

Case report

Abnormal gel flotation caused by contrast media during adrenal vein sampling

Gabriel Lima-Oliveira^{*1}, Giuseppe Lippi², Gian Luca Salvagno¹, Matteo Gelati¹, Antonella Bassi¹, Alberto Contro³, Francesca Pizzolo⁴, Gian Cesare Guidi¹

¹Laboratory of Clinical Biochemistry, Department of Life and Reproduction Sciences, University of Verona, Italy

²Laboratory of Clinical Chemistry and Hematology, Academic Hospital of Parma, Parma, Italy

³Vascular Interventional Radiology Unit, Institute of Radiology, University of Verona, Italy

⁴Division of Internal Medicine, Department of Medicine, University of Verona, Italy

*Corresponding author: dr.g.lima.oliveira@gmail.com

Abstract

Introduction: During adrenal venous sampling (AVS) procedure, radiologists administer a contrast agent via the catheter to visualize the proper catheter position.

Materials and methods: A patient with primary aldosteronism diagnostic-hypothesis was admitted for AVS. A venogram was performed to confirm the catheter's position with 2mL of Iopamidol 300 mg/mL. Samples were collected with syringe connected to a hydrophilic coated catheter by low-pressure aspiration from each of the four collection sites: inferior vena cava in the suprarenal portion, inferior vena cava in the infrarenal portion, left adrenal vein, and right adrenal vein; then immediately transferred from syringe to tubes with gel separator. All tubes were centrifuged at 1200 x g for 10 minutes.

Results: At the end of centrifugation process, primary blood tubes containing blood from inferior vena cava and left adrenal vein exhibited the standard gel separator barrier, while tubes from right adrenal vein showed abnormal flotation of gel separator. The radiologist confirmed the usage of 2.6 mL instead of 2.0 mL of Iopamidol 300 mg/mL. This iodinated contrast media, with 1.33 g/cm³ of density, was used close to the right adrenal vein due to some difficulty to access it.

Conclusion: The abnormal flotation of gel separator in samples taken from right adrenal vein can be explained by the usage of the iodinated contrast media. We suggest using plain-tubes (without gel separator) for AVS in order to avoid preanalytical nonconformities. Moreover, a blood volume equivalent to twice the catheter extension should be discarded to eliminate residual contrast media before collection of samples for laboratory assays.

Key words: preanalytical phase; blood specimen collection; contrast media; gels; phlebotomy

Received: January 13, 2016

Accepted: August 13, 2016

Introduction

Adrenal venous sampling (AVS) is recommended by current guidelines to identify surgically curable causes of hyperaldosteronism (1). However, selective adrenal blood sampling is frequently viewed as difficult and time consuming by radiologists due to three different reasons: a) the small size of the right adrenal vein; b) the difficult in recognizing the specific vascular characteristics, and particularly in distinguishing it from other affluent veins to the posterior wall of the inferior vena cava; and 3) the length of the segment of the inferior vena

cava, due to the high variance of adrenal vein anatomy, can extend from the 10th to the 12th intercostal space (2).

The simultaneous laboratory measurement of cortisol concentrations during catheterization in samples from the adrenal vein allows a correction for eventual dilution, and is an objective evidence of the proper cannulation (e.g., by selectivity index). The selectivity index – plasma cortisol concentration from side divided by plasma cortisol concentration from inferior vena cava – with values great-

er than the cut-off confirms that the blood sample was obtained from the adrenal vein (1). Briefly, laboratory should report selectivity index as soon as possible (e.g., within less than half hour). This procedure allows any improperly collected adrenal samples to be immediately re-collected, thus reducing the frequency of repeat procedures and thereby reducing the cost as well as the discomfort to the patient and avoiding delay in diagnosis (3).

