Practical Laboratory Medicine 7 (2017) 36–40

Contents lists available at ScienceDirect

Practical Laboratory Medicine

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

Impact of serum-clot contact time on lactate dehydrogenase and inorganic phosphorus serum levels

Olivier Bausset[a,*], Olivier Juvet[a], Caroline Staller-Gobelli[b], Emilie Milano[c], Jean-Paul Bausset[a]

[a] SYCAR Clinical Laboratories, Sainte-Maxime, France
[b] SYCAR Clinical Laboratories, Cogolin, France
[c] Pharmacy Unit, Frejus-Saint-Raphaël Hospital, Fréjus, France

A B S T R A C T :

Objectives: The aim of this study is to determine the longest acceptable serum-clot contact time before centrifugation in lactate dehydrogenase and inorganic phosphorus analysis.
Materials and methods: The LDH and inorganic phosphorus serum levels from 103 adults were analyzed at three different storage times. The three measures were done immediately (T0), after a 2-h serum-clot contact (T2) and after a 4-h serum-clot contact (T4). A paired two-tailed Student *t*-test evaluated the impact of the serum-clot contact time on the serum levels. Another approach using analytical reproducibly and intra-individual variability was used. Furthermore, we have compared the mean percentage deviation to the measurement uncertainty.
Results: The LDH serum level is not significantly impacted by the three different studied serum-clot contact times.

The immediate Phosphorus serum level is not significantly different from the 2-h serum-clot contact condition. However, after a 4-h serum-clot contact, the phosphorus serum level is significantly lower than the immediate phosphorus serum level. Considering the reference change value approach, an acceptable mean variation was shown for inorganic phosphorus serum level after a 4-h serum-clot contact time. After a 4-h serum-clot contact, LDH and phosphorus mean percentage deviation are below our measurement uncertainties.
Conclusion: This study evidences that in our daily practices a 4-h serum-clot contact time for LDH and inorganic phosphorus analysis is acceptable.

1. Introduction

Clinical laboratory services are a vital part of healthcare systems [1]. The preanalytical phase is a critical step in the testing process. The time between blood collection and centrifugation is one of the many important factors [2,3] that may influence the reliability of test results and, thereby, affect the diagnostic outcome, follow-up, or even the therapeutic management of patients [4].

Under ISO 15189, an accredited medical laboratory must control the pre-analytical process, including the time between venipuncture and centrifugation [5]. Lactate dehydrogenase (LDH) and inorganic phosphorus are two biochemical analytes which are strongly influenced by serum-clot contact time before centrifugation [6].

In the context of consolidated laboratory networks, transport conditions and delay in transport are important variables. [7] The stability of the specimen dictates conditions for transport from remote collection sites (satellite draw stations) to the testing location.

* Correspondence to: SYCAR Laboratory, BP56, 20 place Louis Blanc, 83120 Sainte-Maxime, France.
E-mail address: olivier.bausset@orange.fr (O. Bausset).

http://dx.doi.org/10.1016/j.plabm.2017.01.002
Received 31 October 2016; Received in revised form 17 January 2017; Accepted 19 January 2017
Available online 03 February 2017

When an uncentrifuged whole blood specimen is sent to the laboratory for testing, it must reach the laboratory in time to be processed with serum/plasma separation occurring within the time necessary to protect the stability of the analytes [8].

The product information for the Siemens Healthcare Diagnostics lactate dehydrogenase assay states that serum or plasma should be physically separated from red blood cells as early as possible with a maximum limit of two hours from collection time [8]. The product information for the Siemens serum inorganic phosphorus assay states that serum or plasma should be separated from cells within one hour [9]. These two target times are difficult to adhere to in routine clinical practice.

The aim of this study was to determine the longest acceptable serum-clot contact time before centrifugation for lactate dehydrogenase and inorganic phosphorus analysis. This study should help laboratories to define the acceptable pre-centrifugation delay for determination of LDH activity and phosphorus concentration in serum.

