Serum-Constituents Analyses: Effect of Duration and Temperature of Storage of Clotted Blood

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We studied the effects on 25 analytes of duration of contact of serum with non-anticoagulated blood and of temperature. Serum was separated after blood was allowed to stand, for 0, 2, 4, 6, 8, 24, or 48 h at 4, 23, or 30 °C. Results obtained for bilirubin, albumin, zinc sulfat turbidity, thymol turbidity, cholinesterase (EC 3.1.1.8), alkaline phosphatase (EC 3.1.3.1), leucine aminopeptidase (EC 3.4.11.1), amylase (EC 3.2.1.2), total cholesterol, triglycerides, β-lipoprotein, serum urea nitrogen, creatinine, uric acid, and γ-glutamyltransferase (EC 2.3.2.2) were not influenced by storage at 4, 24, or 30 °C for as long as 48 h. Negligible differences were seen for potassium in sera in contact with cells as long as 24 h at 23 °C and for inorganic phosphorus after 48 h at 4 °C. However, at 4 °C we noted an increase at 8 h, a slight decrease at 30 °C. Statistically significant changes were seen for total protein and calcium after 48 h at 30 °C; for aspartate aminotransferase (EC 2.6.1.1), and alanine aminotransferase (EC 2.6.1.2), between 8 and 24 h at 23 °C and as soon as 6 h at 30 °C; for lactate dehydrogenase (EC 1.1.1.27) after 8 h at 30 °C and between 8 and 24 h at 23 °C; for glucose at 24, 4, or 2 h of storage at 4, 23, or 30 °C, respectively; for inorganic phosphorus after 48 h at 23 °C or 8 h at 30 °C; for potassium after 4 h at 4 °C or 24 h at 30 °C; and for sodium after 48 h at 4 °C or 6 h at 23 or 30 °C.

Additional Keyphrases: variation, source of - sample preparation and handling

To avoid time-dependent changes in the release of cellular constituents, it is standard practice to separate serum from the blood clot by centrifugation as soon as the clot forms. The effects of hemolysis, on some of the analytes in sera are well known (1), and approximate corrections can be applied (2). To assess the changes that occur in serum constituents with time when sera are subject to three temperatures (4, 23, and 30 °C), we assayed 25 analytes in sera obtained from apparently healthy adult volunteers. The clotted specimens were allowed to stand at 4, 23, or 30 °C for 2, 4, 6, 8, 24, or 48 h, then assayed.

Materials and Methods

The subjects were healthy women volunteers, about 18 years old, from a nurses’ training school. The volunteers were instructed to fast overnight (until blood collection was completed), and to avoid vigorous exercise upon waking. Two hundred milliliters of blood was collected from each volunteer without the use of an anticoagulant, and 5-mL aliquots of it were dispensed into 38 tubes. Two of the tubes were centrifuged within 20 min of dispensing the aliquots and the separated sera were stored at −20 °C until assay. These sera represented initial (zero time) values. The remaining 36 aliquots were divided into three groups; each group was stored at one of the following temperatures: 4 ± 1 °C (range) in a refrigerator, 23 ± 1 °C at a relative humidity of 50%, or 30 ± 0.5 °C in an incubator.

At 2, 4, 6, 8, and 48 h from initial zero time sampling, two aliquots from each temperature group were centrifuged, and the separated sera were kept frozen at −20 °C until assay.

When 380 samples of sera from 10 volunteers had been processed in this manner, all sera were thawed and analysis was performed with an Autochemist Multichannel Analyzer (Autochem Instruments AB, Lindigo, Sweden). Each serum was assayed in duplicate for 25 constituents. A pooled serum sample and a commercial reference serum (Hyland, Div. of Travenol Lab., Inc., Costa Mesa, CA 92626) were included between every 10 serum samples, to monitor instrument drift. The methods involved in Autochemist assays are detailed elsewhere (3).

Results

Within-day precision, judged by the results obtained on the pooled serum included between every 10 samples, was such that the coefficient of variation (CV) did not exceed 2.5% for any of the 25 constituents assayed.