Hayden *et al.* achieved 89 % success rate in diagnostic sampling of the right and left adrenal veins after implementing rapid cortisol method. Successful AVS was defined as an adrenal/peripheral cortisol ratio > 4. Moreover, these authors defined AVS as essential procedure to patient management, since 50 % of their patients had laboratory results discordant with imaging (e.g., elevated cortisol-corrected aldosterone ratio from the adrenal gland without a nodule) (4). Furthermore, once achieved a correct cannulation, as assessed by the proper selectivity index in both adrenal veins, the lateralization index (LI) should be calculated (Table 1) (1). Lateralization Index is obtained from the ratio aldosterone/cortisol in both adrenal veins. A LI higher than the adopted cut-off (it ranges from 2 to 4) evidences a lateralized aldosterone excess, thus a dominant adrenal that is eligible for surgical ablation in order to treat primary aldosteronism. By contrast, if LI is under the cut-off, both adrenals are responsible for aldosterone excess, and the patient is candidate to medical therapy (1, 5).

Preanalytical variability, especially regarding blood sampling, still drives an important issue in laboratory diagnostics (6). Moreover, selection and procurement of tubes for blood collection (i.e., tubes used during AVS procedure) in healthcare facilities is often an underestimated issue. Furthermore, na-

tional, regional and local tenders are frequently plagued by policies, guided primarily by price savings rather than by quality of tubes for blood collection (7,8). All devices (i.e., tubes for blood collection) should be standardized and managed by the laboratory in order to avoid different brands from the same kind of tubes at hospital. Some laboratory managers prefer to employ tubes with gel separator to perform blood collection for immunochemistry assay, since this kind of tube is theoretically considered able to ensure greater analytes stability over time, regardless of the storage conditions (9,10). Briefly, the gel is displaced and moves upward to form a barrier between serum (or plasma) and blood cells upon centrifugation. Moreover, gel barrier formation is due to differences in density of three components (6):

1. serum or plasma (e.g., density range from 1.026 to 1.031 g/cm³);
2. thixotropic polymer gel as the separator gel (e.g., density range from 1.040 to 1.050 g/cm³); and
3. cellular components of the blood (e.g., density range from 1.092 to 1.095 g/cm³).

This case report aims to demonstrate an abnormal gel flotation caused by contrast media during adrenal vein sampling.

Material and methods

Case report

A day-hospital inpatient (male, 50 years old) with primary aldosteronism diagnosis was admitted in Verona University Hospital (Azienda Ospedaliera Universitaria Integrata Verona, Verona, Italy) to perform a sequential catheterization adrenal vein sampling. The patient was initially evaluated for a

TABLE 1. Clinical importance of selectivity index and lateralization index calculated from laboratory results.

Description	Formula	Interpretation
Selectivity index (SI)	$\text{cortisol}_{\text{side}} / \text{cortisol}_{\text{inferior vena cava}}$	Values higher than cut-off confirm that blood sample is properly collected from adrenal vein
Lateralization index (LI)	$(\text{aldosterone}^* / \text{cortisol}^*) / (\text{aldosterone}^\# / \text{cortisol}^\#)$	Values higher than cut-off confirm lateralized aldosterone excess.

* – results from side with higher concentration. # – results from side with lower concentration.

resistant hypertension, and resulted positive at both: the screening test (aldosterone-to-renin ratio), and confirmatory test (intravenous salt loading test) for primary aldosteronism, according to previously described procedures (11). During the diagnostic work-up, and at the time of AVS, interfering anti-hypertensive drugs were avoided, and the patient was taking no drugs other than verapamil. An informed consent was obtained before the AVS, after a careful explanation of risk, benefits, and possible inconclusive results because of difficult adrenal vein cannulation. The AVS was performed by an expert radiologist, according to standardized operational procedures arranged by a multidisciplinary team involving radiologists, specialists in laboratory medicine, and general medicine physicians, in agreement with international recommendations (1). The patient was in recumbent position for more than one hour before the cannulation, being the AVS performed without cosyntropin stimulation, and normokalemia was verified. The venogram – an X-ray test that shows the blood flow through the veins – was performed to confirm the catheter position with 2 mL of Iopamidol 300 mg/mL (Iopamiro®, Bracco, Milan-IT). Samples (10mL) were collected with syringe connected to a hydrophilic coated catheter (Glidecath 5FR C1, Terumo Europe N. V., Leuven, Belgium) by low-pressure aspiration from each of the four collection sites: inferior vena cava in the suprarenal portion, inferior vena cava in the infrarenal portion, left adrenal vein, and right adrenal vein. Samples from each collection site were immediately transferred from syringe into two tubes: one 3.5 mL tube with 52.5 USP U of lithium heparin and gel separator; and one 3.5 mL serum tube with clot activator and gel separator (both from Terumo Europe N. V., Leuven, Belgium). All eight samples were immediately delivered to our laboratory, and were centrifuged together using the same centrifuge Rotanta 460R (Hettich Lab Technology, Tuttlingen, Germany) at 1200 x g for 10 minutes, half an hour after the adrenal vein sampling procedure. When trying to aspirate plasma and serum from the tubes, we observed an abnormal flotation of gel in the tubes collected from the right adrenal vein that hampered further process-

