Clinical Biochemistry 45 (2012) 464–469

Contents lists available at SciVerse ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Stability study of 81 analytes in human whole blood, in serum and in plasma

Christiane Oddoze *, Elise Lombard, Henri Portugal

Clinical Laboratory, Department of Clinical Biology, CHU Timone, Marseille, France

ARTICLE INFO

Article history:
Received 4 August 2011
Received in revised form 28 December 2011
Accepted 6 January 2012
Available online 18 January 2012

Keywords:
Stability of laboratory tests
Temperature
Time
Tubes
Whole blood
Serum
Plasma

ABSTRACT

Objective: We studied the pre-analytical stability of 81 analytes based on the variables of delay before processing, storage as whole blood or serum/plasma, the storage temperature and the type of tube the sample was stored in.

Design and methods: The mean difference between assays for samples from 10 subjects was calculated with the samples being kept under different storage conditions and for different times between sampling time and analysis: up to 24 h for biochemistry, coagulation and hematology, and up to 72 h for hormonology. This difference was compared to the acceptable limits derived from the analytical and the intra individual biological variation.

Results: Most of the analytes investigated remained stable up to 24 h under all storage conditions prior to centrifugation. However, some analytes were significantly affected either by delay, tube type or temperature, such as potassium, inorganic phosphorus, magnesium, LD, glucose, lactate, mean corpuscular volume, mean corpuscular hemoglobin, activated partial thromboplastin time, insulin, C-peptide, PTH, osteocalcin, C-telopeptide and ACTH.

Conclusion: This study may be useful to help define acceptable delay times and storage conditions when a short time between sample collection and processing is not possible.

Introduction

As part of a laboratory accreditation process and to address the pre analytical requirements of ISO 15189 [1], a study was conducted in our laboratory to look at the effect of different pre-analytical storage conditions of blood samples. They included storage temperature, type of blood collection tube and whether the serum or plasma was separated immediately or whether centrifugation was delayed. Post-centrifugation delays were also investigated. The impact on the stability of 81 biochemical, hormonal, hematological analytes and routine coagulation tests was evaluated.

Some of the recommendations from WHO [2] and CLSI [3], are difficult to apply in routine practice as the analyte stability times described are often not compatible with the time taken to transport blood samples from the place of collection to the laboratory. As such, a delay before plasma separation from red blood cells occurs frequently which can modify analyte stability (for example WHO states that potassium and phosphate stability in the whole blood is less than 1 h).

In the literature, the stability of numerous analytes following prolonged contact of serum with cells has been described [2–8], but information is often incomplete and contradictory. Some reports included data at different times and temperatures; studies are generally dedicated to one specific discipline; the number of samples in analyte studies is sometimes very low [9,10]; samples are centrifuged and subsequently aliquoted and frozen before testing which can introduce a bias into the results [5,11]. In these studies, the observed variations were analyzed in different ways: by statistical analysis such as Student's *t*-test [5], ANOVA [12], Wilcoxon [13], the Bonferroni *t* test [10], a change more than 10% relative to baseline concentrations [11,14] or allowable variability as given by the German Federal Medical Council recommendations [8], or from an analytical SCL (significant change limits) approach [15] or combined analytical and biological variation [9]. These various methods give considerably different performance limits [16]. It is difficult, because of these differences in approach, to define standard limits for the stability of most analytes. To our knowledge, no studies have been conducted using the same criteria for the investigation of the stability of 81 analytes.

Our study was designed to provide information relevant to our current testing methods, on:

(a) delays before analysis (2 h, 4 h, 6 h and 24 h for biochemistry, coagulation and hematology and up to 72 h for hormonology),
(b) different storage temperatures (4 °C +/−2 or Room Temperature (RT) 25 °C +/−2),
(c) choice of collection tubes (serum plain glass tube, serum separator tube, K3 EDTA (tri potassium ethylenediaminetetraacetic acid), lithium heparin, sodium fluoride, CTAD (sodium citrate–theophylline–adenosine–dipyridamol)),

* Corresponding author at: Laboratoire de Biochimie Endocrinienne, Service Pr Portugal, Hôpital Timone Adulte 2ème étage, 264 rue St Pierre, 13385 Marseille cedex 05, France.
E-mail address: christiane.oddoze@ap-hm.fr (C. Oddoze).

0009-9120/$ – see front matter
doi:10.1016/j.clinbiochem.2012.01.012

(d) immediate separation versus delayed centrifugation before analysis.

Materials and methods

Subjects and collection tubes

Blood specimens were collected from a total of 50 apparently healthy donors following ethics committee approval (10 donors per analyte). Specimens from donors were used for multiple analytes.

Specific Becton Dickinson (BD) Vacutainer™ blood collection tubes were selected for each of the following analysis types:

Biochemistry analyses: plain glass tubes (ref 367614); serum separator tubes with clot activator BD SST™ II (ref 367957) and lithium heparin tubes (ref 368884).
Glucose and lactate: sodium fluoride tubes (ref 368521).
Hematology analysis: K3 EDTA tubes (ref 368860).
Coagulation analysis: CTAD tubes (ref 367599).
Hormonology analysis: plain glass tubes (ref 367614), BD SST™ II (ref 367957) and K3 EDTA tubes (ref 368860).

