Analysis of the stability of alanine aminotransferase (ALT) levels in donor samples

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Abstract: This study was designed to 1) analyze the stability of serum alanine aminotransferase (ALT) activity in nonseparated donor samples stored at room temperature (RT) over 72 hours and 2) compare ALT levels of paired samples stored at RT and 4°C over the same time period. The study showed that mean ALT activities for samples stored at RT increased at all assay times and that those stored at 4°C decreased (0.54 IU/L over 72 hours). Although the mean increase in activity of the RT samples over 72 hours (1.45 IU/L) was statistically significant, it was of little practical significance. For example, based on the frequency distribution of the 311 RT samples from this study, and using a cut-off value of 69 IU/L, only one unit of blood would have been eliminated based on the difference between time zero and 72 hours. It was concluded that RT would be the storage of choice for 72 hours, since the tendency of ALT levels to decrease during storage at 4°C could make possible the inclusion of borderline donor units in the blood supply.

In 1981, two studies\(^1,2\) established a relationship between elevated alanine aminotransferase (ALT) levels in donor blood and the incidence of non-A, non-B (NANB) hepatitis in recipients of such blood.

The study by Aach et al.\(^1\) showed that 5 percent of all patients who received units of blood with ALT levels < 30 IU/L developed NANB hepatitis and that this incidence increased substantially to > 90 percent when two or more units with ALT levels in excess of 45 IU/L were transfused. The observations suggested that about 40 percent of the cases of NANB posttransfusion hepatitis (PTH) among recipients in the study might have been prevented by using ALT levels as a surrogate marker and by discarding donor blood with ALT levels above 45 IU/L in their analytic system.\(^1\)

The study by Alter et al.\(^2\) confirmed the significant association between elevated ALT levels in donor blood and the development of recipient PTH. The study also predicted that a donor exclusion value of 53 IU/L in their analytic system "could prevent 29 percent of the PTH cases at a loss of 1.6 percent of donor units."\(^2\)

Further studies by Alter and Hoofnagle\(^3\) showed that an inordinately high percentage of NANB patients developed chronic hepatitis, some with evidence of cirrhosis of the liver. Because there was no specific serologic method to identify NANB carriers, some investigators proposed that surrogate ALT screening be instituted on all donor units as an interim attempt to reduce the occurrence of NANB PTH.\(^4,5\) To implement this, appropriate ALT cutoff values had to be determined, and the test methods for determining the ALT levels had to be standardized.

Cuccherini et al.\(^6\) tested serums obtained from normal individuals and from patients with elevated ALT levels. The serums had been stored at RT, 4°C, and -40°C for up to one month. They found that all ALT activity declined markedly, beginning at 24 hours, in samples other than those from the normal individuals. ALT activity in those samples, possibly because of the relative importance of ALT released from red cells in samples with low (normal) ALT activity, showed a rise during the first few days, followed only later by a decline below baseline values.

In 1986, a study by Williams et al.\(^7\) examined the effect of handling and storage on the stability of serum ALT levels, since testing immediately after collection of blood was not always feasible. Williams and co-workers tested serum samples stored for up to 24 hours at RT, 4°C, -20°C, and -80°C. They also tested serum samples stored on the red cells at RT and 4°C. Serum was removed both proximal and distal to the red cells to determine effects of ALT leakage from red cell lysis. From their results, they recommended that for storage up to one week, serum should be separated early and maintained at 4°C. As expected, ACT levels were higher in some samples taken from near
Stability of ALT levels

the red cell interface due to leakage of ALT from the red cells.

ALT testing on all donor samples was implemented in the American Red Cross Blood Services, Northern Ohio Region, in 1986. The large number of donor samples processed by the blood center each day, the number of different tests required on each sample, and other delays encountered from collection to actual analysis of the samples made it impossible to test all donor samples immediately upon arrival at the center.

The directive of the American Red Cross (BSD 6.53) states that samples may be stored at room temperature for up to 24 hours after collection or at 2-8°C for up to 72 hours after collection. If not tested within 72 hours, serum or plasma separated from the cellular components may be stored at 2-8°C for up to seven days following collection.