ing of the samples. In order to clarify such an unusual sample condition, we decided investigate the causes of such a phenomenon.

Methods

Peripheral venous blood sample was collected (with vacuum tube system from the same lot and kind of evacuated tubes used during adrenal venous sampling procedure), in order to exclude the hypotheses of any possible cause of abnormal gel flotation: e.g. high total protein and high immunoglobulin concentrations.

Total protein was assayed by Biuret method on cobas 6000 c501 (Roche Diagnostics GmbH, Penzberg, Germany). Protein electrophoresis was performed on Capillarys 2 (Sebia, Paris, France), whereas serum immunofixation electrophoresis was performed on Hydrasys 2 Scan with Hydragel 4 IF (Sebia, Paris, France). Briefly, the Hydragel 4 IF assay is based on the principle of agarose gel electrophoresis followed by immunofixation. After the separation of serum proteins according to their charge, the gel was incubated with different specific antisera targeted against: *gamma* - (IgG), *alpha* - (IgA), *mi* - (IgM) heavy chains; whereas, free and bound *kappa* (κ) and *lambda* (λ) light chains were assayed with the specific antibodies. The gel was then processed in order to remove the antisera excess prior to the final staining step. The gel was interpreted visually to characterize all immunoglobulins.

Results

At the end of centrifugation process primary blood tubes with blood from inferior vena cava, and left adrenal vein exhibited the standard gel separator barrier, whereas unpredictably both tubes (serum and plasma) from right adrenal vein showed abnormal flotation of gel separator (Figure 1). We made some vain attempts to access both serum and plasma from right adrenal vein through the gel barrier by a micropipette, however we did not succeed, due to the occlusion of pipette-tip by gel. A similar situation was described by Gerin *et al.* (12). Such an attempt was required in order to

