Original Article

Stability of common biochemical analytes in serum gel tubes subjected to various storage temperatures and times pre-centrifugation

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Abstract

Background: Blood samples collected in rural and remote areas of Australasia are often exposed to a range of environmental conditions prior to analysis in a laboratory. The aim of this study was to determine analyte stability of venous blood specimens in serum gel tubes exposed to a range of storage temperatures and times prior to centrifugation.

Methods: Thirty healthy adult volunteers were enrolled in the study. Blood was collected into 11 serum gel separator tubes. All samples were allowed to clot at room temperature for 30 min. Two samples were centrifuged and analysed as controls. Nine samples were stored at 15, 25 or 35°C for 4, 8 or 24 h, respectively, before centrifugation. Thirty-five biochemical analytes were measured on each sample.

Results: Most analytes remained stable in all storage conditions including sodium, total protein, albumin, bilirubin, alanine transferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transferase, creatinine kinase, lipase, cholesterol, triglycerides, transferrin, urate, C-reactive protein, vitamin B_{12}, thyroid-stimulating hormone, free thyroxine, free triiodothyronine, follicle-stimulating hormone, oestradiol, prostate-specific antigen, cortisol and vitamin D. Potassium, glucose, phosphate, creatinine, urea, ferritin, iron, lactate dehydrogenase, magnesium and calcium were not stable in at least one of the storage conditions.

Conclusions: These results can be used to determine which analytes produce valid results despite exposure to variable storage conditions for up to 24 h prior to centrifugation. The majority of analytes were unaffected by a delay in centrifugation at a variety of temperatures, however, some important analytes were significantly affected.

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Introduction

The development of blood collection tubes that contain gel and form a barrier after centrifugation has markedly improved serum analyte stability in the primary tube and removed the need for aliquoting the serum.[1–3] However, there are few published studies on analyte stability where there is a delay before centrifugation. Other studies have looked at the stability of various analytes in whole blood, however, the conditions to which the specimens were exposed have usually been within manufacturer's defined limits.[1,4–7]

The Asia Pacific region has unique challenges arising from its vast geography and climatic conditions. Regional and remote health-care workers often do not have access to a centrifuge. This results in a delay of centrifugation and exposure of the specimen to variable temperatures and times during transport before being centrifuged.

The aim of this study is to determine the analyte stability of venous blood specimens in serum gel tubes exposed to a range of storage temperatures and times prior to centrifugation. The data will determine which commonly requested biochemical analytes are unaffected by adverse environmental temperatures and prolonged times before processing of the specimen, and which analytes are significantly affected and cannot be reliably analysed.

Methods

Study design

For each volunteer, morning venous blood samples were collected into 11 5 mL-serum gel separator tubes (Becton Dickinson Vacutainer® Systems SST™II, Plymouth, UK). The order of collection was randomized to different

storage conditions prior to centrifugation for each subject. There were nine storage conditions – three temperatures (15, 25 or 35°C) for each of the three time periods (4, 12 or 24 h). Two tubes (time 0a and 0b) were used as controls and to assess tube-to-tube variability. The study was approved by the Sir Charles Gairdner Hospital Human Research Ethics Committee. All volunteers gave written informed consent to participate in the study.

Volunteers

Thirty volunteers were enrolled in the study. Volunteers were healthy adults who were not known to be anaemic, were not on anticoagulant therapy and did not undergo a regular phlebotomy for any reason. There were 15 male and 15 female subjects aged between 19 and 57 years – mean age was 40 years.

Specimen collection and processing

Approximately 55 mL of venous blood was collected from each subject into the gel collection tubes. Phlebotomists performed the venipuncture in accordance with the established Standard Operating Procedure using BD Vacutainer® Safety Lok™ winged collection set with 21G needle. All specimens were mixed by gentle inversion at least five times following collection and then allowed to clot for 30 min at a temperature of 18–25°C.

The two time 0 tubes were then centrifuged and analysed. The other nine collection tubes were stored for time periods of 4, 12 or 24 h at a temperature of 15, 25 or 35°C prior to centrifugation and analysis. Storage temperatures were maintained within ±1°C. All collection tubes were centrifuged at 1500 *g* for 10 min at 18–25°C.

