Letter to the Editor

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Assessment of *in vitro* stability: a call for harmonization across studies

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To the Editor,

According to the ISO Guide 30, stability is defined as 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)" [1].

The first objective of the study was to assess the stability of 14 parameters at room temperature (RT) in both decanted and non-decanted plasma (still in contact with blood cells). As there is no standardized way to interpret *in vitro* stability, the second objective was to compare our results through different calculation methods reported in the literature and to evidence potential discrepancies.

For that purpose, multiple parameters sodium (Na), potassium (K), chloride (Cl), creatinine (Cr), calcium (Ca), phosphorus (P), total proteins (TP), estradiol (E2), luteinizing hormone (LH), 25-hydroxyvitamine D (25[OH] D), intact parathyroid hormone (PTH) and immunoglobulins A (IgA), M (IgM) and G (IgG) were studied in 20 healthy volunteers. Five samples were collected from each subject: two lithium-heparin tubes, two serum tubes and one serum-gel tube (S-Monovette® 7.5 mL tubes, Sartstedt, Nümbrecht, Germany). Within 15 min of blood collection, all the samples were spun at RT for 10 min at 3500 rpm (≈2369×*g*) (Eppendorf® 5810 benchtop centrifuge [Hamburg, Germany]). For 10 healthy volunteers, the plasma (or serum) was immediately separated from blood cells, and for the 10 other volunteers, the plasma (or serum) was not separated from the blood cells after the centrifugation process. All parameters were measured on the Cobas 8000® (Roche Diagnostics, Basel, Switzerland). Once baseline results were obtained, multiple measurements of each parameter were performed after 3, 6, 9, 24 and 50 h. All samples were left at RT during the stability study. Effect of stoppers was also evaluated.

The bias from the reference was calculated as follows: bias = value at specific condition – reference value. Calculated bias was then compared to multiple criteria found in literature: Royal College of Pathologists of Australasia (RCPA) [2], desirable specification for allowable total error (TEa) [3], reference change value (RCV) [4], total change limit (TCL) [5], acceptable change limit (ACL) [6], analytical coefficient of variation (CVa, homemade), arbitrary CV (10%), ANOVA analysis (Dunnet's test) and significant change limit (SCL) [7] (Figure 1 and Table 1). We left the criterion in percentage or in absolute value depending on the cutoff proposed in the literature (Table 1). To simplify the interpretation, we attributed for each time point an arbitrary score (<3 h = 1, 3 h = 2, 6 h = 3, 9 h = 4, 24 h = 5 and 50 h = 6). The mean score of each criterion per parameter (with/without stoppers or blood cells) was calculated (Table 1). Finally, different presentations of data (mean, mean ± CI 95%, median and median ± IQR) were compared and illustrated for the sodium (Figure 1). Statistical analysis was conducted using GraphPad Prism® software (Version 6.0e, California, USA). A p-value <0.05 was considered as statistically significant in regard to the reference. The Ethical principles provided by the Declaration of Helsinki were fulfilled.

Our results showed that some parameters were less affected by the criterion used (e.g. LH, TP or Ig) than others (e.g. K, Na or Ca) (Table 1). For example, the stability of potassium varied widely across the experiment (50, 9 or 6 h according to RCPA, ACL or ANOVA criteria,