Lithium heparin tubes were used only for biochemistry analytes and then only tested up to a delay of 4 h. This was to investigate, particularly, the stability of electrolytes (especially potassium) and glucose, to identify any differences in stability versus plain glass tubes.

The SST™ II tubes were not tested in the whole blood portion of the study because we considered that analyte stability in whole blood in SST II tubes would be equivalent to analyte stability in plain glass serum tubes. The real advantage of SST™ II tubes is serum conservation after centrifugation thanks to cells being separated from the serum.

Filling and mixing of the tubes collected in the laboratory were performed according to the manufacturer's recommendations to avoid any collection order effect.

The collection duration (maximum 5 min) for each donor was sufficiently short to avoid any bias between first and last collected tube. In accordance with safe practice, a maximum of 115 mL (23 tubes) of blood was drawn from each donor.

Centrifugation also complied with BD's recommendations: 2000 *g* for 10 min at 20 °C except tubes for ACTH analysis where tubes were centrifuged at 2600 *g* for 10 min at + 5 °C.

After collection, the "T0" specimen (first time point) from each volunteer was centrifuged after 30 min for blood clotting for serum samples, and immediately after phlebotomy for plasma samples, and then analyzed. The mean of these results represented the initial value (T0) for each analyte.

The mean results, "Tx", from all ten volunteers for each analyte, each delay and each tube were obtained according to the two following methods.

For whole blood analyte stability, tubes were stored for the chosen delay either at 4 °C or RT and then were centrifuged and immediately analyzed.

For serum or plasma analyte stability, tubes were centrifuged either immediately or 30 min after phlebotomy, depending on whether the sample was plasma or serum, and stored either at 4 °C or RT and then analyzed after the chosen delay.

Each sample was analyzed in a primary tube without removing an aliquot or storage in a frozen state.

Instruments

The analytes with their abbreviations and the instruments included in the study are summarized in Table 1.

Table 1
Analytes and instruments.

Biochemistry				Hematology and coagulation		Hormonology	
Modular® PP (Roche Diagnostics)	BNProspec® (Siemens)	RxLDimension® (Siemens)	G5® (Tosoh)	Advia 120® (Siemens)	STAR® (Stago)	Cobas® 6000 e601 (Roche Diagnostics)	Liaison® (Diasorin)
Alanine aminotransferase (ALT), albumin (AL), alkaline phosphatase (ALP), amylase (AM), aspartate aminotransferase (AST), total bilirubin (TB), bicarbonate (BIC), calcium (Ca), chloride (Cl), total cholesterol (TC), creatinine (CREA), creatine kinase (CK), fructosamin (FRU), γ-glutamyltransferase (GGT), glucose (GLU), iron (Ir), lactate (LAC), lactate déshydrogénase (LD), lipase (LIP), magnesium (Mg), phospholipids (PLIP), inorganic phosphorus (P), potassium (K), total protein (TP), sodium (Na), triglyceride (TG), urea (BUN), uric acid (UA), cholesterol component in HDL (HDL) and LDL	Apolipoprotein A1 (ApoA1), Apolipoprotein B (ApoB), haptoglobine (HAP), α2 macroglobulin (AMG), transferrin (TF), soluble transferrin receptor (STFR)	Myoglobin (MG), C-Reactive Protein (CRP)	HbA1c	Red blood cells counts (RBC), hemoglobin concentration (HG), hematocrit (HT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), white blood cell counts (WBC), neutrophil (N:C), eosinophil (E:C), monocyte (M:C), lymphocyte (L:C) counts, platelet counts (P:C)	Fibrinogen (FG), Prothrombin Time (PT), Factors II, X, V, Activated partial Thromboplastin Time (APTT), Anti Thrombin III (AT)	Adrenocorticotrophic hormone (ACTH), cortisol (COR), C-peptide (C P), C-telopeptide (CTELO), Insulin (INS), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), osteocalcin (OST), parathyroid hormone (PTH), progesteron (PROG), folate (FOL), vitamin B12 (vB12), DHEA sulfate (DHEAS), prolactin (PROL), testosterone (TEST), TSH, free T3 (FT3), free T4 (FT4), SHBG	Vitamin D (vit D), growth hormone (Gh), insulin –like growth factor (IGF-1).

Statistics

According to ISO GUIDE 30 [17]: "stability is the ability of a sample to maintain the initially measured value, within specified limits, of a constituent over a period of time under specified storage conditions. The instability is given as an absolute difference, as a quotient or as a percentage deviation of results obtained from measurements at initial time (T0) and after a given period of time (Tx)".

The ten donors mean percentage deviation (or difference), $[(Tx - T0)/T0] \times 100$, was calculated.