The effects of time and temperature of storage on results for the following assays were not statistically significant as determined by Student’s t-test (n = 0.05): total bilirubin, direct bilirubin, cholinesterase, urea nitrogen, albumin, zinc sulfat turbidity, thymol turbidity, alkaline phosphatase (EC 3.1.3.1), leucine aminopeptidase (EC 3.4.11.1), γ-glutamyltransferase (EC 2.3.2.2), amylase (EC 3.2.1.2), total cholesterol, triglycerides, β-lipoprotein, creatinine, and uric acid. Statistically significant changes as compared with the initial zero time values were noticed for nine constituents (Figures 1–5).

For total protein (Figure 1) there was virtually no effect on samples stored at 23 °C for as long as 8 h. At this temperature there is an upward trend in values at 24 and 48 h, but it is by no means clinically significant. The values for samples stored at 4 °C decrease between 6 and 8 h and remain lower during 48 h of storage. The values at 30 °C are relatively higher than those at 23 °C and 4 °C, presumably a combination of several effects, including turbidity which could affect the biuret procedure. The values at this temperature begin to drift upwards at 24 and 48 h. The changes depicted in this figure for each temperature, even at 48 h of storage, cannot be considered clinically significant.

Figure 2 depicts results for aspartate aminotransferase and alanine aminotransferase. For both enzymes the effect of storage at 4 °C for as long as 48 h results in a slight decrease in activity, which may not be clinically significant. Storage of specimens at 23 °C for 8 h has a negligible effect on enzyme activity; between 8 and 24 h the values increase, and the specimen is clearly unsatisfactory when assayed at 48 h. Storage of specimens at 30 °C activates both enzymes by as early as 4 h of storage and has the effect of artificially increasing the activities of these enzymes when they are assayed between 8 and 48 h of storage. The effect of storage for more than 8 at ambient temperature (23 °C) or 30 °C on enzyme activity

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activation is apparently greater for alanine aminotransferase than for aspartate aminotransferase.

For lactate dehydrogenase (Figure 3) storage at 4 °C for as long as 24 h did not result in any changes in enzyme activity that would be considered clinically significant. The effect at 23 °C is noticed by as early as 2 h of storage; beyond 6 h the activity increases so much that the results obtained at subsequent time intervals clearly are overestimates. Storage of specimens at 30 °C for even short periods (2 to 4 h) would produce an artifactual and clinically significant effect on enzyme activity.

Glycolysis at 4 °C storage is minimal for 8 h (Figure 3). Blood specimens stored at 23 °C reach unacceptable amounts as early as 4 to 6 h of storage. The rate of glycolysis at 30 °C would render invalid the storage of a blood specimen for even 2 h.

Blood stored at 4 °C for as long as 8 h maintains its initial concentration of serum sodium (Figure 4), but after that values decrease to a point of clinical significance at 48 h of storage. The effects of storage at 23 and 30 °C on sodium are similar during the first 6 h of storage, but values higher than those obtained at 4 °C for this time-span. The greatest divergence from initial (time zero) sodium values is seen at 24 h of storage for both 23 °C and 30 °C; values at 48 h approximate the values obtained at 2 h storage at 23 °C and initial storage at 30 °C.

Results obtained for potassium (Figure 4) at 4 °C reveal a slight inhibition of the sodium-potassium pump up to 6 h. Beyond 8 h of storage the inhibition of the sodium-potassium pump is so marked as to render the potassium determination invalid. This is in line with the results of other investigators (4). Storage of blood samples at 23 °C and up to 24 h results in a slight but clinically acceptable decrease in potassium values. The effect of prolonged contact with erythrocytes becomes evident at 48 h at 23 °C; at 30 °C the effect is noticeable at 24 h, drastically marked at 48 h. Interestingly, for the first 8 h of storage, samples at 30 °C showed a greater decrease in potassium values than those stored at 23 °C.