Since the standard operating procedure for specimen handling was to maintain the donor samples at RT, unseparated from the red cells, the purpose of this study was to assess the stability of ALT under those conditions. A 72-hour time period was chosen to conduct the analysis, to simulate any possible delay in testing (i.e., sample collection on Friday and analysis on Monday). A comparison of the ALT levels between paired samples stored at RT and 4°C was also conducted.

Materials and Methods

Specimen collection and handling

All samples were free of visible hemolysis, and no attempt was made to select serum either distal or proximal to the red cells.

The study evaluated random donor samples taken from units of blood obtained from a total of 311 volunteer donors. In order to evaluate paired samples, two clotted samples were obtained from 114 of the units by collecting peripheral blood from the bag tubing into two 7-mL Vacutainer tubes (Becton-Dickinson, Rutherford, NJ) containing no anticoagulant. Upon arrival at the blood center (within 3-8 hours of collection), one set of tubes was placed at 4°C; the other set remained at RT. The 4°C samples were analyzed at 24-hour intervals; the RT samples were tested at 12-hour intervals for the entire 72-hour time period.

One clotted sample was obtained from the remaining 197 units of blood at the time of donation using the same procedure described above. Upon arrival at the blood center, these single samples were stored at RT and tested at 12-hour intervals, as above.

Prior to initial testing, all donor samples were centrifuged at 2500 rpm for 10 minutes at RT. The 4°C samples were returned to the refrigerator immediately, where they remained for the 72-hour time period. Aliquots were obtained from each 4°C sample immediately prior to testing at each 24-hour interval. Once the 4°C serum was aliquoted, no attempt was made to maintain the sample at 4°C during testing.

The time lag between collection and initial analysis for the 425 specimens ranged from 4.5 to 14.5 hours (mean=10 hours). The first analysis of ALT levels represented time zero, and the batch included all 425 samples (311 RT and 114 4°C). The time intervals selected for testing the samples over the 72-hour period were based on preliminary studies performed by this investigator.

Enzyme activity

Serum ALT activity was analyzed using a chemical analyzer (Altaire, Electro-Nucleonics, Inc., Fairfield, NJ). SysteMate reagents (EM Diagnostic Systems, Inc., Gibbstown, NJ) were used for testing. The decrease in absorbance due to consumption of nicotinamide adenine dinucleotide, which is directly proportional to ALT activity in donor samples, was measured spectrophotometrically at 340 nm in a kinetic assay. Standard controls were run at regular intervals. A cut-off value of ≤69 IU/L was used.

Statistics

Changes in ALT activity at RT were evaluated statistically by using the t test. Comparisons between the paired RT and 4°C mean ALT levels were analyzed by the paired sample t test for each assay time. The probability (p) values were ascertained using standard methods and a confidence level of 95%.

Results

Mean ALT levels for the 114 samples stored at 4°C were slightly lower at all assay times than the paired samples stored at RT (Table 1). Levels at RT showed an increase of 1.45 IU/L over 72 hours, while levels at 4°C showed a decrease of 0.54 over the same period of time.

Therefore, the mean difference between RT and 4°C ALT levels progressively increased from a time-zero value of 0.42 IU/L to a 72-hour value of 2.41 IU/L, as shown in Table 1. The mean ALT levels of the RT and 4°C serum samples were significantly different at time zero (p=0.0012). Significant differences in ALT activity were
also observed at hours 24 (p = 0.0001), 48 (p = 0.0001), and 72 (p = 0.0001) (Table 1). The mean ALT levels of the 311 samples stored at RT and analyzed at 12-hour intervals for a 72-hour time period increased from a time-zero value of 22.24 IU/L to a 72-hour value of 23.78 IU/L (Table 2). Significant differences were seen for each interval except the 48- to 60-hour interval. The t value at this interval was not statistically significant (-0.72).

ALT activities for the 311 RT samples and the 114 4°C samples, at both time zero and 72 hours, showed that the majority of samples (≥88%) maintained ALT levels of 11–20 IU/L.