We considered 2 approaches for establishing outcome-related analytical performance goals:

(a) The mean percentage deviation was compared to the Acceptable Change Limit (ACL), according to ISO 5725-6 [18]. The ACL for interpreting a measured difference is based on the analytical imprecision (CVa), using the formula ACL = 2.77 CVa. The factor 2.77 is derived from $Z\sqrt{2}$, where Z = 1.96, determined by the 95% of confidence interval value for bi-directional changes, and $\sqrt{2}$ as we are comparing two results with the same CVa. We concluded that a mean percentage deviation greater than 2.77 CVa represents a probable difference in analyte concentration. The CVa was obtained from in house routine mean data of QC values accumulated over a six month period for each analyte.

(b) The second approach had taken into account the acceptable imprecision based on the intra individual biological variation. According to the College of American Pathologists recommendations [19,20], the imprecision of a method, for individual single and multipoint testing, should be equal or less than one-half of the average within-subject variation (CVb), and this should be the goal for short-term laboratory imprecision ($\leq$0.5 CVb). The CVb of each analyte was taken from the listing of biological variation for 316 analytes by Ricos et al. [21]. This database was most recently updated in 2010 but some analytes are still missing [22].

For monitoring changes due to the instability of an analyte, in the same sample for a same individual, we combined the two approaches (analytical and intra-individual imprecisions) by the estimation of the square root of the sum of the squared analytic and biologic imprecisions, defined as the total change limit (TCL):

$$TCL = \ddot{O}(2.77\ \mathrm{CVa})^2 + (0.5\ \mathrm{CVb})^2.$$

If the results for an analyte had a mean percentage difference which exceeded the TCL, then the difference was judged to be significant and not to meet the stability criteria.

Results

Tables 2a and 2b show the statistical analysis for biochemistry, Tables 3a and 3b for hematology and coagulation and Tables 4a and 4b for hormonology. The tables compare the mean percentage differences with the TCL, with a "+" for an increase and a "–"for a decrease. These tables only show results for analytes exceeding the TCL because of prolonged contact with cells or delayed analysis.

For the 39 biochemistry analytes, the tests that were suitable for analysis after 24 h storage at 4 °C and RT in whole blood and in plasma glass tubes, BD SST™ II serum tubes or lithium heparin tubes were: sodium, chloride, total protein, albumin, calcium, urea, total bilirubin, uric acid, creatinine, total cholesterol, triglyceride, phospholipids, HDL and LDL cholesterol, fructosamine, iron, ALP, ALT, AST, CK, amylase, GGT, CRP, lipase, ApoA1, ApoB, haptoglobin, α2macroglobulin, transferrin, soluble transferrin receptor, myoglobin. HbA1c is stable in whole blood for 24 h stored at 4 °C and RT in K3 EDTA tubes.

Stability of hematological analytes is not affected by storage at 4 °C or RT in K3 EDTA up to 24 h, except for MCV and MCH which increase and decrease respectively after 6 h at RT.

The clotting factors investigated, except APTT, are remarkably stable in CTAD whole blood and plasma.

Thirteen of the twenty three tested hormones were not significantly affected up to 72 h in whole blood and in serum or plasma at 4 °C and RT in glass, in BD SST™ II and in K3 EDTA tubes: cortisol, IGF_1, GH, vitamin D, TSH, FT_4, FT_3, LH, progesterone, testosterone, DHEA sulfate, vitamin B12, and SHBG (K3 EDTA is not recommended for SHBG by Roche Diagnostics).

Discussion

In this large study we investigated the effects that the time, the temperature, the type of tube and the delay before centrifugation have on the subsequent concentration of 81 analytes (about 20,000 tests).

Our definition of a change exceeding the analytic and biologic imprecision limit, defined as the TCL, tended to reflect the pre-analytical instability.

Most tested analytes remained stable up to 24 h at all storage conditions prior to centrifugation, using our statistical approach. However, some important analytes were significantly affected because of:

– prolonged contact of plasma and serum with cells and leakage of intracellular constituents such as potassium, inorganic phosphorus, magnesium, LD.

Table 2a
Stability of biochemical analytes on whole blood.