Analysis

Roche Modular PPE analyser (Roche Diagnostics Ltd, Basel, Switzerland) was used to assay the following analytes (between-run coefficient of variation); sodium (0.5% at 136 mmol/L), potassium (0.5% at 3.9 mmol/L), urea (2.4% at 5.5 mmol/L), creatinine (2.7% at 74 μmol/L), glucose (1.7% at 4.8 mmol/L), total protein (0.7% at 69 g/L), albumin (1.8% at 44 g/L), bilirubin (2.2% at 15.7 μmol/L), alanine aminotransferase (ALT, 2.4% at 22 U/L), aspartate aminotransferase (AST, 3.6% at 36 U/L), alkaline phosphatase (ALP, 1.4% at 76 U/L), gamma glutamyl transferase (GGT, 2.8% at 32 U/L), creatine kinase (CK, 1.2% at 162 U/L), troponin T (3.3% at 0.12 μg/L), lipase (1.6% at 32 U/L), magnesium (2.1% at 0.71 mmol/L), phosphate (1.6% at 0.99 mmol/L), calcium (1.4% at 1.95 mmol/L), cholesterol (1.5% at 3.4 mmol/L), triglycerides (TG, 1.3% at 2.0 mmol/L), iron (2.9% at 11.2 μmol/L), transferrin (1.0% at 31.8 μmol/L), lactate dehydrogenase (LDH, 1.4% at 152 U/L), urate (0.67% at 0.24 mmol/L) and C-reactive protein (CRP, 2.4% at 14.8 mg/L). Abbott Architect i4000 analyser (Abbott Laboratories, IL, USA) was used to assay vitamin B_{12} (4.9% at 615 ng/L), ferritin (8.1% at 155 μg/L), thyroid-stimulating hormone (TSH, 3.8% at 0.39 mU/L), free thyroxine (fT4, 4.8% at 10.5 pmol/L), free triiodothyronine (fT3, 5.6% at 3.6 pmol/L), follicle-stimulating hormone (FSH, 5.0% at 6 U/L), oestradiol (6.1% at 330 pmol/L) and prostate-specific antigen (PSA, 5.6% at 0.9 μg/L). DPC Immulite 2000 analyser (Diamond Diagnostics, MA, USA) was used to measure cortisol (4.4% at 570 nmol/L). A Diasorin equilibrium radioimmunoassay (Diasorin S.P.A., Sallugia, Italy) was used to analyse vitamin D (10.8% at 37 nmol/L). All collection tubes were assessed for haemolysis, icterus and lipaemia using indices calculated from calibration factors and spectrophotometric measurements at two wavelengths on the Roche Modular PPE analyser.

Statistical analysis

Data were analysed using two-way repeated measures analysis of variance. The result of each storage condition was compared with the mean result of the control specimens – time 0a and 0b. A clinically acceptable limit (CAL) was defined by a group of chemical pathologists and biochemists for each analyte. This was based on analytical variation from laboratory data, biological variation[8] and the minimal change considered clinically relevant. Although these values were subjective, they were similar to or exceeded calculated significant change intervals based on the analytical variation as shown in Table 1. The change produced by the storage conditions was considered to be clinically significant if it exceeded the CAL and was statistically significant when compared with control samples.

Results

Baseline data and CALs are shown in Table 1. There was no statistically significant difference between the two control samples ($P > 0.05$). Haemolysis index (H index) was between 20 and 52 in six of the 330 collection tubes and <20 in all other collection tubes (H index of 100 is approximately equal to free haemoglobin of 1 g/L). Based on manufacturer's kit information and laboratory data, H index of 20–100 causes a significant positive interference in the measurement of LDH, but does not have a significant effect on other analytes measured. The results of the six LDH samples with H index >20 were not included in the analysis. Icteric index (I index) was <100 for all samples which does not cause a significant interference with any of the analytes measured (I index of 100 is approximately equal to bilirubin of 100 μmol/L). Lipaemia (L index) was not detected in any of the samples. Internal quality control results were within laboratory limits through out the study.