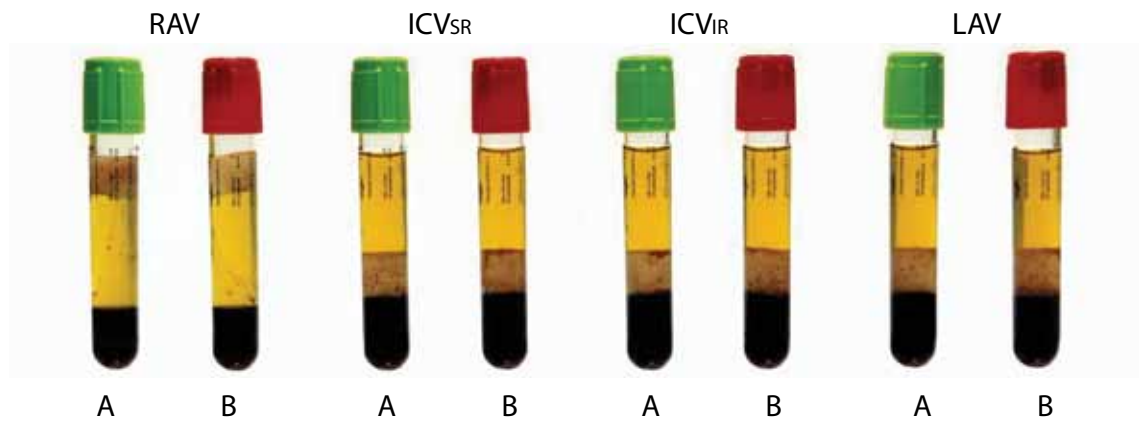


FIGURE 1. Abnormal gel flotation caused by contrast media during adrenal vein sampling. A – plasma sample in 3.5 mL tube with 52.5 USP U of lithium heparin and gel separator. B – serum sample in 3.5 mL serum tube with clot activator and gel separator. RAV – right adrenal vein. ICSV_{SR} - inferior vena cava in the suprarenal portion. ICSV_{IR} - inferior vena cava in the infrarenal portion. LAV - left adrenal vein.

measure the laboratory parameters needed to adopt appropriate treatments (i.e. the selectivity index, and the lateralization index) (1). Without both cortisol and aldosterone concentrations from right adrenal vein, the endocrinologist lacks the expected results from the adrenal venous sampling procedure performed, resulting in a missed diagnostic procedure.

The total protein concentration from venous blood sample was 62.5 g/L, with normal electrophoresis performance (i.e. 40.7 g/L of albumin, 2.1 g/L of alpha 1, 5.3 g/L of alpha 2, 6.0 g/L of beta,

and 8.4 g/L of gamma globulin). Serum immunofixation showed a normal polyclonal pattern of immunoglobulins (Figure 2 and Figure 3).

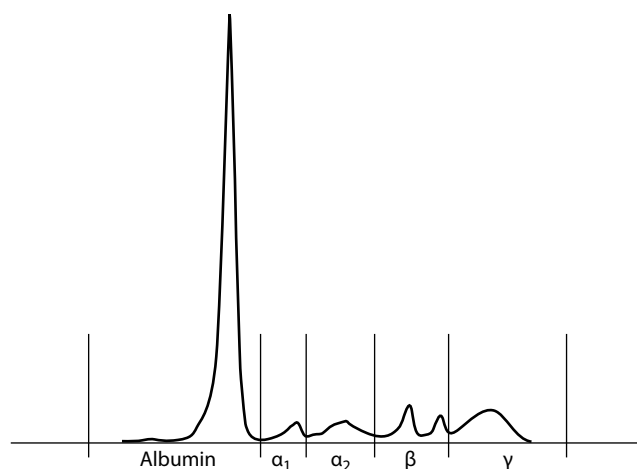


FIGURE 2. Electropherogram of serum from peripheral venous sample. All electrophoretic fractions are within normal limits.

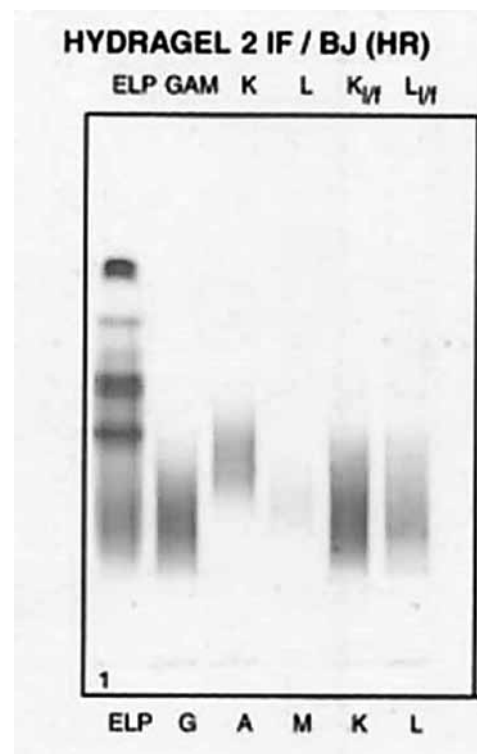


FIGURE 3. Immunofixation electrophoresis of serum from peripheral venous sample. Normal polyclonal pattern of immunoglobulins was found.