2. Materials and methods

2.1. Subjects and samples collection

Blood specimens were collected from 103 donors (45 men, 58 women; mean age 63 years [SD 16 years], range 18–90)) from June to August 2016. Each donor was informed of the purpose of this investigation and signed a consent form before blood was collected. Venipunctures were performed after 12 h of fasting. For each donor, three serum tubes of capacity 4.5 mL with clot activator (Vacuette*, Greiner Bio-One GmbH, Kremsmunster, Austria, REF 454027) were collected.

Venipunctures in the median cubital vein were carefully done to avoid hemolysis, using a 22-gauge needle. The cubital fossa was cleaned before tourniquet application. The tourniquet was applied between 7.5 and 10 cm above the puncture site. The tourniquet application time was as short as possible, with a maximum of 1 min.

Collection and mixing of tubes were performed according to the manufacturer's recommendations by gently inverting the tubes five times.

After collection, all tubes were left in the upright position for 30 min at room temperature.

(22 ± 2 °C) before centrifugation.

The first tubes (T0) were then centrifuged and sera were analyzed immediately. The second tubes (T2) were stored at room temperature (22 ± 2 °C) for 2 h then centrifuged and sera analyzed. The third tubes (T4), were stored at room temperature (22 ± 2 °C) for 4 h.

then centrifuged and sera analyzed.

Centrifugation (ThermoScientific SL16R centrifuge, Waltham, MA, USA) was performed according to Greiner Bio-One recommendations: 2000*g* for 10 min at 20 °C.

Haemolysed samples were excluded from study..

3. Analytes and instruments

Lactate dehydrogenase activiry and inorganic phosphorus concentration were determined using a Siemens Dimension RXL Max analyzer (Siemens HealthCare Diagnostics, Newark, DE, USA). Our laboratory is accredited under ISO 15189 [10] for theses two analytes (Accreditation N°8–3100).

The LDH method is standardized according to the International Federation of Clinical Chemistry (IFCC) lactate dehydrogenase primary method procedure at 37 °C [11].

The phosphorus method is a modification of the classical phosphomolybdate method of Fiske and Subbarow [12].

Sera were analyzed every day during the study period. The same reagent lots were used. Quality control was performed before and after each series of analyses. The instrument was calibrated prior to use with manufacturers reagents and calibrators.

4. Statistical analysis

The Student *t*-test and GraphPad Prism 7.01 (GraphPad Software, La Jolla, CA, USA) were used for statistical analyses. The first two time points (T0 and T2) and the first and third time points (T0 and T4) were compared using a paired two-tailed Student *t*-test in order to determine whether the effects of sample storage time before centrifugation of 2 h and 4 h were statistically significant. A p value of 0.05 was considered to be significant.

Following recommendations of Simundic et al. [13], another approach was also used. This approach included consideration of the analytical reproducibility CV_A (determined at the initial installation of the equipment) and biological variations CV_I (found in the database of Ricos et al. [14,15]). The clinically significant change in consecutive results from an individual, taking into consideration both analytical and biological variations, was calculated for each test using the reference change value (RCV) equation as follows:

$$RCV = 2^{1/2} \times Z \times (CV_A^2 + CV_I^2)^{1/2},$$

where: Z is a constant depending on the probability used for significance. Z=1.96 is most commonly used, corresponding to $P < 0.05$.

The mean percentage deviation between T0 (no storage) and T4 (4 h serum-clot contact time before centrifugation) was compared to the reference change value (RCV). If the mean percentage deviation of an analyte exceeds the RCV, then this difference is judged to be clinically significant and is taken as proof of the influence of serum-clot contact time before centrifugation on the

Table 1
Statistical analysis of serum-clot contact effects.

Test (Units)	N	CV_A %	CV_I %	RCV %	Measurement uncertainty %	T0 Mean ± SD [min-max]	T2 Mean	Mean % deviation	P value	T4 Mean	Mean % deviation	P value
LDH (U/L)	103	2.13	8.60	24	4.5	201 ± 160 [109–1141]	203	0.91	0.934	208	3.56	0.748
P (mg/L)	103	1.90	8.15	23.2	5.7	1.25 ± 0.18 [0.85–1.68]	1.22	−2.24	0.249	1.19	−4.04	0.039*

N: number of donors; CV_A: analytical coefficient of variation; CV_I: intra-individual coefficient of variation (Ricos database; [14,15]); RCV: Reference Change Value; SD: standard deviation; Measurement uncertainty included imprecision, and systematic bias obtained with internal quality control and external quality assurance. T0: no storage; T2: 2 h serum-clot contact time; T4: 4 h serum-clot contact time; P values represent the significance obtained by paired *t*-test ($p < 0.05$).
* Statistically significant difference ($P < 0.05$).

result.