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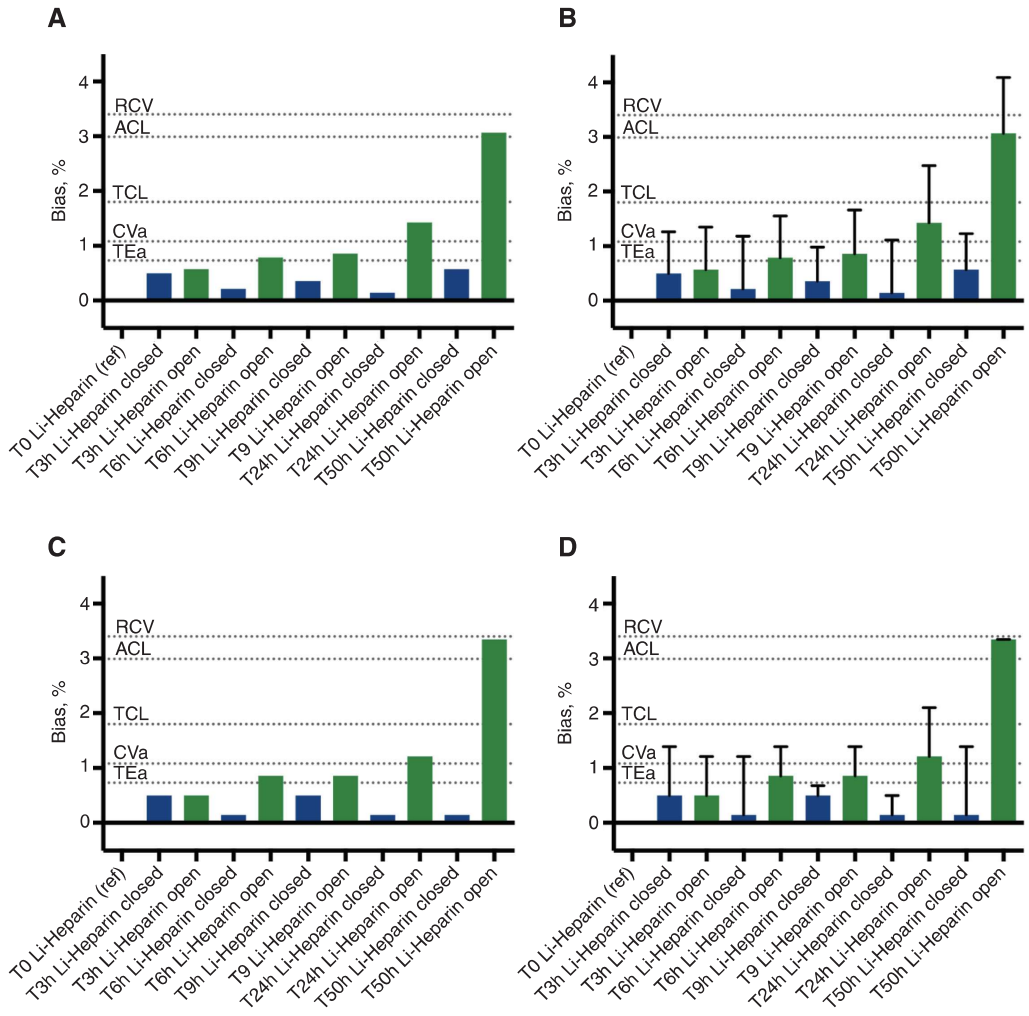

Figure 1: Stability of sodium according (1) to different criteria (RCV, ACL, TCL, CVa, TEa) and (2) to data presentation.
(A) Mean, (B) mean ± CI 95%, (C) median and (D) median ± IQR. The bias (%) from the reference was calculated as followed: bias = value at specific condition – reference value.

respectively). Except for Cl, LH, E2, TP, IgG, IgA and IgM, the stability period was affected by the presence of stoppers and by the contact of blood cells (calculated scores pretty much similar, Table 1). For example, the sodium concentration did not change much with a stopper in decanted samples (Figure 1, in blue), but a progressive increase was observed without stoppers (Figure 1, in green). CVa and ANOVA analysis always showed lower stability period in comparison to other criteria (score of 4.2 and 4.6, respectively). By contrast, RCV and arbitrary CV (10%) both presented the highest score of 5.8. The use of the confidence interval may also impact the period of stability. For example, sodium was more stable considering the mean (or median) in comparison to mean ± CI 95% (or median ± IQR) (Figure 1).

The assessment of *in vitro* stability is mandatory for the appropriate use of laboratory tests and is required for accreditation bodies. The use of *in vitro* stability data from literature could not always be transfer in every laboratory as differences exist according to time before centrifugation, temperature of centrifugation and of storage as well as the precision of assays and the use of stoppers or not. Even if everyone agrees about the bias presentation to assess stability [1, 5, 8, 9], different criteria may impact the interpretation (Table 1). The presentation of data may also impact the stability periods. In case of utilization of 95% CI and IQR along with a mean or median bias, the number of patient included is very important. The more patients included, the lower the CI 95% or IQR is. As a matter of fact, minimal number and type of subjects (healthy vs. ill patients) vary across study [5, 7, 8, 10] and is not defined yet.

In our opinion, the CVa or ANOVA approaches are too stringent to be used as a stability criterion alone. Furthermore, arbitrary CV (e.g. 10%) should be discouraged

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Table 1: Stability period of 14 parameters according to different criteria.