Discussion

Our case report showed an unexpected abnormal flotation of gel separator only in samples from right adrenal vein. Abnormal flotation of gel separator could be induced by high protein concentration, high density, and/or high viscosity (12-19). Faught *et al.* had experimentally demonstrated that samples with high protein concentration induce inappropriate flotation of gel separator (19). Furthermore, Gerin and colleagues had properly shown samples with high plasma density due to elevated immunoglobulin concentration could also induce abnormal gel flotation (12). Based on our results, we can exclude the above key-causes of abnormal flotation of gel separator, i.e. high total protein, high immunoglobulin concentration, and abnormal pattern of immunoglobulins.

During adrenal venous sampling procedure, radiologists usually administer a contrast agent via the catheter to visualize the proper catheter position. Media contrast could be responsible for both laboratory tests interference, and abnormal flotation of gel separator (12). The abnormal flotation of gel separator was to be expected in all the samples obtained; however, this was not observed. To investigate why only samples from the right adrenal vein were affected, we interviewed the radiologist. The radiologist confirmed that 2.6 mL instead of 2.0 mL of lopamidol 300 mg/mL was used close to the right adrenal vein due to some difficulty to access it. Lopamidol is a non-ionic, low-osmolar iodinated contrast media with density of 1.33 g/cm³. This fact can explain the abnormal flotation of gel separator only on samples taken from the right adrenal vein. Therefore, the excess of contrast media used near the right adrenal vein could be the cause of the increased blood density, explaining the abnormal gel flotation after centrifugation.

Laboratory managers mainly prefer to use vacuum tubes with gel separator, since it reduces the need to aliquot specimens and allows a greater amount of sample after centrifugation, with virtually absent risk of contamination from the cell pellet thanks to the gel barrier (20). Moreover, samples from gel-tubes are more stable than plain tubes. Leino and Koivula showed that cortisol is stable up

to 6 hours from collection in lithium-heparin plasma specimens collected with lithium-heparin gel tube from Terumo (same kind of tube used at own University Hospital) (21). However, the gel in Becton Dickinson's tubes was reported to interfere with LC-MS assays of steroid molecules (i.e. 17-hydroxyprogesterone, and aldosterone) (22). Furthermore, both serum- and lithium heparin-vacuum tubes with gel separator produced by different companies showed different laboratory results for clinical chemistry tests (23,24). Before adopting and standardizing every kind of in vitro devices (i.e. blood tube) for diagnostic use, all laboratories should obtain information from the manufacturer/method developer (i.e. tube manufacturer) to confirm the performance characteristics of the device/procedure. In addition, the independent verification by the laboratory should confirm, through objective evidence (in the form of performance characteristics), that the performance claims for the examination procedure have been met (7,8). The performance claims for the examination procedure, as confirmed during the verification process, shall be those relevant to the intended use of the examination results. Our non-conformity was due to impaired performance of the gel tube when 2.6 mL of lopamidol 300 mg/mL were used by the radiologist during adrenal vein sampling procedure.

At present, primary aldosteronism is the most frequent cause of secondary hypertension, often undiagnosed because of the complexity of the diagnostic work-up (5). In patients with primary aldosteronism, AVS is the only reliable technique to distinguish between unilateral and bilateral autonomous production of aldosterone, allowing to offer to the patient the best available targeted therapy, either unilateral adrenalectomy or medical treatment with a mineralocorticoid receptor antagonist. AVS is an invasive and sometimes difficult procedure because the cannulation of the right adrenal vein requires very well trained radiologist and thus is not performed in all centres. AVS procedure can also lead to rare complications, such as bleeding at the cannulation site or adrenal vein rupture. For this reason the identification of possible laboratory interferences with the AVS re-

sults are of paramount importance in order to avoid the AVS diagnostic failure.