Most of the analytes remained stable over the 24-h period at all three temperatures. For these stable analytes, the greatest change from baseline that occurred as a result of storage conditions was as follows: sodium −1.0%, +1.4%, total protein +3.2%, albumin +3.6%, bilirubin −1.0%, +6.8%, ALT −0.1%, +3.1%, AST −0.9%, +5.1%, ALP −0.2%, +3.7%, GGT +8.2%, CK +3.2%, lipase +2.9%, cholesterol −1.2%, +6.0%, TG +6.4%, transferrin +3.0%, urate −7.7%, +3.5%, CRP −10.7%, +4.2%, vitamin B_{12} −0.2%,

Table 1 Baseline data and significant change intervals

	0a	0b	LSC (%)	CAL
Haemolysis index	4 ± 1	4 ± 0		
Icteric index	19 ± 1	19 ± 1		
Lipaemic index	0	0		
Sodium	139.9 ± 0.3	139.7 ± 0.3	1.7	2
Potassium	4.17 ± 0.05	4.14 ± 0.04	2	7
Urea	5.0 ± 0.3	5.0 ± 0.3	7	10
Creatinine	80.5 ± 2.6	80 ± 2.6	7	10
Glucose	4.95 ± 0.15	4.96 ± 0.16	5	10
Total protein	76.3 ± 0.9	76.4 ± 0.9	2	5
Albumin	46.6 ± 0.5	46.6 ± 0.5	5	5
Bilirubin	14.5 ± 1.6	14.5 ± 1.6	6	10
Alanine aminotransferase	28.6 ± 6.0	28.7 ± 6.0	7	10
Aspartate aminotransferase	27.0 ± 2.6	26.8 ± 2.6	10	10
Alkaline phosphatase	64.9 ± 2.9	64.9 ± 2.9	4	10
Gamma glutamyl transferase	21.6 ± 2.7	21.5 ± 2.7	8	10
Creatine kinase	160.9 ± 18	160.3 ± 18	3	15
Troponin	0	0	9	10
Lipase	32.2 ± 1.2	32 ± 1.2	4	10
Magnesium	0.832 ± 0.01	0.830 ± 0.01	6	10
Phosphate	1.145 ± 0.03	1.147 ± 0.03	4	10
Calcium	2.397 ± 0.01	2.404 ± 0.01	4	5
Cholesterol	4.97 ± 0.17	4.96 ± 0.16	4	10
Triglycerides	1.18 ± 0.12	1.17 ± 0.12	4	10
Iron	17.7 ± 1.1	17.7 ± 1.1	8	10
Transferrin	33.7 ± 0.9	33.6 ± 1.0	3	10
Lactate dehydrogenase	170.4 ± 4.4	169.5 ± 4.2	4	15
Urate	0.328 ± 0.015	0.327 ± 0.015	2	10
C-reactive protein	1.7 ± 0.4	1.7 ± 0.4	7	20
Vitamin B_{12}	411.6 ± 34	418.0 ± 36	14	15
Ferritin	114.4 ± 18	115.7 ± 17	22	25
Thyroid stimulating hormone	1.405 ± 0.11	1.397 ± 0.11	11	15
Free thyroxine	11.9 ± 0.3	11.9 ± 0.3	13	15
Free triiodothyronine	4.11 ± 0.09	4.14 ± 0.10	15	15
Follicle stimulating hormone	9.5 ± 3.1	9.2 ± 3.0	14	30
Oestradiol	205 ± 53	201 ± 52	17	20
Prostate specific antigen	0.39 ± 0.11	0.38 ± 0.11	16	25
Cortisol	318 ± 26	334 ± 28	12	30
Vitamin D	64.0 ± 4.9	62.1 ± 4.6	30	30

Mean ± SEM for control samples (0a and 0b), least significant change (2.77$\sqrt{}$analytical coefficient of variation) and clinically acceptable limit for all analytes

+4.7%, TSH −6.0%, +3.5%, fT4 −3.5%, +4.2%, fT3 −1.2%, +2.5%, FSH +12.3%, oestradiol −6.4%, +4.3%, PSA −4.1%, +2.3%, cortisol −1.9%, +5.4% and vitamin D −4.1%, +1.1%.

PSA, FSH and oestradiol all remained stable over 24 h at all temperatures; however, the analysis was performed using subgroups. For PSA, only the 15 men were included as women had undetectable concentrations of PSA. For oestradiol, the eight women not on the oral contraceptive pill (OCP) and premenopausal were included as all the remaining subjects (men, women on the OCP and postmenopausal women) had low oestradiol concentrations. For FSH, the 15 men and the 8 women not on the OCP and premenopausal were included. There was an adequate statistical power in each of these groups to enable statistically significant changes to be identified. Troponin T was not analysed as all specimens had undetectable concentrations and therefore inadequate power to detect change.