Analytes	To	Tubes	TCL	Mean difference%								Acceptable delays	
				T2h		T4h		T6h		T24h			
				4 °C	25 °C	4 °C	25 °C	4 °C	25 °C	4 °C	25 °C	4 °C	25 °C
K^+	4.3 mmol/L	Glass tube	±2.8%	+8.7[a]	+2.4	+18.3[a]	+2.4	+36.1[a]	+3.8[a]	+132.0[a]	+18.7[a]	<2 h	4 h
		Li heparin		+5.8[a]	+3.5[a]	+13.8[a]	+4.3[a]	NT	NT	NT	NT	<2 h	<2 h
Bicarbonate	28.9 mmol/L	Glass tube	±8.6%	−1.3	+0.9	−1.4	+1.4	−5.0	−6.6	−0.9	−5.1	24 h	24 h
		Li heparin		−0.2	+0.1	−0.6	−1.0	NT	NT	NT	NT	>4 h	>4 h
Inorganic phosphorous	1.09 mmol/L	Glass tube	±5.2%	+1.6	0.0	+1.9	−2.1	−0.5	−3.8	−2.2	+20.1[a]	24 h	6 h
		Li heparin		+1.3	−4.4	+1.6	−7.9[a]	NT	NT	NT	NT	>4 h	2 h
Lactate	1.33 mmol/L	Fluoride	±17.8%	NT	NT	NT	NT	+10.6	+9.9	+16.2	+31.7[a]	24 h	6 h
LD	158 UI/L	Glass tube	±6.4%	+5.1	+6.5[a]	5.2	+6.8[a]	+6.8[a]	+11.3[a]	+12.3[a]	+14.0[a]	4 h	<2 h
Glucose	4.73 mmol/L	Glass tube	±4.5%	−4.0	−9.5[a]	−7.5[a]	−17.0[a]	−8.8[a]	−19.7[a]	−33.0[a]	−62.8[a]	2 h	<2 h
		Li heparin		−4.3	−10.0[a]	−6.7[a]	−15.8[a]	NT	NT	NT	NT	2 h	<2 h
		Fluoride		NT	NT	NT	NT	−0.3	−0.6	0.7	−0.9	24 h	24 h
Magnesium	0.86 mmol/L	Glass tube	±5.5%	−0.1	+0.8	−0.7	+2.1	+0.5	+1.4	+2.7	+6.4[a]	24 h	6 h
		Li heparin		−0.3	+0.1	−0.7	+0.5	NT	NT	NT	NT	>4	>4 h

NT: non tested; To: initial value; TCL: Total Change Limit.
[a] Exceeds the TCL.

Table 2b
Stability of biochemical analytes on serum or plasma.

Analytes	To	Tube	TCL	Mean difference%								Acceptable delays	
				T2h		T4h		T6H		T24h			
				4 °C	25 °C	4 °C	25 °C	4 °C	25 °C	4 °C	25 °C	4 °C	25 °C
K^+	4.3 mmol/L	Glass tube	±28%	+0.7	−0.6	+1.0	+1.5	+2.8	−1.0	+20.6[a]	+0.1	6 h	24 h
		SST		+1.1	+0.6	+1.3	NT	+0.6	+1.3	+0.9	+1.1	24 h	24 h
		Li heparin		+08	+1.2	+14	−1.8	NT	NT	NT	NT	>4 h	>4 h
Bicarbonate	28.9 mmol/L	Glass tube	±8.6%	+2.6	+0.3	+1.7	0.0	−6.5	−7.5	−2.0	−5.5	24 h	24 h
		SST		+2.8	+0.6	+2.7	0.0	−7.3	13.0[a]	−8.8[a]	−13.2[a]	6 h	4 h
		Li heparin		+1.0	−0.1	−0.5	−0.8	NT	NT	NT	NT	>4 h	>4 h
Inorganic phosphorous	1.09 mmol/L	Glass tube	±5.2%	+0.3	+1.9	+0.4	+0.9	−18	0.0	−0.4	+7.0[a]	24 h	6 h
		SST		+0.8	+1.1	+0.5	+2.1	+0.1	−0.8	+2.6	+3.8	24 h	24 h
		Li heparin		+0.6	+0.5	+0.9	+0.7	NT	NT	NT	NT	>4 h	>4 h
Lactate	1.33 mmol/L	Fluoride	±17.8%	NT	NT	NT	NT	+8.8	+3.1	+11.8	+6.8	24 h	24 h
LD	158 UI/L	Glass tube	±6.40%	NT	NT	NT	NT	−0.6	+1.5	+1.2	+4.1	24 h	24 h
		SST		NT	NT	NT	NT	+3.8	+5.8	4.2	+6.9[a]	24 h	6 h
Glucose	4.73 mmol/L	Glass tube ±4.5%	±4.5%	−0.5	+1.7	−0.6	0.0	−2.6	−6.6[a]	−11.9[a]	−17.7[a]	6 h	4 h
		SST		+1.2	+0.8	+0.9	+2.0	+0.9	+1.7	+1.1	+0.6	24 h	24 h
		Li heparin		−1.1	0.0	−1.9	−2.8	NT	NT	NT	NT	>4 h	>4 h
		Fluoride		NT	NT	NT	NT	0.0	0.0	0.0	0.0	24 h	24 h
Magnesium	0.86 mmol/L	Glass tube	±5.5%	−0.3	−0.7	−0.8	−0.1	−0.1	+0.6	+0.5	+2.5	24 h	24 h
		SST		−0.8	−0.6	0.0	−1.0	−0.4	+1.1	+0.1	+1.4	24 h	24 h
		Li heparin		−0.5	−0.6	−0.5	+0.1	NT	NT	NT	NT	>4 h	4 h

NT: no tested; To: initial value; TCL: Total Change Limit.
[a] Exceeds the TCL.

