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Research Institute

Minimal Infectious Dose via Transfusion

HEV

Effects of early ART treatment and PrEP on laboratory indices of HIV infection: Implications for blood safety

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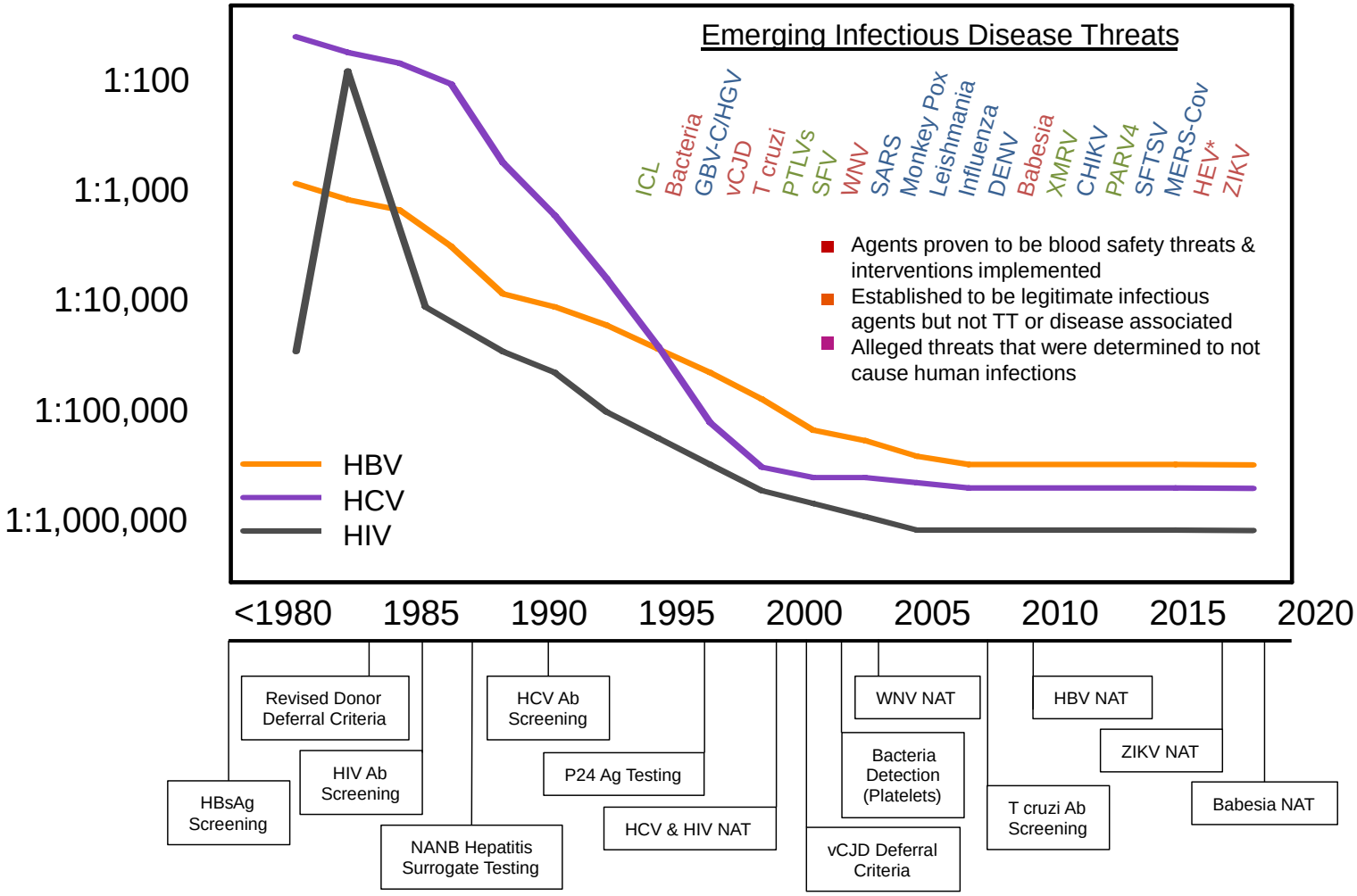
Risks of major transfusion-transmitted viral infections and emerging infectious agents of concern to blood safety



Busch MP, Bloch EA, Kleinman S. Prevention of Transfusion Transmitted Infections. Blood 2019

Infectivity of human immunodeficiency virus-1, hepatitis C virus, and hepatitis B virus and risk of transmission by transfusion

Steven H. Kleinman, Nico Lelie, and Michael P. Busch

TABLE 1. Theoretical factors that could influence the infectivity of a transfusion

Viral properties	Viral load
	Viral genotype/clade
	Genome mutations
	Ratio of detectable nucleic acid equivalents to infectious particles
	Stage of viral infection (acute versus chronic)
	Binding of virus to endogenous neutralizing antibodies
Transfusion factors	Diminishing viral replicative capacity with component storage
	Transfusion of other components with neutralizing antibody
Recipient factors	Existing immunity through natural infection or vaccination (HBV)
	Lack of viral receptors (i.e., resistance to infection)
	Degree of immunosuppression
	Body weight and/or blood volume

Kleinman SH, Lelie N, Busch MP. Transfusion 2009:49:2454-89

Infectivity of human immunodeficiency virus-1, hepatitis C virus, and hepatitis B virus and risk of transmission by transfusion

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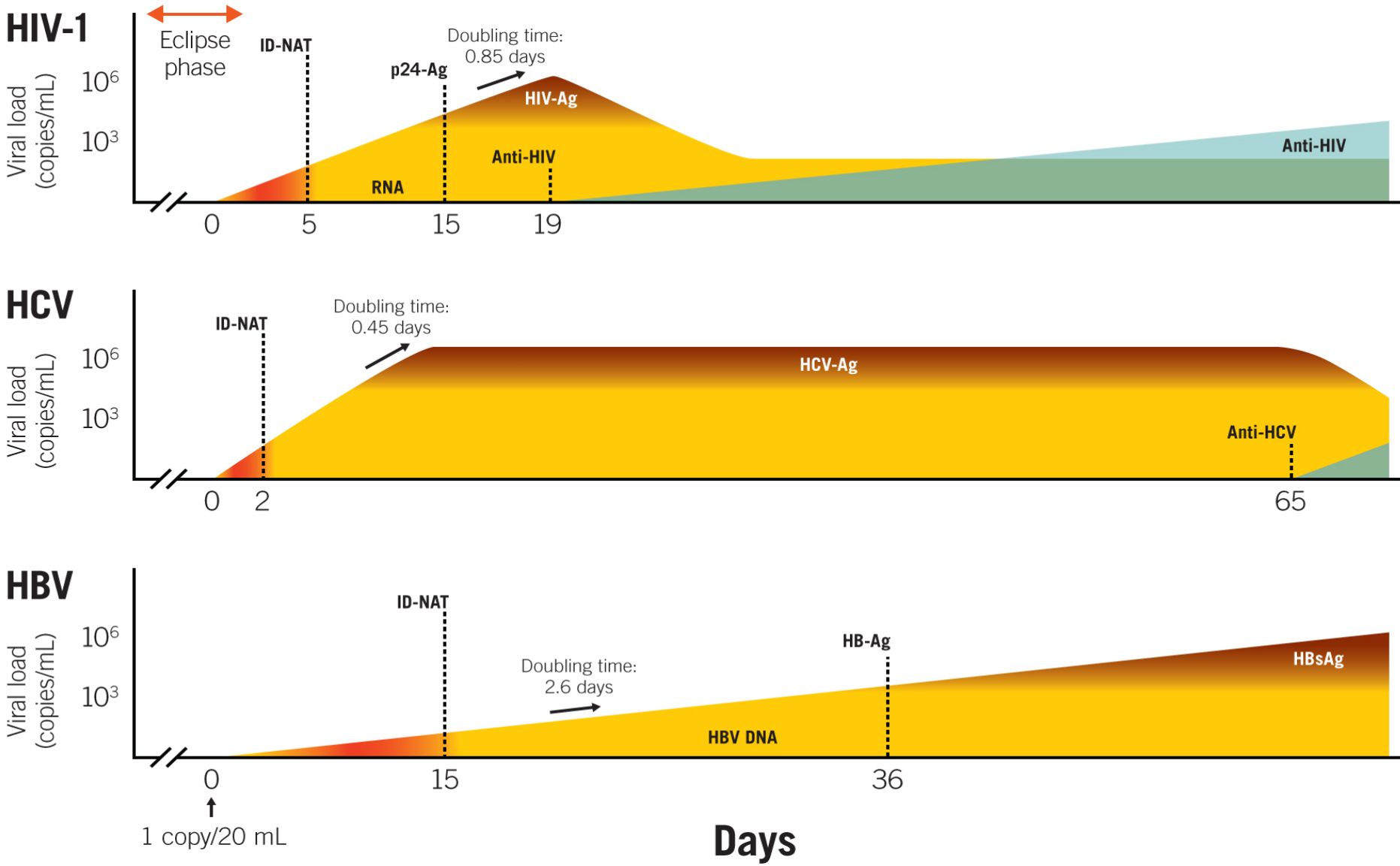
TABLE 3. Application of various methods of study to the infectivity of HIV-, HCV-, and HBV-infected blood components

Method	HIV	HCV	HBV
In vitro cell culture systems	Initial experiments performed in late 1980s	NA	NA
Animal model systems	Experiments with macaques inoculated intravaginally with SIV	Chimp experiments with human strains	Chimp experiments with human strains: human liver chimeric (transgenic) mice infected with human strains passed through chimps
Human case investigation (individual case lookback)	At least 10 donor units from early window period infection have been evaluated (see Tables 5A and 5B)	At least 4 donor units from early window period infection have been evaluated (see Table 7)	
Systematic transfusion transmission or lookback studies	Performed for transfused HIV antibody–positive components	Performed for transfused HCV antibody–positive components	Performed for a limited number of window phase and chronic occult carrier units

Kleinman SH, Lelie N, Busch MP. Transfusion 2009:49:2454-89

Early dynamics of viral markers & infectivity



Fiebig EW et al. Aids. 2003;17: 1871-9. Bruhn R et al. Transfusion 2013:53:2399-2412
Glynn SA, et al. Transfusion 2005;45: 994-1002. Bruhn R et al Transfusion. 2015 [Epub ahead of print]
Tsoi W-C et al. Transfusion 2013;53:2477-2400. Weusten J et al. Transfusion 2011;51:203-15

Interpretation of pathogen load in relationship to infectivity and pathogen reduction efficacy

Jeffrey McCullough,[1] Harvey J. Alter,[2] and Paul M. Ness[3]

TABLE 1. Factors that could influence the infectivity of a transfusion

Viral factors
Viral load
Viral genotype/clade
Genome mutations
Ratio of detectable nucleic acid equivalents to infectious particles
Stage of viral infection (acute vs. chronic)
Binding of virus to endogenous neutralizing antibodies
Diminishing viral replicative capacity with component storage
Donor factors
Stage of infection
Presence of antibody to the agent
Transfusion factors
Transfusion of other components with neutralizing antibody
Blood component being transfused (see below)
Product factors
Specific component (eg RBCs or FFP)
Storage duration of RBCs
Patient related factors
Existing immunity through natural infection or vaccination (HBV)
Lack of viral receptors (i.e., resistance to infection)
Degree of immunosuppression
Body weight and/or blood volume

Adapted from Kleinman et al.[4]
FFP = fresh frozen plasma; HBV = hepatitis B virus.

TABLE 2. Levels of circulating agent at different stages of infection and infectivity for HIV (+ indicated infections have been reported but rate not specified)

Infectious agent	Number of agents/mL: Range	Median	Units of measurement		Reference
Circulating					
Window	82 to 3×10^7		NR		20,26
	1–2400		Copies		6
Seroconversion	<1600 to 1.6×10^6		Copies		27
	10^3 and 10^8		Copies		16,26,27
	<1600 – 3×10^7		Copies		20,26
	1–106		Copies		28
Infectivity				Infectivity	
	5 to 246		Copies	90	4
	187; 126		geq	+	26
	10 3.6		Copies	+	15
	3.7 and 7.5		IU	+	29
	20 and 1000		Copies	+	6
	5–1000		Copies	+	4,6,30
		400	Virions	50%	4
		10^3	Copies	None	15
		400	Virions	50%	4
	10^4–10^6		Copies	None	4

geq = genome equivalent; IU = international unit; NR = not reported.

TABLE 3. Levels of circulating agent at different stages of infection and infectivity for HBV

Infectious agent	Number of agents/mL: Range	Median	Units of measurement	Reference
Circulating Levels				
Pre–ramp-up	5–100		geq/mL	32
Ramp-up	10–10^6		geq/mL	32
Window		1	IU	33
	1–2,400		Copies	6
	10–860		Copies	23
	10–100		Copies	34
	12–1,460	126	IU	35
		66% < 20	IU	35
		1.3	Copies	34
Seroconversion	3,000–15,000		NR	6,27
		<11	IU	36
	200–61,000		Copies	4
		<70	IU	37
	<20–1,460		IU	35
Anti-HBc neg	10–1460??	126	IU	35
MP+ ID+		17,000	Copies	38
MP– ID+		1,470	Copies	38
MP– ID–		200	Copies	38
Chronic	1.3–400		Copies	39
	12–310		IU	35
		<300	Copies	23
		95% < 100	Copies	23
	1–166		IU	33
	10–860		Copies	23
	20,000 – 90,000		Copies	4
Anti-HBc pos:	10–100		Copies	34,40
		1.3	Copies	34

Anti-HBc = antibody to hepatitis B core antigen; ID = individual donor; IU = international units; MP = mini-pool; NR = not reported.

TRANSFUSION 2019;59;1132–1146

HIV-1 breakthrough transmission cases

Test Failure Breakthrough Cases											
Country	**Blood Product**	**Screening Assay**	**50% LOD**	**95% LOD**	**Pool size**	**Cp/mL**	**Viral load method**	**plasma mL**	**Estimated copies**	**Recipient Infected**	**Reference**
Germany	RBC	In-house			96	6400	TaqMan	20	**128,000**	**Yes**	Schmidt M et al. Transfusion 2009;49:1836-1844
Germany	RBC	In-house	4.2	22.1	96	12800	TaqMan	20	**256,000**	**Yes**	Chudy M et al. Transfusion 2012;52:431-9
Viral Load Below Detectable Level											
Japan	FFP	TaqScreen	4.1	28.0	20	4.1	50% LOD	240	**984**	**Yes**	Shinohar N. Transfusion 2014;54:2361-2
USA	FFP	Duplex	2.1	15.6	16	33.6	50% LOD	200	**6720**	**Yes**	Phelps R et al. Transfusion 2004;44:929-933
USA	FFP	Duplex	2.1	15.6	16	33.6	50% LOD	200	**6720**	**Yes**	Stramer S et al. Transfusion 2003;43:Supplement :40-41A
Spain	FFP-MB	Ampliscr	4.3	22.1	44	135	Monitor	261	**35235**	**Yes**	Alvarez et al. Transfusion 2016;56;831–836
Germany	PLT	In-house			24	0	TaqMan	240	**0.001**	**No**	Kalus U et al. , Transfusion 2009;49:435-439
Singapore	PLT	No NAT				50	Probit	25	**1250**	**Yes**	Ling AE et al. JAMA 2000; 28:4:210-214
Denmark	PLT	In-house			96	246	Abbott RT	25	**6150**	**Yes**	Harritshoj Let al. Transfusion 2008;48:2026-28
Spain	PLT	Ampliscr	4.3	22.1	44	135	Monitor	65	**8775**	**Yes**	Alvarez et al. Transfusion 2016;56;831–836
S. Africa	RBC	Ultrio	2.7	18.4	1	2.7	50% LOD	20	**54**	**Yes**	Vermeulen et al. Transfusion 2019
Japan	RBC	TaqScreen	4.1	28.0	20	4.1	50% LOD	20	**82**	**No**	Shinohar N. Transfusion 2014;54:2361-2
Brazil	RBC	in house		22.6	24	4.4	probit	20	**87**	**Yes**	Salles NA. Transfusion 2013;53:2593-5
Greece	RBC	No NAT	2.1	15.6	4	8.4	50% LOD	20	**168**	**Yes**	Hatzakis et al. (personal communication, 2006)
Germany	RBC	Gfe Blut			96	23.6		20	**472**	**No**	Muller B. Transfusion. 2013;53:2422-30
Thailand	RBC	TaqScreen	4.1	28.0	6	24.6	50% LOD	20	492	Yes	Rujirojindakul P. Vox Sang 2015:109 Suppl 1 226 P-408
S. Africa	RBC	No NAT				31	Probit	20	620	No	Ferriera MCet al. Transfusion 2006;46:156-157
S. Africa	RBC	No NAT				31	Probit	20	620	No	Ferriera MCet al. Transfusion 2006;46:156-157
USA	RBC	Duplex	2.1	15.6	16	33.6	50% LOD	20	**672**	**Yes**	Phelps R et al. Transfusion 2004;44:929-933
USA	RBC	Duplex	2.1	15.6	16	33.6	50% LOD	20	**672**	**No**	Stramer S et al. Transfusion 2003;43:Supplement :40-41A
USA	RBC	Duplex	2.1	15.6	16	33.6	50% LOD	20	**672**	**Yes**	Laffoon et al, MMWR 2010;59:1335-6
France	RBC	NuclAmpl	1.9	15.5	24	45.6	50% LOD	20	**912**	**Yes**	Najiouallah F et al. J Med Virol 2004;73:347-349
Singapore	RBC	No NAT				50	Probit	20	**1000**	**Yes**	Ling AE et al. JAMA 2000; 28:4:210-214
Italy	RBC	No NAT				98	TaqMan	20	**1960**	**No**	Zanetti et al. Transfusion 2007;47:1328-1329
Spain	RBC	Ampliscreen	4.3	22.1	44	135	Monitor	20	**2700**	**No**	Alvarez et al. Transfusion 2016;56;831–836
USA	RBC	In-house			24	180	NGI [b]	20	**3600**	**Yes**	Delwart EL et al. Vox Sang 2004;86:171-177
Denmark	RBC	In-house			96	246	Abbott RT	25	**4920**	**Yes**	Harritshoj Let al. Transfusion 2008;48:2026-28
Japan	RBC	AmpliNAT	10.4	43.6	50	520	50% LOD	20	**10400**	**Yes**	Satake M et al. Journal of Hematology 2004;80:306-10

MID_{50} estimated at ~ 50 virions by probit analysis

Nico Lelie, Lelie Research

Hepatitis E risks: pigs or blood—that is the question

TRANSFUSION 2017;57;267–272

Richard S. Tedder,[1,2,3] Samreen Ijaz,[1] Alan Kitchen,[2] Ines Ushiro-Lumb,[2] Kate I. Tettmar,[2] Patricia Hewitt,[2] and Nick Andrews[4]

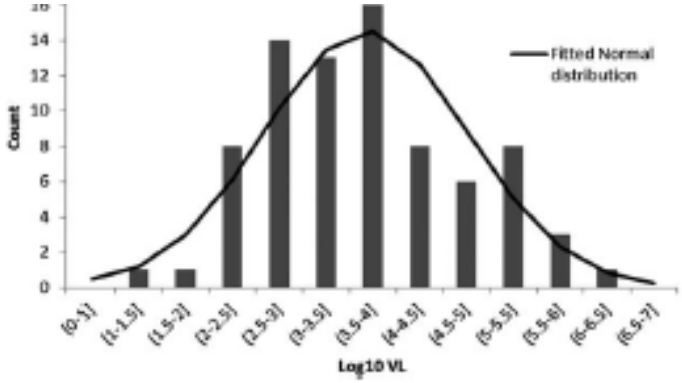


Fig. 1. Log normal distribution of the HEV level (log IU/mL) detected at pickup in 79 donors found to have HEV RNA in their plasma at the time of donation.