Potassium, urea, creatinine, glucose, magnesium, phosphate, calcium, LDH, iron and ferritin did not remain stable throughout the 24-h period. The mean changes for these analytes are shown in Table 2. For all results that exceeded the CAL, the change compared with the controls was statistically significant ($P < 0.05$).

Discussion

The Clinical and Laboratory Standards Institute recommends that serum samples are separated within 2 h of collection for most analytes.[9] They also note that temperature may affect the stability of some analytes. Our study assessed the effects of temperature and time on samples collected into serum gel tubes prior to centrifugation. Overall, most analytes were stable and provided valid results even when centrifugation was delayed by up to 24 h across a range of temperatures. Ten of the 35 analytes were significantly affected by at least one of the storage conditions. The two most susceptible analytes, as expected, were glucose and potassium, which were both significantly different when compared with control samples at all but one of the storage temperatures and times.

Table 2 Analytes significantly affected by storage conditions

Temperature (°C)	Time	K (mmol/L)	Urea (mmol/L)	Cr (μmol/L)	Glucose (mmol/L)	Mg (mmol/L)	Phosphate (mmol/L)>	Ca (mmol/L)	LDH (U/L)	Iron (μmol/L)	Ferritin (μg/L)
	0a	4.2 ± 0.05	5.0 ± 0.3	80 ± 3	5.0 ± 0.1	0.83 ± 0.01	1.14 ± 0.03	2.40 ± 0.01	170 ± 4	18 ± 1	114 ± 18
	0b	4.2 ± 0.04	5.0 ± 0.3	80 ± 3	5.0 ± 0.2	0.83 ± 0.01	1.15 ± 0.03	2.40 ± 0.01	169 ± 4	18 ± 1	116 ± 17
15	4 h	4.5 ± 0.05*←	5.1 ± 0.3	80 ± 3	4.6 ± 0.1	0.84 ± 0.01	1.15 ± 0.03	2.40 ± 0.01	172 ± 4	18 ± 1	118 ± 18
	8 h	4.9 ± 0.05*←	5.1 ± 0.3	80 ± 3	4.3 ± 0.1*←	0.84 ± 0.01	1.14 ± 0.03	2.38 ± 0.01	174 ± 5	18 ± 1	121 ± 18
	24 h	6.2 ± 0.11*←	5.2 ± 0.3	80 ± 3	3.5 ± 0.1*←	0.87 ± 0.01	1.10 ± 0.03	2.39 ± 0.01	179 ± 5	18 ± 1	117 ± 18
25	4 h	4.0 ± 0.04	5.1 ± 0.3	80 ± 3	4.2 ± 0.2*←	0.85 ± 0.01	1.08 ± 0.03	2.39 ± 0.01	178 ± 24	18 ± 1	115 ± 18
	8 h	3.8 ± 0.04*←	5.2 ± 0.3	81 ± 3	3.7 ± 0.1*←	0.86 ± 0.01	1.09 ± 0.03	2.40 ± 0.02	185 ± 5	19 ± 1	121 ± 18
	24 h	4.4 ± 0.07†	5.4 ± 0.3	90 ± 3*←	2.5 ± 0.1*←	0.91 ± 0.01	2.40 ± 0.06*←	2.44 ± 0.01	198 ± 5*←	20 ± 1*←	127 ± 19*←
35	4 h	3.8 ± 0.05*←	5.2 ± 0.3	83 ± 3	3.6 ± 0.1*←	0.86 ± 0.01	1.36 ± 0.03*←	2.42 ± 0.01	191 ± 5	19 ± 1	122 ± 19*←
	8 h	4.2 ± 0.07†	5.3 ± 0.3	87 ± 3	3.0 ± 0.1*←	0.89 ± 0.01	2.27 ± 0.05*←	2.43 ± 0.01	198 ± 26*←	20 ± 1*←	128 ± 19*←
	24 h	8.0 ± 0.15*←	5.7 ± 0.3*←	97 ± 3*←	2.0 ± 0.1*←	0.95 ± 0.01*←	4.36 ± 0.10*←	2.22 ± 0.02*←	222 ± 5*←	22 ± 1*←	144 ± 20*←

Mean ± SEM
*Exceeded clinically acceptable limit (CAL)
†Exceeded CAL at earlier time point

A sample was considered to be significantly affected by the storage condition if the difference compared with the control samples was outside CAL, and the difference was statistically significant. As the two control samples were not statistically different, the changes seen can be solely attributed to the effect of the storage condition rather than analytical variation.

