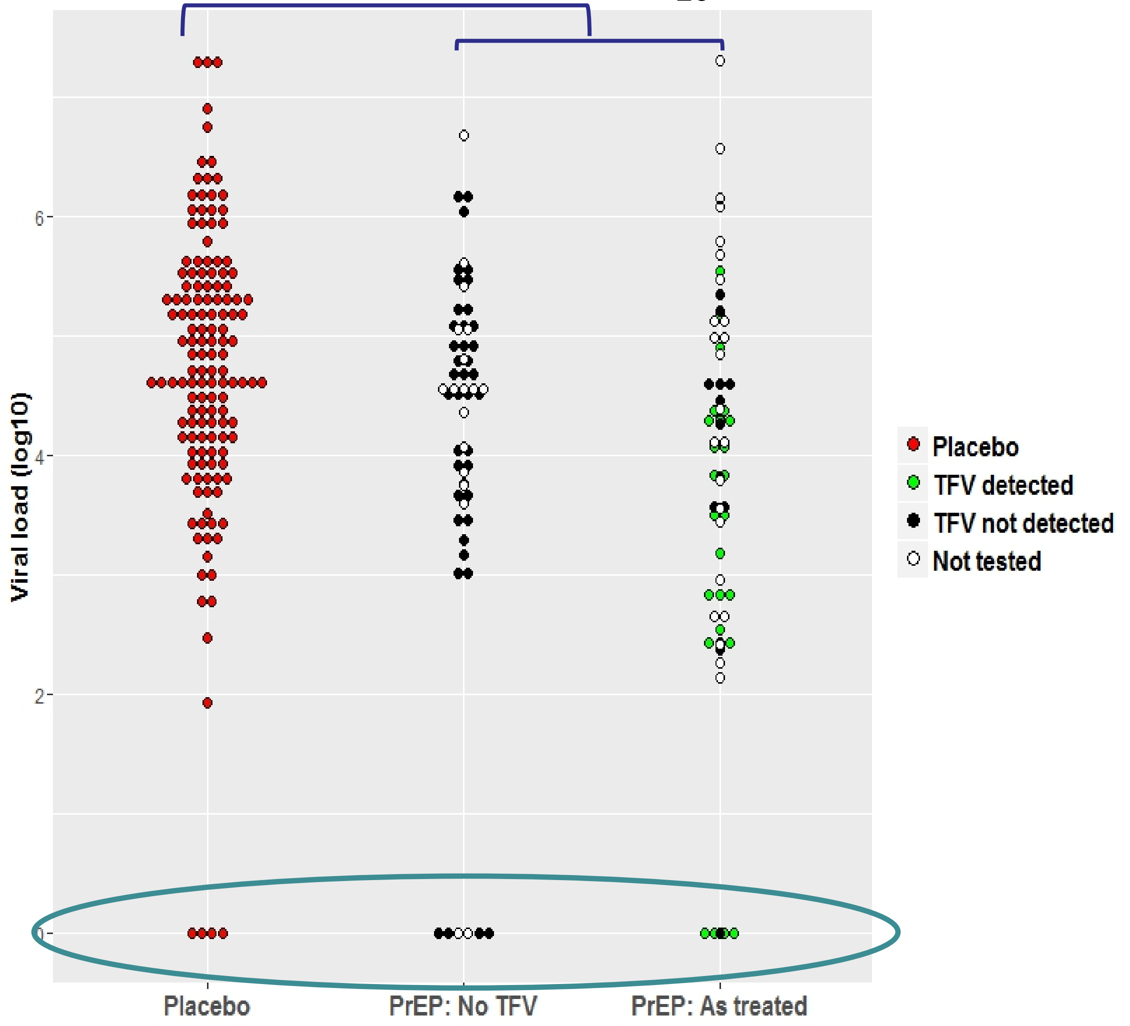
N=138 (113 included for analysis)

Exclusion of 25:
16 had no HIV-uninfected samples;
9 had >100 days with no site HIV test

HIV-1 RNA level for all samples during seroconversion

As treated: $\log_{10}$ VL change = -0.74, $p < 0.001$ (5.5-fold)

