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Integrity of serum samples is changed by modified centrifugation conditions

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Abstract

Background: Serum samples should be centrifuged for at least 10 min at 1300–2500 × *g*. Changed centrifugation conditions could compromise sample quality. The objective of this study was to compare the serum quality and turnaround time (TAT) using different centrifugation conditions.

Methods: The study was done in four different periods (A, B, C and D) at different conditions: for 10, 5 and 7 (A, B and C, respectively) at 2876 × *g*, and 7 (D) min at 4141 × *g*. Sample quality was assessed as the proportion of samples with: (a) aspiration errors, (b) H index >0.5 g/L and (c) suppressed reports of potassium (K) due to hemolysis. TAT was calculated for emergency samples. The proportions of samples (a), (b) and (c) were compared according to period A.

Results: The number of aspiration errors was significantly higher in samples centrifuged at 2876 × *g* for 5 min ($p = 0.021$) and remained unchanged when centrifuged for 7 min ($p = 0.066$ and 0.177, for periods C and D, respectively). In periods B, C and D, the proportion of samples with hemolysis was higher than that in period A (p-values 0.039, 0.009 and 0.042, respectively). TAT differed between all periods ($p < 0.001$), with the lowest TAT observed for B and D. The lowest number of samples exceeding 60-min TAT was observed in period D ($p = 0.011$).

Conclusions: The integrity of serum samples is changed with different centrifugation conditions than those recommended. Our study showed that shorter centrifugation at higher force (7 min at 4141 × *g*) significantly decreases TAT, with unchanged proportion of samples with aspiration errors.

Keywords: centrifugation; hemolysis; residual fibrin strands; sample quality; turnaround time.

Introduction

Modern technology provides fast analyzers that are usually capable of reporting accurate and precise results for numerous biochemistry and immunochemistry tests. However, despite almost perfect performances in the analytical phase, laboratories still struggle with the preanalytical phase, especially with sample quality. To provide optimal samples for analytical systems, the blood should be sampled in vacuum collection tubes and treated adhering to special conditions provided by the manufacturers, including clotting time, centrifugation time and force. The Clinical and Laboratory Standard Institute (CLSI) guidelines and the World Health Organization (WHO) recommend that serum samples should be centrifuged for at least 10 min at 1300–2500 × *g* after spontaneous clotting [1–4].

Although recommended by guidelines and manufacturers, a sample centrifugation time of 10 min (or more) prolongs the turnaround time (TAT). In order to reduce TAT, studies have been done with abbreviated centrifugation times. It was demonstrated that shorter centrifugation at higher force did not impact the results of the most frequently ordered analytes in serum or plasma samples. The stability, accuracy and precision of analytes in specimens were also unaffected [5–8]. Furthermore, as expected, TAT was significantly lower when the time of centrifugation was reduced to 7 or 5 min (6). However, these studies did not investigate the effect of a shorter centrifugation time on sample quality. In order to reduce TAT, plasma samples could be used, but serum has several advantages. The stability of analytes in serum is longer than that in plasma [9]. Serum samples contain lower number of cells (leukocytes and thrombocytes) that might affect some measurements (e.g. lactate dehydrogenase). Also, serum is the suitable sample for all chemistry and immunology tests; plasma would

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require additional samples for tests that are only measured in the serum.

According to some studies, the highest rejection rate of samples in emergency laboratory is due to fibrin clots [10]. For serum samples, fibrin residues are most commonly caused by inadequate centrifugation conditions. The remaining fibrin strands could lead to poorer sample quality [11, 12], and results could be erroneous and compromise patients' healthcare and safety [13]. Additionally, leftover fibrin strands could cause various problems with analyzers, mainly obstruction of sample aspiration systems. This could result in greater delays of the total laboratory process, because every sample with leftover fibrin should be manually managed after first pass through the system, fibrin strands should be removed and samples should be re-centrifuged. After re-centrifugation, as well as after inadequate centrifugation, a problem of increased hemolysis might occur [14, 15].