Table 1
Comparison of ALT levels on 114 paired donor samples stored at RT or 4°C

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Mean ALT RT IU/L</th>
<th>Mean ALT 4°C IU/L</th>
<th>Mean difference IU/L</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>23.14</td>
<td>22.72</td>
<td>0.42</td>
<td>3.33</td>
<td>0.001</td>
</tr>
<tr>
<td>24</td>
<td>23.67</td>
<td>22.68</td>
<td>0.98</td>
<td>6.08</td>
<td>0.0001</td>
</tr>
<tr>
<td>48</td>
<td>24.18</td>
<td>22.50</td>
<td>1.68</td>
<td>12.27</td>
<td>0.0001</td>
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<tr>
<td>72</td>
<td>24.59</td>
<td>22.48</td>
<td>2.11</td>
<td>13.49</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Table 2
Comparison of 311 ALT levels of donor samples stored at RT

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Mean RT IU/L</th>
<th>Mean difference IU/L</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>22.24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>22.55</td>
<td>0.31</td>
<td>5.64</td>
<td>0.0001</td>
</tr>
<tr>
<td>24</td>
<td>22.67</td>
<td>0.12</td>
<td>2.52</td>
<td>0.0213</td>
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<td>36</td>
<td>22.83</td>
<td>0.46</td>
<td>2.60</td>
<td>0.0037</td>
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<tr>
<td>48</td>
<td>23.28</td>
<td>0.45</td>
<td>6.81</td>
<td>0.0001</td>
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<tr>
<td>60</td>
<td>23.21</td>
<td>-0.07</td>
<td>-0.72</td>
<td>0.4750</td>
</tr>
<tr>
<td>72</td>
<td>23.78</td>
<td>0.57</td>
<td>5.52</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Discussion
This study shows that when donor serum is stored at RT for 72 hours, unseparated from the red cells, mean ALT activity increases. Although the mean increase of 1.54 IU/L is statistically significant, from a practical standpoint the increase is not of a large enough magnitude to be operationally important.

Based on the frequency distribution of the 311 RT samples (data not shown), and using the cut-off value of ≤69 IU/L, one unit of blood would have been eliminated based on the difference observed between time zero and 72 hours. This unit had a borderline ALT level of 68 IU/L at time zero. The level fluctuated sporadically throughout the 72-hour testing period, with an ALT level of 69 IU/L at the time of final testing. Whether the apparent exclusion of this unit would have been due to a true increase in its ALT activity or to inherent imprecision in the analytic system was not determined.

This study also shows that when unseparated donor samples are stored at 4°C for 72 hours, the mean ALT activity decreases. The mean difference between time zero and 72 hours was -0.54 IU/L. Again, from a practical standpoint, the mean difference is not operationally important, but the tendency of ALT levels to decrease during storage at 4°C makes possible the inclusion of borderline donor units in the blood supply. Thus, prolonged 4°C storage of unseparated donor samples may be undesirable.

Although 72-hour RT storage of unseparated donor samples may eliminate an occasional donor who truly satisfies acceptance criteria, we conclude from the results of this study that RT is the storage temperature of choice for up to 72 hours.

This study did not test ALT activity in serum separated from the clot before storage, as is detailed in the study by Williams et al.7 While their study documented a gradual loss of activity over time in both separated and unseparated serum, we found a loss of ALT levels at 4°C and a small but gradual increase in ALT levels in unseparated serum samples stored at RT over a period of 72 hours. Williams et al.7 recommended that serum be separated from the clot for storage periods of up to one week but stated that both separated and unseparated samples could safely be stored at RT for 24 hours.

The results from both the Williams' report7 and this study support the conclusion that no alterations are necessary at this time in the common standard operating procedure for specimen handling to accommodate ALT stability over a 24-hour period. Our study demonstrates that RT storage for 72 hours may also be acceptable.

References
4. International Forum. Based on your analysis of the benefits and costs of routine donor screening for ALT-GPT to reduce...
the incidence of posttransfusion non-A, non-B hepatitis in your blood services region, what action would you recommend on this matter? Vox Sang 1983;44:48-64.

LITERATURE REVIEWS

Red cell membrane chemistry (part 4)

Red cell membrane antigens

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