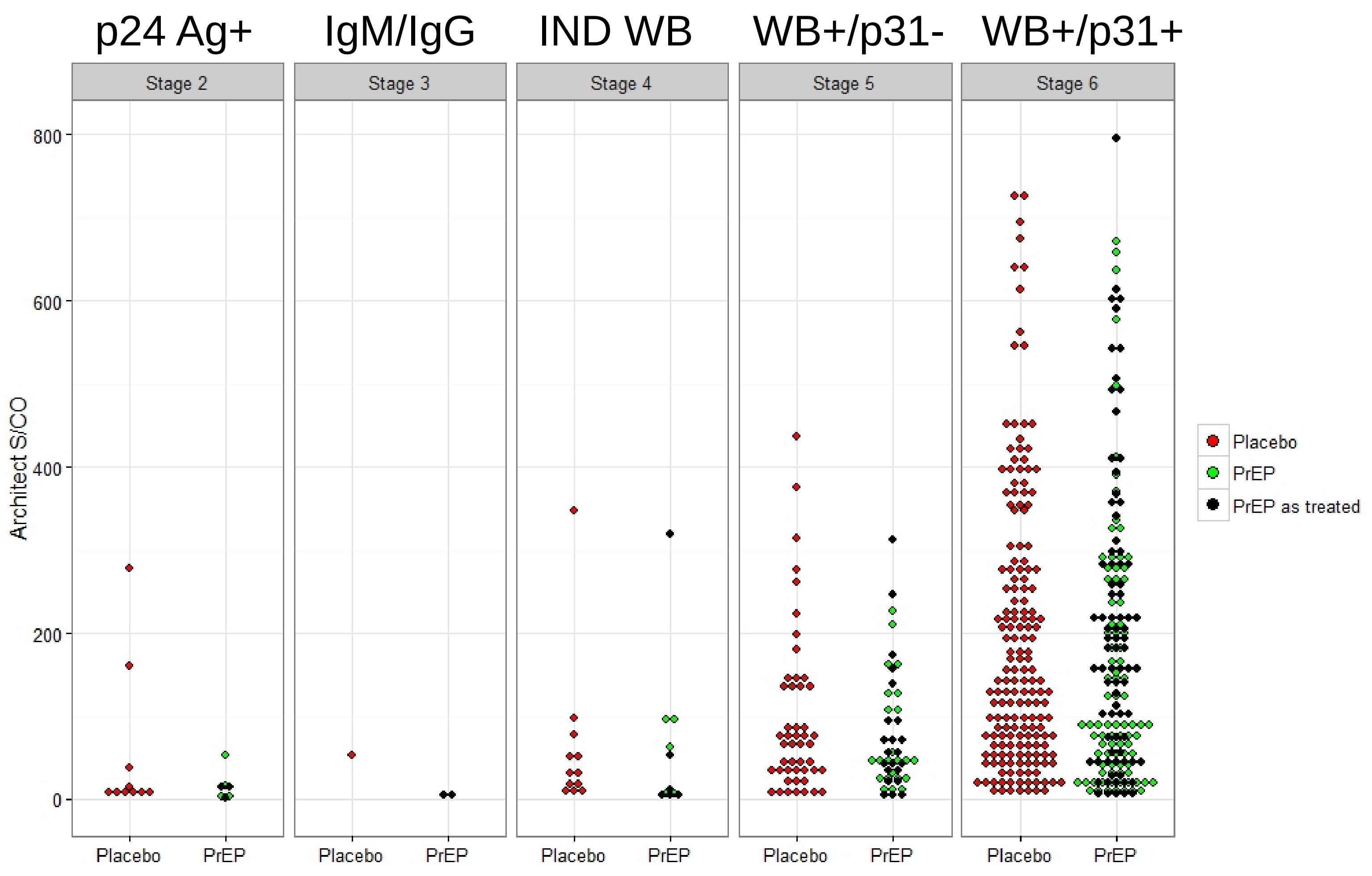
As randomized: $\log_{10}$ VL change = -0.64, $p < 0.001$ (4-fold)