Figure 5 summarizes our results for calcium and phosphorus. For calcium, temperature-related effects are apparent when blood is stored beyond 24 h. Thus at 48 h calcium values are significantly less than the initial value in blood stored at 30 °C. In contrast, blood stored at 4 or 23 °C shows an upward shift for this analyte. Storage of blood for 48 h at 4 °C negligibly affects values for phosphorus. At 23 °C results are comparable to the initial value for the first 24 h, although values between 4 and 8 h are slightly less than those at 4 °C. Results at 23 and 30 °C are several-fold greater than the initial value, and are clinically unacceptable. At the latter temperature values for phosphorus are comparable to the initial values for only up to 6 h.

We also assessed the extent of turbidity and hemolysis in
sera obtained from blood stored at the three temperatures. The former was followed by measuring the absorbance of the sample at 620 nm (5), and the latter by the measurement of hemoglobin (6). Temperature-dependent increases in turbidity were observed, but did not affect the measured values for serum constituents. Thus the absorbance at 4 °C in the 48 h stored serum samples was 0.66 (SD 0.04), identical with that for fresh serum. For 23 and 30 °C storage the respective values were 0.79 (SD 0.13) and 0.9 (SD 0.1). Hemoglobin values for sera from blood stored at all temperatures for as long as 48 h did not exceed 340 mg/L, and did not affect the assayed values of the constituents.

Discussion

In designing this study we took into account considerations that have already been covered in the literature in regard to the proper handling and storage of samples (7), minimization of preinstrumental errors (8, 9), and errors associated with sample evaporation (10). Because the intra-individual differences in the values of serum constituents are reasonably small (11, 12), good assay precision is a prerequisite. The coefficients of variation of the assays in this study are similar to those reported previously (13, 14).

The increased values for total protein at 30 °C are in line with the reported increase of apparent serum protein concentrations during shipment (15), apparently related to a combination of several effects, including turbidity, that would affect the total protein procedure that we used. The increase in the activity of the intracellular enzymes aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase is perhaps related to changes in cell permeability, and to the fragility of erythrocyte membrane during prolonged storage (16), and may reflect a decrease in ATP concentration in the erythrocytes (17). It has been noted that lactate dehydrogenase in normal human serum is least stable at 0 °C, and that stability increases with increasing temperature up to 22 °C, the maximum temperature used in that study (18). Our results indicate lower stability of this enzyme at 30 °C as compared to 0 and 24 °C. The relative stability of this enzyme at 0 °C might be related to the effect of leaving the serum in contact with the cells. Alanine aminotransferase was less stable than aspartate aminotransferase. Glucose concentrations in blood from adults reportedly decrease at a rate of 36 mg/L per hour at room temperature (19), which is twice the value we report for healthy young women. Refrigeration at 4 °C minimizes the decrease to 3.9 mg/L.

Carothers et al. (20) recommend that serum be separated from the clot within 3 h of blood collection and stored at room temperature if inorganic phosphorus concentrations are to be valid. Our results indicate that blood can be stored at 4 °C for as long as 48 h without affecting inorganic phosphorus values. The slight decrease in inorganic phosphorus in blood stored at 23 °C for 8 h is perhaps related to the consumption of inorganic phosphorus during phosphorylation of glucose in the cell. However, as glycolysis proceeds during prolonged storage, inorganic phosphorus is released from the acid-soluble organic phosphate metabolites, which might explain the marked increase in inorganic phosphorus after storage for 24 h.

In conclusion, our intent in reporting this study is in no way to encourage deviation from the standard practice of separating the serum from the clot as soon as it is practicable to do so. Rather, we hope that the results we have presented will help assess which of the constituents may be assayed in blood stored for prolonged times under commonly encountered storage conditions, when such prolonged storage occurs inadvertently or is unavoidable.

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Fig. 4. Time and temperature dependency of Na (top) and K (bottom) concentrations on storage of whole blood without anticoagulant

Fig. 5. Time and temperature dependency of Ca (top) and inorganic phosphorus (bottom) concentrations on storage of whole blood without anticoagulant
References