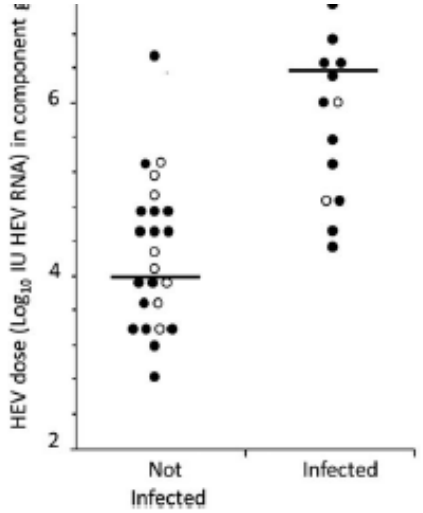


Fig. 2. Spread of the HEV dose (log IU HEV RNA) in those transfused components that gave rise to infection and those that did not. (○) Presence of detectable antibody to HEV in the donation; solid bar indicates the median viral load.

TABLE 1. Blood components ranked by included plasma volume, showing the minimum donor plasma viral load that could be expected to constitute the observed lowest infectious dose of 20,000 IU

Component	Included plasma volume	Minimum load (IU/mL) in donor for infection to occur in recipient
Pooled granulocytes	10	2000
RBCs in AS	Mean, 12.5	1600
PLT individual preparation	25	800
Apheresis PLTs	180	111
Plasma contributing to a PLT pool	225	89
FFP	275	73

Cost-effectiveness of the screening of blood donations for hepatitis E virus in the Netherlands

TRANSFUSION 2017;57;258–266

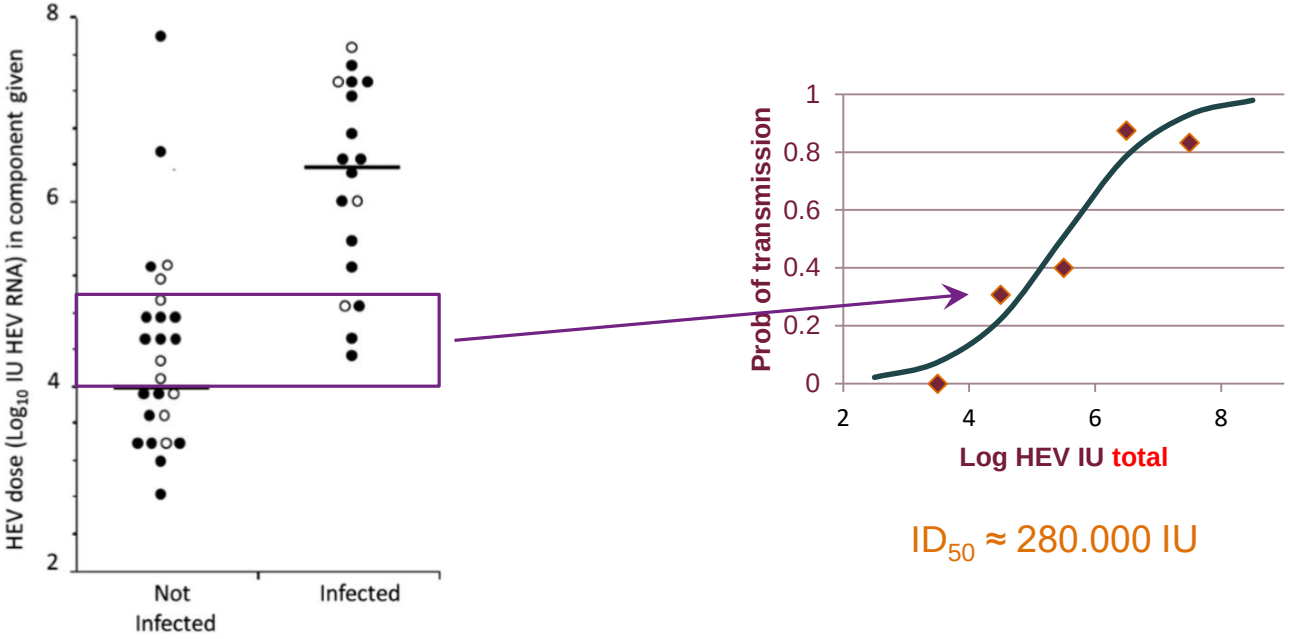
Anneke S. de Vos,[1] Mart P. Janssen,[1] Hans L. Zaaijer,[2] and Boris M. Hogema[2]

Viral load dependent probability of HEV transmisison

42% of transfused HEV positive blood products caused transmission

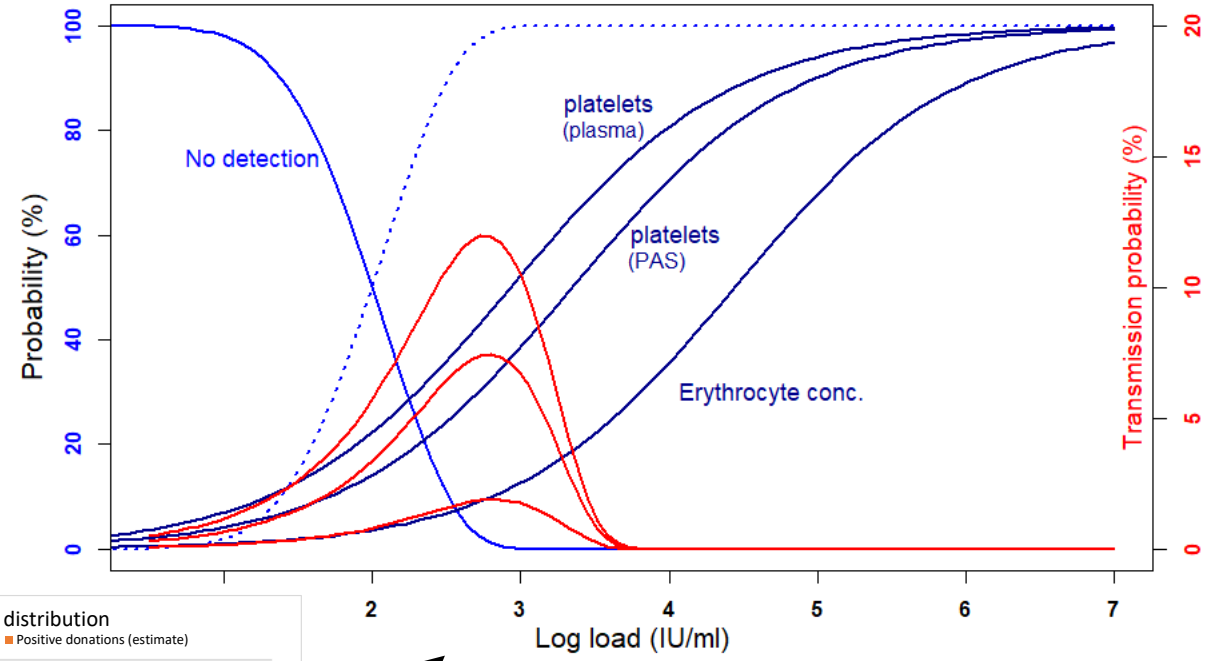
Infectious dose is higher in infectious transfusion

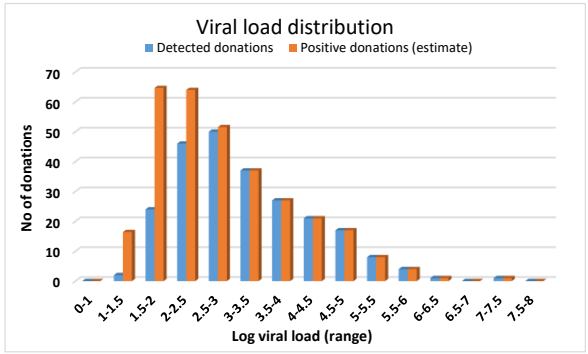
Estimated probablitly of transmission can be calculated



$ID_{50} \approx 280.000$ IU

Residual risk of HEV transmission by NAT screened blood products





Plasma volumes:

- 10 ml (ery)
- 124 ml (platelets stored in PAS
- 322 ml (platelets stored in plasma)

- Unknown what products would have been made from HEV+ donations
- We do know the percentage of donations processed into each type of blood product

Transfusion-Transmitted Hepatitis E: NAT Screening of Blood Donations and Infectious Dose

Front. Med. 5:5.
doi: 10.3389/fmed.2018.00005

Jens Dreier, Cornelius Knabbe and Tanja Vollmer*

Institut für Laboratoriums- und Transfusionsmedizin, Herz- und Diabeteszentrum Nordrhein- Westfalen, Universitätsklinik der Ruhr-Universität Bochum, Bad Oeynhausen, Germany

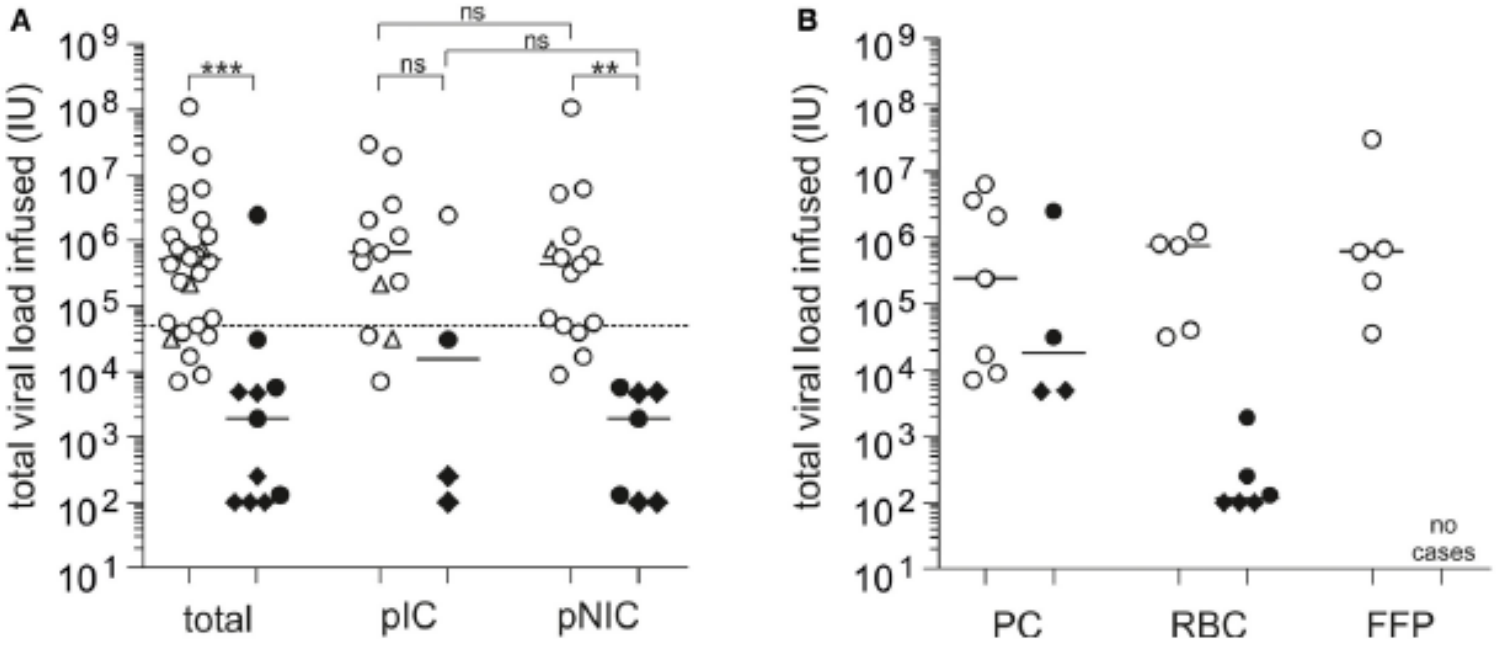


FIGURE 3 | Systematic case review analysis of the total viral load transfused observed in individual case studies (Tables S1 and S2). **(A)** Displayed is the total viral load transfused resulting in posttransfusion hepatitis E virus (HEV) infection or no posttransfusion HEV infection, independently from and depending on the immune status of the recipients ($n = 39$). pIC, possibly immunocompromised; pNIC, possibly not immuno-compromised. **(B)** Displayed is the total viral load transfused resulting in posttransfusion HEV infection or no posttransfusion HEV infection depending on the transfused blood product ($n = 25$). RBC, red blood cell concentrates; PC, apheresis or pooled PCs; FFP, fresh frozen plasma. ◊: values specified with <IU/mL, viral loads for these cases are placed at the maximum possible value, Δ: estimated infectious dose, solid bars indicate median viral load. The solid horizontal line represents median values, and the dotted horizontal line represents the minimum infectious dose. White symbols: HEV infection and black symbols: no HEV infection. ***$p < 0.0001$, **$p = 0.0002$, and *$p < 0.05$, ns, not significant.

Hepatitis E and blood donation safety in selected European countries: a shift to screening?

