Twenty Year Stability Study of HIV, HBV, and HCV Antibodies, Antigen and Nucleic Acids in Plasma

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INTRODUCTION

Plasma samples that are stored frozen for prolonged periods are important for retrospective and epidemiological studies in infectious disease. Antibody, antigen, and nucleic acid detected in retention samples can also provide important information for treatment, assessment of disease progression, and drug discovery. Frozen human blood from 1959 has been used to trace the spread of HIV group M in Africa.[1] Frozen serum samples collected from military recruits in 1948-1954 were found to contain HCV RNA and antibody, demonstrating that these can be detected 45 years after collection and storage at -20°C.[2]

Seroconversion panels (undiluted, minimally processed serial bleeds collected from individual donors while markers of an infection are emerging) have been used for twenty years for research in early infection and in the development of assays for markers of infection.

HIV and HCV antibodies, HBsAg, and viral RNA and DNA were evaluated in seroconversion panels collected between 1981 and 2000 to determine the stability of these markers after prolonged frozen storage.

MATERIALS & METHODS

Plasma: Serial bleeds from plasma donors collected prior to and during very early infection between 1981 and 2000 were characterized, aliquoted, and stored at -20ºC (prior to 1996) or -70ºC (after 1996) in 0.25 to 1.5 mL aliquots or larger volumes, and retested in 2007 and 2008.

Test Methods: Panels were tested at SeraCare in 2007 and 2008 using current serology or NAT methods. Serology tests were performed with Abbott EIA, following manufacturer's instructions; data are reported as s/co. Western and RIBA blots (from Medmira and Ortho) were performed for HIV and HCV respectively. Serology comparison was available for similar or identical methods used to test antibody or antigen in 1988 through 1996.

Series collected in 1981, 1989, 1990 and 1995 were tested for HIV RNA with Roche PCR methods in 1995-6 (qualitative) and 2007-8 (quantitative). Panels collected in 1993, 1995, 1996 and 2000 were tested for HCV RNA with Roche PCR in 1994-5 (qual) and 2007-8 (quant). Panels collected in 1990 and 1991 were tested in 1994 with an in-house method for HBV DNA or in 2005 by Roche PCR (both qual), and panels from 1993 and 1997 were tested in 1998 and in 2007-8 by Roche PCR (quant).

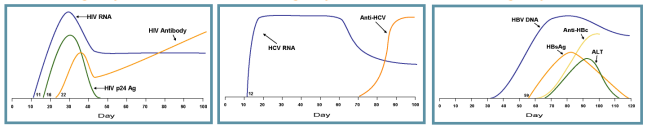
EIA and nucleic acid tests were performed in duplicate while blot tests (Western and RIBA) were single assays.

Data Analysis: Correlation was determined by comparing initial test results (1988-1996) to 2007-8. Correlation was positive if results agreed, and negative if results did not agree.

TABLE 1. SEROLOGY RESULTS COMPARED 1988 - 1996 vs. 2007

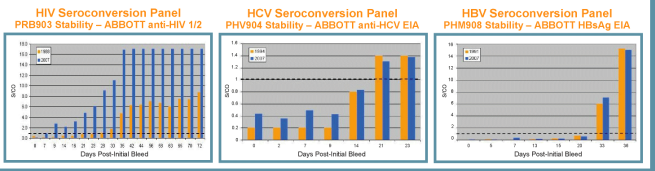
Marker	Panel ID	Initial Test Date	# Members	Correlated Results	% Correlation
Anti-HIV	PRB903	1988	18	18	100
	PRB904	1992	5	5	100
	PRB910	1992	7	7	100
	PRB916	1992	6	6	100
HBsAg	PHM904	1991	3	3	100
	PHM908	1991	8	8	100
	PHM911	1991	25	24	96
	PHM912	1991	9	8	89
Anti-HCV	PHV904	1995	7	7	100
	PHV905	1996	9	9	100
	PHV908	1996	13	13	100
Overall			110	108	98

HIV AND HEPATITIS SEROCONVERSION



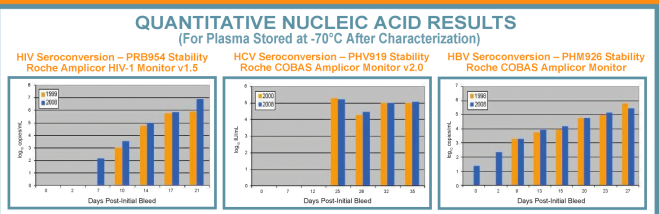
Time to antibody response (HIV, HCV) or antigen appearance (HBV) following virus exposure and detection of viral nucleic acid in plasma is on average shortest for HIV, longest for HCV and intermediate for HBV.