- glycolysis in cells which consumes glucose and produces lactate.
- some changes were temperature dependent such as for potassium (more stable at 25 °C), phosphorus, magnesium, and glucose (more stable at 4 °C) and for hematological parameters: MCV and MCH were more stable at 4 °C.
- degradation of proteins and peptides by blood enzymes; samples collected in tubes with EDTA as anticoagulant and transported at 4 °C were practical for the measurement of hormones because EDTA is known to protect peptides from proteolysis.

The percentage change in potassium (+6 to 9% after 2 h) is greatest at 4 °C because the lower temperature induced inhibition of Na–K ATPase pump that leads to increased release of potassium from cells. The change became clinically significant within 6 h of serum-clot contact at RT which is in agreement with some authors [4,9], but not with others [5,6,8].

Glucose is stable for 24 h in whole blood stored at 4 °C and 25 °C only in sodium fluoride tubes because glycolysis is inhibited by this additive [23]. In all the other tubes we observed a significant decrease according to the time the sample was left in contact with cells. This decrease was greater at RT (−10%) than at 4 °C (−4%) after 2 h, as previously described in the literature because glycolysis is slowed by low temperature. The decrease of glucose is mirrored by a time – and temperature – dependent increase in lactate concentration [24].

The increase of LD activity during the first 2 h is due to change in cell membrane activity. This increase at RT is very small (+6.5%) during the first 4 h, but exceeded our analytical and biological limits (6.4%). Zhang et al. [9] observed values remaining within the clinically acceptable limits until 24 h because his limit was 9.5%. The question is to know if such a small increase will cause changes in clinical interpretation. Beyond 6 h the activity increases (>10%) so that the results overestimate LD. Storage is better at 4 °C (4 h) than RT (<2 h).

For hematological analytes, MCV and MCH increase (+4.9%) and decrease (−4.8%) respectively at 24 h at RT. These observations are consistent with those of Imeri et al. [12].

For coagulation tests, APTT increases after 6 h at 4 °C and RT (respectively 10.1 and 8.5% at 24 h) as previously described, but Zurcher et al. [25] do not observe a significant difference because they decided to consider a 10% change limit which is larger than our TCL (5.28%).

The estradiol instability has been reported previously and seems to be collection volume dependent [26–28].

We observed a temperature effect on several hormonological analytes: insulin is stable only for 6 h at RT in glass tubes and then decreases, but is stable 72 h at 4 °C. However, insulin is also very sensitive to hemolysis providing liberation of intra-erythrocyte insulinase.

Except in K3 EDTA, stability of C-peptide and PTH is lower at RT than at 4 °C, folate shows a better stability at 4 °C, and osteocalcin is stable 48 h at 4 °C, but not at RT.

This study demonstrated that for all these hormones, except ACTH (24 h) and osteocalcin (48 h), collection into K3 EDTA, transport and storage in whole blood at 4 °C is practical up to 72 h. K3 EDTA is the recommended sample type in which ACTH is stable for 24 h [14].

Our results in whole blood are consistent with WHO for several analytes as LD, LH, FSH, prolactin, PTH, TSH, cortisol, DHEAS, testosterone and vitamin D. However, it should be noted that we observe improved stability in our study: bicarbonate is stable 24 h in glass tubes at RT, instead of 30 min in WHO, and, in K3 EDTA at RT, insulin is stable 72 h versus 15 min, C peptide 24 h versus 6 h, and osteocalcin

Table 3a
Stability of hematological and coagulation analytes on whole blood.

Analytes	To	Tube	TCL	Mean difference%				Acceptable delays	
				T 6 h		T 24 h			
				4 °C	25 °C	4 °C	25 °C	4 °C	25 °C
MCV	85.2 fL	K3 EDTA	±1.7%	+0.4	+1.2	+0.8	+4.9[a]	24 h	6 h
MCH	21.1 mmol/L Er	K3 EDTA	±2.8%	−0.5	−1.3	−1.0	−4.8[a]	24 h	6 h
APTT	32 s	CTAD	±5.3%	+3.9	+3.6	+10.0[a]	+5.4[a]	6 h	6 h

To: initial value; TCL: Total Change Limit; Er: erythrocytes.
[a] Exceeds the TCL.

Table 3b
Stability of coagulation analytes on plasma.

Analytes	To	Tube	TCL	Mean difference%				Acceptable delays	
				T 6 h		T 24 h			
				4 °C	25 °C	4 °C	25 °C	4 °C	25 °C
APTT	32 s	CTAD	±5.3%	+4.5	+3.5	+10.1[a]	+8.5[a]	6 h	6 h

To: initial value; TCL: Total Change Limit; Er: erythrocytes.
[a] Exceeds the TCL.

Table 4a
Stability of hormonological analytes on whole blood.

