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Controlled storage conditions prolong stability of biochemical components in whole blood

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Abstract

Blood specimens from primary care centres are normally transported to central laboratories by mail. This necessitates centrifugation and separation, especially since the potassium ion concentration in whole blood changes during storage at ambient temperature. Thus, because of the growing awareness of and concern for pre-analytical contributions to the uncertainty of measurements, we investigated 27 components and their stability under controlled temperature conditions from 17 to 23°C. We found that storage of whole blood can be prolonged by up to 8–12 h for all components examined, including potassium ions, when stored at 20±0.2°C. We conclude that this opens the possibility for establishing a pick-up service, by which whole blood specimens stored at 20–21°C can be collected at the doctor's office, making centrifugation, separation and mailing superfluous. In addition, the turn-around time from sample drawing to reporting the analytical result would be shortened. After investments in thermostatted boxes and logistics, the system could reduce costs for transporting blood samples from general practice centres to central laboratories.

Keywords: centrifugation; potassium ion; storage; whole blood stability.

Introduction

Blood specimens taken from patients in general practice need transport to central laboratories for analysis. To preserve the initial concentration of components, a combination of separation of cells from serum or plasma and cooling or freezing during transportation is used. This is based on the knowledge that proteins, enzymes, hormones, coagulation factors and biochemical substances are generally more stable at lower temperature, and they are more stable in serum/plasma than in whole blood.

It has therefore been a rule to separate serum/plasma before sending it to a central laboratory for analyses. The general requirement for separation within 3 h of drawing the blood from the patient is especially important when analysing for potassium ion, one of the frequent variables used for monitoring patients on diuretic treatment. (Aspartate aminotransferase and lactate dehydrogenase are other examples of components with large difference between intra- and extracellular concentrations.)

Because of the growing awareness of and general concerns for medical errors, the pre-analytical step of analysis is currently in focus. We were interested to investigate whether whole blood could be stabilised at a defined temperature, making the centrifugation of specimens before transportation unnecessary, thereby reducing both pre-analytical variation and costs.

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Materials and methods

Tubes

The tubes used were Li-heparin Vacutainer tubes (Becton Dickinson, Brøndby, Denmark, Ref 368884).

Blood samples

Blood samples were drawn from five volunteer employees in the morning. From each person, six samples were collected, giving a total of 30 tubes for experiment at one temperature setting.

One sample from each person was immediately centrifuged and plasma was carefully separated. A maximum of 3/4 of the plasma layer was taken, thus avoiding contamination with platelets and red cells. This sample was the reference, called ''0''. The other samples were stored in a temperature-controlled cabinet for up to 12 h. At given time intervals one sample was centrifuged and the plasma separated as above.

Centrifugation

All blood samples were routinely centrifuged for 10 min at $3000 \times g$ as standard procedure. The effect of lower centrifugal force was investigated in a preliminary experiment by comparing results for albumin, calcium, glucose, lactate dehydrogenase, potassium, sodium and total protein from two samples drawn from six persons and centrifuged at $1200 \times g$ and $3000 \times g$, respectively.

Storage effect

The effect of storage was investigated at 17°C, 20°C, 23°C and 25±0.2°C on six samples drawn from each donor. Storage time was from 0 to 12 h before centrifugation. All plasma samples were kept at +4°C until analysed.

Transport Two samples were taken from five donors and stored at 12 h at 20°C. Then, one sample from each person was centrifuged and separated. The second was transported

Table 1 Clinically acceptable changes in concentration of whole blood components during storage.