To the best of our knowledge, this is the first report of abnormal gel flotation regarding contrast media (i.e., iodinate) used during adrenal vein sampling. However, it is known that iodinate contrast media cause abnormal peaks in capillary zone electrophoresis of serum proteins and positive bias in assessment of troponin I (25). Further studies should evaluate the impact of media contrast on laboratory assays (i.e. liquid chromatography–mass spectrometry and immunochemistry). This case report was discussed at our own University Hospital within a multidisciplinary committee involving radiologists, specialists in laboratory medicine and general medicine physicians. We wanted to define a new procedure directed at replacing tube types (i.e. from gel- to plain-plasma tubes) and schedule an internal program to teach radiology residents to perform AVS in agreement with international recommendations. This strategy

is in agreement with International Organization for Standardization 15189:2012 standard and aimed to avoid possible future nonconformity in this field (26).

In conclusion, we suggest using plain plasma tubes (without gel separator) for adrenal vein sampling in order to avoid potential preanalytical nonconformities in the diagnostic procedure. Moreover, radiologist staff should discard a blood volume equivalent to twice the catheter extension to eliminate residual media contrast before collection of samples for laboratory assays.

Acknowledgments

Our special thanks to Lucia Stefani, BSc and Gabriella Righetti, BSc for their technical skills on protein electrophoresis, and serum immunofixation electrophoresis.

Potential conflict of interest

None declared.

References

- Rossi GP, Auchus RJ, Brown M, Lenders JW, Naruse M, Plouin PF, et al. An expert consensus statement on use of adrenal vein sampling for the subtyping of primary aldosteronism. *Hypertension* 2014;63:151-60. <http://dx.doi.org/10.1161/HYPERTENSIONAHA.113.02097>.
- Blondin D, Quack I, Haase M, Kücükköylü S, Willenberg HS. Indication and technical aspects of adrenal blood sampling. *Fortschr Röntgenstr* 2015;187:19-28.
- Mengozi G, Rossato D, Bertello C, Garrone C, Milan A, Pagni R, et al. Rapid cortisol assay during adrenal vein sampling in patients with primary aldosteronism. *Clin Chem* 2007;53:1968-71. <http://dx.doi.org/10.1373/clinchem.2007.092080>.
- Hayden JA, Kwan SW, Valji K. Implementation of rapid cortisol during adrenal vein sampling. *Hypertension* 2014;63:e88. <http://dx.doi.org/10.1161/HYPERTENSIONAHA.113.03064>.
- Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, et al. The management of primary aldosteronism: Case detection, diagnosis, and treatment: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* 2016;101:1889-916. <http://dx.doi.org/10.1210/jc.2015-4061>.
- Lima-Oliveira G, Lippi G, Salvagno GL, Picheth G, Guidi GC. Laboratory diagnostics and quality of blood collection. *J Med Biochem* 2015;34:288-94. <http://dx.doi.org/10.2478/jomb-2014-0043>.
- Lippi G, Cornes MP, Grankvist K, Nybo M, Simundic AM. EFLM WG-Preanalytical phase opinion paper: local validation of blood collection tubes in clinical laboratories. *Clin Chem Lab Med* 2016;54:755-60. <http://dx.doi.org/10.1515/cclm-2015-1274>.
- Giavarina D, Banfi G, Daves M, Dolci A, Farci Santarcangeli D, Lima-Oliveira G, et al (in press). Validation of blood collection tubes in clinical laboratories: local adaptation of the guidelines of the European Federation of Laboratory Medicine by the SIBioC working group on extra-analytical variability. *Biochimica Clinica*.
- O'Keane MP, Cunningham SK. Evaluation of three different specimen types (serum, plasma lithium heparin and serum gel separator) for analysis of certain analytes: clinical significance of differences in results and efficiency in use. *Clin Chem Lab Med* 2006;44:662-68. <http://dx.doi.org/10.1515/CCLM.2006.099>.
- Chance J, Berube J, Vandersmissen M, Blanckaert N. Evaluation of the BD Vacutainer PST II blood collection tube for special chemistry analytes. *Clin Chem Lab Med* 2009;47:358-61. <http://dx.doi.org/10.1515/CCLM.2009.072>.
- Pizzolo F, Zorzi F, Chiecchi L, Consoli L, Aprili I, Guarini P, et al. NT-proBNP, a useful tool in hypertensive patients undergoing a diagnostic evaluation for primary aldosteronism. *Endocrine* 2014;45:479-86. <http://dx.doi.org/10.1007/s12020-013-0028-6>.