Therefore, the aim of this study was to compare serum quality (as the proportion of samples with a high hemolysis index and leftover fibrin strands) in different settings of centrifugation time and force. The additional objective was to determine if sample TAT could be significantly reduced with shorter centrifugation time, without compromising patient safety.

Materials and methods

This study was done in the Department of Clinical Chemistry, Sestre milosrdnice University Hospital Center in four different periods of approximately 10 days: July 20th–August 4th (period A), September 20th–30th (period B), October 28th–November 8th (period C) and December 18th–27th (period D) 2017.

Samples and setting

This study included only venous serum samples drawn in 4.5- or 6-mL tubes with clot activator without a separator (Greiner Bio-One GmbH, Kremsmünster, Austria).

Outpatient samples or samples from emergency and hospital wards were delivered to the laboratory by the hospital staff (walking on foot) using delivering boxes. After 30 min of clotting, all samples were put on the total laboratory automation (TLA) system, Abbott Accelerator a3600 (Abbott, Abbott Park, IL, USA), consisting of an input-output module, two integrated centrifuges (Hettich Rotanta 46RSC; Hettich, Tuttlingen, Germany), decapper, two biochemistry analyzers Architect c8000, one immunochemistry analyzer Architect i2000, aliquoter and sealer.

According to the manufacturer (Greiner Bio-One), serum samples should be centrifuged at 1800–2200 × *g* for 10–15 min.

In the first period (A), all samples were centrifuged at 2876 × *g* for 10 min, which were considered as the reference conditions. After the initial period, the conditions were changed for other periods as follows: in period B and C, the centrifugation time was reduced to 5 and 7 min, respectively, with the same centrifugation force (2876 × *g*). In period D, the centrifugation force was increased to 4141 × *g*, while the time was set to 7 min.

After centrifugation, the samples were automatically decapped and sent to the Architect c8000 for measurement of biochemistry parameters (if requested) and indices (for all samples).

Methods

The index of hemolysis was measured using the Architect c8000 for all emergency and routine samples received in the laboratory, regardless of the sample type (venous serum, capillary serum and plasma with anticoagulants).

According to the manufacturer's declarations, Architect c8000 used saline solution to dilute the samples and measure the spectra at four pairs of wavelengths (500/524 nm, 572/604 nm, 628/660 nm and 524/804 nm). The H index is then calculated using all the wavelengths and constant coefficients.

The H index corresponds to the concentration of free hemoglobin (Hb). According to the manufacturer's declaration and own laboratory validation, serum with a H index of only 0.5 g/L was considered hemolyzed due to interference with potassium concentration. Potassium results from hemolyzed samples (H > 0.5 g/L) were suppressed, and the results were not reported and only the comment about hemolysis was reported.

The analyzers Architect c8000 and Architect i2000 recognize samples with clot or leftover fibrin strands as aspiration errors. The same errors could appear in pediatric capillary samples due to the low volume in tubes. To exclude possible confounding factors, i.e. low fillings of tube, in this part of the study, only venous serum samples with properly filled tubes were included. After one of the analyzers recognized an aspiration error, such samples were sent to the input-output module, visually inspected for fibrin leftovers, then removed from the TLA and re-centrifuged. All aspiration errors were saved in the analyzers (and are available for download as the number of errors).

Sample quality parameters

The quality of samples was defined with the following parameters: number of samples with aspiration errors and number of samples with the H index above 0.5 g/L against the total number of serum samples received in the laboratory in all the studied periods. Additionally, the quality of samples was defined as the number of samples with suppressed reports of potassium (K) due to a high H index (>0.5 g/L) against the total serum samples with the requested potassium received. We chose two indicators to reflect the degree of hemolysis. All samples received in the laboratory are processed on the TLA system Abbott Accelerator a3600 and the H index is measured. Some samples are only aliquoted for other work stations which are less sensitive to hemolysis, and mildly hemolyzed samples (H: 0.5–1.0 g/L) are processed for immunology, while these samples are discarded for biochemistry (potassium) measurement. Therefore, the number of suppressed potassium results corresponds to the

percentage of biochemistry samples (measured on Architect analyzers) with requested potassium, and the H index corresponds to all samples received in the laboratory.