Component	Bias		Clinically acceptable change
	Required	Actual	
ALT, U/L	±2.2	±1.0	±3
ALP, U/L	±4.4	±2.0	±4
Amylase (pancreas), U/L	±3.4	±1.5	±4
Bilirubin, μmol/L	±1.1	±0.5	±3
Calcium, mmol/L	±0.03	±0.03	±0.04
Cholesterol, mmol/L	±0.24	±0.05	±0.3
HDL-C, mmol/L	±0.09	±0.04	±0.1
LDL-C, mmol/L	±0.18	±0.08	±0.2
CK, U/L	±8.8	±4.0	±5
Cobalamin, pmol/L	±44.0	±20.0	±25
Creatinine, μmol/L	±5.0	±2.0	±4
Estradiol, nmol/L	±0.01	±0.005	±0.05
Ferritin, pmol/L	±14.3	±6.0	±10
FSH, IU/L	±0.75	±0.30	±1.0
FT_3, pmol/L	±0.18	±0.08	±1.0
FT_4, pmol/L	±0.75	±0.30	±2.0
GGT, U/L	±4.1	±2.0	±5
Iron, μmol/L	±1.1	±1.0	±2
LDH, U/L	±6.3	±5.0	±8
LH, IU/L	±0.63	±0.33	±1.0
Magnesium, mmol/L	±0.01	±0.05	±0.03
Phosphate, mmol/L	±0.04	±0.02	±0.1
Potassium, mmol/L	±0.08	±0.03	±0.4
Progesterone, nmol/L	±1.0	±0.4	±1.0
Sodium, mmol/L	±0.81	±3.0	±1.0
TSH, mU/L	±0.24	±0.10	±0.1
Urea, mmol/L	±0.26	±0.10	±0.4

Bias required is the sum of analytical bias and bias introduced by storage and was calculated as 1/16 of the reference range (2). Actual bias shows the typical analytical bias in our laboratory. Clinically acceptable changes were defined by us and estimated on the basis of our experience and knowledge of clinical application in general practice centres.

by car in the insulated box for 1 hour, then centrifuged and separated. All 10 samples were analysed in the same run.

The components investigated were a mixture of general biochemical components requested by general practitioners: alanine transaminase (ALT), alkaline phosphatase (ALP), amylase (pancreas specific), bilirubin, calcium, cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) cobalamin, creatine kinase (CK), creatinine, estradiol, ferritin, follicle-stimulating hormone (FSH), γ-glutamyl transferase (GGT), iron, lactate dehydrogenase (LDH), lutropin hormone (LH), magnesium, phosphate, potassium, progesterone, sodium, free triiodothyronine (FT_3), free thyroxine (FT_4), thyrotropin (TSH) and urea. This combination represents 60% of the requirements for biochemical analyses (the rest are haematology and coagulation analyses).

All analyses were carried out on a Modular P+E system from Roche Diagnostics (Hvidovre, Denmark) in the same series and with use of Roche reagent kit and calibrators.

Calculations For each combination of storage time and temperature the mean value for all participants and absolute difference from the initial ''0'' value (mean change) was calculated in the relevant units.

Stability The stability of blood samples during storage was defined as the capacity of sample material to retain the initial value of the quantity measured within specified limits and under specified conditions (1, 2).

The quality demand limits for analytical bias (Table 1) were defined as the sum of analytical bias and bias introduced by storage and was calculated as 1/16 of the reference interval (3, 4).

Clinically acceptable changes were defined by us and estimated on the basis of our experience and knowledge of clinical application in general practice offices.

The changes in concentration observed during the storage time were evaluated in relation to these clinical specifications.

Analytical imprecision and bias

All analyses were quality controlled according to rules applied in the laboratory. The control materials used were Seronorm Human from Sero a/s (Billingstad, Norway) and Immunoassay Plus from BioRad Laboratories (Herlev, Denmark).

Analytical quality determined as the coefficient of variation ($CV_{analytical}$) for ALT, ALP, amylase (pancreas specific), bilirubin, calcium, cholesterol, HDL-C, LDL-C, CK, creatinine, GGT, iron, LDH, magnesium, phosphate, potassium, progesterone, sodium and urea was less than 3%. $CV_{analytical}$ for cobalamin, estradiol, ferritin, FSH, LH, FT_3, FT_4, and TSH was less than 5%.

Absolute analytical bias was eliminated, since all samples were analysed in the same run and, furthermore, we were only interested in relative changes, not exact values.

Results

Centrifugal force

Albumin, calcium, glucose, LDH, potassium, sodium and total protein measured in plasma centrifuged at

Table 2 Stability of biochemical components in whole blood at different temperatures.