	RCPA RCPA table	TEa, % Ricos's table	RCV, % $2^{1/2}(CVA^2 + CVI^2)^{1/2}$	TCL, % $[(2.77\ CVa)^2 + (0.5\ CVi)^2]^{1/2}$	ACL, % CVA*2.77	CVa, % 20 Last days	CV, % Arbitrary	ANOVA Dunnett's test	SCL, abs Initial value ± 2,8 SD	
Na, mM	3 mM	0.73	3.4	1.8	2.99	2.08	10	p < 0.05	4.65 mM	Score
	50 h	50 h	50 h	50 h	50 h	50 h	50 h	50 h	50 h	6
	24 h	6 h	50 h	24 h	24 h	9 h	50 h	9 h	50 h	4.9
	50 h	3 h	50 h	50 h	50 h	6 h	50 h	3 h	50 h	4.8
	9 h	<3 h	24 h	6 h	9 h	3 h	50 h	6 h	24 h	3.7
K, mM	0.05	5.61	13.2	3.8	3.24	1.17	10	p < 0.05	0.14 mM	
	50 h	50 h	50 h	50 h	9 h	9 h	50 h	6 h	50 h	5.2
	50 h	50 h	50 h	50 h	24 h	3 h	24 h	6 h	24 h	4.9
	9 h	9 h	24 h	9 h	9 h	3 h	24 h	9 h	9 h	4.0
	6 h	9 h	9 h	6 h	3 h	<3 h	9 h	9 h	3 h	3.0
Cl, mM	0.03	1.5	4.6	2.1	3.19	1.15	10	p < 0.05	3.05 mM	
	50 h	9 h	50 h	9 h	50 h	9 h	50 h	9 h	50 h	5.1
	50 h	50 h	50 h	50 h	50 h	50 h	50 h	50 h	50 h	6
	50 h	24 h	50 h	24 h	50 h	24 h	50 h	24 h	50 h	5.6
	50 h	24 h	24 h	50 h	50 h	24 h	50 h	24 h	50 h	5.6
Ca, mM	50 h	2.55	6.6	2.4	3.05	1.1	10	p < 0.05	0.06 mM	
	50 h	50 h	50 h	50 h	50 h	50 h	50 h	50 h	50 h	6
	50 h	24 h	50 h	50 h	24 h	9 h	50 h	24 h	24 h	5.3
	50 h	24 h	50 h	24 h	24 h	9 h	50 h	9 h	24 h	5.1
	50 h	9 h	50 h	9 h	9 h	6 h	50 h	9 h	9 h	4.6
P, mM	0,08	10,11	24,3	7,9	9,03	3,26	10	p < 0,05	0,084 mM	
	50 h	50 h	50 h	50 h	50 h	9 h	50 h	3 h	50 h	5.3
	50 h	50 h	50 h	50 h	50 h	50 h	50 h	24 h	50 h	5.8
	24 h	24 h	24 h	24 h	24 h	9 h	24 h	9 h	24 h	4.8
	9 h	9 h	9 h	9 h	9 h	9 h	9 h	9 h	9 h	4
Creatinine, mg/dL	0.09 mg/ dL	8.87	33.3	11.2	13.38	4.83	10	p < 0.05	1.016 mg/dL	
	50 h	50 h	50 h	50 h	50 h	24 h	50 h	50	50	5.8
	50 h	50 h	50 h	50 h	50 h	50 h	50 h	50	50	6
	50 h	50 h	50 h	50 h	50 h	9 h	50 h	50	50	5.8
	9 h	24	50 h	24	50 h	<3	24	50	50	4.9
25OH-D, ng/mL			36.7	12.4	14.93	5.39	10	p < 0.05	3.612 ng/mL	
	NA	NA	50 h	50 h	50 h	<3 h	50 h	3 h	50 h	4.7
			50 h	24 h	24 h	3 h	24 h	3 h	24 h	4.3
			NT	NT	NT	NT	NT	NT	NT	NA
			NT	NT	NT	NT	NT	NT	NT	NA
PTH, pg/mL			33.43	72.4	19.1	9.28	3.35	10	p < 0.05	3.472 pg/mL	Score
	NA		50 h	50 h	50 h	50 h	<3 h	50 h	50 h	50 h	5.4
			50 h	50 h	50 h	50 h	<3 h	50 h	<3 h	50 h	5.5
			NT	NT	NT	NT	NT	NT	NT	NT	NA
			NT	NT	NT	NT	NT	NT	NT	NT	NA
LH, IU/L			27.92	63.9	16.5	4.71	1.7	10	p < 0.05	0.224 UI/L	
	NA		50 h	50 h	50 h	50 h	50 h	50 h	50 h	50 h	6
			50 h	50 h	50 h	50 h	50 h	50 h	50 h	50 h	6
			50 h	50 h	50 h	50 h	50 h	50 h	24 h	50 h	5.9
			50 h	50 h	50 h	50 h	24 h	50 h	50 h	50 h	5.9
E2, ng/L			26.86	66.4	21	22.88	8.26	10	p < 0.05	9.436 ng/ L	
	NA		50 h	50 h	50 h	50 h	50 h	50 h	50 h	50 h	6
			50 h	50 h	50 h	50 h	50 h	50 h	50 h	50 h	6
			50 h	50 h	50 h	50 h	6 h	50 h	6 h	50 h	5.3
			50 h	50 h	50 h	50 h	50 h	50 h	6 h	50 h	5.6
TP, g/L	0.05		3.63	8.6	3.1	3.93	1.42	10	p < 0.05	2.66 g/ L	
	50 h		50 h	50 h	50 h	50 h	50 h	50 h	50 h	50 h	6
	50 h		50 h	50 h	24 h	50 h	9 h	50 h	50 h	50 h	5.7
	NT		NT	NT	NT	NT	NT	NT	NT	NT	NA
	NT		NT	NT	NT	NT	NT	NT	NT	NT	NA
IGG, g/L			13.5	13.2	4.1	4.35	1.57	10	p < 0.05	0.2 g/ L	
	NA		50 h	50 h	50 h	50 h	9 h	50 h	50 h	9 h	5.5
			50 h	50 h	50 h	50 h	24 h	50 h	50 h	24 h	5.8
			NT	NT	NT	NT	NT	NT	NT	NT	NA
			NT	NT	NT	NT	NT	NT	NT	NT	NA
IGA, g/L			16.8	15.4	4.4	3.6	1.3	10	p < 0.05	0.03 g/ L	
	NA		50 h	50 h	50 h	50 h	50 h	50 h	50 h	50 h	6
			50 h	50 h	50 h	24 h	9 h	50 h	9 h	24 h	5.3
			NT	NT	NT	NT	NT	NT	NT	NT	NA
			NT	NT	NT	NT	NT	NT	NT	NT	NA
IGM, g/L			8	18.1	6.3	7.81	2.82	10	p < 0.05	0.03 g/L	
	NA		50 h	50 h	50 h	50 h	50 h	50 h	50 h	50 h	6
			50 h	50 h	50 h	50 h	9 h	50 h	9 h	9 h	5.3
			NT	NT	NT	NT	NT	NT	NT	NT	NA
			NT	NT	NT	NT	NT	NT	NT	NT	NA
Score	5.5		5.3	5.8	5.5	5.7	4.2	5.8	4.6	5.5	

