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HIV Seroconversion Panels

Tools to Study Evolving Infection

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Abstract

Seroconversion is the evolution of plasma markers of infection following pathogen exposure. Plasma collection (for fractionation into immune globulins, etc.) gives rise to unique circumstances that allow the identification of serial samples collected at short intervals from a recently infected donor:

- Plasma can be donated by plasmapheresis twice a week.
- Plasmapheresis collections are 600-880 ml.
- Every unit collected is tested for anti-HIV-1/2, anti-HCV, and HBsAg; most are tested for HIV, HBV and HCV nucleic acid.
- Nucleic acid tests are performed on pools of 24-512 units.
- Donors who test positive for HIV or HCV are permanently deferred from donation.
- Units with a positive test result, and previous units from the same donor, are removed from the fractionation supply.

HIV Seroconversion Panels (SCP) were first identified in 1987. A data sheet comparing most test methods then available was developed, and SCP were made available to researchers worldwide. New information on viral dynamics and human immune response in very early infection has resulted from study of more than 100 SCP for HIV alone, but new SCP are becoming rare. Results from 7 new SCP, 3 collected in 2006, will be presented.

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Materials

Serial plasma donations were acquired from a plasma collection company after the donor tested positive for HIV and was permanently deferred from further donation. Seven series consisting of 6-10 bleeds, each with ~800 ml volume, were assembled for characterization. Table 1 lists some attributes of each panel.

Introduction

The first seroconversion panels were developed in 1986, after testing to screen blood donations for HIV began (anti-HIV, 3/85). As test methods for HIV p24 antigen (HIVAg, 1996), HIV Ag/Ab (1998) and HIV RNA (1999) became available, we learned that HIV infections develop with a specific pattern of marker evolution (Figure 1).

In a typical HIV seroconversion profile, HIV RNA becomes detectable first using amplification methods, rises rapidly, then begins to fall nearly as rapidly as the immune response begins, and settles at a plateau level prognostic for disease progression in the absence of treatment. HIVAg becomes detectable with current methods when HIV RNA reaches about 1E+5, follows the time course of HIV RNA rise and fall, and becomes undetectable when anti-HIV appears. Anti-HIV becomes detectable 7-14 days after HIV RNA is first detected by screening methods.

This average picture can mask much variation based on the strain of the infecting virus, the immune system of the person infected, the test methods' sensitivity and specificity, and the interactions among all of these. Characterization to date of seven newly-developed HIV seroconversions collected in 1997, 2000, and 2006 illustrates some of this variation.

Methods

Characterization to date has included testing with commercially available methods for HIV RNA (2 methods), HIV antigen EIA (4), anti-HIV 1/2 EIA (5), combination HIVAg/Ab EIA (6), and anti-HIV Western blot (1). Test method names and product numbers are available on data sheets for each panel at: www.seracare.com/bbidx/hiv_panels.htm.
Table 2 lists examples from panel data sheets for PRB960, 964, 965 and 966. Additional characterization is planned to acquire genotype, drug resistance mutation patterns and CCR5 tropism for each panel, as well as data from other test methods for RNA, HIVAg and anti-HIV.

Results

HIV seroconversion during regular plasma donation is a relatively rare event. Seven such series collected over 9 years will add to the approximately 80 series currently available for study and use in test method development. Observations from these series include:

- HIV RNA rising to peak at 30 million copies over 7 days in one panel (PRB960), and to a peak 2 orders of magnitude lower over 12 days in another (PRB965) (See Figure 2.)
- HIV antibody appearing 7 days after 1st RNA detection in one series (PRB963), and 13 days in another (PRB966)
- HIV antigen appearing quite consistently when HIV RNA reaches 1E+5 copies/ml
- One series (PRB960) where HIV antigen is not detected by one HIVAg/Ab test that is consistent with five other methods in all other series
- One series (PRB964) where HIV antibody appears almost simultaneously with HIV antigen, rather than 4 to 7 days later
- Two series (PRB965 and PRB966) that illustrate differing sensitivities among HIVAg/Ab combination methods, and that these methods can produce a negative result (s/co<1.0) when HIVAg levels decrease rapidly as anti-HIV levels rise

Acknowledgements

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Conclusions

- Each new seroconversion found can add to our understanding of early HIV infection and of comparative test method sensitivity.
- These seroconversion panels confirm previous findings of an average initial RNA ramp up rate of just over 2 doublings per day, or 10 fold increase in viral load every other day, until a peak is reached and a decrease begins.
- Monitoring test methods with newly identified seroconversions provides manufacturers and users with important information on the performance of their assays, when continuing virus mutation, individual immune responses and new drug therapies can impact that performance.
- Seroconversion panels for HIV and other infectious diseases represent a unique resource for evaluating test sensitivity and specificity for markers of these diseases.