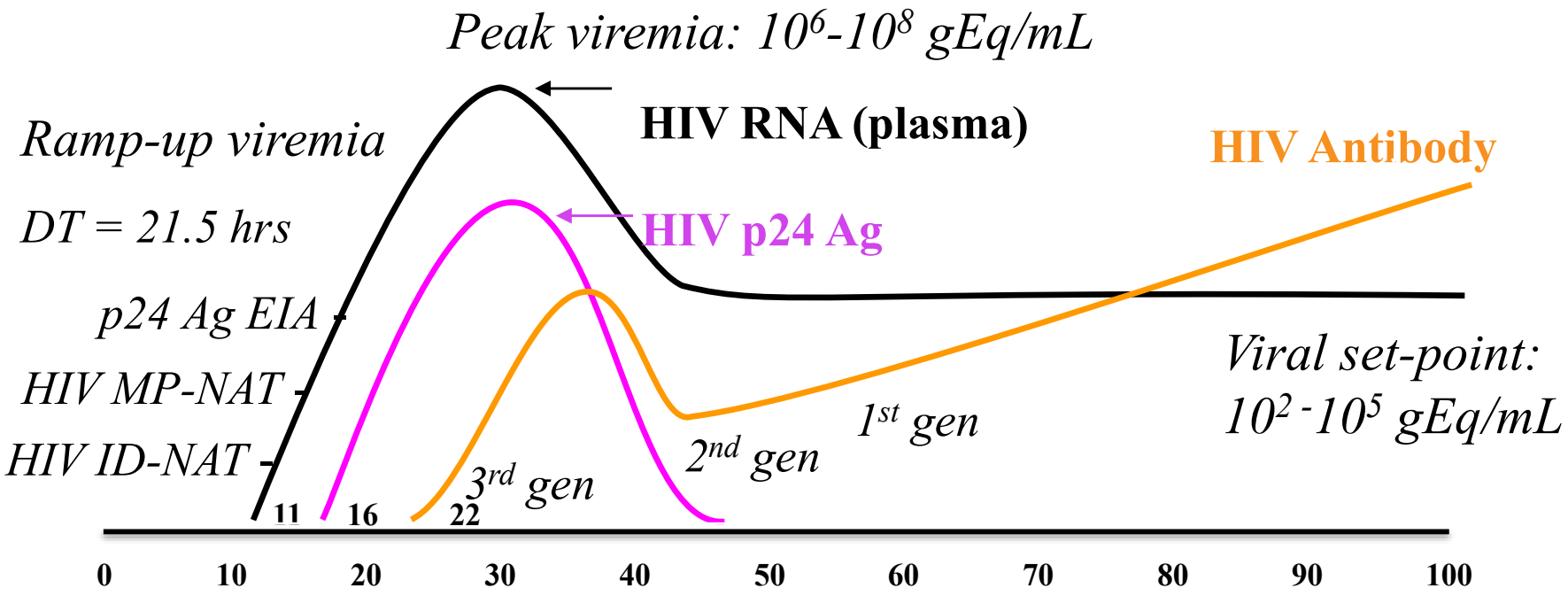
D Domanović [1], R Tedder [2], J Blümel [3], H Zaaijer [4], P Gallian [5], C Niederhauser [6], S Sauleda Oliveras [7], J O'Riordan [8], F Boland [8], L Harritshøj [9], MSJ Nascimento [10], AR Ciccaglione [11], C Politis [12], C Adlhoch [1], B Flan [13], W Oualikene-Gonin [14], G Rautmann [15], P Strengers [16], P Hewitt [17]

1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

Prevalence of hepatitis E virus RNA positive donations, population of transplanted patients at risk, reported cases of transfusion-transmitted hepatitis E virus and screening of blood donations in 11 European countries

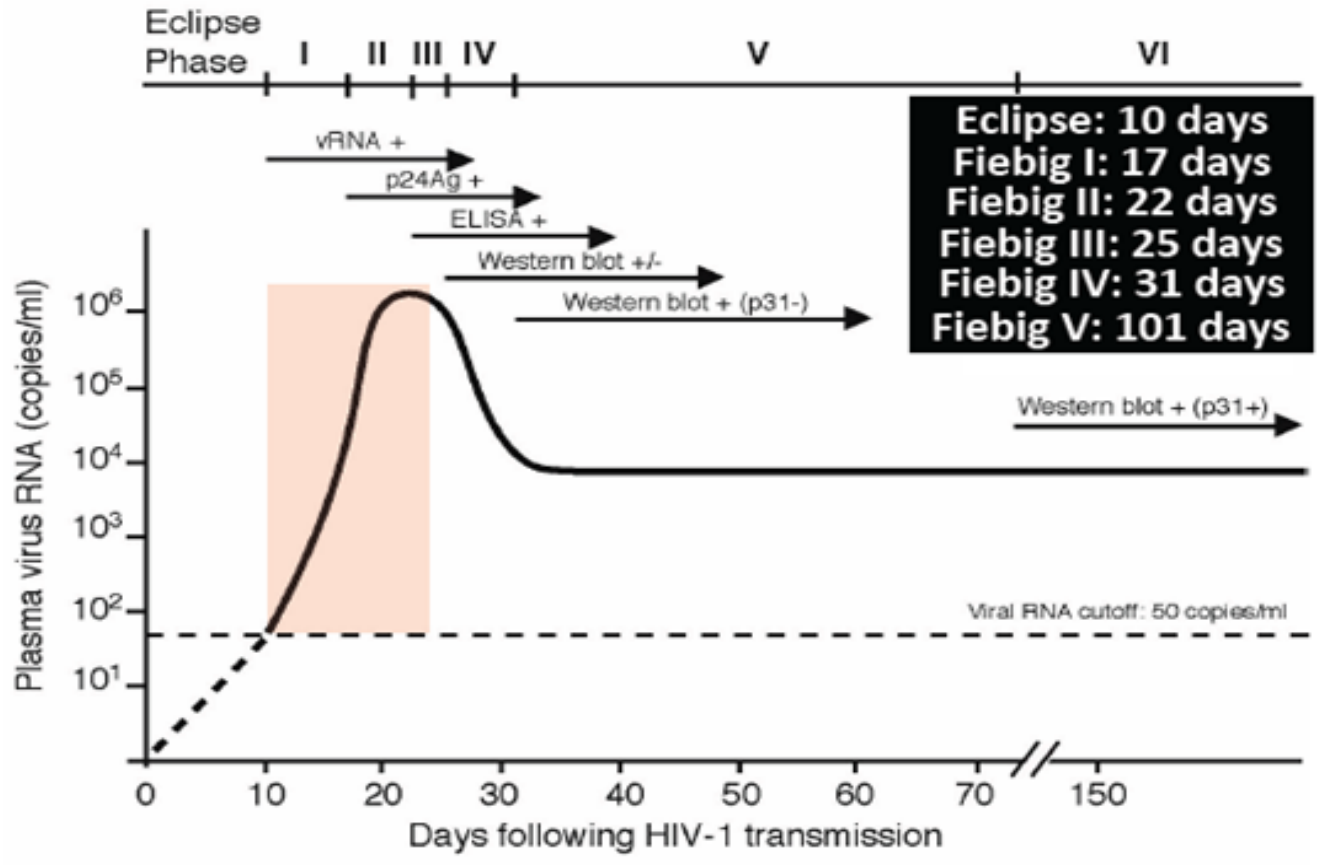
Country	HEV RNA positive donations	Population at risk		Reported TT HEV infections	Screening of blood donations			
		allo-HSCT [51] AN (AR/p1omp)	SOT [52] AN (AR/pmp)		Implemented	Under Consideration	In evaluation	Not recommended
Denmark	1:2,331(2016) [16]	144 (201 – 300)	356 (63.6)					x
France	1:2,218 (2012–3) [18]	1,724 (201 – 300)	5,141(79.6)	x		x[a]		
Germany	1:1,241 (2012) [24]	2,892 (›300)	3,710 (44.9)	x		x[b]		
Greece	NA	169 (151 – 200)	171 (15.4)				x	
Ireland	1:2,778 (2016)	77(151 – 200)	246 (52.3)		x[c]			
Italy	NA	1,625 (201 – 300)	3,252 (53.2)				x	
The Netherlands	1: 726 (2016) [7]	1175 (›300)	1,315 (78.3)			x [d/e]		
Portugal	NA	137 (101 – 150)	739 (69.7)				x	
Spain	1:3,333 (2014) [53]	1,072 (201 – 300)	4,247 (90.2)	x			x	
Switzerland	NA	191 (201 – 300)	504 (61.5)			x		
United Kingdom	1:1,340–5,000 (2016)	1,602 (201 – 300)	4,561 (71.8)	x	x[e/f]			

HIV Viremia and seroconversion during early infection



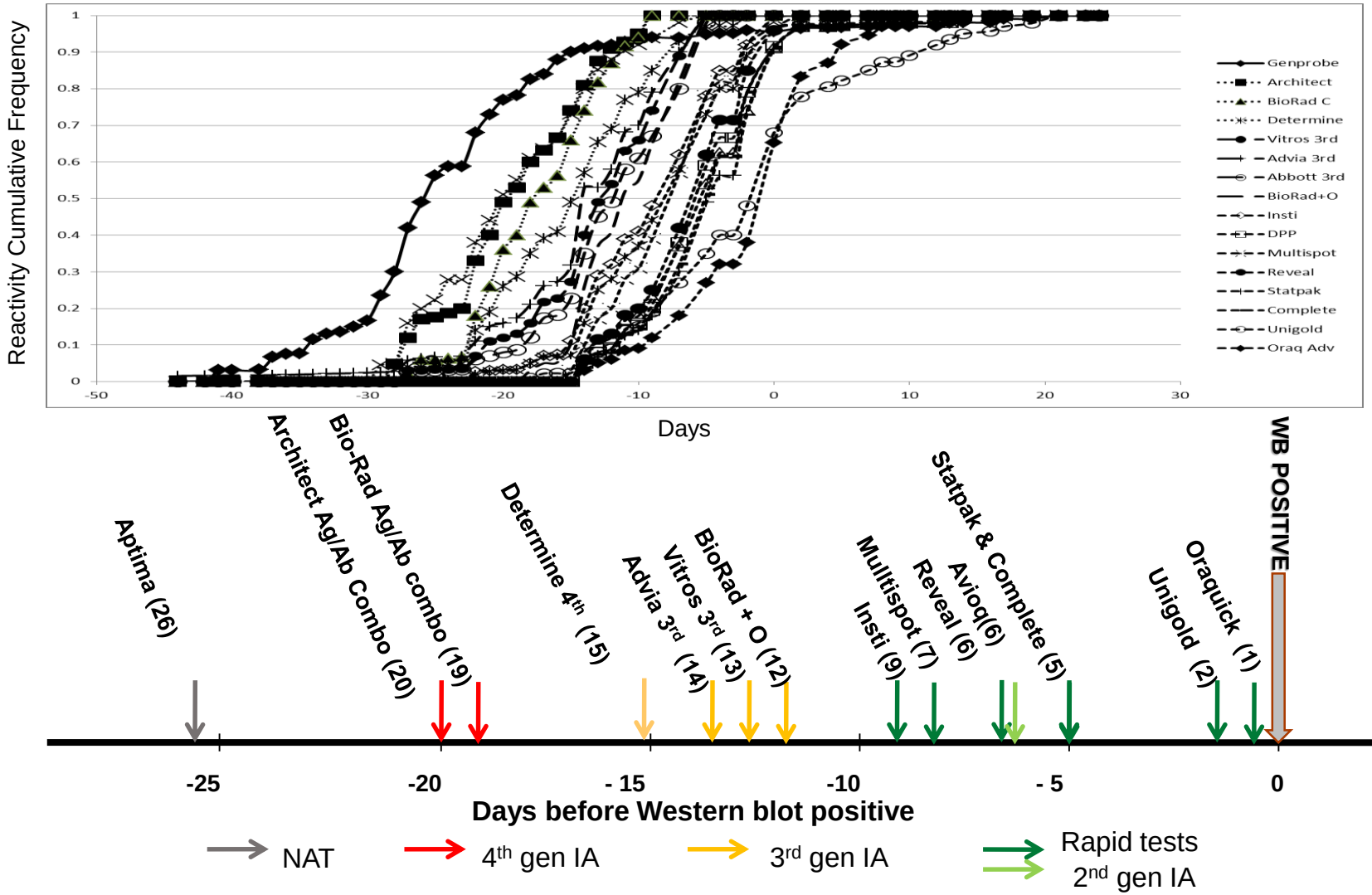
Busch et al. Am J Med. 1997

Fiebig Stages of Acute HIV Infection



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

Data from 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

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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.

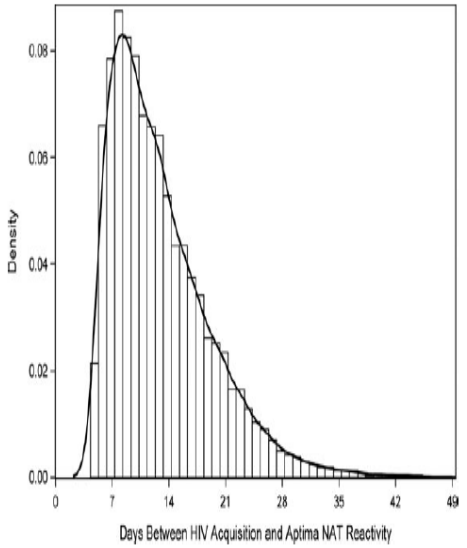
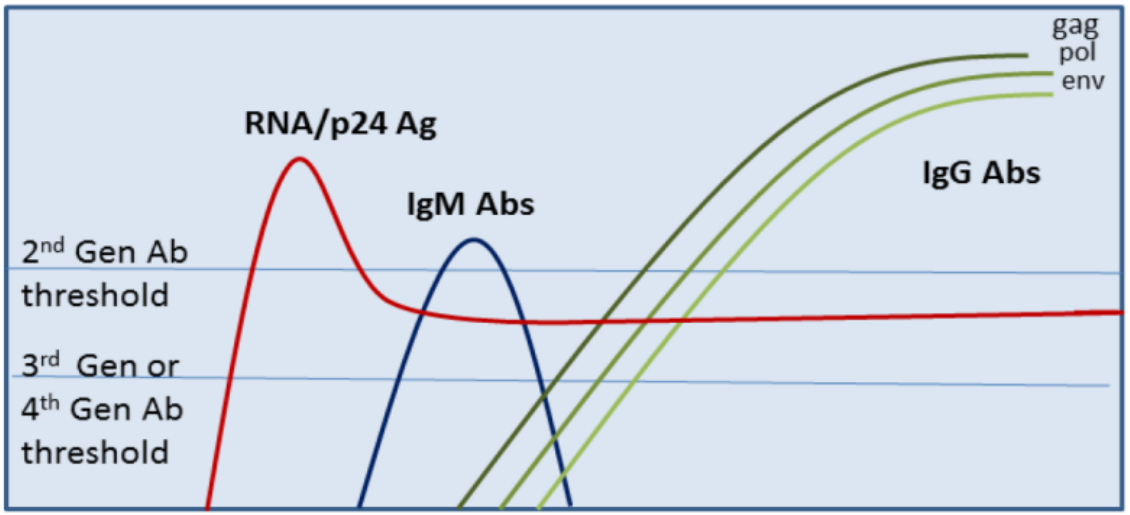
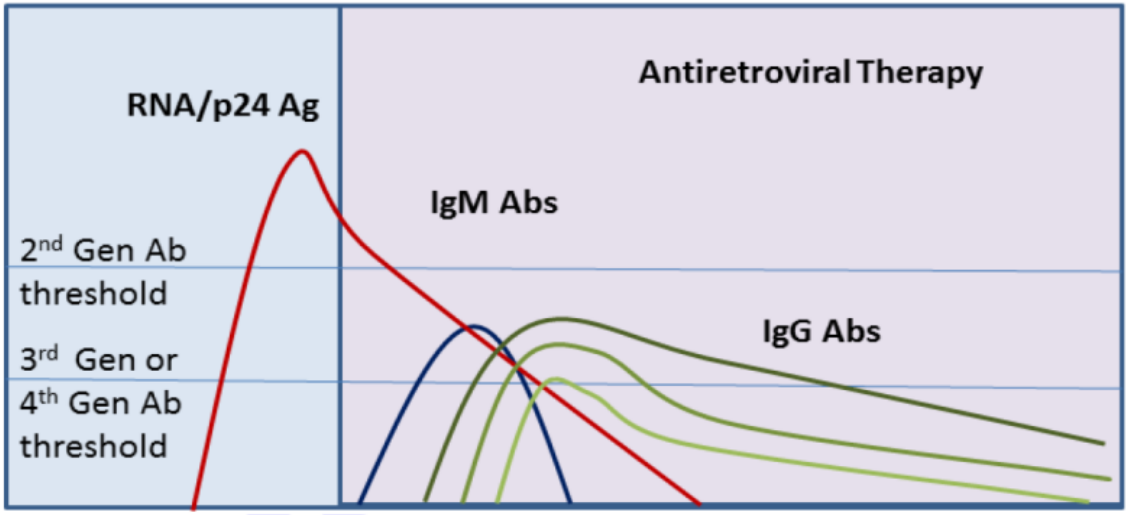


Figure 1. Simulated eclipse period probability density function (PDF) translated from a 3-parameter Weibull prior distribution. Parameters for location, shape, and

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

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





Using Seroreversion as a Marker of Viral Suppression

The Effects of Early Antiretroviral Therapy and Its Discontinuation on the HIV-Specific Antibody Response

M. SCOTT KILLIAN,[1] PHILIP J. NORRIS,[1,2] BHUPAT D. RAWAL,[2] MILA LEBEDEVA,[2] FREDERICK M. HECHT,[1] JAY A. LEVY,[1] and MICHAEL P. BUSCH[1,2]