N=134

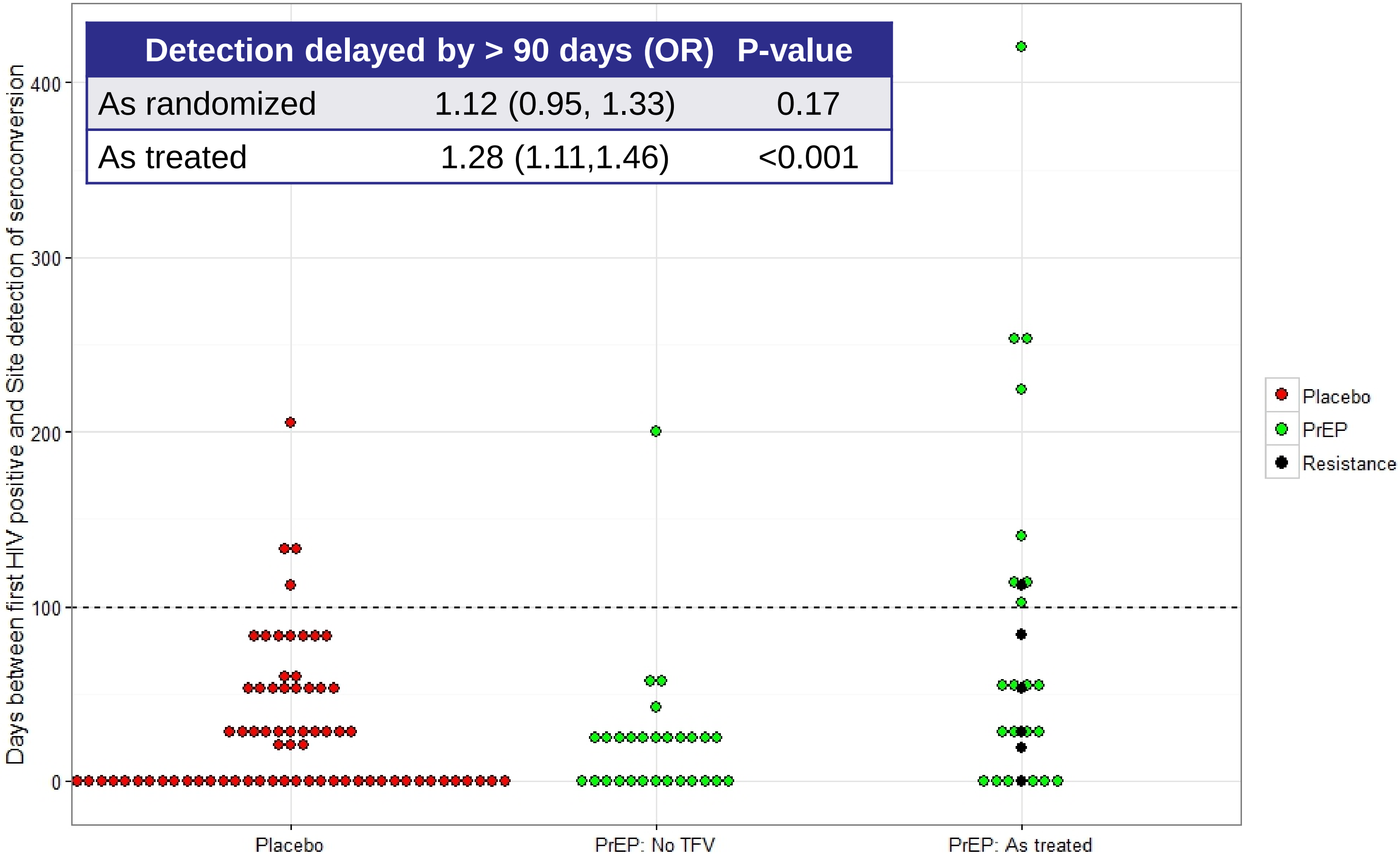
N=121

HIV-1 antibody response was not affected by PrEP



Time between first HIV-infected sample tested positive by Central Laboratory and Site detection of seroconversion before and after 90 days (N=129)

Detection delayed by > 90 days (OR)		P-value
As randomized	1.12 (0.95, 1.33)	0.17
As treated	1.28 (1.11,1.46)	<0.001



Summary

- PrEP during seroconversion
  - Slightly slower progression through Fiebig stages
  - Modest decrease in viral load
  - Some participants had delayed seroconversion
- Antibody response not changed by PrEP
- Regardless of PrEP, rapid tests were not highly sensitive for detection of acute/early infection

Antigen/Antibody HIV Screening Tests are Crucial for Pre-Exposure Prophylaxis Programs

Delaugerre et al. JID in press

- 488 high-risk MSM in IPERGAY Trial (949 pys)
- 31 diagnosed with HIV infection (Architect Ag/Ab Combo)
  - 13 infected at screening or at randomization visit
  - 19 acquired infection during PREP monitoring (q1 month)
    - 16 placebo; 2 TDC/FTC; one during open phase;
  - Prior samples tested for RNA and 2 positive
- Sensitivity of Ag/Ab combo assays:
  - EIA 4G Architect and BioPlex: 83% (95% CI: 76-99%)
  - VIKIA: 54% (34-72%)
  - AUTOTEST: 50% (31-69%)
  - ALERE: 78% (59-91%)

Acknowledgements

BSRI
Sheila Keating
Mila Lebedeva
Dylan Hampton
Celine Chang
Mars Stone
Mohamed Mohsen
Satish Pillai

CDC
Kelly Curtis
Michelle Owen

NHLBI
Simone Glynn
Steve Kleinman

SACEMA
Alex Welte
Eduard Grebe

Public Health England
Gary Murphy

UCSF
Chris Pilcher
Steve Deeks
Peter Bacchetti
Sulggi Lee
Rick Hecht
Shelley Facente
Kara Marson