TAT calculation

TAT was calculated only for emergency samples. TAT was defined as the time from sample registration to the laboratory information system (LIS) to the time of releasing the results. All results are released to the LIS when all requested tests are measured. The median time for TAT with the interquartile range (IQR) and the 90% completion time were calculated. Also, the proportion of emergency samples exceeding 60 min was calculated.

Specific data for serum samples received in defined periods were collected from the LIS.

Statistical analysis

Normality of all data was investigated using the D'Agostino-Pearson test. TAT and the H index were not normally distributed, so results were presented with medians and IQR. Also, non-parametric tests were used for comparison of data. Comparison of TAT in different periods was done using the Kruskal-Wallis test with *post-hoc* test according to Conover [16].

Categorical data are presented as numbers (n) and percentages. Differences in proportions of samples with leftover fibrin, H index above 0.5 and suppressed K results in all tested time periods were compared using the χ^2 test. *Post-hoc* testing against the initial period (reference group A: 10 min at $2876 \times g$) was done with comparison of proportions. The Bonferroni correction was used to adjust p-values due to multiple testing. TAT between all periods was compared with the Kruskal-Wallis test and *post-hoc* testing was done. All calculations were done in MedCalc (v.11.5.1.0, Ostend, Belgium). A p-value < 0.05 was considered statistically significant.

Results

The H values for the investigated periods were 0.04 (0.02–0.08), 0.04 (0.02–0.08), 0.04 (0.02–0.09) and 0.05 (0.02–0.11) g/L for A, B, C and D, respectively. As all values were below clinically significant criteria, we categorized hemolytic samples as below and above the cutoff value of 0.5 g/L, which is also recommended by EFLM WG-PRE [17].

The results of different centrifugation times and force on the sample quality are presented in Table 1.

The number of samples with leftover fibrin strands was significantly higher in samples centrifuged for 5 min vs. recommended 10 min at the same centrifugation force of $2876 \times g$ (p = 0.021). Centrifugation for 7 min at both forces (2876 and $4141 \times g$) showed no significant increase

Table 1: Sample quality indicators in different conditions of centrifugation time and force.

Period	Centrifugation conditions		n Total received	n (%) Aspiration errors	p-Value[a]	p-Value[b]	n (%) of Hemolyzed samples	p-Value[a]	p-Value[b]	n Total requested K	n (%) of Suppressed K results	p-Value[a]	p-Value[b]
	Time, min	Force, $\times g$											
A	10	2876	5944	139 (2.34)		/	219 (3.68)			/	4050		/
B	5	2876	5024	161 (3.20)	**<0.001**	**0.021**[c]	233 (4.64)	**0.011**	**0.039**[c]	3261	201 (6.16)	0.144	0.087[c]
C	7	2876	4946	152 (3.07)		0.066[c]	240 (4.85)		**0.009**[c]	3321	182 (5.48)		1.000[c]
D	7	4141	3919	69 (1.76)		0.177[c]	184 (4.70)		**0.042**[c]	2678	156 (5.83)		0.399[c]

K, potassium; min, minutes; n, number; [a]χ^2-test, [b]*post-hoc* comparison of proportions against period A, [c]Bonferroni adjusted p-values. Statistically significant values are bolded.

in aspiration errors (with p-values of 0.066 and 0.177, respectively).

All centrifugation settings different from the recommended conditions showed higher proportion of samples with the H index above 0.5 (p-values 0.039, 0.009 and 0.042 for periods B, C and D, respectively). However, when considering the number of suppressed results of potassium, differences were not observed with p-values of 0.087, 1.000 and 0.399 for periods B, C and D, respectively.

TAT differed significantly between all tested periods ($p < 0.001$). *Post-hoc* analysis showed that the lowest TAT was observed for both periods B (5 min at lower force) and D (7 min at higher force), with medians of 32 (28–37) min for both periods against 36 (32–42) min for period A (reference conditions). The 90% completion time was also reduced for all tested periods (45–47 against 50 in period A). When the number of samples exceeding a TAT of 60 min was compared, significant reduction was observed only in period D ($p = 0.011$).