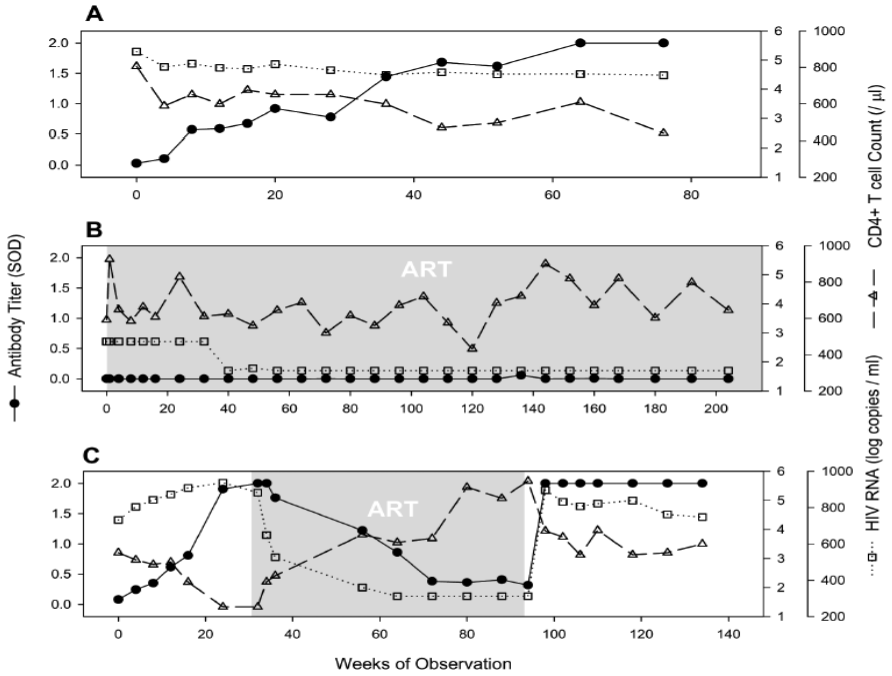


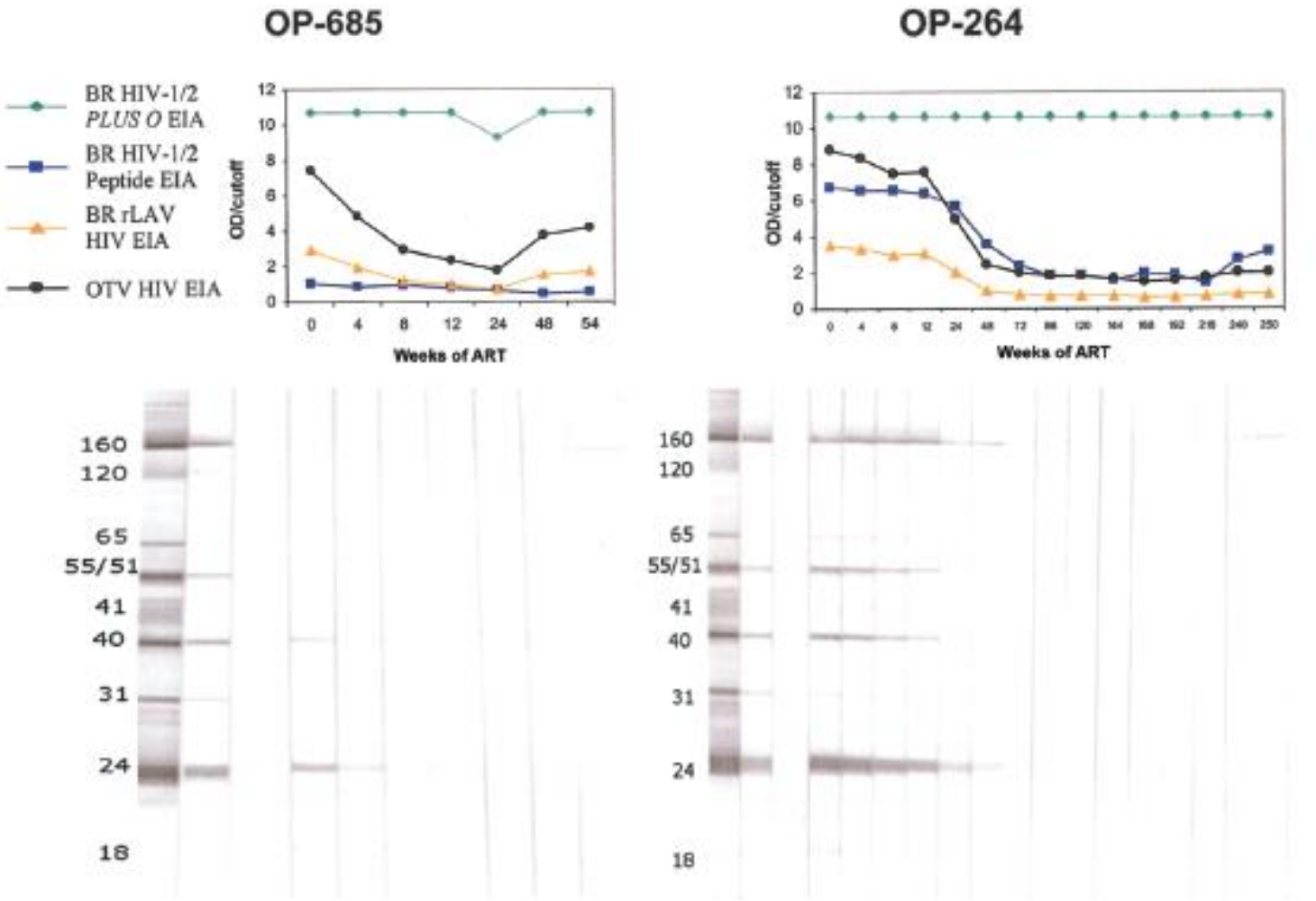
FIG. 1. Representative cases. Shown are antibody levels (closed circles), virus titers (open squares), and $CD4^+$ T cell counts (open triangles) for three representative subjects. Data are provided for (A) a therapy naive subject who exhibited an indeterminate Western blot result at week 0 and chose not to receive ART; (B) a subject undergoing primary HIV-1 infection and initiated ART upon entry into the study; and (C) a subject who exhibited negative Western blot and standard EIA test results at week 0, initiated ART after 35 weeks of observation, and then 60 weeks later, discontinued therapy.

AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 22, Number 7, 2006, pp. 640–647

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



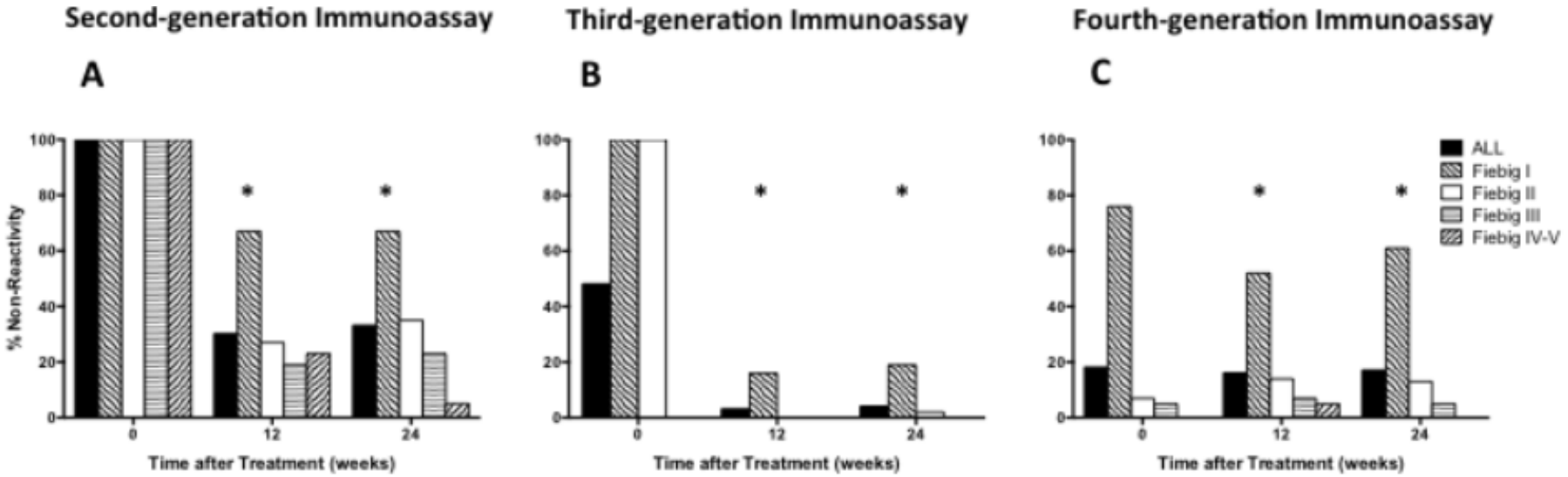
Clinical Infectious Diseases 2006; 42:700–8

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

Clinical Infectious Diseases®

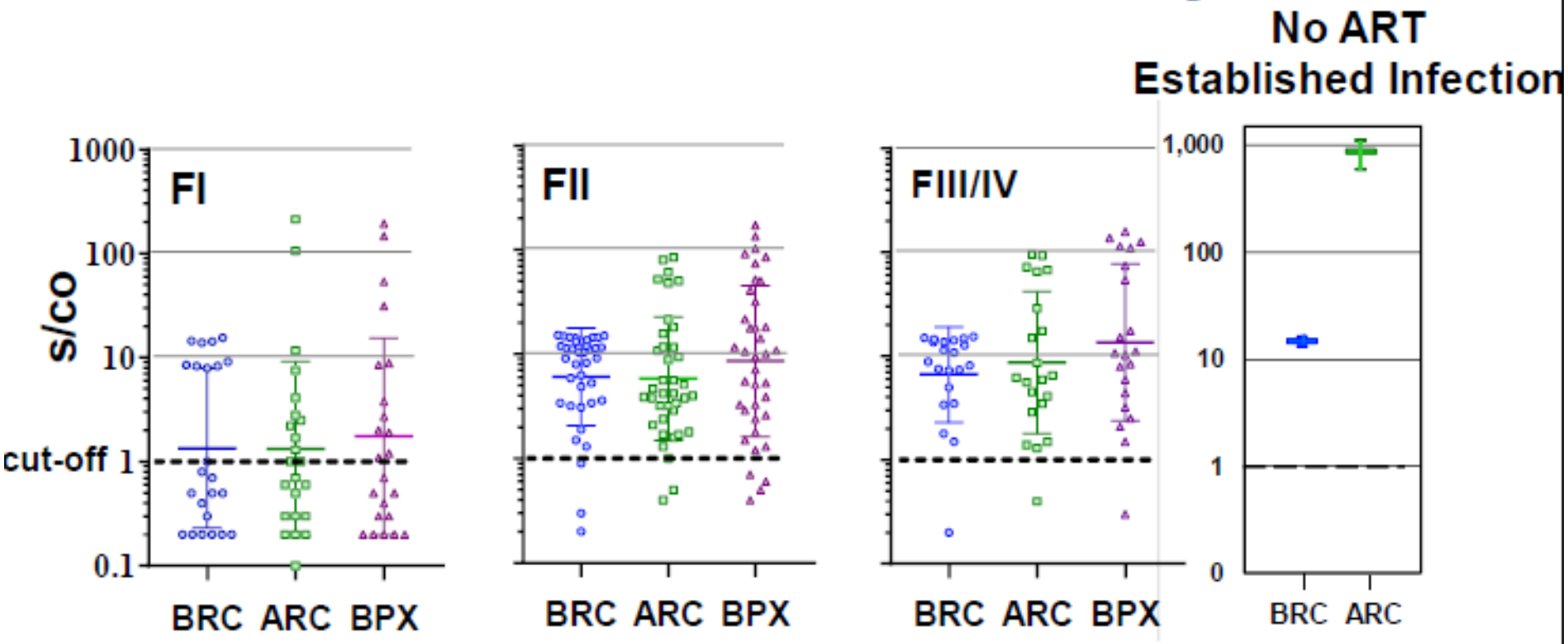
Mark S. de Souza,[1,2,3] Suteeraporn Pinyakorn,[4,5] Siriwat Akapirat,[6] Supanit Pattanachaiwit,[2] James L. K. Fletcher,[1] Nitiya Chomchey,[1] Eugene D. Kroon,[1,2] Sasiwimol Ubolyam,[7] Nelson L. Michael,[5,8] Merlin L. Robb,[4,5] Praphan Phanuphak,[1,2] Jerome H. Kim,[9] Nittaya Phanuphak,[1,2]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

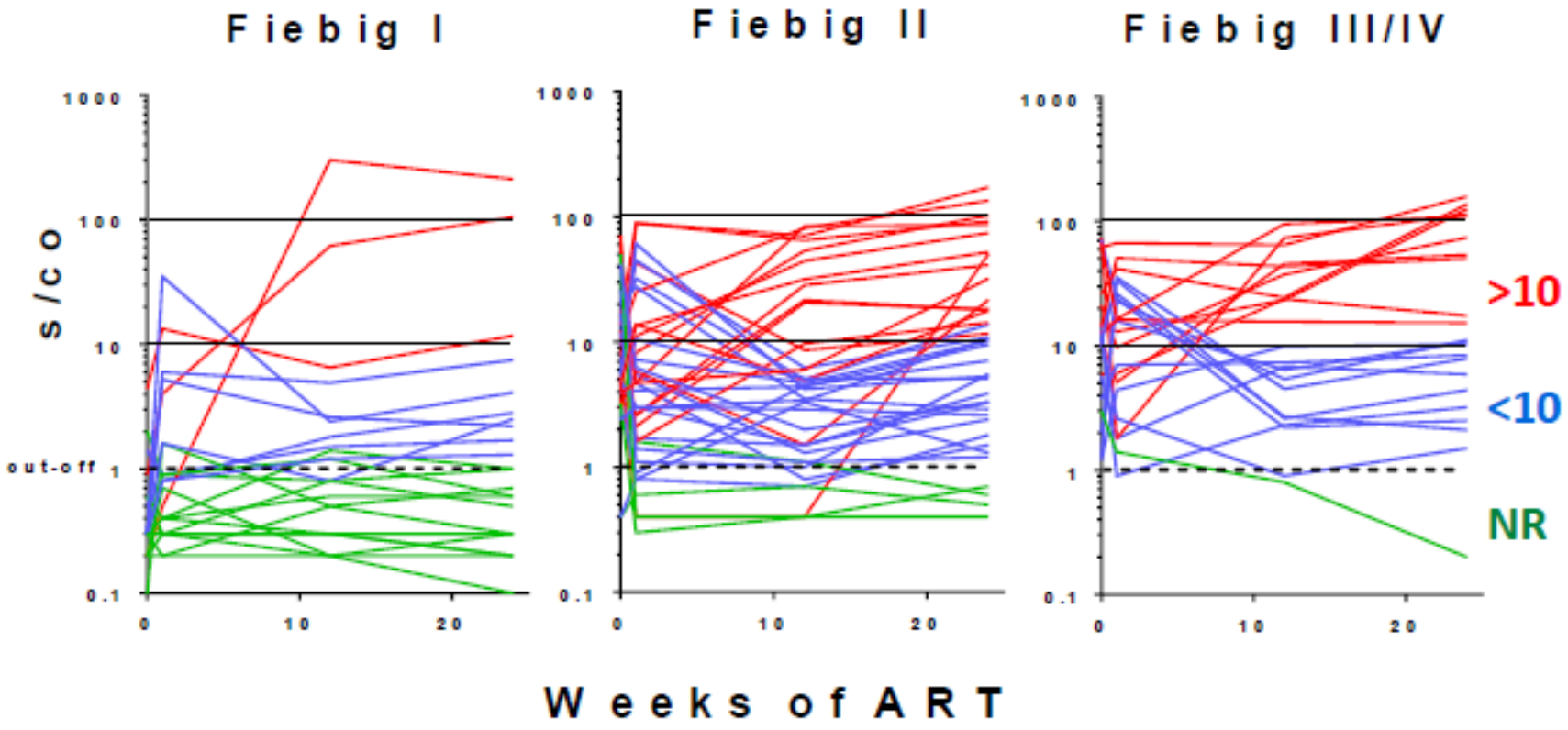
S/CO at 24 Weeks After Early ART



- FI S/CO remains close to cut-off, most NR
- FII Increased S/CO compared to FI, Fewer NR
- FIII/IV Low, but Reactive S/CO, Very few NR

- No ART Established Infection;
  S/CO BRC 13.5-15.0; ARC 800-1200: BPX >200

Time Course of BPX Reactivity After ART

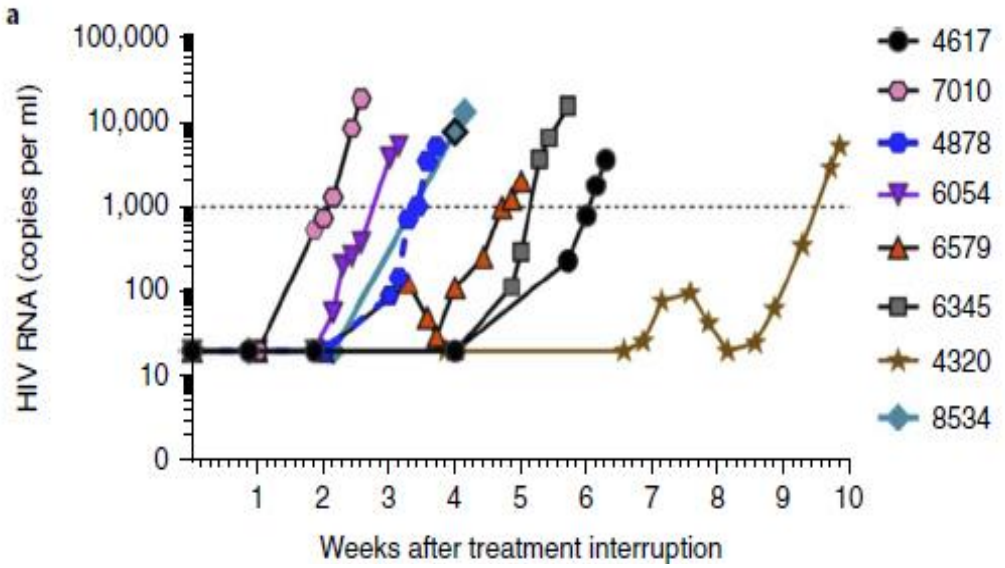


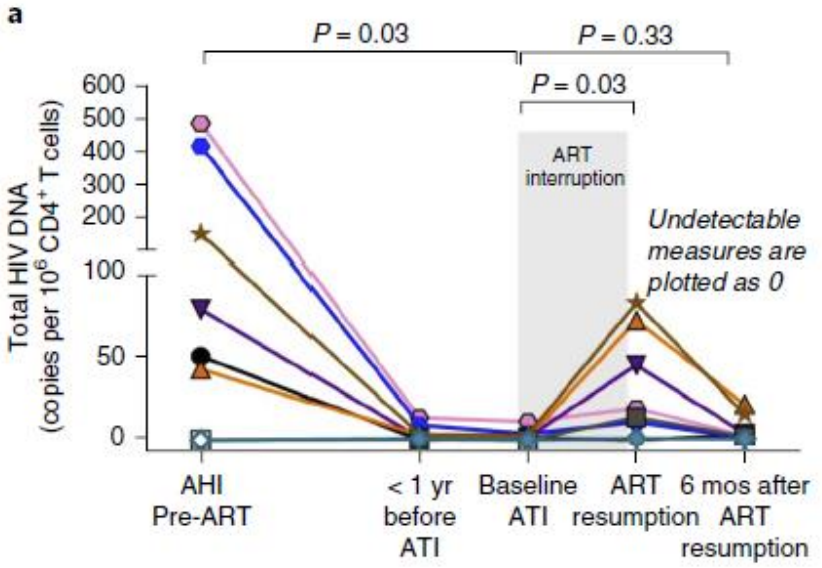
- Transient increase in signal in some individuals at week 1
- Decrease to low level and little increase thereafter
- Seroreversion observed in some individuals

