Long-Term Stability of Viral Markers in Plasma

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Abstract

Seroconversion panels and performance panels are assembled from minimally processed plasma. That is, panels are assembled from units of source or recovered plasma that have at most been filtered at 0.2 microns, subjected to fewer than 5 freeze-thaw cycles during processing, and aliquoted into small volumes for use in research or test method development or evaluation. No preservatives have been added, and in some cases the original plasma units had been stored for years at -20° C in temperature cycling freezers.

Since the units that comprise these panels are large volumes (600-880mL), the resulting 1 mL panels are often available for many years. If the tests are also available for many years, real-time stability can be readily established for viral markers and potentially for other components.

Seroconversion panels for HIV, HBV and HCV that had been collected as early as 1981 and as recently as 1996 (18 panels in total) were evaluated by comparing the earliest test results available to test results generated on the same plasma in 2007. Antibodies to HIV and HCV, HBsAg, HIV and HCV RNA and HBV DNA were tested.

Results demonstrate that antibodies to HIV and HCV, and HBsAg, show no deterioration over more than 20 years even when stored in less than ideal conditions. These results, not unexpected, allowed us to propose 25 year dating for these plasma products.

Somewhat more surprising were the results for HIV and HCV RNA. Early quantitative test results were not available, but qualitative comparisons indicated that for HIV and HCV RNA, all panel members that were positive in the earliest test results available were still positive when tested in 2007, indicating a minimal degradation of RNA.

HBV DNA appeared stable between 1995 and 2007 when test results from similar methods were compared. Some panels tested by an in-house method in the early 1990s yielded lower results in 2007, possibly due to DNA degradation or to test method differences.

Introduction

- 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.
- Beginning in 1986, panels consisting of a set of aliquots from such serial plasma collections and a data sheet allowing test method comparisons were commercialized as seroconversion panels.
- Seroconversion panels and some other plasma products for diagnostics are developed using 'minimally processed plasma' (MPP) to maintain the closest similarity possible to patient samples.
- Minimally processed plasma is defined for this purpose as plasma that has undergone no more processing than 0.2 micron filtration, up to five freeze-thaw cycles, and aliquoting or dispensing.
- The use of seroconversion panels to track the sensitivity improvement in test methods requires that the panels themselves are stable over time. See Table 1 for an earlier example.
- The large plasma volumes available for each seroconversion panel member, and the consistent patterns of marker evolution, allow us to track the stability of these markers in frozen plasma.

Materials

HIV, HCV and HBV seroconversion panels were selected from current SeraCare Life Sciences inventory using the earliest collection and test dates available. Panels were intended to be representative of these MPP products, and had data for serological and nucleic acid tests. Serial collections from 1981 and from other years through 1996 were available. Bulk and dispensed plasma were stored at -20º C prior to 1997 and subsequently at -70ºC.

Methods

Panels were tested at SeraCare in 2007 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 and compared to previous blots. 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 (quantitative). Panels collected in 1993, 1995 and 1996 were tested for HCV RNA with Roche PCR in 1994-5 (qual) and 2007 (quant). Panels collected in 1990 and 1991 were tested in 1994 with an in-house method or in 1995 by Roche PCR (both qual) and in 2007 by Roche PCR (quant).

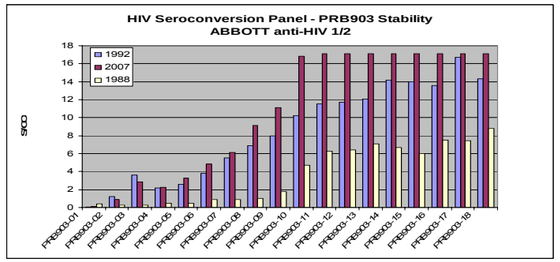
EIA and nucleic acid tests were performed in duplicate while the blots tests (Western and RIBA) were single assays.