To introduce the concept of bias to our study, we compared the mean percentage deviation to our measurement uncertainty. Measurement uncertainty is a non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand [16]. Our laboratory has analyzed and estimated the main sources of uncertainty and they are shown to result from pre-analysis and analysis. The main sources of uncertainty included imprecision, within-subject biological variation, calibrator uncertainty, and systematic bias assessed using internal quality control and external quality assurance.

5. Results

The results of this investigation are shown in Table 1.

For serum LDH activity, no significant difference was detected between the T0 result and the 2 h serum-clot contact time (p=0.9338), and between the T0 condition and the 4 h serum-clot contact time (p=0.7466).

The mean percentage deviations between T0 and T2 and T0 and T4 were below the RCV.

For serum inorganic phosphorus concentration, no significant difference was detected between the T0 result and the 2-h serum-clot contact time (p=0.2490). However, a significantly lower serum phosphorus concentration was observed after the 4 h serum-clot contact (p=0.0390). Using the other statistical approach, the mean percentage deviations of the three different time points were below the RCV.

Fig. 1 shows that the LDH and phosphorus mean percentage deviations are both below the respective measurement uncertainties.

6. Discussion

This study was performed on 103 donors over 3 months (June–August 2016) taken from our laboratory's routine workload. We obtained a large range of values with both normal and pathological levels.

The T4 time after collection selected for study is a reality in our daily practice. A 4-h delay can occur between venipuncture

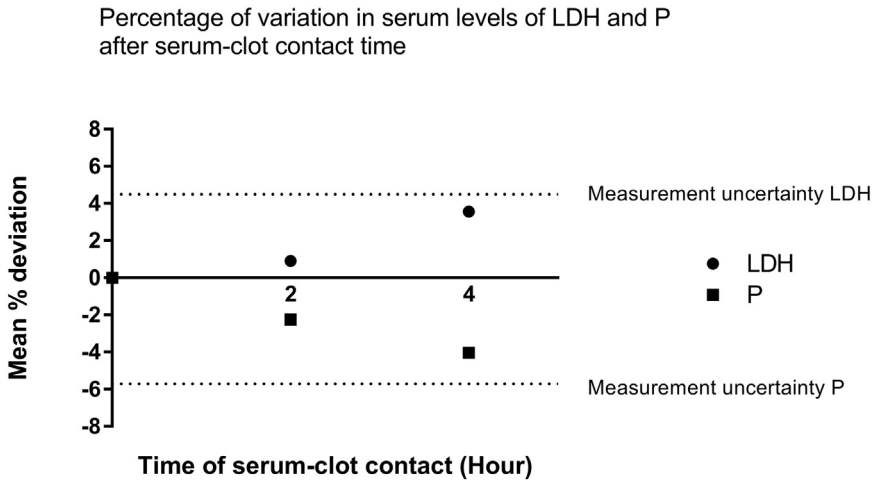

Fig. 1. Mean percentage deviation of lactate dehydrogenase activity (LDH) and inorganic phosphorus concentration (P) without storage, after 2 h serum-clot contact time and after 4 h serum-clot contact time.

outside the laboratory and reception of the whole blood samples in the testing laboratory. Transport conditions from remote collection sites (satellite draw stations) to the testing location must also be considered. In our study, samples were stored at room temperature without transport after collection. Long distance road transport before reaching the laboratory increases the risk of hemolysis [7,17,18]. These conditions have not been studied in this work. It is obvious that no analysis can be done on haemolysed serum. Haemolysed serum indices should be taken into account as well as the time between venipuncture and centrifugation. Each Laboratory should define its own cut off for haemolysed serum indices in order to perform LDH and phosphorus analysis.

This study shows that a longer serum-clot contact time than the one recommended by the manufacturer can still give valid results for LDH activity in serum.