NATURE MEDICINE | VOL 24 | JULY 2018 | 923–926 |

Rapid HIV RNA rebound after antiretroviral treatment interruption in persons durably suppressed in Fiebig I acute HIV infection

Donn J. Colby[1], Lydie Trautmann[2,3], Suteeraporn Pinyakorn[2,3], Louise Leyre[4], Amélie Pagliuzza[4], Eugène Kroon[1], Morgane Rolland[2,3], Hiroshi Takata[2,3], Supranee Buranapraditkun[2,3,5,6], Jintana Intasan[1], Nitiya Chomchey[1], Roshell Muir[7], Elias K. Haddad[7], Sodsai Tovanabutra[2,3], Sasiwimol Ubolyam[8], Diane L. Bolton[2,3], Brandie A. Fullmer[9], Robert J. Gorelick[9], Lawrence Fox[10], Trevor A. Crowell[2,3], Rapee Trichavaroj[11], Robert O'Connell[11], Nicolas Chomont[4], Jerome H. Kim[2,13], Nelson L. Michael[2], Merlin L. Robb[2,3], Nittaya Phanuphak[1], Jintanat Ananworanich[1,2,3,12*]





VIEWPOINT

HIV Viral Load and Transmissibility of HIV Infection
Undetectable Equals Untransmittable

Carl W. Dieffenbach, PhD
Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.

Anthony S. Fauci, MD
Office of the Director, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.

JAMA February 5, 2019 Volume 321, Number 5

Box. Principles to Achieve and Maintain an Undetectable Viral Load

- In order for antiretroviral therapy (ART) to provide maximum benefit, taking medication as prescribed is essential.
- Achieving an undetectable viral load can take up to 6 months of ART. Once achieved, continued adherence is required.
- According to guidelines from the Department of Health and Human Services, viral load testing should be performed every 3-4 months after the plasma HIV-1 RNA level reaches undetectable (<200 copies/mL). If viral suppression and stable immunologic status are maintained for >2 years, the viral load testing can be extended to every 6 months thereafter.
- Stopping therapy negates the validity of assuming that U = U.

Risk of HIV transmission through condomless sex in serodifferent gay couples with the HIV-positive partner taking suppressive antiretroviral therapy (PARTNER): final results of a multicentre, prospective, observational study

Alison J Rodger, Valentina Cambiano, Tina Bruun, Pietro Vernazza, Simon Collins, Olaf Degen, Giulio Maria Corbelli, Vicente Estrada,

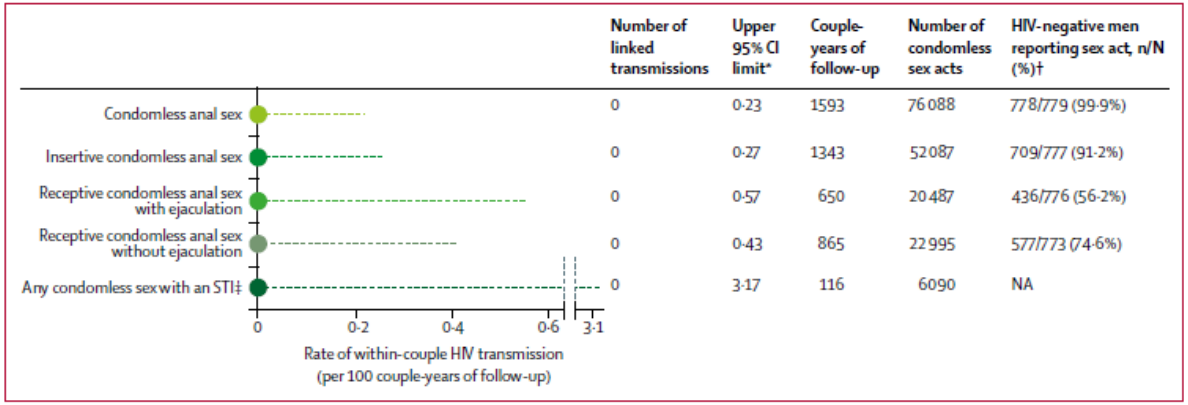
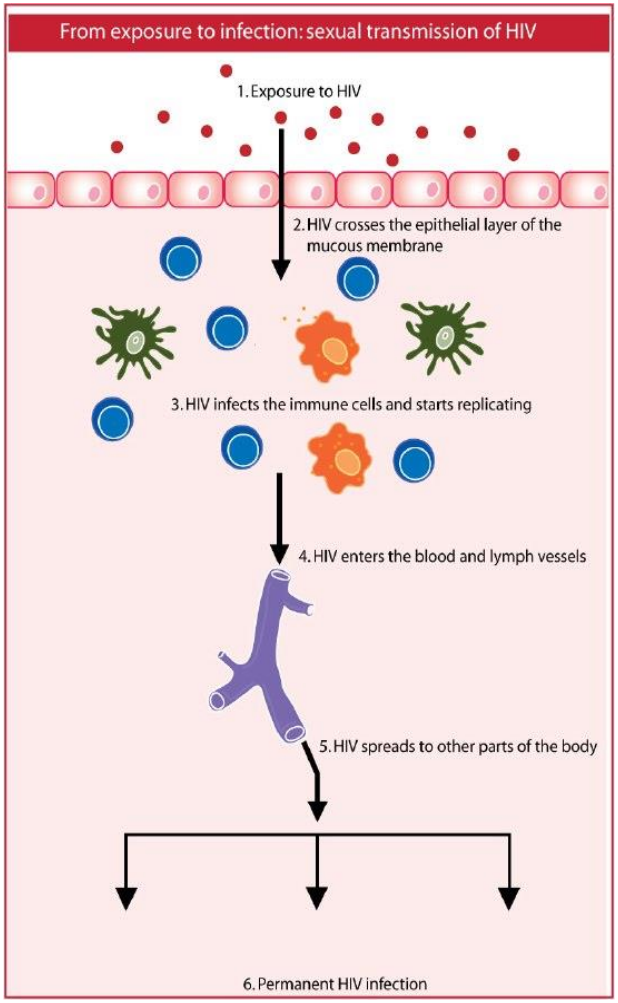


Figure 1: **Rate of within-couple HIV transmission through condomless sex according to sexual behaviour reported by the HIV-negative partner**
STI=sexually transmitted infection. NA=not applicable. *Estimated using the exact Poisson method. †Numerator is the number of HIV-negative men within the eligible couples ever reporting that specific sexual act and denominator is the group-specific number of HIV-negative participants who contributed eligible couple-years of follow-up. ‡Refers to STIs (excluding HIV) self-reported by the HIV-negative partner.

Implications of all the available evidence
The results from the PARTNER studies in addition to evidence from other studies in serodifferent couples indicate that the risk of transmission of HIV through condomless sex in the context of virally suppressive ART is effectively zero for both gay men and heterosexual couples. These results support the U=U (undetectable equals untransmittable) message, as well as promoting the benefits of early testing and treatment.

www.thelancet.com **Published online May 2, 2019**

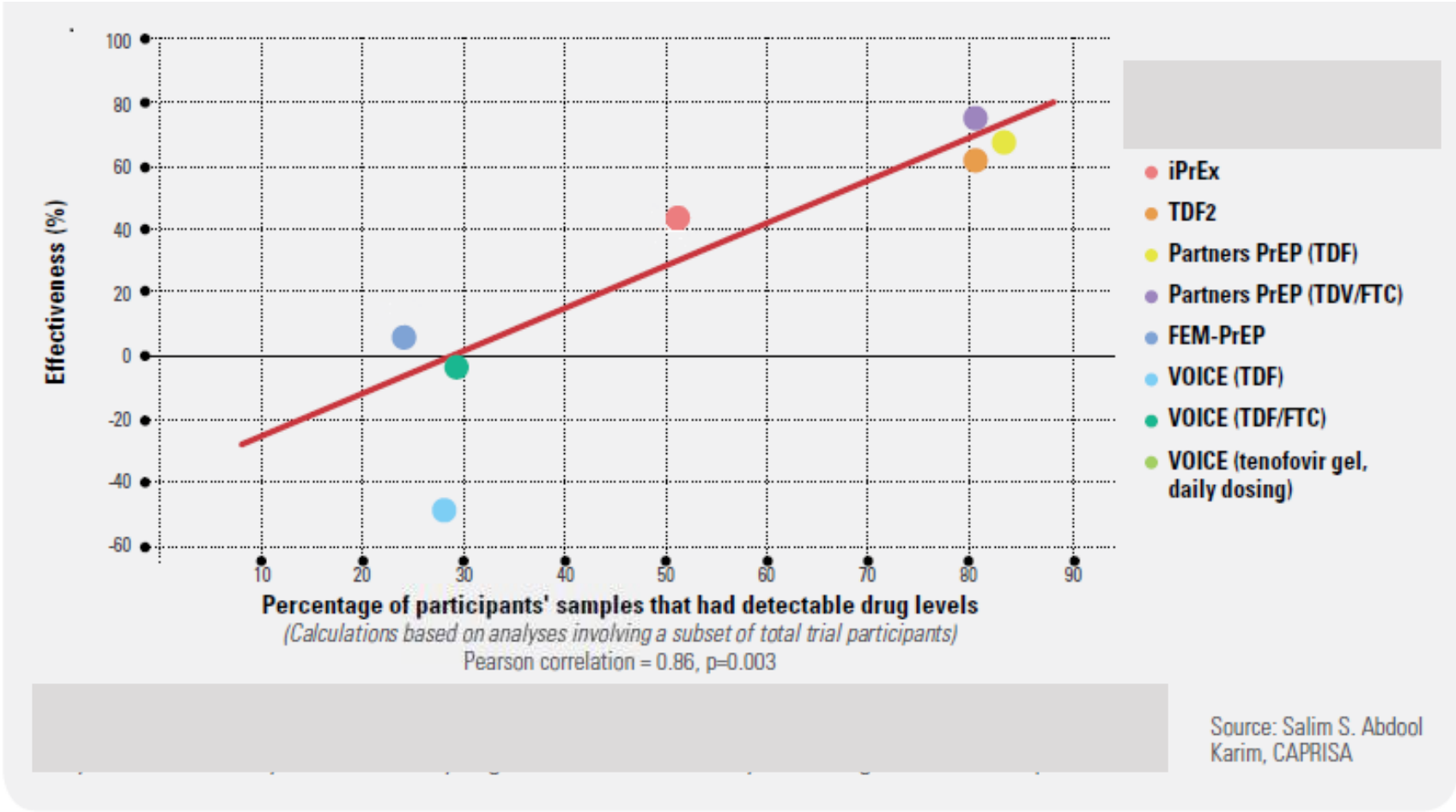
Pre-exposure Prophylaxis (PrEP)



How PrEP works

- **TDF/FTC enters immune cells and are converted into active forms**
- **Two levels of protection**
  - Mucosal tissues
  - Lymph nodes

PrEP RCT Findings – Incidence



Source: Salim S. Abdool Karim, CAPRISA

Uptake of pre-exposure prophylaxis, sexual practices, and HIV incidence in men and transgender women who have sex with men: a cohort study

Robert M Grant, Peter L Anderson, Vanessa McMahan, Albert Liu, K Rivet Amico, Megha Mehrotra, Sybil Hosek, Carlos Mosquera, Martin Casapia, Orlando Montoya, Susan Buchbinder, Valdilea G Veloso, Kenneth Mayer, Suwat Chariyalertsak, Linda-Gail Bekker, Esper G Kallas, Mauro Schechter, Juan Guanira, Lane Bushman, David N Burns, James F Rooney, David V Glidden, for the iPrEx study team

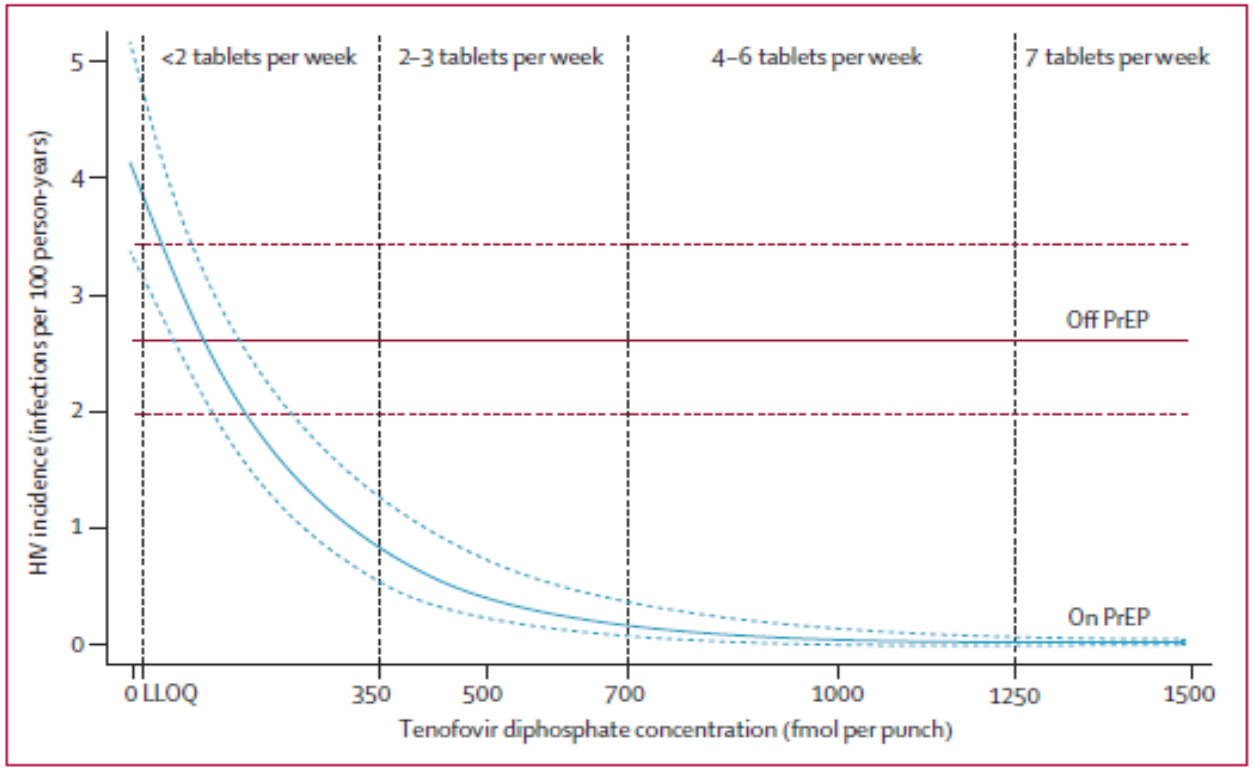


Figure 2: **Pre-exposure prophylaxis and HIV incidence**
For those visits on PrEP, the incidence of HIV is estimated by exponential regression by tenofovir diphosphate in dried blood spots. The incidence for the concomitant off-PrEP group is depicted as a constant for reference. The dotted lines represent the estimate bounded by 1 SE. Dosing for each interval is estimated by pharmacokinetic modelling. LLOQ=lower limit of quantitation.