Storage time, h	ALT 22.8 U/L Difference, U/L			ALP 60.2 U/L Difference, U/L			AMYL 29.6 U/L Difference, U/L		
	17°C	23°C	25°C	17°C	23°C	25°C	17°C	23°C	25°C
2	−1.0	−1.2	−0.6	0.4	0.2	0.4	0.4	0.6	0.6
4	−1.0	−2.4	−1.4	0.4	−0.8	0.4	0.4	0.2	0.8
6	−1.6	−2.8	−1.2	0.6	−0.4	−0.6	0.6	0.8	1.0
8	−0.4	−1.8	−0.6	0.2	−0.4	−1.2	0.4	0.6	0.8
	Bilirubin 9.8 μmol/L Difference, μmol/L			CK 104.0 U/L Difference, U/L			GGT 20.4 U/L Difference, U/L		
2	0.0	0.2	0.2	0.2	0.4	0.8	0.2	0.8	0.8
4	0.0	0.2	0.4	1.8	0.2	2.2	0.8	1.0	1.0
6	0.0	0.4	0.4	1.6	0.6	1.6	0.0	1.2	1.4
8	−0.2	0.6	0.2	1.6	2.0	2.2	1.0	1.8	1.4
	Iron 18.4 μmol/L Difference, μmol/L			Magnesium 0.81 mmol/L Difference, mmol/L			LDH 165.8 U/L Difference, U/L		
2	0.2	0.4	0.6	0.00	0.01	0.01	−9.8	−5.4	−8.4
4	0.4	0.4	0.8	0.00	0.01	0.02	−1.6	−9.2	−4.6
6	0.4	0.4	1.0	0.01	0.01	0.02	−1.6	−2.2	−10.2
8	1.0	1.4	1.0	0.01	0.02	0.03	1.4	−7.2	−7.4
	Potassium 3.82 mmol/L Difference, mmol/L			Sodium 141.4 mmol/L Difference, mmol/L					
2	0.12	−0.10	−0.16	−0.2	0.2	0.2			
4	0.28	−0.02	−0.22	−0.6	0.4	0.6			
6	0.42	−0.12	−0.28	0.0	0.6	0.6			
8	0.48	−0.18	−0.26	−0.6	0.0	0.0			

Whole blood specimens from five persons were stored for different periods of time and at different temperatures before centrifugation. The Table shows mean initial values and mean storage-induced changes observed in the concentration of 11 blood components.

the widely used value of 1200×*g* or at our routine 3000×*g* showed no differences exceeding the analytical variation.

The same result was previously found for glucose measurements in plasma (5). This suggests that the results presented in this paper were not influenced by the higher *g*-force centrifugation used in our laboratory.

Storage of blood

Initially we investigated the stability of potassium, as it is well known that its concentration in blood samples undergoes significant changes depending on the pre-analytical conditions, such as time and temperature before centrifugation (6, 7).

Since the purpose of our investigation was to design a system for transport of uncentrifuged blood samples, we also measured other components that could be influenced by prolonged storage on the same occasion. We chose bilirubin, iron, magnesium, sodium and the enzymes ALT, ALP, amylase (pancreas specific), CK, GGT and LDH.

Blood samples were stored at 17, 23 and 25°C from 0 to 8 h. We used plasma for all measurements as it is more convenient for the laboratory (no need to await coagulation) and is in agreement with recommendations from tube and kit producers.

Table 2 summarises the measurement results for all components and the mean changes in concentrations (as absolute units) during storage for different times and at different temperatures.

All components except potassium were stable according to demands defined on the basis of known analytical performance and clinical requirements.

Figure 1 shows changes in potassium concentration as a function of storage time and temperature. Our findings confirm what has already been shown – the concentration of potassium decreases as storage temperature increases, and increases with decreasing temperature (6). This is usually explained by the temperature dependence of K/Na-ATPase activity.

The sequence of 17, 23 and 25°C curves inspired us to explore if we could determine a storage temperature for blood samples at which transport of potassium in and out of cells was in balance.

On the basis of the results shown in Figure 1, potassium measurements were repeated at 20°C and the duration of storage was prolonged up to 12 h. Other analytical components frequently undergoing transport were also examined for stability at this temperature.

The results for potassium ion at 20°C are inserted in Figure 1 as the stippled line. Results for all components stored at 20°C are summarised in Table 3.