Discussion

The main finding of our study is that a shortened centrifugation time of 5 min at $2876 \times g$ decreases TAT and 90% completion time; however, it significantly affects sample integrity by increasing the number of samples with leftover fibrin. Optimal conditions in our laboratory are obtained at a centrifugation time of 7 min/$4141 \times g$, with the highest reduction of TAT, lowest percentage of samples exceeding 60 min TAT and without increase in the number of aspiration errors according to the original protocol. All investigated centrifugation conditions significantly increased the percentage of hemolyzed samples; however, the increase was not high (from 3.7% to 4.6%, 4.9% and 4.7%, respectively).

In the laboratory, the quality of serum samples is one of the prerequisites to deliver reliable results. Sample quality is most commonly compromised with hemolysis, unsuitable sample volume, wrong tube selection or inappropriate clotting [18]. That was the reason for choosing hemolysis and residual fibrin for the assessment of sample quality in this research. Hemolysis is usually caused by inappropriate sampling techniques, or sample management and transport [19]. Centrifugation conditions different from those recommended, especially longer time and/or higher force, as well as re-centrifugation could also induce *in vitro* hemolysis of samples [20]. In our study, we confirmed that centrifugation conditions can significantly contribute to the proportion of hemolyzed samples.

Previously published studies dealt mostly with comparison of results in samples processed in different centrifugation conditions [5, 21]. Møller and Cadamuro with coauthors in their researches did not find differences in test results for more than 70 biochemistry and immunology parameters in samples centrifuged at shorter times or higher force [8, 22]. Cadamuro et al. found that only the concentration of free Hb was significantly higher in serum samples with changed centrifugation conditions [8]. Our study is consistent with those results. The percentage of samples with the H index (or concentration of free Hb) above 0.5 g/L was significantly higher when samples were centrifuged for a shorter time and at higher force. This could be due to insufficient settling of cells in serum when centrifuged for less time or due to breakage of cells when centrifuged at a higher force than recommended. Cadamuro's research was done on samples without hemolysis with very low H index and, consequently, showed a very small (maximum of 0.03) increase in free Hb concentration. If the concentration of free Hb in samples included in that research was about 0.5 g/L, even small changes could lead to suppression of results and accordingly to incorrect or delayed medical decisions [15]. Emergency physicians usually (in more than 50% of patients) request potassium for diverse conditions and symptoms as well as for screening or diagnosis. The percentage of suppressed K results therefore could correspond to the proportion of hemolyzed samples. Our research showed that incomplete or delayed coagulation in the serum tube could also lead to a sample of lower quality. Moreover, invisible clots or residual fibrin in serum samples could interfere with the quality and accuracy of the test results [23]. Residual fibrin strands in serum can affect immunoassays and lead to false-positive results for critical emergency tests as, for example, troponin [24]. Changed results due to fibrin strands could significantly affect patient care. Fibrin strands could be aspirated by analyzers and result with errors not only on specific immunoassays, but for all tests due to obstruction of the aspiration system and necessity for re-centrifugation of the sample [18, 25]. Re-centrifugation of the sample could lead to pseudohyperkalemia [26] and falsely increased concentration of troponin [27] and should therefore be avoided [28]. Those errors not only affect sample quality, but also the overall management of analyzers in the laboratory. Our study demonstrated that the frequency of aspiration errors was higher when samples were centrifuged at a lower force ($2876 \times g$) for 5 min than was done initially.

An increased centrifugation force is beneficial for the sample because it is more capable of sedimenting cells that remain in the supernatant. For potassium,

pseudohyperkalemia can occur when an increased number of leukocytes is present in the sample. It is therefore important to provide as clean a sample as possible.