Analytes	To	Tubes	TCL	Mean difference%								Acceptable delays	
				T 6 h		T 24 h		T 48 h		T 72 h			
				4 °C	25 °C	4 °C	25 °C	4 °C	25 °C	4 °C	25 °C	4 °C	25 °C
FSH	12.7 UI/L	Glass tube	±9.8%	+1.1	+2.0	+1.8	+3.6	+2.7	+6.1	+3.9	+8.2	72 h	72 h
		K3 EDTA		+0.4	+0.9	+1.2	+3.4	+2.1	+8.1	+3.4	+10.5[a]	72 h	48 h
Estradiol	168.4 ng/mL	Glass tube	±13.9%	−2.2	+2.8	+0.2	+3.8	+9.6	+9.3	+7.2	+10.6	72 h	72 h
		K3 EDTA		+4.4	+4.7	+4.8	+7.6	+2.6	+3.9	+5.5	+1.5	72 h	72 h
Prolactin	236 mUI/L	Glass tube	±6.6%	−0.8	+0.3	+0.1	+0.1	−0.2	+2.0	−0.1	+4.1	72 h	72 h
		K3 EDTA		−1.6	+0.1	−0.8	+2.5	+1.2	+7.7[a]	+3.2	+10.6[a]	72 h	24 h
Folate	14.1 nmol/L	Glass tube	±22.4%	+7.7	+2.4	+6.2	−4.7	+2.8	−15.1	+4.3	−27.4[a]	72 h	48 h
Insulin	10.6 mUI/L	Glass tube	±14.4%	−3.7	+0.1	−9.3	−22.1[a]	−10.2	NT	−11.4	NT	72 h	6 h
		K3 EDTA		−8.9	−6.4	−8.1	−5.5	−5.5	−7.6	−8.2	−11.2	72 h	72 h
C-peptide	0.73 nmol/L	Glass tube	±9.5%	−0.6	−2.0	−2.1	−18.4[a]	−1.6	NT	−3.9	NT	72 h	6 h
		K3 EDTA		−1.0	−1.9	−1.5	−6.5	−0.3	−23.7[a]	−2.0	−41.3[a]	72 h	24 h
PTH	5.85 pmol/L	Glass tube	±16.0%	+0.5	−7.7	−1.1	−29.0[a]	−5.8	NT	−6.6	NT	72 h	6 h
		K3 EDTA		+0.8	+2.9	+1.4	+1.7	+0.9	−3.2	−0.2	−7.8	72 h	72 h
Osteocalcin	27.0 ng/mL	Glass tube	±8.9%	−5.3	−9.7[a]	−6.1	−30[a]	−9.9[a]	NT	−14.6[a]	NT	24 h	<6 h
		K3 EDTA		−5.7	−8.1	−8.4	−19[a]	−7.8	−29[a]	−10.9[a]	−38[a]	48 h	6 h
C-telopeptide	0.41 ng/mL	Glass tube	±8.4%	−7.8	−11.0[a]	−14.8[a]	−41[a]	−15.8[a]	NT	−23.2[a]	NT	6 h	<6 h
		K3 EDTA		−4.5	−2.4	−6.8	−5.8	−3.0	−1.1	−0.6	−4.4	72 h	72 h

Analyte	To	Tube	TCL	Mean difference%								Acceptable delays	
				T 4 h		T 8 h		T 12 h		T 16 h		T 24 h			
ACTH	4.72 pmol/L	K3 EDTA	±7.5%	−1.6	−6.7	−4.2	−10.4[a]	−6.6	13.3[a]	−3.5	−14.2[a]	−4.8	−23.7[a]	24 h	4 h

NT: Not tested; To: initial value; TCL: Total Change Limit.
[a] Exceeds the TCL.

6 h versus 15 min. For glucose and lactate WHO do not indicate the stability in sodium fluoride tubes. Elsewhere, results presented in WHO are mainly evaluated at RT whereas we demonstrated that storage at 4 °C sometimes improves the stability as for ACTH 24 h at 4 °C versus 1–4 h at RT. Finally, WHO is a meta-analysis which combines the results of several stability studies with lack of homogeneity.

In summary, it is important to know whether a parameter is stable or not. Stable parameters, if processed in 24 h, don't need to be centrifuged prior to the shipment to a central laboratory. The reduction of a pre-analytical step limits the number of uncontrolled variables in the laboratory and leads to a gain in time. Both contribute to the continuous improvement of the laboratory workflow.

Table 4b
Stability of hormonological analytes on serum or plasma.