It is evident that for all components measured, including potassium, the stability can meet the clinical demands defined in Table 1 when samples are stored at 20°C for 8 h, or even longer. The mean differences from initial values observed for most components are smaller than our bias demands and did not exceed

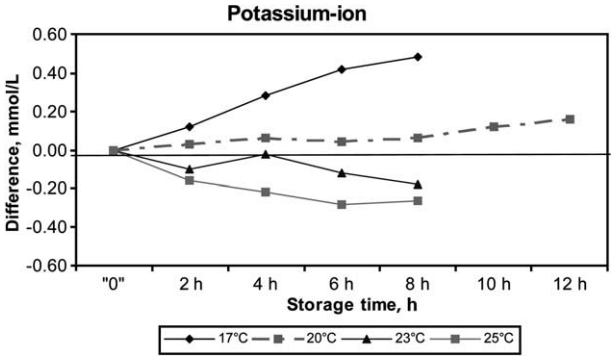

Figure 1 Stability of potassium ion under storage at different temperatures. The highest change observed for a single person was +0.7, −0.4, −0.3 and +0.2 mmol/L for storage at 17, 23, 25 and 20°C, respectively.

the clinically acceptable change defined for any patient. Phosphate was an exception – stability decreased after 8 h storage.

Discussion

The problem of medical errors has recently received a great deal of attention. For laboratory medicine, the most frequent errors occur in the pre- and postanalytical phases. Up to 75% of total errors occur during pre-analytical processing of samples, with incorrect samples as the most frequent cause (8–10). The belief that the composition of in vitro samples analysed in the laboratory reflects the true value of in vivo material is crucial for interpretation of the results. This means that the composition and concentration of any components measured should not change during the pre-analytical phase. The time span between sample drawing and analysis in the laboratory is of special importance and it seems to be generally accepted that blood specimens should be separated from cells with minimal delay and, in the meantime and thereafter, cooled.

Many papers have been published on the topic of stability of serum, plasma and whole blood components (2, 7, 11–14) and have described stability limits for different components. None of these papers, however, suggested a solution for the stabilisation of whole blood.

There are also several ''stability'' definitions. According to Berg et al. (13) a sample is stable if ''a change of the mean concentration of the constituent is less than the amount equal to one standard deviation of the analytical method used''. One may accept or disagree with this definition, depending on whether the effect of this change is for a single patient or the whole population. A systematic deviation can move a patient population from a group of ''healthy'' to a group of ''diseased'' patients (15).

Zawta (1) defines stability as ''the maximum permissible instability corresponding to the maximum

Table 3 Stability of biochemical components in whole blood at 20°C.

Storage	Difference					
	Calcium, mmol/L	Creatinine, μmol/L	Cholesterol, mmol/L	HDL, mmol/L	LDL, mmol/L	Potassium, mmol/L
Initial	2.344	89.4	4.90	1.904	2.800	3.96
4 h	0.002	−2.1	0.04	−0.018	0.024	0.06
6 h	−0.018	−2.0	0.00	−0.022	0.002	0.04
8 h	−0.006	−1.3	0.06	0.004	0.012	0.06
10 h	−0.020	−1.2	0.02	−0.014	0.020	0.12
12 h	0.006	−1.5	0.06	−0.008	0.028	0.16
MC	−0.03 → +0.02	−5 → +1	0.0 → 0.1	−0.02 → +0.0	−0.03 → +0.08	+0.0 → +0.4
	Magnesium, mmol/L	Phosphate, mmol/L	Urea, mmol/L	Cobalamin, pmol/L	Estradiol, nmol/L	Ferritin, pmol/L
Initial	0.798	1.132	4.50	304.4	0.439	78.7
4 h	0.002	−0.046	0.06	−0.7	0.038	1.7
6 h	−0.004	−0.072	0.04	8.9	−0.017	1.3
8 h	−0.002	−0.076	0.02	11.8	0.044	2.6
10 h	0.014	−0.134	0.02	17.4	0.009	0.8
12 h	0.009	−0.158	−0.02	15.8	−0.007	−1.0
MC	+0.00 → +0.02	−0.19 → −0.12	−0.2 → +0.3	−13 → +26	−0.03 → +0.01	−6 → +7
	FSH IU/L	FT_3, pmol/L	FT_4, pmol/L	LH IU/L	Progesterone, nmol/L	TSH, mU/L
Initial	17.7	5.35	16.1	14.8	7.08	2.16
4 h	0.3	0.15	0.9	0.2	0.33	0.07
6 h	0.4	0.19	1.1	0.1	0.60	0.06
8 h	0.5	0.18	1.2	0.1	0.09	0.06
10 h	0.5	0.28	1.5	0.7	0.04	0.09
12 h	0.4	0.20	0.8	0.8	−0.17	0.06
MC	+0 → +1	+0.1 → +0.4	+0 → +2	+0 → +2	−1.2 → +0.2	+0.0 → +0.1

Whole blood specimens from five persons were stored at 20°C for different periods of time before centrifugation. The Table shows mean initial values and mean storage-induced changes observed in the concentration of 18 blood components, as well as the maximum change in concentration (MC) observed for a single patient.