Glucose was lower when compared with baseline in all storage conditions except 15°C for 4 h where it was within the CAL and not statistically different from the control samples. The most pronounced effects were seen in the 35°C and 24-h samples. This is a well-known effect that has been seen previously in many studies.[1,10,11] Glucose is required for cellular metabolism and the rate at which glucose is depleted is dependent on the temperature and time, as well as leukocyte count. At higher temperatures, there is a higher metabolic rate and glucose is depleted quickly, whereas at lower temperatures it is depleted more slowly.

The effects on potassium are more variable.[12–14] At lower temperatures, activity of the cellular Na-K-ATPase pump is decreased that leads to an increased efflux of potassium from cells. This increase in potassium was seen in samples kept at 15°C for all three time periods. At higher storage temperatures, there is a higher metabolic rate with an increased cellular potassium uptake. This decrease in serum potassium was seen in samples stored at 25°C for 8 h and at 35°C for 4 h. Later, as glucose is depleted, the activity of Na-K-ATPase pump decreases and then potassium is released from the cells. This results in serum potassium increasing to normal as seen in samples stored at 25°C for 24 h and at 35°C for 8 h, and then increasing further as seen in samples stored at 35°C for 24 h. This process occurs earlier at higher temperatures as glucose is depleted at a faster rate. The only sample where potassium did not change significantly was that stored at 25°C for 4 h. Other studies have found no statistically significant difference with delayed separation of 1 or 2 h.[9,12]

Phosphate was significantly increased in all samples stored at 35°C and in samples stored at 25°C for 24 h. Phosphatases as well as changes in cell membrane integrity cause increased efflux of phosphate out of the cells. Magnesium and LDH are other intracellular analytes that can also efflux from cells. In this study, both analytes were more stable than phosphate with an increase in LDH seen in samples stored at 25°C for 24 h and 35°C for 8 h, and an increase in both LDH and magnesium seen at 35°C for 24 h. Calcium was found to decrease in samples stored at 35°C for 24 h. The mechanism for this is not clear. It may be due to precipitation of calcium with the significantly increased serum concentrations of phosphate. Boyanton *et al.*[1] found calcium to be stable in serum on cells for up to 24 h at 25°C, but did not assess the effects at 35°C.

Creatinine was increased in samples stored at 25°C and 35°C for 24 h. Recently, Shepherd *et al.*[15] found creatinine to increase after 24 h at room temperature when using Jaffe methods, which can lead to misclassification of chronic kidney disease stage using estimated glomerular

filtration rate (eGFR). Creatinine measured by enzymatic methods was not affected. Urea was increased in samples stored at 35°C for 24 h and iron and ferritin were increased at 25°C for 24 h and 35°C for 8 and 24 h.

I and L indices were all below concentrations at which an interference may arise. H index was increased in six collection tubes to a concentration that can cause a positive interference with the measurement of LDH. The six results with H index >20 were removed from the analysis for LDH.

Given the results of this study, analytes including sodium, total protein, albumin, bilirubin, ALT, ASP, ALP, GGT, CK, lipase, cholesterol, TG, urate, CRP, transferrin, vitamin B_{12}, TSH, fT4, fT3, FSH, oestradiol, PSA, vitamin D and cortisol, are stable when stored at 15, 25 or 35°C for up to 24 h prior to centrifugation using our analytical methods. Valid results should be produced for these analytes despite exposure to variable storage conditions for up to 24 h. Potassium, glucose, phosphate, magnesium, LDH, calcium, creatinine, urea, iron and ferritin have variable stability when centrifugation is delayed. The storage temperature and time data from this study can be used to determine which storage conditions will produce valid results. When temperature is not known, the data can be used to determine the time period prior to centrifugation in which the result will be valid over a range of temperatures.

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