Analytes	To	Tubes	TCL	Mean difference%								Acceptable delays	
				T 6 h		T 24 h		T 48 h		T 72 h			
				4 °C	25 °C	4 °C	25 °C	4 °C	25 °C	4 °C	25 °C	4 °C	25 °C
FSH	12.7 UI/L	Glass tube	±9.8%	+1.1	+1.0	+3.0	+3.5	+1.5	+2.8	+2.9	+4.5	72 h	72 h
		SST		+0.8	+2.3	+4.0	+6.4	+2.6	+4.2	+3.7	+5.7	72 h	72 h
		K3 EDTA		−0.1	+0.6	+1.8	+2.1	+1.8	+1.9	+2.3	+2.9	72 h	72 h
Estradiol	168.4 ng/mL	Glass tube	±13.9%	−3.2	+0.8	+2.0	+2.5	+8.7	+8.5	+8.3	−0.6	72 h	72 h
		SST		−1.3	−3.1	+8.5	NT	−1.0	+4.2	−1.3	−15.0[a]	72 h	48 h
		K3 EDTA		+2.3	+3.8	+5.7	+5.2	+5.0	+2.6	+0.4	+1.0	72 h	72 h
Prolactin	236 mUI/L	Glass tube	± 6.6%	−1.7	−1.4	−0.5	−0.6	−1.1	−1.7	−1.0	−2.2	72 h	72 h
		SST		−1.5	−0.9	+0.1	−1.6	−0.4	−2.0	−0.5	−2.6	72 h	72 h
		K3 EDTA		−1.3	−1.2	0.0	+0.4	+0.1	+0.05	+0.1	−0.03	72 h	72 h
Folate	14.1 nmol/L	Glass tube	±22.3%	+3.8	+0.2	+6.1	+2.9	−3.5	−9.2	−0.5	−11.1	72 h	72 h
		SST		−3.2	−5.5	+1.3	−2.5	−4.5	−12.8	−3.4	−30.4[a]	72 h	48 h
Insulin	10.6 mUI/L	Glass tube	±14.3%	−0.5	−7.7	−4.4	−19.0[a]	−5.2	−37.5[a]	−8.8	−48.8[a]	72 h	6 h
		SST		−2.5	−5.8	−4.2	−25.7[a]	−0.2	−21.4[a]	−1.8	−32.2[a]	72 h	6 h
		K3 EDTA		−0.2	−4.8	−2.0	−8.3	−1.9	−12.7	−2.8	−18.3[a]	72 h	48 h
C−Peptide	0.73 nmol/L	Glass tube	±9.4%	−0.7	−1.7	−1.8	−6.5	−1.2	−12.0[a]	−2.7	−21.9[a]	72 h	24 h
		SST		−0.6	−0.4	−0.6	−1.7	+0.2	−7.1	−0.6	−13.3[a]	72 h	48 h
		K3 EDTA		−0.2	−1.4	+0.8	−5.3	+0.1	−12.5[a]	−1.0	−29.7[a]	72 h	24 h
PTH	5.85 pmol/L	Glass tube	±16%	+3.4	−3.7	−1.7	−20.9[a]	+0.3	NT	−3.4	NT	72 h	6 h
		SST		−1.6	−5.6	−6.6	−26.0[a]	−2.3	−34[a]	−3.9	−44[a]	72 h	6 h
		K3 EDTA		+2.6	+9.1	+2.3	+6.8	−2.8	−3.0	−2.3	−14.1	72 h	72 h
Osteocalcin	27.0 ng/mL	Glass tube	±8.9%	−2.4	−13[a]	−8.4	−28[a]	−7.6	NT	−14.3[a]	NT	48 h	<6 h
		SST		−3.3	−10.6[a]	−10.6[a]	−20[a]	−6.8	−29[a]	−12.8[a]	−32[a]	6 h	<6 h
		K3 EDTA		−2.8	−7.8	−7.0	−16[a]	−1.7	−23[a]	−5.4	−30[a]	72 h	6 h
C-Telopeptide	0.41 ng/mL	Glass tube	±8.4%	−8.1	−10.6[a]	−13.9[a]	−25[a]	−15.3[a]	NT	−20.4[a]	NT	6 h	<6 h
		SST		−7.4	−10.3[a]	−14.2[a]	−21[a]	−12.3[a]	−31[a]	−16.3[a]	−38[a]	6 h	<6 h
		K3 EDTA		−3.5	−4.5	−5.5	−6.0	−2.4	−5.4	−1.3	−6.3	72 h	72 h

NT: Not tested; To: initial value; TCL: Total Change Limit.
[a] Exceeds the TCL.

A standardized approach is proposed in this publication to investigate the impact of pre-analytical conditions for most of the analytes and this approach can easily be applied by other laboratories in order to complete these data. The CVa used, the methods and the instruments are those of our own laboratory and could be extended to others using their own analytical imprecisions. We assume that this study was performed on healthy donors and that these outcomes should be applicable for a large majority of specimens. Follow-on studies would benefit from the inclusion of pathological subjects, to increase the analyte data range, and by being extended to include other analyzers.

Acknowledgments

Financial support was provided by the Becton Dickinson and Roche Diagnostics. We gratefully acknowledge the clinical laboratory personnel for accommodating and helping with this project.