Modern laboratories encounter increased number of different laboratory tests. Nevertheless, the number of test requests is growing on a daily basis, and physicians request all results to be not only accurate but also fast, especially in emergency departments. One of the solutions to reduce TAT is the introduction of fully automated systems for total laboratory processes [29]. Previously published researches showed significant TAT reduction when the centrifugation time was reduced from 15 to 10 min [21]. Moreover, Holland and coauthors demonstrated that even a shorter centrifugation time of 4 min is enough to provide analytically comparable results and, of course, to shorten TAT (5). However, although they did not find differences between the results in those samples, their research lack information about sample quality. Our study showed that median TATs were lower for 2–4 min in all modifications (5 and 7 min at 2876 and 4141×*g*, respectively), but the percentage of samples that exceeded 60 min of TAT was significantly lower only when the centrifugation time was shortened at 7 min, but at higher centrifugation force. This could be due to the necessity to re-centrifuge samples centrifuged at lower force for 5 min because of higher proportion of aspiration errors. With re-centrifugation, the number of samples that exceeded 60 min was higher.

This study has some limitations. One of the biggest limitations is that it was not performed using paired samples but in four different periods with different centrifugation conditions. Such a setting could introduce several confounding factors such as tube filling or sample collection. Tube filling was excluded by using only serum samples in 4.5–6 mL tubes, checked for blood volume before putting on the TLA. It is possible that different tube volumes could affect results and change the number of aspiration errors. This should be investigated in a new study. We assume that conditions at the wards and emergency departments are similar in studied periods, as, during all selected periods, there were no major changes in patient load, hospital organization, laboratory staff and no major interventions or maintenance procedures were done on analytical systems. Additionally, we are aware of the fact that validation of centrifugation condition could not only be related to the rate of hemolysis. Therefore, we introduced another parameter, residual fibrin strands. As previously published papers investigated the concentration of biochemistry parameters at different centrifugation conditions [8, 22], we omitted comparison of biochemistry parameters and addressed only the issue of sample integrity.

Our work strongly emphasizes the importance of sample quality verification when sample processing conditions are changing. The integrity of serum samples is affected when centrifugation conditions are different from those recommended. Based on the results of our study, 5 min of centrifugation at lower force can increase the percentage of samples with leftover fibrin strains. Centrifugation for 7 min at a higher force (4141×*g*) provides the lowest TAT, without increasing the proportion of samples with aspiration errors. Each laboratory should investigate the optimal centrifugation conditions in their own laboratory setting.

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References

1. Clinical Laboratory Standards Institute. Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline – Third Edition. Wayne, PA: Clinical and Laboratory Standards Institute, 2004. CLSI document H18-A3.
2. Greiner Bio-One. Vacuette blood collection system – handling recommendations. 980183 rev 05, February 2016. http://www.gbo.com/preanalytics. Accessed: 25 Feb 2019.
3. Becton Dickinson. BD Diagnostics – Preanalytical Systems. 2009. https://www.bd.com/resource.aspx?IDX=10155. Accessed: 25 Feb 2019.
4. World Health Organization. Use of anticoagulants in diagnostic laboratory investigations, 2nd ed. Geneva: World Health Organization, 2002.
5. Holland LL, DomBourian MD. Evaluation of an abbreviated centrifugation protocol for chemistry testing. Lab Med 2012;43:78–81.
6. Koenders MM, van Hurne ME, Glasmacher-Van Zijl M. The analytic impact of a reduced centrifugation step on chemistry and immunochemistry assays: an evaluation of the modular pre-analytics. Ann Clin Biochem 2012;49:468–74.
7. Minder EI, Schibli A, Mahrer D. Effects of different centrifugation conditions on clinical chemistry and Immunology test results. BMC Clin Pathol 2011;11:6.
8. Cadamuro J, Mrazek C, Leichtle AB, Kipman U, Felder TK, Wiedemann H, et al. Influence of centrifugation conditions on the results of 77 routine clinical chemistry analytes using standard vacuum blood collection tubes and the new BD-Barricor tubes. Biochem Med (Zagreb) 2018;28:010704.
9. Boyanton BL, Blick KE. Stability studies of twenty-four analytes in human plasma and serum. Clin Chem 2002;48:2242–7.