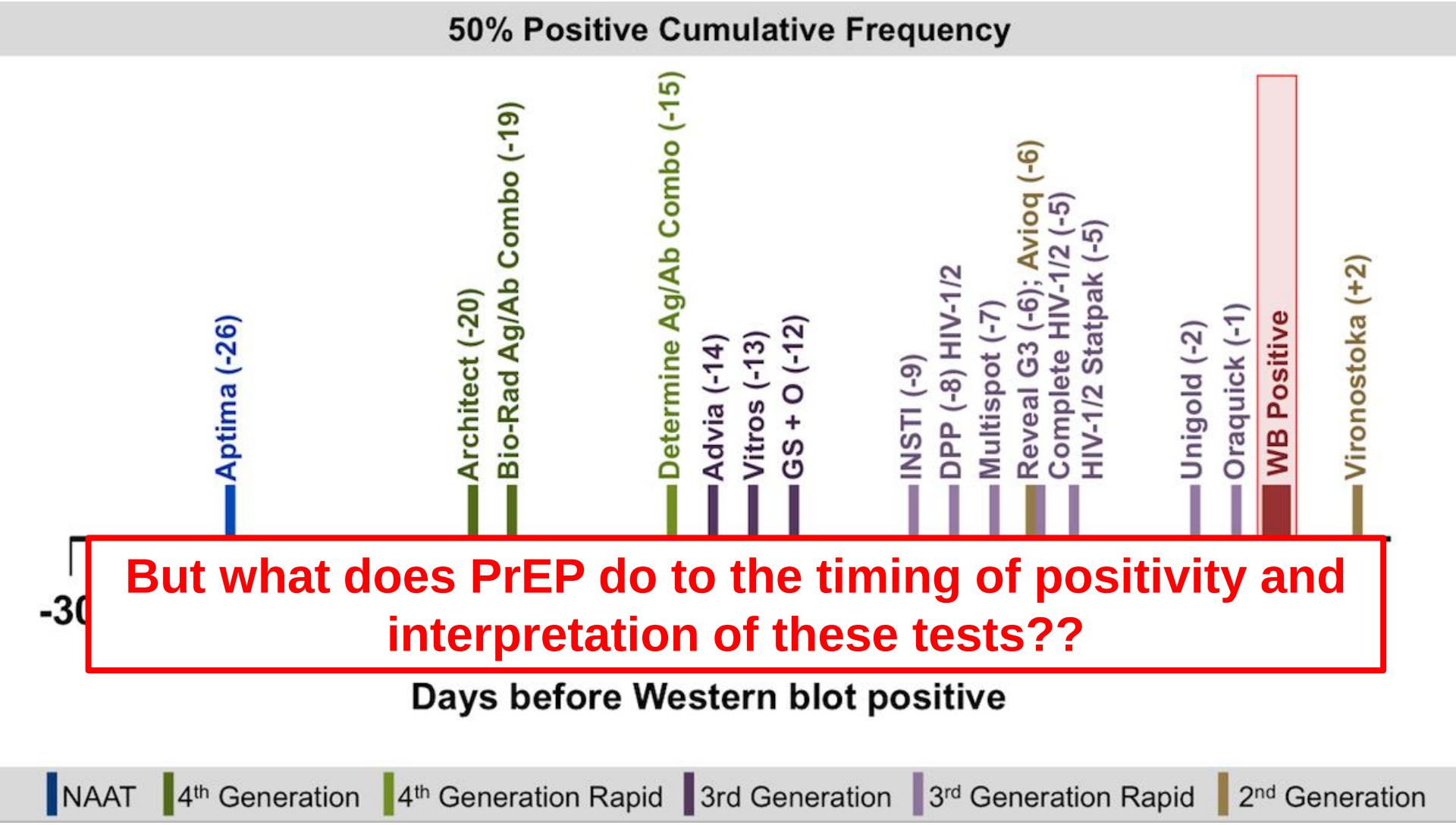
HIV Infections in Persons Prescribed PrEP

Observational Studies:

- **Kaiser Permanente North**
  - **0 seroconversions in 5104 py of follow-up**
  - **11 seroconversons among 1301 who no longer had PrEP in possession at end of follow-up**
- **Demo Project (SF, DC, Miami)**
  - **2 serconversions in 481 py of follow-up**
  - **Both in persons who had not been taking prescribed PrEP**
- **SHIPP Study (Chicago, Philadelphia, DC, Jackson MS)**
  - **10 seroconversion in 1411 py of follow-up**
  - **All in persons who had not been taking prescribed PrEP**

Sources: Marcus Jl et al.. Clinical Infectious Diseases. 2017;65(10):1768-9;
Liu Ay et al. JAMA internal medicine. 2016;176(1):75-84.
Smith DK. Unpublished data

Incidence Assay Performance in Early HIV Infection



National HIV Curriculum at https://www.hiv.uw.edu/go/screening-diagnosis/diagnostic-testing/

A Strategy for PrEP Clinicians to Manage Ambiguous HIV Test Results During Follow-up Visits

Dawn K. Smith, William M. Switzer, Philip Peters, Kevin P. Delaney, Timothy C. Granade, Silvina Masciotra, Luke Shouse, and John T. Brooks

Division of HIV/AIDS Prevention, National Center for HIV, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia

Prompt determination of HIV infection status is critical during follow-up visits for patients taking pre-exposure prophylaxis (PrEP) medication. Those who are uninfected can then continue safely taking PrEP, and those few who have acquired HIV infection can initiate an effective treatment regimen. However, a few recent cases have been reported of ambiguous HIV test results using common testing algorithms in PrEP patients. We review published reports of such cases and testing options that can be used to clarify true HIV status in these situations. In addition, we review the benefits and risks of 3 antiretroviral management options in these patients: (1) continue PrEP while conducting additional HIV tests, (2) initiate antiretroviral therapy for presumptive HIV infection while conducting confirmatory tests, or (3) discontinue PrEP to reassess HIV status after a brief antiretroviral-free interval. A clinical consultation resource is also provided.

Table 1. Case Reports of HIV Test Results of Men Who Have Sex With Men Who Acquired HIV Infection While Taking Tenofovir for Hepatitis Treatment or TDF/FTC for PrEP, US, Germany, Canada, and the Netherlands, 2017–2018

First Author of Case Report	HIV Tests Before PrEP Initiation	Time After Last Negative HIV Test	Antigen/Ab	Supplemental or Confirmatory Tests	Qualitative NAT	Quantitative NAT, Copies/mL	Antiretroviral Management	Resistance Mutations
Seroconversions on PrEP with ambiguous HIV test results								
Knox [4]	Ag/Ab-nonreactive	3 mo	Reactive	Negative WB		28 000 copies (3 mo + 7 d)	TDF/FTC for PrEP, darunavir, ritonavir, raltegravir added for treatment regimen at 3 mo + 4 d	41I, 67G, 69D, 70R, 184V, 215E, 181C, 10L, 51Y, 92Q
Markowitz [5]	Ag/Ab-nonreactive, qualitative NAT–nonreactive	19 wk	Reactive	Nonreactive multispot	Reactive	Not detected	TDF/FTC for PrEP until week 22	K65R, M184V, K103S, E138Q, Y199L (25 wk)
		21 wk	Reactive	Nonreactive multispot	Reactive	<20 copies		
		22 wk					Dolutegravir added to TDF/FTC for treatment regimen	
		25 wk	Reactive	Nonreactive multispot	Nonreactive	Not detected		
Zucker [6]	POC antibody–nonreactive, Ag/Ag-nonreactive, pooled NAT–negative	28 d	Reactive	Negative WB	Reactive		TDF/FTC for PrEP until 32 d	Not assessed
		32 d	Nonreactive		Nonreactive	<20 copies	Dolutegravir added to TDF/FTC for treatment regimen	
		46 d	Reactive	Indeterminate WB	Reactive	Not detected		
Hoornenborg [7]	Ag/Ab-nonreactive, pooled NAT–negative	10 wk	Reactive Ab, nonreactive p24 Ag				TDF/FTC for PrEP until 11 wk	None
		11 wk	Reactive	Negative WB	Nonreactive	Not detected		
		12 wk				Not detected		
		14 wk				12 882 copies		
		15 wk		Weak positive WB		101 156 copies	Started TDF/FTC, ritonavir, darunavir, dolutegravir for treatment regimen	
		19 wk		Positive WB				
Seroconversions on PrEP with unambiguous HIV test results								
Thaden [8]	Ag/Ab-nonreactive	16 mo	Reactive			27 316 copies	TDF/FTC for PrEP until month 16, started on dolutegravir, rilpivirine, darunavir/cobicistat for treatment	M184V, K65R, K70T, K103N, V109, V197I
Streek [9]	Ag/Ab-nonreactive	103 d	Reactive			59 copies	TDF for hepatitis B treatment until day 103, FTC and ritonavir-boosted darunavir added for treatment regimen	K65R

The effect of oral preexposure prophylaxis on the progression of HIV-1 seroconversion

Deborah Donnell[a,c], Eric Ramos[b], Connie Celum[c,d,e], Jared Baeten[c,d,e], Joan Dragavon[b], Jordan Tappero[g], Jairam R. Lingappa[c,e,f], Allan Ronald[h], Kenneth Fife[i], Robert W. Coombs[b], for the Partners PrEP Study Team*

Results: There was a significant increase in delayed site detection of infection associated with PrEP (odds ratio = 3.49, $P = 0.044$). Delay in detection was not associated with increased risk of resistance in the PrEP arm (odds ratio = 0.93, $P = 0.95$). Estimated time to each Fiebig stage was elongated in seroconverters with evidence of ongoing PrEP use, significantly for only Stage 5 (28 versus 17 days, $P = 0.05$). Adjusted for Fiebig stage, viral RNA was ~2/3 log lower in those assigned to PrEP compared with placebo; no differences were found in Architect signal to cut-off at any stage.

Conclusion: Ongoing PrEP use in seroconverters may delay detection of infection and elongate seroconversion, although the delay does not increase risk of resistance.

AIDS 2017, **31**:2007–2016

RESEARCH ARTICLE

HIV-1 persistence following extremely early initiation of antiretroviral therapy (ART) during acute HIV-1 infection: An observational study

Timothy J. Henrich[1]*, Hiroyu Hatano[2], Oliver Bacon[2,3], Louise E. Hogan[1], Rachel Rutishauser[1,2], Alison Hill[4], Mary F. Kearney[5], Elizabeth M. Anderson[5], Susan P. Buchbinder[2,3], Stephanie E. Cohen[2,3], Mohamed Abdel-Mohsen[2,6], Christopher W. Pohlmeyer[7], Remi Fromentin[8], Rebecca Hoh[2], Albert Y. Liu[2,3], Joseph M. McCune[1], Jonathan Spindler[5], Kelly Metcalf-Pate[7], Kristen S. Hobbs[1], Cassandra Thanh[1], Erica A. Gibson[1], Daniel R. Kuritzkes[9,10], Robert F. Siliciano[11,12], Richard W. Price[13], Douglas D. Richman[14,15], Nicolas Chomont[8], Janet D. Siliciano[10], John W. Mellors[16], Steven A. Yukl[17,18], Joel N. Blankson[7], Teri Liegler[2], Steven G. Deeks[2]

Participant in SF PrEP Demo Project for at-risk MSM

HIV-uninfected at 2 pre-enrollment visits

- 21 and 13 days prior to PrEP baseline
- Pooled RNA neg, 4th gen EIA neg, rapid Ab neg
- Ongoing risk for HIV infection

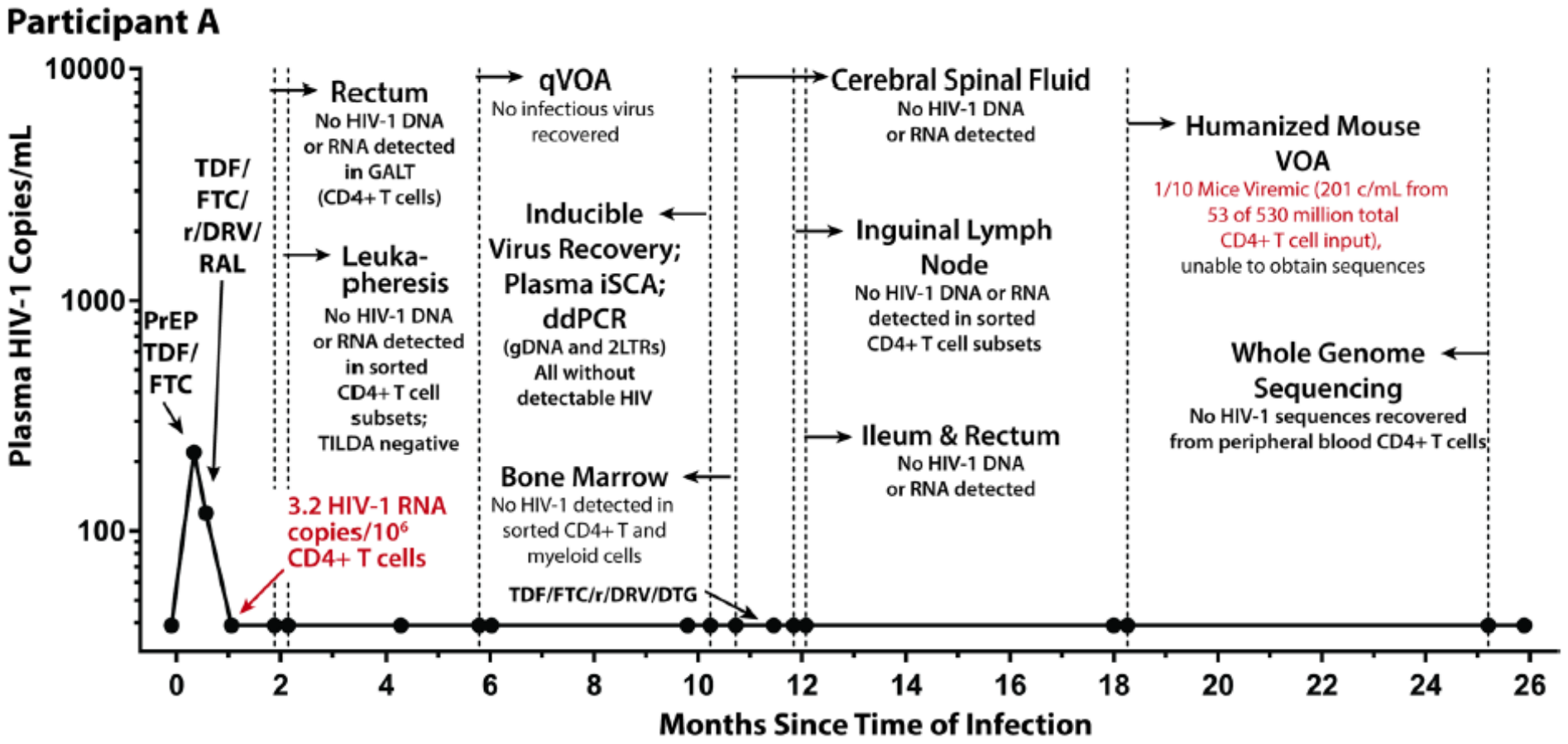
“Eclipse” Phase HIV infection detected at PrEP baseline visit

- RNA 220 copies/mL, 4th gen EIA neg, rapid Ab neg
- *Estimated ~10 days after HIV infection*

Received PrEP (TDF/FTC) for 7 days, at which time PrEP baseline test results returned and conventional ART was initiated

PLOS Medicine | https://doi.org/10.1371/journal.pmed.1002417 November 7, 2017

SF Participant #1: Lack of Detectable HIV DNA in a PrEP Study Participant Treated Immediately After "Eclipse" Phase



SF Participant #1: Lack of Detectable HIV DNA in a PrEP Study Participant Treated Immediately After "Eclipse" Phase

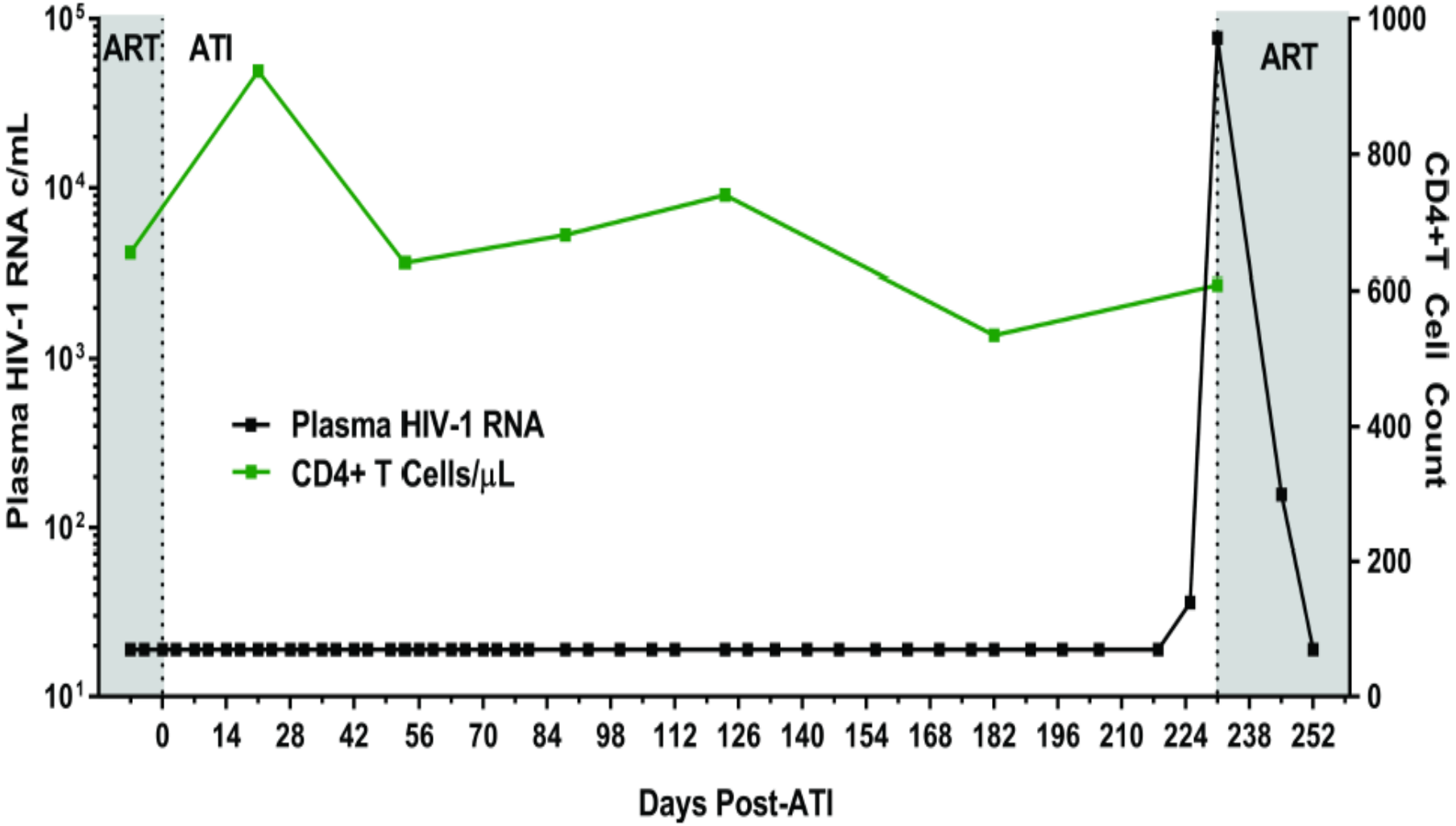


Fig 2. Summary timeline of plasma viral load and CD4+ T cell counts following analytical ART treatment interruption in Participant A.
Abbreviations: ART, antiretroviral therapy; ATI, analytical treatment interruption; c/mL, copies/mL.