References

[1] ISO 15189. Medical laboratories—particular requirements for quality and competence; 2007.
[2] Quality of diagnostic samples. Recommendations of the Working Group on Preanalytical Quality of the German Society for Clinical Chemistry and Laboratory Medicine. WHO/DIL/LAB.99.1 Rev, 2; 2009. 85 pp.
[3] Clinical and Laboratory Standards Institute (CLSI). Procedures for the handling and processing of blood specimens for common laboratory tests; approved guideline. Document H18-A4, Vol. 30 N°10; 2010.
[4] Laessig RH, Indrikson AA, Hassemer DJ, Paskey TA, Schwartz TH. Changes in serum chemical values as a result of prolonged contact with the clot. Am J Clin Pathol 1976;66:598–604.
[5] Ono T, Kitaguchi K, Takehara M, Shiiba M, Hayami K. Serum-constituents analyses: effect of duration and temperature of storage of clotted blood. Clin Chem 1981;27(1):35–8.
[6] Chu SY, MacLoed J. Effect of three-day clot contact on results of common biochemical tests with serum. Clin Chem 1986;32:2100.
[7] Rehak NN, Chlang BT. Storage of whole blood. Effect of temperature on the measured concentration of analytes in serum. Clin Chem 1988;34(10):2111–4.
[8] Heins M, Heil W, Withold W. Storage of serum or whole blood samples? Effect of time and temperature on 22 serum analytes. Eur J Clin Chem Biochem 1995;33:231–8.
[9] Zhang DJ, Elswick RK, Miller WG, Bailey JL. Effect of serum-clot contact time on clinical chemistry laboratory results. Clin Chem 1998;44(6):1325–33.
[10] Zwart SR, Wolf M, Rogers A, Rodgers S, Gillman PL, Hitchcox K, et al. Stability of analytes related to clinical chemistry and bone metabolism in blood specimens after delayed processing. Clin Biochem 2009;42:907–10.
[11] Evans MJ, Livesey JH, Ellis MJ, Yandle TG. Effect of anticoagulants and storage temperatures on stability of plasma and serum hormones. Clin Biochem 2001;34:107–12.
[12] Imeri F, Herklotz R, Risch L, Arbetsleitner C, Zerlauth M, Risch GM, et al. Clin Chim Acta 2008;397:68–71.
[13] Parent X, Alenabi F, Brignon P, Souberbielle JC. Delayed measurement of PTH in patients with CKD: storage of the primary tube in the dialysis unit, which temperature, which kind of tube? Nephrol Ther 2009;5:34–40.
[14] Ellis MJ, Livesey JH, Evans MJ. Hormone stability in human whole blood. Clin Biochem 2003;36:109–12.
[15] Boyanton BL, Blick KE. Stability studies of twenty-four analytes in human plasma and serum. Clin Chem 2002;48(12):2242–7.
[16] Klee GG. Establishment of outcome-related analytic performance goals. Clin Chem 2010;56(5):714–22.
[17] ISO Guide 30. Terms and definitions used in connection with reference materials; 1992.
[18] ISO 5725-6. Accuracy (trueness and precision) of measurement methods and results. Part 6: use in practice of accuracy values; 1994.
[19] Fraser CG, Petersen PH, Ricos C, Haeckel R. Proposed quality specifications for the imprecision and inaccuracy of analytical systems for clinical chemistry. Eur J Clin Chem Clin Biochem 1992;30:311–7.
[20] Elevitch FR, Noce PS, editors. Data recap 1970–1980. Northfield, IL: College of American Pathologists; 1981.
[21] Ricos C, Alvarez V, Cava F, Garcia-Lario JV, Hernandez A, Jimenez CV, et al. Current databases on biologic variation; pros, cons and progress. Scand J Clin Lab Invest 1999;59:494–500.
[22] Ricos C, Alvarez V, Cava F, Garcia-Lario JV, Hernandez A, Jimenez CV, et al. Desirable quality specifications for total error, imprecision, and bias, derived from biological variation. http://www.Westgard.com/biodatabase1.htm Accessed on june 5, 2009.
[23] Chan AYW, Swaminathan R, Cockram CS. Effectiveness of sodium fluoride as a preservative of glucose in blood. Clin Chem 1989;35:315–7.
[24] Astles R, Williams CP, Sedor F. Stability of plasma lactate in vitro in the presence of antiglycolytic agents. Clin Chem 1994;40:1327–30.
[25] Zurcher M, Sulzer I, Barizzi G, Lammle B, Alberio L. Stability of hemostasis assays performed in plasma from citrated whole blood transported at ambient temperature. Thromb Haemost 2008;99:416–26.
[26] Reinartz JJ, Ramey ML, Fowler MC, Killeen A. Plastic vs. glass SST evacuated serum separator blood drawing tubes for endocrinologic analytes. Clin Chem 1993;39:2535–6.
[27] Ferry JD, Collins S, Sykes E. Effect of serum volume and time of exposure to gel barrier tubes on results for progesterone by Roche Elecsys 2010. Clin Chem 1999;45:1574–5.
[28] Bush VJ, Janu MR, Bathur F, Wells A, Dasgupta A. Comparison of BD Vacutainer SST™ plus tubes with BD SST™ II plus tubes for common analytes. Clin Chim Acta 2001;306:139–43.