12. Gerin F, Ramazan DC, Baykan O, Sirikci O, Haklar G. Abnormal gel flotation in a patient with apparent pneumonia diagnosis: a case report. *Biochem Med (Zagreb)* 2014;24:180-2. <http://dx.doi.org/10.11613/BM.2014.021>.
13. Fatás M, Franquelo P, Franquelo R. Anomalous flotation of separator gel: density or viscosity? *Clin Chem* 2008; 54:771-2. <http://dx.doi.org/10.1373/clinchem.2007.093716>.
14. Van Der Ouweland JMW, Church S. High total protein impairs appropriate gel barrier formation in BD Vacutainer blood collection tubes. *Clin Chem* 2007;53:364-5. <http://dx.doi.org/10.1373/clinchem.2006.081653>.
15. William J, Goodwin F, Banatwala N. Poor performance of serum separator. *Ann Clin Biochem* 1995;32:332. <http://dx.doi.org/10.1177/000456329503200222>.
16. Spiritus T, Zaman Z, Desmet W. Iodinated contrast media interfere with gel barrier formation in plasma and serum separator tubes. *Clin Chem* 2003;49:1187-190. <http://dx.doi.org/10.1373/49.7.1187>.
17. Srivastava R, Murphy MJ, Card J, Severn A, Fraser CG. The case of the floating gel. *J Clin Pathol* 2004;57:1333-4. <http://dx.doi.org/10.1136/jcp.2004.020495>.
18. Daves M, Lippi G, Cosio G, Raffagnini A, Peer E, Dangella A et al. An unusual case of a primary blood collection tube with floating separator gel. *J Clin Lab Analysis* 2012;26:246-7. <http://dx.doi.org/10.1002/jcla.21512>.
19. Faught RC, Marshall J, Bornhorst J. Solution densities and estimated total protein contents associated with inappropriate flotation of separator gel in different blood collection tubes. *Arch Pathol Lab Med* 2011;135:1081-84. <http://dx.doi.org/10.5858/2010-0488-OAR.1>.
20. Bowen RA, Hortin GL, Csako G, Otañez OH, Remaley AT. Impact of blood collection devices on clinical chemistry assays. *Clin Biochem* 2010;43:4-25. <http://dx.doi.org/10.1016/j.clinbiochem.2009.10.001>
21. Leino A, Koivula MK. Stability of chemical and immunochemical analytes in uncentrifuged plasma samples. *Ann Clin Biochem* 2009;46:159-61. <http://dx.doi.org/10.1258/acb.2008.008212>.
22. Buse JD, Orton DJ, Boyd J, Sadrzadeh H. The influence on analytical interference introduced by separator gel blood collection tubes on steroid molecules analysis. *Clin Chem* 2015; 61(10 Suppl): S43.
23. Lima-Oliveira G, Salvagno GL, Lippi G, Brocco G, Voi M, Montagnana M, et al. Quality management of preanalytical phase: impact of lithium heparin vacuum tubes changes on clinical chemistry. *Accred Qual Assur* 2013;18:429-34. <http://dx.doi.org/10.1007/s00769-013-0995-6>.
24. Lima-Oliveira G, Lippi G, Salvagno GL, Montagnana M, Picheth G, Guidi GC. Pre analytical management: serum vacuum tubes validation for routine clinical chemistry. *Biochem Med (Zagreb)* 2012;22:180-6. <http://dx.doi.org/10.11613/BM.2012.021>.
25. Lippi G, Daves M, Mattiuzzi C. Interference of medical contrast media on laboratory testing. *Biochimica Medica (Zagreb)* 2014;24:80-8. <http://dx.doi.org/10.11613/BM.2014.010>.
26. ISO 15189:2012: *Medical laboratories: requirements for quality and competence*. Geneva, Switzerland: International Organization for Standardization, 2012.