Cellular immune correlates analysis of an HIV-1 preexposure prophylaxis trial

Peter J. Kuebler[a,1], Megha L. Mehrotra[b,2], J. Jeff McConnell[b,2,3], Sara J. Holditch[a,2], Brian I. Shaw[a,2], Leandro F. Tarosso[c], Kaitlyn S. Leadabrand[a], Jeffrey M. Milush[a], Vanessa A. York[a], Rui André Saraiva Raposo[a,d], Rex G. Cheng[a], Emily M. Eriksson[a,4], Vanessa McMahan[b], David V. Glidden[e], Stephen Shiboski[e], Robert M. Grant[b,f,5], Douglas F. Nixon[a,d,5], and Esper G. Kallás[c,5]

HIV-1–specific T-cell responses in exposed seronegative subjects suggest that a viral breach of the exposure site is more common than current transmission rates would suggest and that host immunity can extinguish subsequent infection foci. The Preexposure Prophylaxis Initiative (iPrEx) chemoprophylaxis trial provided an opportunity to rigorously investigate these responses in a case–control immunology study; 84 preinfection peripheral blood mononuclear cell samples from individuals enrolled in the iPrEx trial who later seroconverted were matched with 480 samples from enrolled subjects who remained seronegative from both the placebo and active treatment arms. T-cell responses to HIV-1 Gag, Protease, Integrase, Reverse Transcriptase, Vif, and Nef antigens were quantified for all subjects in an IFN-γ enzyme-linked immunospot (ELISpot) assay. IFN-γ responses varied in magnitude and frequency across subjects. A positive response was more prevalent in those who remained persistently HIV-1–negative for Gag ($P = 0.007$), Integrase ($P < 0.001$), Vif ($P < 0.001$), and Nef ($P < 0.001$). When correlated with outcomes in the iPrEx trial, Vif- and Integrase-specific T-cell responses were associated with reduced HIV-1 infection risk [hazard ratio (HR) = 0.36, 95% confidence interval (95% CI) = 0.19–0.66 and HR = 0.52, 95% CI = 0.28–0.96, respectively]. Antigen-specific responses were independent of emtricitabine/tenofovir disoproxil fumarate use. IFN-γ secretion in the ELISpot was confirmed using multiparametric flow cytometry and largely attributed to effector memory CD4+ or CD8+ T cells. Our results show that HIV-1–specific T-cell immunity can be detected in exposed but uninfected individuals and that these T-cell responses can differentiate individuals according to infection outcomes.

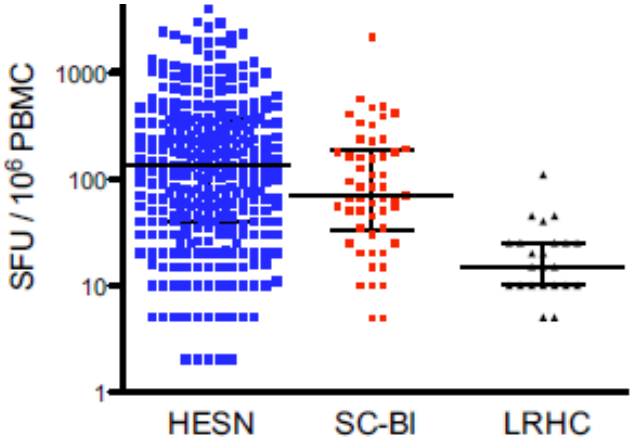
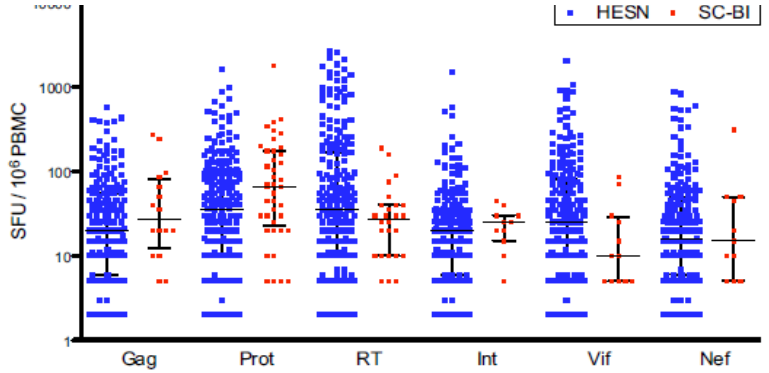


Fig. 1. Distribution of cumulative anti-HIV-1 SFU counts in responders only. Cumulative nonzero SFU counts are shown with medians and interquartile ranges.



Ending the HIV Epidemic: A Plan for America

HHS is proposing a once-in-a-generation opportunity to eliminate new HIV infections in our nation. The multi-year program will infuse 48 counties, Washington, D.C., San Juan, Puerto Rico, as well as 7 states that have a substantial rural HIV burden with the additional expertise, technology, and resources needed to end the HIV epidemic in the United States. Our four strategies – diagnose, treat, protect, and respond – will be implemented across the entire U.S. within 10 years.

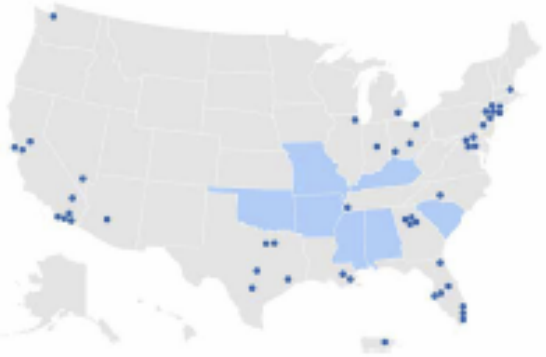
GOAL:

75% reduction in new HIV infections in 5 years and at least 90% reduction in 10 years.

Our goal is ambitious and the pathway is clear – employ strategic practices in the *places* focused on the right *people* to:

- **Diagnose** all people with HIV as early as possible after infection.
- **Treat** the infection rapidly and effectively to achieve sustained viral suppression.
- **Protect** people at risk for HIV using potent and proven prevention interventions, including PrEP, a medication that can prevent HIV infections.
- **Respond** rapidly to detect and respond to growing HIV clusters and prevent new HIV infections.
- **HIV HealthForce** will establish local teams committed to the success of the Initiative in each jurisdiction.

The Initiative will target our resources to the 48 highest burden counties, Washington, D.C., San Juan, Puerto Rico, and 7 states with a substantial rural HIV burden.



Geographical Selection:
Data on burden of HIV in the US shows areas where HIV transmission occurs more frequently. More than 50% of new HIV diagnoses* occurred in only 48 counties, Washington, D.C., and San Juan, Puerto Rico. In addition, 7 states have a substantial rural burden – with over 75 cases and 10% or more of their diagnoses in rural areas.

*2016-2017 data

Ending the HIV Epidemic

www.HIV.gov

Emerging concerns over blood safety in detecting HIV infection if treated by ART/PrEP

Identifying HIV-infected persons and facilitating early ART and PrEP use is a major public health goal -- Ending the HIV Epidemic: A Plan for America Program

Early ART leads to delayed antibody response or seronegativity, as well as suppression of viral RNA

There is delayed seroconversion among people who used PrEP but become infected

Although blood donors are not supposed to donate if known to be HIV-infected or on antiretrovirals (PrEP or ART), evidence from REDS-III South Africa and FDA TTIMS US studies that persons on ART donate

Vox Sanguinis (2017) **112**, 473–476

DOI: 10.1111/vox.12516

SHORT REPORT

Blood safety implications of donors using HIV pre-exposure prophylaxis

C. R. Seed,[1] H. Yang[2] & J. F. Lee[1]
[1]*Australian Red Cross Blood Service, Perth, WA, Australia*
[2]*Australian Red Cross Blood Service, Sydney, NSW, Australia*

NHLBI REDS-III Monitoring and Acute Treatment of HIV Study (MATHS)

MATHS Goals:

1. Determine Fiebig stage at donation. For those identified as Fiebig I or II initiate ART, and ascertain Fiebig stage at therapy initiation.
2. Establish the size of the peripheral blood viral reservoir at the initiation of ART and at defined time points on ART.
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.

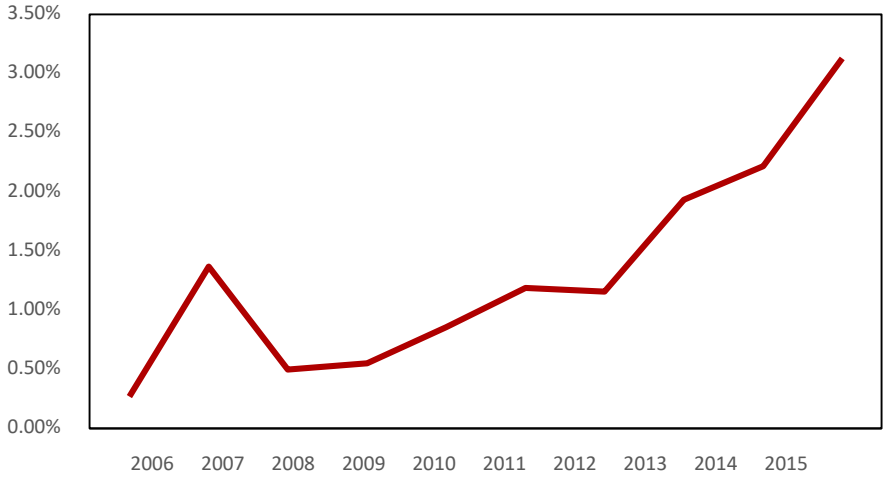
- Discovered that Potential Elite Controllers (HIV antibody+ but RNA-negative) were actually not Elite Controllers but rather HIV infected donors on ART

REDS-III

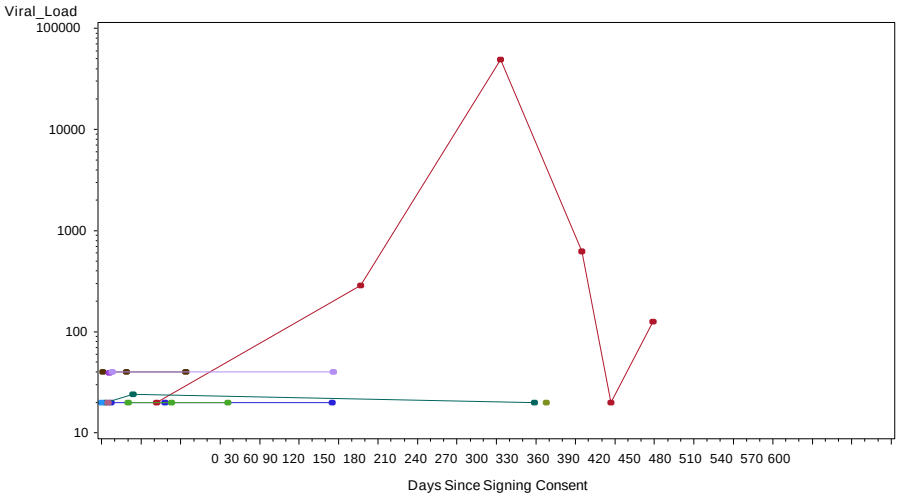
Discovery of "False Elite Controllers in SANBS

- Apparent increase in potential Elite Controllers since 2013
- Anecdotal evidence of Elite Controllers reporting knowledge of HIV status and ART usage at enrolment into MATHS cohort
- Loss of "Elite Control" by a MATHS participant

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



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



REDS-III

Discovery of "False Elite Controllers in SANBS

- Led to a study to evaluate Potential Elite Controllers identified by the South Africa National Blood System between 2010-2016 for the presence of ART

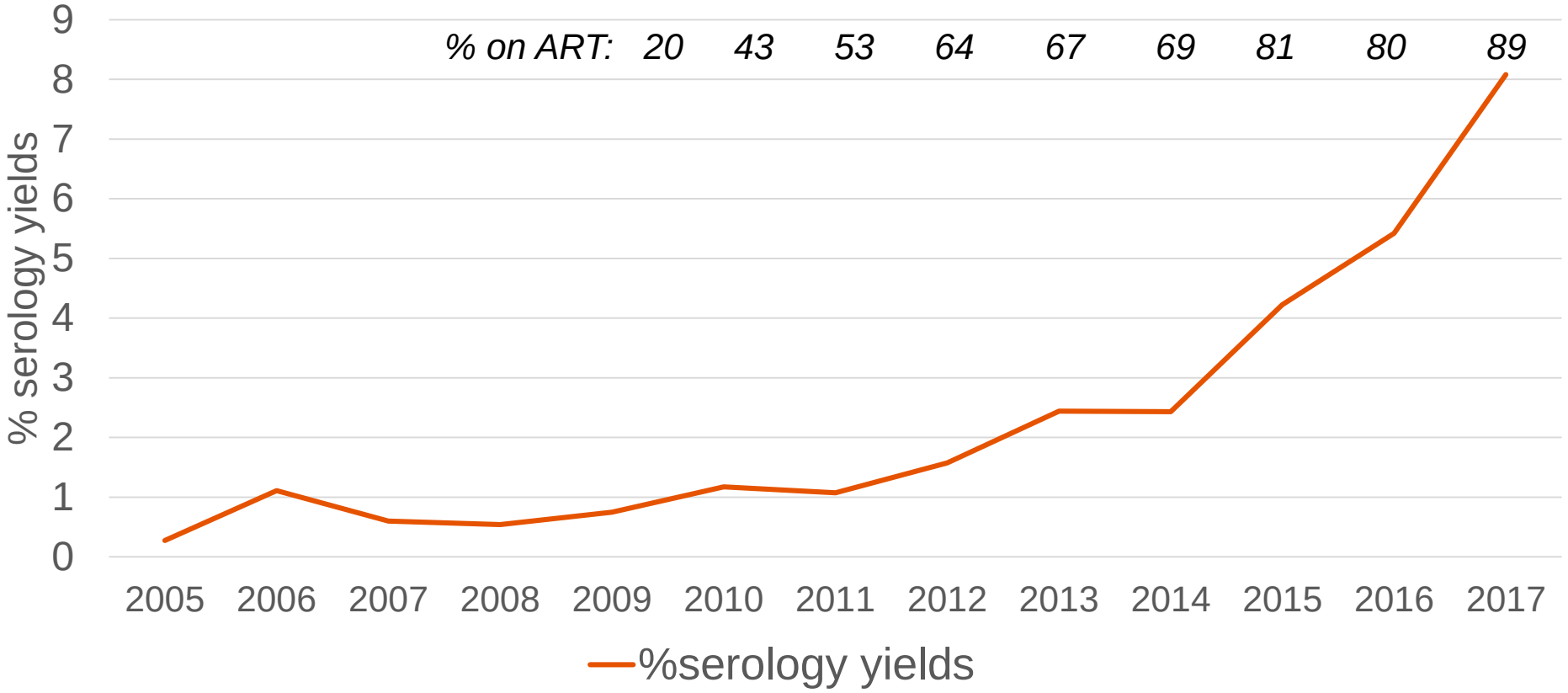
- Frozen plasma from 226 Potential Elite Controllers tested using qualitative liquid chromatography tandem mass spectrometry (sensitivity 0.02µg/mL) for:
  - Nevirapine, Efavirenz, Darunavir, Atazanavir, Lopinavir

➢ **150 (66%) tested positive for at least one ART drug**

SYKES W ET AL. DISCOVERY OF "FALSE ELITE CONTROLLERS": HIV ANTIBODY-POSITIVE RNA-NEGATIVE BLOOD DONORS FOUND TO BE ON ANTIRETROVIRAL TREATMENT. JID 2019

REDS-III

Potential Elite Controllers as a percentage of all HIV positive donations



Vermeulen M. Personal communication

Emerging Concern for Blood Safety in the U.S. Detecting HIV infection if treated by ART/PrEP

Issue: May not be able to detect HIV infections with usual HIV antibody and mini-pool NAT blood donor screening for donors on ART/PrEP

Emerging Questions for the US blood supply:

- What is the proportion of ART use among blood donors in the US?
- What is the proportion of PrEP use among blood donors in the US?
- Can the current HIV assays used in blood donor screening be further optimized?