Table 1. Use of HIV seroconversion panels to track improved test sensitivity

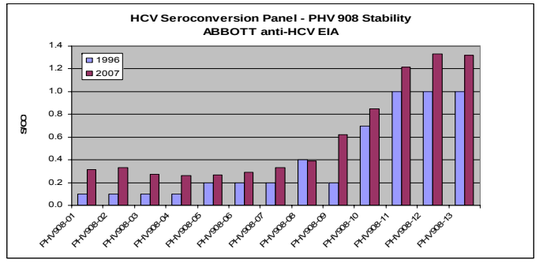
HIV-1 Seroconversion Panel C (PRB903)
Data Sheet

			-------- U.S. FDA-Licensed Anti-HIV EIA Tests, results expressed as s/co --------					
			1988	1992	1988	1992	1988	1992
Member I.D. #	Bleed Dates	Days Since 1st Bleed	Abbott HIV BBI	Abbott HIV BBI	Gen. Sys. HIV BBI	Gen. Sys. HIV BBI	Org. Tek. HIV BBI	Org. Tek. HIV BBI
PRB903-01	16-Jul-85	0	0.4	0.2	0.3	0.1	0.4	0.4
PRB903-02	23-Jul-85	7	0.3	0.2	0.1	0.3	0.5	0.4
PRB903-03	25-Jul-85	9	0.3	0.3	0.2	0.3	0.7	0.4
PRB903-04	30-Jul-85	14	0.5	0.6	0.4	0.5	1.0	1.0
PRB903-05	01-Aug-85	16	0.5	0.7	0.8	0.5	1.2	1.2
PRB903-06	06-Aug-85	21	0.9	1.6	0.8	1.2	1.6	2.1
PRB903-07	08-Aug-85	23	0.9	2.0	0.8	1.2	2.0	2.3
PRB903-08	13-Aug-85	28	1.0	2.8	1.1	1.8	2.2	3.2
PRB903-09	15-Aug-85	30	1.8	3.2	2.0	2.3	2.3	3.6
PRB903-10	20-Aug-85	35	4.7	5.5	2.6	4.0	2.7	4.5
PRB903-11	27-Aug-85	42	6.3	7.3	3.3	4.6	3.0	4.7
PRB903-12	29-Aug-85	44	6.4	7.2	3.3	4.5	2.7	4.8
PRB903-13	10-Sep-85	56	7.1	7.3	3.3	4.5	2.9	5.3
PRB903-14	12-Sep-85	58	6.7	7.3	3.7	5.0	2.9	5.1
PRB903-15	17-Sep-85	63	6.0	7.2	3.0	4.9	3.0	5.1
PRB903-16	19-Sep-85	65	7.5	7.3	2.8	4.4	3.0	5.4
PRB903-17	24-Sep-85	70	7.4	7.3	4.1	5.0	3.0	5.5
PRB903-18	26-Sep-85	72	8.8	7.3	3.7	5.1	2.9	5.2

Graph 1. Comparison (s/co) for PRB903 tested with Abbott anti-HIV-1 in 1988, and Abbott anti-HIV-1/2 in 1992 and 2007



Graph 2. Comparison (s/co) for panel PHV908 tested with Abbott anti-HCV in 1996 and 2007



Results and Discussion

➢Seroconversion panels have been in wide use since 1987, and are considered 'gold standards' for the assessment of serology test sensitivity, particularly for anti-HIV, anti-HCV and HBsAg. The stability of the markers themselves in plasma is therefore of interest.

➢Tracking the long term stability of viral markers in minimally processed plasma (or any analyte in any medium) requires, in addition to a sufficient supply of the plasma, consistent availability of the assay or test method. For this purpose, the continued availability of test methods considered by many to be 'old technology' is helpful.

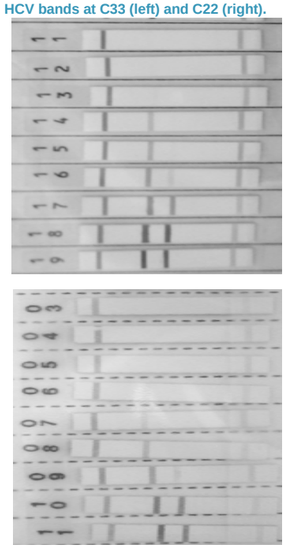
➢**Table 1** exemplifies early efforts to improve anti-HIV-1 test methods. Organon Teknika was the most sensitive method in 1988 and 1992 and appeared unchanged until recently when the method was discontinued.