Previous studies are contradictory regarding the stability of LDH during prolonged serum-clot contact [18]. Rehak et al. [19] found no significant change of LDH activity up to 24 h, whereas Laessig et al. [20] reported that LDH serum activity increased after 2 h of contact between serum and cells. Fig. 1 shows an increasing trend in LDH concentration over time. Our results show that the increase of LDH serum level after 4 h serum-clot contact is not significant.

Furthermore, the mean percentage deviation of LDH activity after 4 h serum-clot contact time is below the measurement uncertainty for LDH. Thus, in the light of these results, a 4 h serum clot contact can be used as the maximum pre-centrifugation delay for LDH analysis.

Inorganic phosphorus is described as a very sensitive analyte and the recommended serum-clot contact time before centrifugation must be respected. Indeed, our results show a significant difference between the T0 and the T4 serum levels. In many studies, the Student's *t*-test is used to evaluate the statistical significance. However, a statistically significant change may or may not have an impact on the interpretation of the test results. The clinical relevance of the change may be difficult to define. An approach based on RCV is a better tool to provide appropriate informations for deciding whether a clinically significant change has occurred.

The RCV approach shows an acceptable mean variation for inorganic phosphorus after 4 h serum-clot contact time. Similarly, the mean percentage deviation of phosphate after 2 and 4 h serum-clot contact time is below the measurement uncertainty for phosphate.

We agree with Dialma et al. [7] regarding the preanalytical whole blood instability of phosphorus as our results are similar to theirs, with a phosphorus decrease level of 4.04%. In opposition to these observations, the study of Zhang et al. [18] study shows an increase in inorganic serum phosphorus concentration during clot contact. With the RCV approach, our results show a longer acceptable serum-clot contact time than the one recommended by the Clinical and Laboratory Standards Institute and the Siemens technical data sheet. Nikolac et al. have studied [21] the manufacturers' declarations and have shown that manufacturers are using arbitrary limits in declaring the expected effect of interference on laboratory results. We do not believe that the time storage recommendations in the Siemens data sheet are evidence-based quality specifications. Thus, for serum inorganic serum phosphorus, we recommend a maximum 4-h serum-clot contact time.

Many hospital laboratories centralize their analytical activities, for economic and other reasons. Monneret et al. [22] studied the stability of routine biochemical analytes in whole blood and considered the most frequently tested analytes to be stable for up to 6 h using a RCV-based statistical approach.

The consolidated laboratory network often results in a central testing laboratory which may be a long distance from phlebotomy sites. It is essential that this new context can ensure optimal preservation of the samples. Evidence-based pre-analytical recommendations should inform and assist the evolution of laboratory practice, so clinical laboratories can continue to produce high-quality analytical results to help clinicians in their diagnoses and ensure better patient care.

7. Conclusion

Contrary to the reagent manufacturer's recommendations, this study showed that in our daily practice a 4 h serum-clot contact time before LDH and inorganic phosphorus analysis yields acceptable results.

Conflict of interest

The authors state no conflict of interest.

Acknowledgements

Our special thanks to all employees from Laboratory SELAS SYCAR, Sainte-Maxime, France.

References

[1] M. Hallworth, K. Hyde, A. Cumming, I. Peake, The future for clinical scientists in laboratory medicine, Clin. Lab Haematol. 24 (2002) 197–204.
[2] G. Lima-Oliveira, G.L. Salvagno, G. Lippi, M. Montagnana, M. Scartezini, G. Picheth, et al., Elimination of the venous stasis error for routine coagulation testing by transillumination, Clin. Chim. Acta 412 (2011) 1482–1484.
[3] G. Lippi, G. Lima-Oliveira, S.C. Nazer, M.L. Moreira, R.F. Souza, G.L. Salvagno, et al., Suitability of a transport box for blood sample shipment over a long period, Clin. Biochem. 44 (2011) 1028–1029.
[4] D.S. Young, Effects of Preanalytical Variables on Clinical Laboratory Tests, 3rd ed., AACC Press, Washington, DC, 2007.
[5] D. Plumelle, E. Lombard, A. Nicolay, H. Portugal, Influence of diet and sample collection time on 77 laboratory tests on healthy adults, Clin. Biochem. 47 (2014) 31–37.