References

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2. TD Ly et al. Evaluation of the sensitivity and specificity of six HIV combined p24 antigen and antibody assays. J. Virological Methods 2004, **122**(2):185-94.

3. MP Busch et al. Relative sensitivities of licensed nucleic acid amplification tests for detection of viremia in early human immuno-deficiency virus and hepatitis C virus infection. Transfusion 2005, **45**:1853-63.

Results

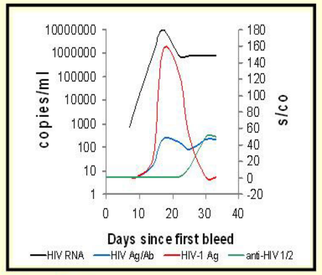

Figure 1. Typical pattern for emergence of HIV markers in plasma

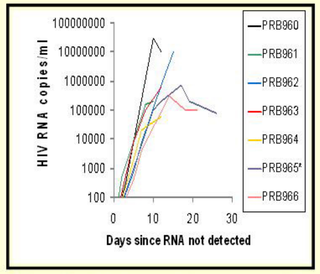

Figure 2. HIV RNA replication in seven HIV seroconverters. *PRB965 has 1000 c/ml HIV RNA in the Day 0 bleed.

Table 1. Properties of newly developed HIV Seroconversion Panels: + indicates that the series has members positive for the indicated marker; – indicates that the marker does not become positive in that series; +/– indicates some members of the series are borderline or low positive for the marker.

				Test Profiles				
Panel ID	# of Bleeds	Year collected	Days spanned	HIV RNA	HIV Ag (p24)	Combi HIVAg Anti-HIV	Anti-HIV	Comments
PRB960	9	1997	30	+	+	+	–	HIV RNA 300-->30 million cp/ml in 7 days
PRB961	9	1997	29	+	+	+	–	p24 band (artifact) on Western blot
PRB962	6	2000	17	+	+	+	–	HIV RNA 800-->10 million cp/ml in 10 days, 4 bleeds
PRB963	7	2000	21	+	+	+	+/-	Anti-HIV positive by the most sensitive methods
PRB964	6	2006	22	+	+	+/-	+/-	Relatively slow HIV RNA progression
PRB965	6	2006	21	+	+/-	+	+	Illustrates differing sensitivity among HIVAg/Ab tests
PRB966	10	2006	51	+	+	+	+	Rapid antibody development

Table 2. Excerpts from data sheets for four HIV seroconversions. Go to www.seracare.com/bbidx/hiv_panels.htm and click on the panel number to see the entire data sheet.