➢**Graphs 1, 2 and 3** illustrate both the improvement in test methods (for anti-HIV) and the stability of the protein markers for HIV, HCV and HBV.

➢**Figure 1** allows comparison of RIBA patterns from 1996 and 2007 for the same 9-member HCV seroconversion panel. Bands actually appear stronger in 2007.

➢ **Figure 2** shows anti-HIV-1 Western blots for PRB910, a seven member HIV seroconversion collected in 1989. Member #3 was collected 12 days after member #2, was weakly to strongly positive by the eight original U.S. anti-HIV EIA methods, strongly positive by the current Abbott anti-HIV-1/2 in 2007, and has a more intense Western blot band pattern in 2007 (the greater intensity is most likely a function of an improved blot, although the 1990 blot interpretation lists presence of a 160 band not visible here, suggesting that the older blots have faded).

Figure 1. RIBA patterns for HCV panel PHV905 in 2007 (top), 1996 (bottom). Anti-HCV bands at C33 (left) and C22 (right).



Graph 3. Comparison (s/co) of HBsAg for panels collected in 1991 (PHM908) and 1990 (PHM912)

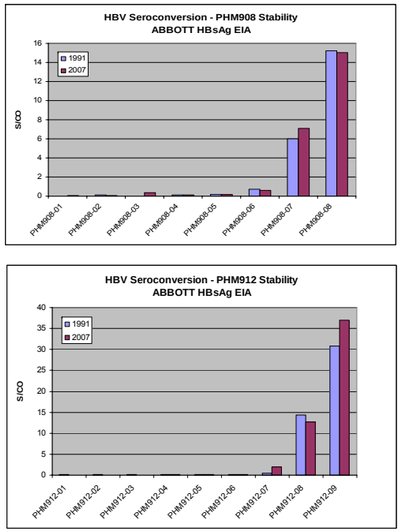
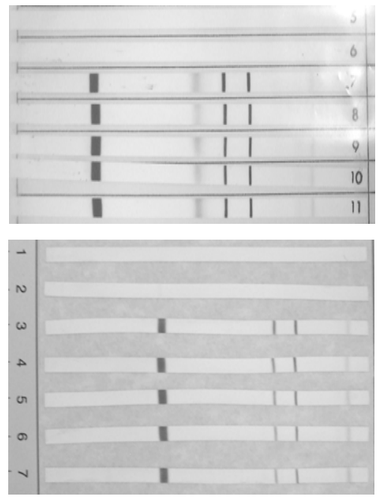


Figure 2. HIV-1 Western blots for PRB910 from 2007 (top) and 1990 (bottom). Visible bands left to right in 2007 are p24, gp41, p51, p65 and gp160. gp41 is not visible in 1990



Conclusions

➢Minimally processed plasma (MPP) is defined as plasma or serum treated with no more that 0.2 micron filtration, aliquoting or dispensing, and up to five freeze-thaw cycles during processing.

➢Seroconversion panels are examples of MPP products, developed to be as similar to patient samples as possible.

➢Tests conducted for anti-HIV, anti-HCV and HBsAg over 11 to 20 years with the same or similar test methods to determine the long-term stability of these analytes in MPP indicated no detectable deterioration of these analytes, and no downward trend in reactivity.

➢Before 1997, seroconversion panels were stored at −20°C, often in frost-free freezers.

➢Based on the test data, a history of continuing usage of seroconversion panels and other MPP products by customers until supplies are exhausted, and no customer complaints concerning panel quality since 2000 (when the current complaint-tracking system began), SeraCare has extended the expiration of MPP products to 25 years.

➢SeraCare plans to continue testing MPP products annually to determine their real-time stability.

➢Quantitative studies of HIV RNA, HCV RNA and HBV DNA in these products are underway. Based on preliminary data and suboptimal storage conditions, expectations are that viral nucleic acids in MPP will be less stable than protein markers.

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