ACTG
Raj Gandhi
Jonathan Li
Ron Bosch
John Mellors
Bob Coombs

WRAIR MHRP
Mark Manak
Sheila Peel

REDS-III

BILL & MELINDA GATES foundation

CEPHIA

Blood Systems Research Institute

Public Health England

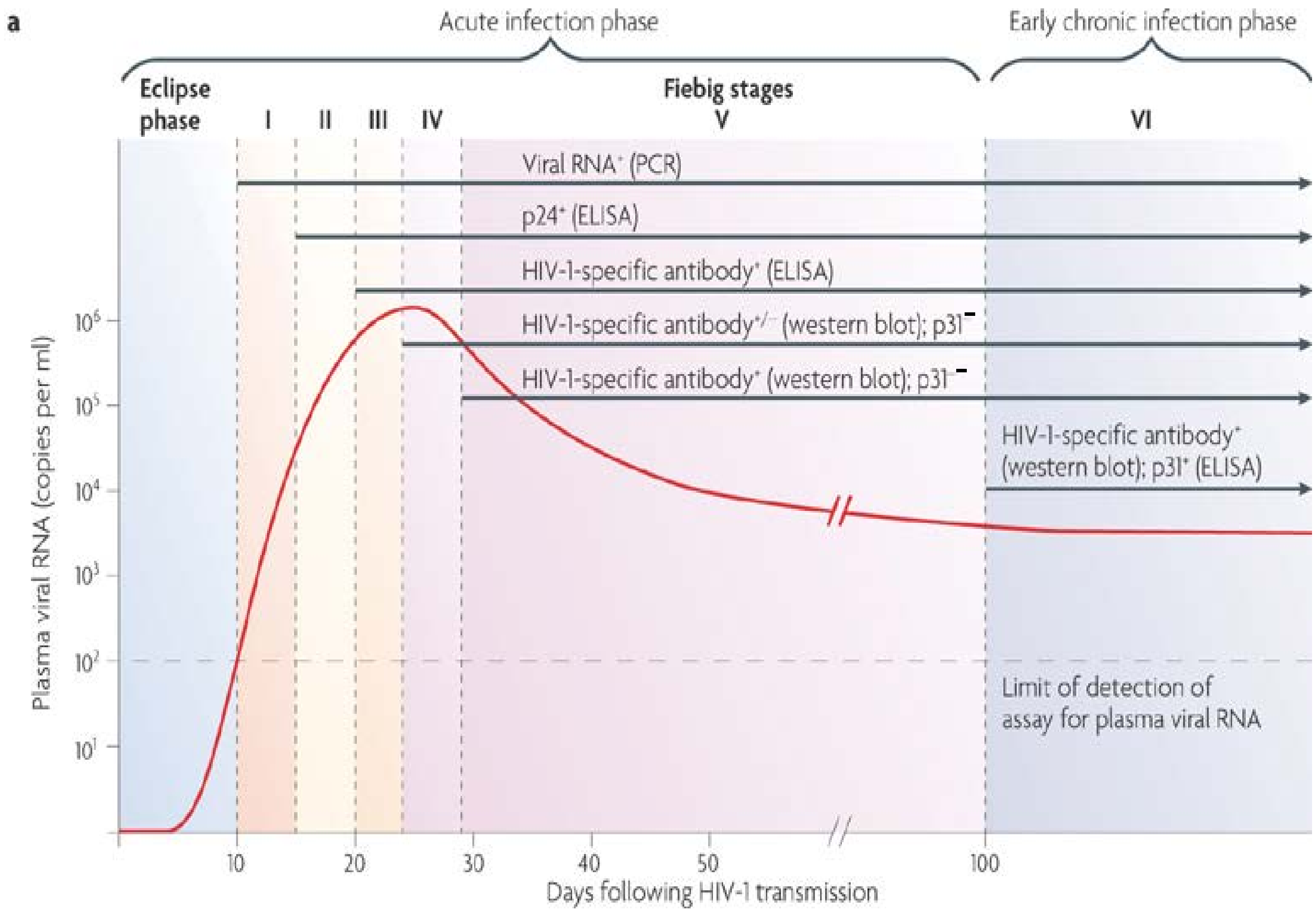
SACEMA

UCSF University of California San Francisco

Delaney AIDS Research Enterprise DARE to find a cure

Fiebig Staging Laboratory Methods (after Fiebig et al., *AIDS* 2003; 17:1871-79)

Period of full seroconversion ~ 3-4 months



HIV-1 Assays

RNA: Abbott m2000rt

P24: ARCHITECT or Bio-Rad HIV-1/2

IgM/IG Ab: Multispot HIV-1/2

WB IND

WB -p31

WB +p31

Genetic Systems