TYPICAL SEROLOGY RESULTS



QUANTITATIVE NUCLEIC ACID RESULTS

(For Plasma Stored at -70°C After Characterization)



QUANTITATIVE NUCLEIC ACID RESULTS

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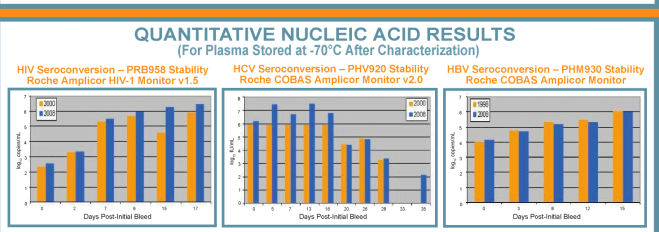
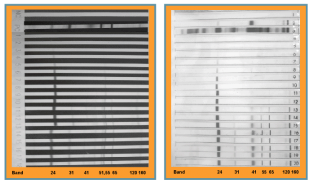


TABLE 2. VIRAL NUCLEIC ACID STABILITY IN PLASMA (Storage: -20°C Before 1997, -70°C After)

Marker	Panel ID	Year Collected	Initial Test Year	# Members	% Correlation Qualitative*	% Correlation Quantitative**
HIV RNA	PRB905	1981	1996	10	100	NT
	PRB916	1989	1995	6	100	NT
	PRB917(M)	1990	1995	5	100	NT
	PRB951	1995	1995	9	100	0
	PRB954	1998	1999	7	100	100
	PRB958	2000	2000	8	100	100
HCV RNA	PHV901	1993	1994	11	64 (2007 negative)	NT
	PHV904	1995	1995	7	0 (2007 negative)	0
	PHV906	1996	1996	13	62 (2007 negative)	0
	PHV919	2000	2000	7	100	100
	PHV920	2000	2000	10	90 (2008 negative)	90 (2008 negative)
HBV DNA	PHM904	1990	1994	3	100	NT
	PHM908	1991	1994	8	25 (2007 negative)	NT
	PHM911	1990	1994	25	84 (2007 negative)	NT
	PHM912	1990	1994	9	11 (2007 negative)	NT
	PHM914	1990	2005	6	100	100
	PHM926	1997	1998	8	100	100
	PHM930	1993	1998	5	100	100

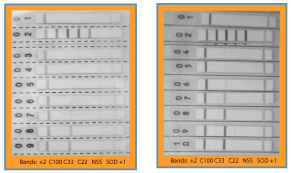
* Qualitative correlation was considered positive if quantitative tests in 2007 detected RNA or DNA.
** Quantitative correlation was considered positive if 2007 or 2008 quantitative results were ±0.3 log from earlier quantitative results.
NT=not tested by quantitative method.

HIV SEROCONVERSION WESTERN Blot



Anti-HIV-1 Western blot in Panel PRB903 from 1990 (left panel) and 2007 (right panel). The antibody profile and intensity were retained for all members. These data are representative of Western blots for other HIV panels tested.

HCV SEROCONVERSION RIBA Results



Anti-HCV was detected in Panel PHV904 from 1995 (left panel) and 2007 (right panel). The antibody profile and intensity were retained. These data are representative of other HCV panels tested.

RESULTS

- As previously reported, anti-HIV, anti-HCV and HBsAg are stable in plasma samples stored frozen at -20°C or colder for 13-20 years.[3]
- No trend toward deterioration over time of anti-HIV, anti-HCV or HBsAg is apparent in these seroconversion series.
- Plasma stored at -20°C for years demonstrates degradation of HCV RNA (most), HIV RNA (significant), and possibly HBV DNA. (1994 in-house HBV DNA assay was not validated/calibrated to current standards.)
- HIV RNA is still detectable in samples stored at -20°C for years, though in much lower concentration than originally found. HCV RNA becomes undetectable in some samples.
- HIV RNA, HCV RNA and HBV DNA in minimally processed plasma are stable for at least eight to ten years after transfer to long-term storage at -70°C.

CONCLUSIONS

- The absence of a trend toward deterioration over time of anti-HIV, anti-HCV and HBsAg, and the literature precedents,[1,2] justify the setting of expiration at 25 years for minimally processed plasma, stored frozen and characterized for these analytes.
- HIV and HCV antibodies in plasma stored frozen produced equivalent staining patterns and intensity in Western blots and RIBA over 15+ years.
- HIV RNA, HCV RNA and HBV DNA in minimally processed plasma were also stable after long-term storage at -70°C.
- Serology tests in 2007-8 are more sensitive than those available in 1988-1996.
- Higher results seen in quantitative nucleic acid tests for HIV and HCV RNA may be due to improved sensitivity or calibration differences.

REFERENCES

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