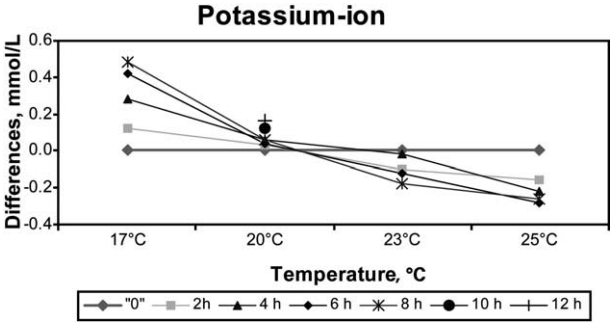

Figure 2 Temperature-induced changes in potassium-ion concentration as a function of storage time. Low temperature has the most pronounced effect. The smallest changes were observed around 20°C.

permissible relative imprecision of the analysis, generally 1/12 of the biological reference interval''. A later paper by Hyltoft Petersen et al. (16) clearly distinguishes between systematic and random variation (bias and imprecision).

We used a different approach. The maximum change from the initial concentration allowed during storage was decided for each component examined on the basis of anticipated clinical needs in usual applications and based on our experience. Furthermore, it was weighed against the analytical requirement of Gowans et al. (3), which is a bias of less than 1/16 of the reference interval.

The actual changes observed for different temperature and storage time combinations were compared with these specifications.

The results shown in Table 2 confirm (6, 7, 14) that potassium is the component of whole blood most influenced by storage. The factor that caused the greatest effect was temperature. All other components examined were not sensitive to storage temperature (changes were within the limits stated in Table 1), and were stable for up to 8 h, so the demand for centrifugation was not obvious.

Table 3 shows that all components investigated including potassium are stable for 8–12 h if blood specimens are stored at a controlled temperature of 20–21°C. This makes centrifugation and separation steps before transport unnecessary, even for potassium. (The cabinet we used was capable of maintaining temperature within 0.2°C, but 20–21°C is sufficient for practical purposes; Figure 2.)

We expect that many other components behave in the same way, but this remains to be examined. However, it should be noted that all data were obtained for healthy persons, and we can only assume that our findings are valid in pathological concentration ranges.

The results presented indicate that standardising and controlling storage conditions for whole blood can reduce the pre-analytical variation. It is possible to prolong the stability of biochemical components and at the same time make handling of specimens easier and cheaper, as the centrifugation step before sample transportation can be omitted.

Our findings on stability of whole blood under standardised conditions allow consideration of a sample pick-up service. A system could be organised in which centrifugation of blood specimens in connection with sampling can be avoided. The solution requires investment in thermostatted boxes for each general practitioner's office and logistics for collection of samples (cars, drivers, wages). Such a courier system could easily be designed in accordance with new EU rules for the transport of diagnostic test specimens (UN Committee of Experts on the Transportation of Dangerous Goods, UN 3373 and ADR-S).

As an additional advantage, the pick-up service would allow analysis and reporting of results on the same day.

Conclusion

The stability of 27 components of blood most frequently requested for analysis by general practitioners was examined.

- Our findings remove the normally required need for centrifugation of blood specimens within 3 h. If whole blood samples are stored at 20–21°C, centrifugation can be carried out after 8–12 h.
- We found that only the potassium-ion concentration was affected by temperature in a way that could influence clinical decisions. When stored at a controlled temperature of 20–21°C the transport of potassium in and out of cells seems to be in balance, whereby storage does not influence the measurement results.

Our work shows that by choosing sample pick-up, the following can be obtained:

- Handling of specimens will be easier (no need for centrifugation, separation of cells or mailing);
- Turn-around time will decrease from 1–2 days to within-day;
- The quality of results will probably improve, because standardised storage conditions reduce pre-analytical variation; and
- Problems with specimens received up to Sundays or holidays will disappear.

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