Long-Term Stability of Viral Markers in Plasma

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Abstraction

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 no more than five 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 (800-900mL), 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, HCV and HBV that had been collected as early as 1986, and as recently as 1995 (18 panels in total) were evaluated by comparing the earliest test results available to test results generated in the same assay 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 standing for these plasma products.

Somewhat more surprising were the results for HIV RNA. Early 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 were still positive when tested in 2007, indicating a minimal degradation of RNA.

HBV DNA appeared stable between 1989 and 2007 when test results from similar panels 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 test method differences.

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.
- 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 ‘old technology’ is helpful.

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

<table>
<thead>
<tr>
<th>Assay</th>
<th>Year Tested</th>
<th>Year Collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott anti-HIV-1</td>
<td>1988</td>
<td>1986</td>
</tr>
<tr>
<td>Abbott anti-HIV-1</td>
<td>1990</td>
<td>1992</td>
</tr>
<tr>
<td>Abbott anti-HIV-1</td>
<td>2007</td>
<td>2005</td>
</tr>
</tbody>
</table>

Though antibody levels are stable over time, it is likely that viral nucleic acids in MPP will be less stable than protein markers.

Materials

- HIV, HCV and HBV seroconversion panels were selected from current SeraCare Life Sciences inventories 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.

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 Medimira and Ortho) were performed for HIV and HCV respectively and RIBA blot for HBV.
- 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 RABA patterns from 1995 and 2007 for the same 3-member HIV seroconversion panel. Bands appear virtually unchanged.
- Figure 2 shows anti-HIV-1 Western blots for PRB910, a seven member HIV seroconversion collected in 1999. band4,4 was weakly to strongly positive by the original U.S. anti-HIV EIA methods, strongly positive by the current Abbott anti-HIV-1 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 150 band not visible here, suggesting that the older bands have faded).
- Graph 1. Comparison (s/co) for PRB903 tested with Abbott anti-HIV-1 in 1988, and Abbott anti-HIV-12 in 1992 and 2007
- Graph 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.
- Graph 3. Comparison (s/co) for panel PHV903 tested with Abbott anti-HCV in 1996 and 2007
- Figure 3. HCV-1 Western blots for PRB910 from 2007 (top) and 1990 (bottom). Visible bands left to right in 2007 are p14, p20, p22 and gp46. gp3 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, adjusting 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 means 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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