[6] B.L. Boyanton Jr, K.E. Blick, Stability studies of twenty-four hours analytes in human plasma and serum, Clin. Chem. 48 (2002) 2242–2247.
[7] P. Dialma, S. Piaulenne, S. Baty, T. Zeitoun, Preanalytical phase and accreditation: acceptance criteria for samples of multisite laboratory, Ann. Biol. Clin. 71 (2013) 121–128.
[8] Clinical and Laboratory Standards Institute/NCCLS. Procedures for the Handling and processing of Blood Specimen; Approved Guideline – Third ed. Wayne, PA: CLSI/NCCLS document H18-A3 [ISBN 1-56238-555-0].
[9] Clinical and Laboratory Standards Institute. Procedures for the Handling and processing of Blood Specimens for Common Laboratory Tests; Approved Guideline – Fourth ed. Wayne, PA: CLSI document GP44-A4 [ISBN 1-56238-724-3].
[10] ISO 15189. Medical Laboratories - Requirements for Quality and Competence; 2012.
[11] L. Siekmann, R. Bonora, C.A. Burtis, F. Ceriotti, P. Clerc-Renaud, G. Férard, et al., IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 degrees C. Part 1. The concept of reference procedures for the measurement of catalytic activity concentrations of enzymes, Clin. Chem. Lab Med. 40 (2002) 631–634.
[12] C.H. Fiske, Y. Subbarow, Phosphorus compounds of muscle and liver, Science 18 (70) (1929) 381–382.
[13] A.M. Simundic, S. Kackov, M. Miler, C.G. Fraser, P.H. Petersen, Terms and symbols used in studies on biological variation: the need for harmonization, Clin. Chem. 61 (2015) 438–439.
[14] C. Ricos, V. Alvarez, F. Cava, J.V. Garcia-Lario, A. Hernandez, C.V. Jimenez, et al., Current databases on biologic variation; pros, cons and progress, Scand. J. Clin. Lab Investig. 59 (1999) 494–500.
[15] C. Ricos, V. Alvarez, F. Cava, J. Garcia-Lario, A. Hernandez, C. Jimenez, et al. Desirable Quality Specifications for Total Error, Imprecision, And Bias, Derived From Biological Variation. ⟨http://www.Westgard.com/biodatabase1.htm⟩; (accessed January 2017)
[16] Working Group 2 of the Joint Committee for Guides in Metrology. JCGM 200:2008. International vocabulary of metrology: basic and general concepts and associated terms (VIM). 3rd ed. JCGM, 2008.
[17] E.A. Jensen, M. Stahl, I. Brandslund, P. Grinsted, Stability of heparin blood samples during transport based on defined pre-analytical quality goals, Clin. Chem. Lab Med. 46 (2008) 225–234.
[18] D.J. Zhang, R.K. Elswick, W.G. Miller, J.L. Bailey, Effect of serum-clot contact time on clinical chemistry laboratory results, Clin. Chem. 44 (1998) 1325–1333.
[19] N.N. Rehak, B.T. Chiang, Storage of whole blood: effect of temperature on the measured concentration of analytes in serum, Clin. Chem. 34 (1988) 2111–2114.
[20] R.H. Laessig, A.A. Indriksons, D.J. Hassemer, T.A. Paskey, T.H. Schwartz, Changes in serum chemical values as a result of prolonged contact with the clot, Am. J. Clin. Pathol. 66 (1976) 598–604.
[21] N. Nikolac, A.M. Simundic, M. Miksa, G. Lima-Oliveira, G.L. Salvagno, B. Caruso, G.C. Guidi, Heterogeneity of manufacturers' declarations for lipemia interference–an urgent call for standardization, Clin. Chim. Acta 426 (2013) 33–40.
[22] D. Monneret, A. Godmer, R. Le Guen, C. Bravetti, C. Emeraud, A. Marteau, et al., Stability of routine biochemical analytes in whole blood and plasma from lithium heparin gel tubes during 6-hr storage, J. Clin. Lab Anal. 30 (2016) 602–609.