- Additional studies warranted to evaluate:
  - FDA Transfusion Transmissible Infections Monitoring System (TTIMS) program
  - Proportions of blood donors who do not disclose ART (N=300 cases and 300 controls) or PrEP use (N=1500 selected based on ZIP codes w high PrEP use).
  - Improved strategies to encourage disclosure of ART/PrEP use, and decrease test-seeking behaviors
  - Need for enhanced assays used for HIV donor screening

Transfusion
Transmissible
Infections
Monitoring
System

Impact of Early Antiretroviral Therapy on Detection of Cell-Associated HIV-1 Nucleic Acid in Blood by the Roche Cobas TaqMan Test

Linda L. Jagodzinski,[a] Mark M. Manak,[b] Holly R. Hack,[b] Ying Liu,[b] Jennifer A. Malia,[a] Joanna Freeman,[b] Nittaya Phanuphak,[c] Mark de Souza,[c] Eugène D. Kroon,[c] Donn J. Colby,[c] Nitiya Chomchey,[c] Michelle A. Lally,[d] Nelson L. Michael,[a] Jintanat Ananworanich,[b,c] Sheila A. Peel,[a] on behalf of the RV254/SEARCH010 Study Team

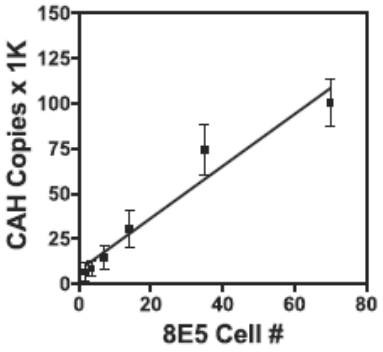


FIG 2 CAP/CTM assay limit of detection for 8E5 cells. Ten replicates of 100-μl aliquots of 1× PBS containing 8E5 cells with CAH at 1, 10, and 100 copies/ml were lysed with SPEX and tested in the CAP/CTM assay. At 1 cell per ml, 1 of 10 replicates resulted in a value at 38 copies/ml (49 copies per 8E5 cell). All aliquots at 10 and 100 cells/ml were detected and gave a broad range of HIV values of from 55 to 2,149 copies per cell, with a mean of 606 and 1,144 copies/cell, respectively. The CAP/CTM assay is capable of detecting CAH in a single 8E5 cell.

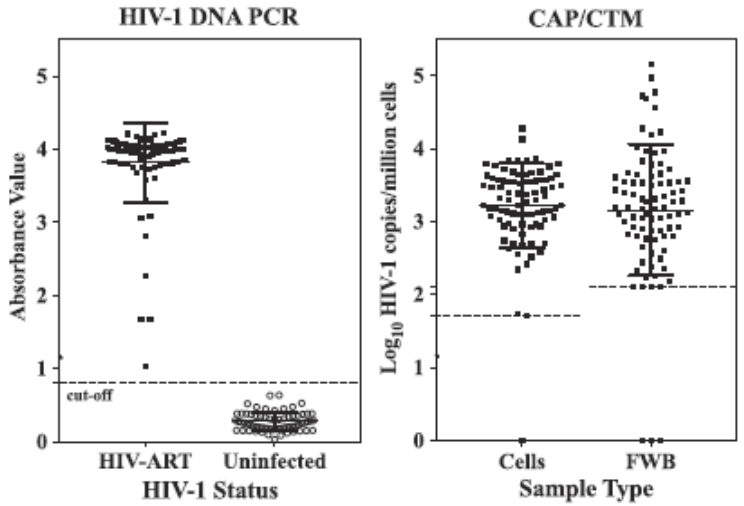


FIG 3 Detection of HIV-1 nucleic acid in frozen whole blood (FWB) and cell pellet samples of ART-treated HIV-1-infected individuals (chronic infection) with no detectable plasma HIV-1 RNA by the Roche Amplicor HIV-1 DNA PCR test, v1.5 (left), and the Roche CAP/CTM assay (right). The dashed lines represent the assay lower-limit cutoff. The CAP/CTM assay results were adjusted for cell input. Samples in which

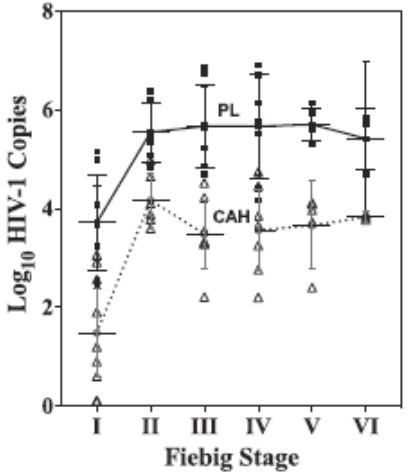


FIG 4 Initial plasma HIV-1 RNA level (plasma load [PL]; in number of $\log_{10}$ HIV-1 RNA copies per milliliter; black circles) and cell-associated HIV (CAH; in number of $\log_{10}$ HIV copies per million cells; open triangles) nucleic acid level prior to initiation of ART.

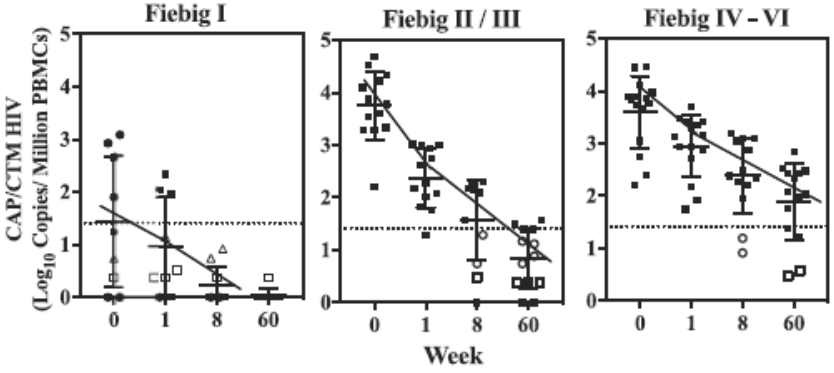


FIG 8 Average decay of cell-associated HIV-1 for participants who initiated ART at Fiebig I, II or III, or IV to VI. Each symbol represents an average of 3 measurements. Values below the LOQ (dotted lines) represent values in which the level in 2 of 3 (open circles) or 1 of 3 (squares) measurements was below the limit of quantitation but detected. Values positioned on the *x* axis were not detected in all three replicates.

Distribution of HIV type 1 (HIV-1) in blood components: detection and significance of high levels of HIV-1 associated with platelets

T.H. Lee, R.R. Stromberg, J.W. Heitman, L. Sawyer, C.V. Hanson, and M.P. Busch

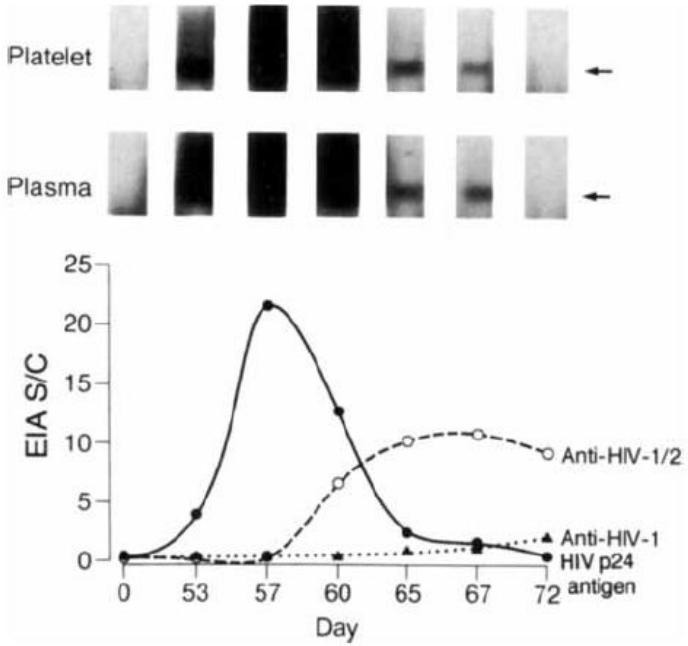


Fig. 3. Platelet-associated HIV-1 before seroconversion.

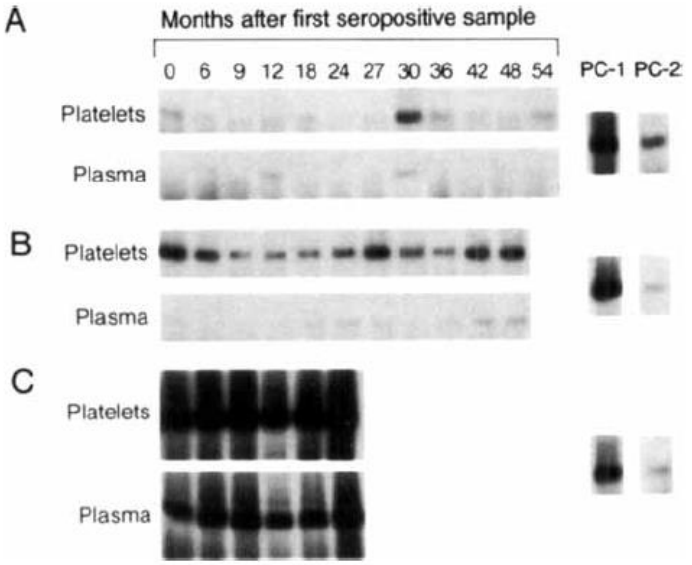


Fig. 4. Comparison of HIV-1 RNA levels in plasma with levels associated with platelets for three seroconvertors with different patterns of disease progression. HIV-1 RNA levels are shown for a subject in whom disease did not progress (A), a subject in whom disease progressed at an intermediate pace (B), and a subject in whom disease progressed rapidly (C; died of AIDS 2 years after seroconversion). The number of platelets processed (20×10^6) at each time point was selected to be approximately equivalent to the amount in 100 µL of whole blood (the volume of plasma processed by immunocapture RT PCR for the corresponding samples). PC-1 = 1000 HIV-1 copies per mL and PC-2 = 100 copies per mL.

TRANSFUSION 1998;38:580-588.

Comprehensive serological profiling of human populations using a synthetic human virome

George J. Xu, Tomasz Kula, Qikai Xu, Mamie Z. Li, Suzanne D. Vernon, Thumbi Ndung'u, Kiat Ruxrungtham, Jorge Sanchez, Christian Brander, Raymond T. Chung, Kevin C. O'Connor, Bruce Walker, H. Benjamin Larman, Stephen J. Elledge*

SCIENCE sciencemag.org 5 JUNE 2015 • VOL 348 ISSUE 6239

Comprehensive Profiling of HIV Antibody Evolution

Susan H. Eshleman,[1,*] Oliver Laeyendecker,[2,3] Kai Kammers,[4] Athena Chen,[5] Mariya V. Sivay,[1] Sanjay Kottapalli,[1] Brandon M. Sie,[1] Tiezheng Yuan,[1] Daniel R. Monaco,[1] Divya Mohan,[1] Daniel Wansley,[1] Tomasz Kula,[6] Charles Morrison,[7] Stephen J. Elledge,[6] Ron Brookmeyer,[8] Ingo Ruczinski,[5] and H. Benjamin Larman[1,9,*]

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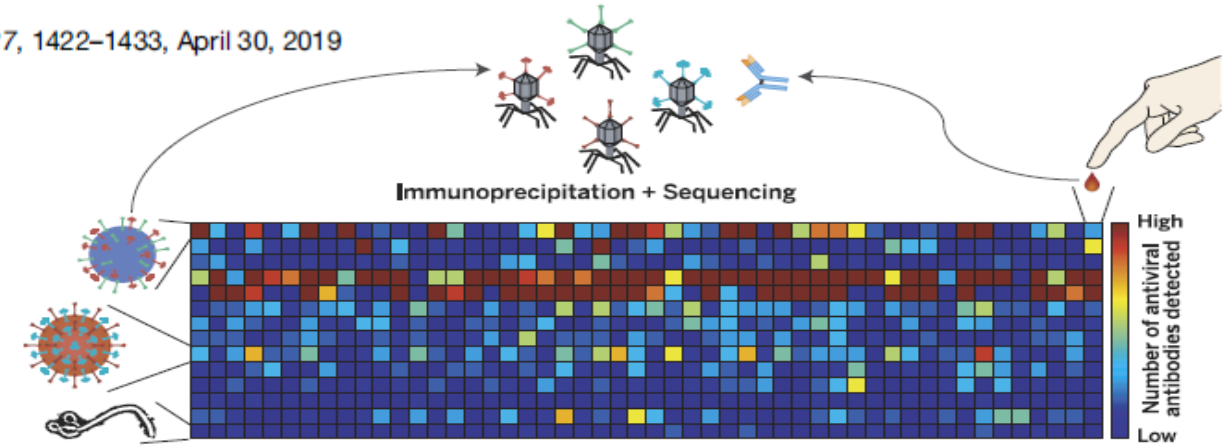
[2]Laboratory of Immunoregulation, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, NIH, Baltimore, MD, USA

[3]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Cell Reports 27, 1422–1433, April 30, 2019



Systematic viral epitope scanning (VirScan). This method allows comprehensive analysis of antiviral antibodies in human sera. VirScan combines DNA microarray synthesis and bacteriophage display to create a uniform, synthetic representation of peptide epitopes comprising the human virome. Immunoprecipitation and high-throughput DNA sequencing reveal the peptides recognized by antibodies in the sample. The color of each cell in the heatmap depicts the relative number of antigenic epitopes detected for a virus (rows) in each sample (columns).

Immunoreactive Proteins on the ADI Pan-HIV Protein Microarray
January 2019

Clade	Gag	Pol	Env	Tat	Rev	Vif	Vpr	Vpu	Nef
A1 (20)	MA, CA	PR, RT-p51, RT-p66, IN	gp120, gp120-euk, V1, V3, gp41, gp41-IE, MPER	Full-length Exon 1 Exon 2	Full-length Exon 2		Vpr		Nef
A2 (28)	MA, CA, NC, p6	PR, RT-p51, RT-p66, IN	gp120, V1, V1-V2, V2, V3, V4, V5, gp41, gp41-IE, MPER	Full-length Exon 1 Exon 2	Full-length Exon 1 Exon 2	Vif	Vpr	Vpu	Nef
B (27)	MA, CA, NC, p6	PR, RT-p51, RT-p66, IN	gp120, gp120-euk, V1-V2, V2, V3, V4, V5, gp41, gp41-IE, MPER	Full-length Exon 1 Exon 2	Full-length Exon 2	Vif	Vpr	Vpu	Nef
C (27)	MA, CA, p6	PR, RT-p51, RT-p66, IN	gp120, gp120-euk, V1, V2, V3, V4, V5, gp41, gp41-IE, MPER	Full-length Exon 1 Exon 2	Full-length Exon 1 Exon 2	Vif	Vpr	Vpu	Nef
D (22)	MA, CA	PR, RT-p51, RT-p66, IN	gp120, gp120-euk, V1, V1-V2, V4, V5, gp41, gp41-IE	Full-length Exon 1 Exon 2	Full-length Exon 2		Vpr	Vpu	Nef
F (17)	MA, CA	PR, RT-p51, RT-p66, IN	gp120, gp120-euk, V3, gp41, gp41-IE	Exon 1	Exon 1 Exon 2		Vpr	Vpu	Nef
G (5)			gp120, gp41, gp41-IE		Full-length			Vpu	
A/E (19)	MA, CA	PR, RT-p51, RT-p66, IN	gp120, gp120-euk, V3, gp41, gp41-IE	Full-length	Full-length Exon 1, 2	Vif	Vpr	Vpu	Nef
A/G (5)			gp120, gp41, gp41-IE		Exon 2			Vpu	
HIV-2A (15)	CA, NC	PR, RT-p51, RT-p66, IN	gp120, gp120-euk, V2, V3, V4, gp41, gp41-IE	Exon 1			Vpx		
HIV-2B (23)	Pr55, MA, CA, NC, p6	PR, RT-p51, IN	gp120, V1, V2, V1-V2, V3, V4, V5, gp41, gp41-IE	Exon 1 Exon 2	Exon 2	Vif	Vpr Vpx		

For several clades we used more than one molecular clone. In total the ADI HIV protein microarray contains almost 300 immunoreactive forms of 208 HIV proteins, protein fragments and epitopes.

Courtesy of David Camerini, Antigen Discovery Inc.

ANTIGEN DISCOVERY INC
the proteome company

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REDS-III

Transfusion Transmissible Infections Monitoring System

BILL & MELINDA GATES foundation

vitalant Research Institute

Public Health England

SACEMA

UCSF University of California San Francisco

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Thank you