The maximal acceptable bias is presented in green. The first line of results corresponds to plasma (or serum) separated from blood cells with a stopper; the second to plasma (or serum) separated from blood cells without a stopper; the third to plasma (or serum) not separated from blood cells with a stopper; and the fourth to plasma (or serum) not separated from blood cells without a stopper. The last column and the last line of the table correspond to arbitrary scores calculated based on the maximal stability period of each criterion (last column) and by criterion (last line). The darkest the color, the longest (or the highest) the stability (or the score). NA, not applicable; NT, not tested.

without proof of evidence. We also think that biological variation component is primordial. Therefore, the TCL definition proposed by Oddoze et al. [5] appeared to be the best. Unfortunately, it is important to notice that data about biological variation do not exist for all parameters (e.g. 25-OHD) [3] and that RCPA criteria are also lacking

(e.g. PTH, LH, E2 or IgG) [2]. In those cases, ACL or SCL definition may represent a valuable alternative. Moreover, some TEa criteria are known to be unachievable with current laboratory platforms [11]. As a matter of proof, our calculated CVa (2.08%) was higher than the TEa (0.73%) for sodium.

In conclusion, utilization of different criteria makes the assessment of *in vitro* stability a challenging task. Furthermore, we showed that misinterpretation may be encountered based on the criteria used. Hence, development of standard procedures for stability studies is needed and could improve these pre-analytical evaluations.

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