Panel Member #	Days since 1st Bleed	HIV RNA	HIVAg	HIV Antigen/Antibody Combination Tests						HIV Antibody Tests			
		Roche	Roche	Abbott	Abbott	Abbott	Dade Behring	Roche	Abbott / Murex	Abbott	Abbott	SIEMENS	Abbott / Murex
		Cobas Ampliprep-Taqman	Elecsys HIV Ag	ARCHITECT HIV Ag/Ab Combo	AxSYM HIV Ag/Ab Combo	Prism HIV Ag/Ab Combo	‡Enzygnost HIV Integral II	Elecsys HIV Combi	HIV Ag/Ab Combination	Abbott Prism HIV O plus	Abbott AxSYM HIV 1/2 gO	Advia Centaur HIV enhanced (EHIV)	Murex HIV 1.2.0
		copies/ml	s/co	s/co	s/co	s/co	s/co	s/co	s/co	s/co	s/co	s/co	s/co
PRB960-01	0	BLD	0.35	0.12	0.52	0.08	0.05	0.26	0.25	0.32	0.47	0.10	0.26
PRB960-02	4	BLD	0.33	0.09	0.52	0.08	0.05	0.23	0.24	0.31	0.44	0.05	0.23
PRB960-03	7	BLD	0.38	0.09	0.49	0.07	0.08	0.23	0.25	0.35	0.45	0.07	0.23
PRB960-04	11	BLD	0.33	0.11	0.48	0.09	0.09	0.22	0.29	0.31	0.44	0.05	0.22
PRB960-05	14	BLD	0.32	0.11	0.65	0.07	0.05	0.21	0.26	0.32	0.71	0.09	0.22
PRB960-06	18	BLD	0.34	0.12	0.54	0.08	0.08	0.26	0.29	0.35	0.46	< 0.05	0.23
PRB960-07	21	2E+2	0.30	0.21	0.49	0.09	0.06	0.23	0.25	0.39	0.42	0.09	0.25
PRB960-08*	28	3E+7	512.30	461.68	40.22	131.77	0.25	69.54	>max	0.30	0.40	0.11	0.26
PRB960-09	30	7E+6	604.00	556.73	43.45	159.62	0.31	90.52	17.87	0.35	0.47	0.12	0.28
PRB964-01	0	BLD	0.27	0.10	0.41	0.06	0.05	0.29	0.28	0.25	0.48	0.09	0.27
PRB964-02	3	BLD	0.29	0.11	0.43	0.06	0.05	0.33	0.27	0.23	0.42	0.09	0.26
PRB964-03	10	BLD	0.27	0.16	0.42	0.06	0.06	0.28	0.28	0.29	0.42	0.12	0.23
PRB964-04	15	2E+3	0.25	0.14	0.42	0.07	0.06	0.27	0.29	0.27	0.40	0.15	0.23
PRB964-05	17	2E+4	0.42	0.43	0.46	0.17	0.15	0.30	0.38	0.29	0.44	< 0.05	0.23
PRB964-06	22	7E+4	0.63	1.05	0.88	0.73	0.43	0.88	1.30	0.67	0.76	0.90	0.46
PRB965-01	0	3E+3	0.27	0.22	0.43	0.09	0.07	0.25	0.28	0.23	0.47	0.06	0.25
PRB965-02	5	2E+5	1.58	2.75	0.72	1.04	0.07	0.34	1.26	0.24	0.43	0.12	0.29
PRB965-03	7	4E+5	2.11	3.82	0.77	1.39	0.06	0.37	1.74	0.30	0.47	0.70	0.38
PRB965-04	12	3E+5	3.01	15.64	14.29	5.99	6.61	33.57	15.78	20.29	10.94	> 50	13.37
PRB965-05	14	2E+5	1.65	22.42	17.14	8.52	13.20	131.40	16.17	31.49	14.90	> 50	13.25
PRB965-06	21	1E+5	0.68	18.78	12.45	8.77	10.31	186.40	15.57	26.52	12.09	> 50	13.38
PRB966-01**	0	BLD	0.24	0.14	0.39	0.05	0.05	0.28	0.29	0.26	0.43	0.31	0.25
PRB966-02	2	BLD	0.23	0.18	0.43	0.06	0.05	0.27	0.30	0.25	0.41	0.27	0.24
PRB966-03	20	BLD	0.24	0.16	0.45	0.08	0.05	0.29	0.28	0.26	0.41	0.08	0.25
PRB966-04	22	BLD	0.23	0.13	0.42	0.06	0.05	0.29	0.27	0.26	0.44	0.09	0.29
PRB966-05	30	BLD	0.26	0.21	0.39	0.08	0.05	0.28	0.27	0.27	0.42	0.21	0.30
PRB966-06	35	4E+2	0.24	0.18	0.43	0.07	0.05	0.28	0.27	0.27	0.43	0.07	0.28
PRB966-07	37	4E+3	0.29	0.30	0.42	0.07	0.06	0.26	0.30	0.29	0.46	0.09	0.27
PRB966-08	44	3E+5	2.57	2.10	1.03	1.10	1.93	0.56	1.90	0.50	0.69	0.87	0.71
PRB966-09	48	1E+5	0.78	2.10	2.50	1.36	0.75	2.03	4.02	5.23	3.28	7.95	4.28
PRB966-10	51	1E+5	1.31	9.62	12.40	3.50	3.99	89.63	15.35	26.00	15.62	> 50	12.77

BLD = below limit of detection *PRB960-08: HIV RNA was quantitated on dilution using the Roche HIV-1 Amplicor v.1.5. **Panel PRB966 was tested with the Roche HIV-1 Amplicor v.1.5. ‡PRB960 test results were repeated in two laboratories.