10. Dikmen ZG, Pinar A, Akbiyik F. Specimen rejection in laboratory medicine: necessary for patient safety? Biochem Med (Zagreb) 2015;25:377–85.
11. Lippi G, Salvagno GL, Montagnana M, Franchini M, Guidi GC. Phlebotomy issues and quality improvement in results of laboratory testing. Clin Lab 2006;52:217–30.
12. Ko DH, Won D, Jeong TD, Lee W, Chun S, Min W-K. Comparison of red blood cell hemolysis using plasma and serum separation tubes for outpatient specimens. Ann Lab Med 2015;35:194–7.
13. Kazmierczak SC, Sekhon H, Richards C. False-positive troponin I measured with the Abbott AxSYM attributed to fibrin interference. Int J Cardiol 2005;101:27–31.
14. Carey RN, Jani C, Johnson C, Pearce J, Hui-Ng P, Lacson E. Chemistry testing on plasma versus serum samples in dialysis patients: clinical and quality improvement implications. Clin J Am Soc Nephrol 2016;11:1675–9.
15. Luksic AH, Nikolac Gabaj N, Miler M, Dukic L, Bakliza A, Simundic AM. Visual assessment of hemolysis affects patient safety. Clin Chem Lab Med 2018;56:574–81.
16. Conover WJ. Practical nonparametric statistics, 3rd ed. New York: John Wiley & Sons, 1999.
17. Lippi G, Cadamuro J, von Meyer A, Simundic AM; European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for Preanalytical Phase (WG-PRE). Practical recommendations for managing hemolyzed samples in clinical chemistry testing. Clin Chem Lab Med 2018;56:718–27.
18. Lippi G, von Meyer A, Cadamuro J, Simundic AM. Blood sample quality. Diagnosis (Berl) 2019;6:25–31.
19. Barnard EB, Potter DL, Ayling RM, Higginson I, Bailey AG, Smith JE. Factors affecting blood sample haemolysis: a cross-sectional study. Eur J Emerg Med 2016;23:143–6.
20. Heireman L, Van Geel P, Musger L, Heylen E, Uyttenbroeck W, Mahieu B. Causes, consequences and management of sample hemolysis in the clinical laboratory. Clin Biochem 2017;50:1317–22.
21. Monneret D, Corlouer C, Bigot J, Atlan G, Alkouri R, Mestari F, et al. Comparison of a 10- vs. 15-min centrifugation time for chemical and immunochemical assays and impact on turnaround time in a hospital laboratory. Clin Chem Lab Med 2016;54:e117–21.
22. Møller MF, Søndergaard TR, Kristensen HT, Münster AB. Evaluation of a reduced centrifugation time and higher centrifugal force on various general chemistry and immunochemistry analytes in plasma and serum. Ann Clin Biochem 2017;54:593–600.
23. Cervellin G, Aloe R, Lippi G. A case of factitious hyponatremia and hypokalemia due to the presence of fibrin gel in serum. Diagnosis 2015;2:73–4.
24. Beyne P, Vigier JP, Bourgoin P, Vidaud M. Comparison of single and repeat centrifugation of blood specimens collected in BD evacuated blood collection tubes containing a clot activator for cardiac troponin I assay on the ACCESS analyzer. Clin Chem 2000;46:1869–70.
25. Lima-Oliveira G, Monneret D, Guerber F, Guidi GC. Sample management for clinical biochemistry assays: Are serum and plasma interchangeable specimens? Crit Rev Clin Lab Sci 2018;55:480–500.
26. Hira K, Shimbo T, Fukui T. High serum potassium concentrations after recentrifugation of stored blood specimens. N Engl J Med 2000;343:153–4.
27. Canovi S, Campioli D, Marcheselli L. Specimen recentrifugation and elevated troponin I levels. Lab Med 2015;46:47–50.
28. Woodworth A, Pyle AL. Chapter 4 – Sample Processing and Specimen Misidentification Issues. In: Dasgupta A, Sepulveda JL, editors. Accurate results in the Clinical Laboratory. Amsterdam, The Netherlands: Elsevier, 2013:35–51.
29. Miler M, Nikolac Gabaj N, Dukic L, Simundic AM. Key performance indicators to measure improvement after implementation of total laboratory automation Abbott Accelerator a3600. J Med Syst 2018;42:28.