In this issue: Improving the preanalytical phase in laboratory medicine

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Detection of haemolysis, a frequent preanalytical problem in the serum of newborns and adults

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ABSTRACT

Background

Preanalytical problems can be more frequent in case of preterm and term newborns as compared to the general patient population. Here we present the leading preanalytical errors in our laboratory, the prevalence of haemolysis and its impact on laboratory test results, and our efforts to improve the diagnostic work-up of newborns' samples.

Methods

Preanalytical quality indicators were analysed in all samples in 2018. The haemolysis index was measured spectrophotometrically in serum samples in the period of 2012-2018, and the ratio of haemolysed samples and the test rejection rates were analysed. The data of newborns and other patients were analysed separately.

Results

During the tested year, the leading preanalytical errors were haemolysis in serum samples, inadequate

sample identification and clotting of anticoagulated blood regarding all samples or newborns. In this seven-year period the ratio of haemolysed serum samples was 4.00% in all patients and 46.4% in newborns, while the test rejection rates due to haemolysis were 0.57% and 3.71%, respectively. Haemolysis indices were significantly higher in case of newborns than in patients with documented severe intravascular haemolysis which suggests that the major reason of elevated haemolysis indices in newborns was *in vitro* haemolysis. Accordingly, all C-reactive protein (CRP) results which were rejected by severe haemolysis became reliable after repeating blood sampling.

Conclusion

Haemolysis is the leading preanalytical problem not only in newborns but also in the general patient population. Our study highlights the importance of automated assessment of serum indices and continuous monitoring of the preanalytical quality indicators and suggests the need for education and blood collection trainings.

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INTRODUCTION

In laboratory medicine, the result of an examination procedure is influenced by the correctness of the preanalytical activities [1]. During the total testing process, preanalytical phase has the highest error rate, since preanalytical errors are estimated to account for up to 70% of all errors in laboratory diagnostics [2].

Problems in the pre-preanalytical phase are particularly relevant when several steps are not performed, and are not under the control of the laboratory staff. The quality of the total testing process can be improved by continuous monitoring of quality indicators; and in clinical laboratories it is also a requirement by the International Organization for Standardization (ISO) 15189: 2012 [2, 3].

On the Consensus Conference entitled „Harmonization of Quality Indicators in Laboratory Medicine: 4 years later" organised in Padova, in 2016, a new version of the model of quality indicators was released, and more than half of the quality indicators of this model, monitor the most vulnerable part, the preanalytical phase [4]. Haemolysis was recognized as the most frequent preanalytical error and *in vitro* haemolysis is the leading cause of test or sample rejection in clinical laboratories [5].

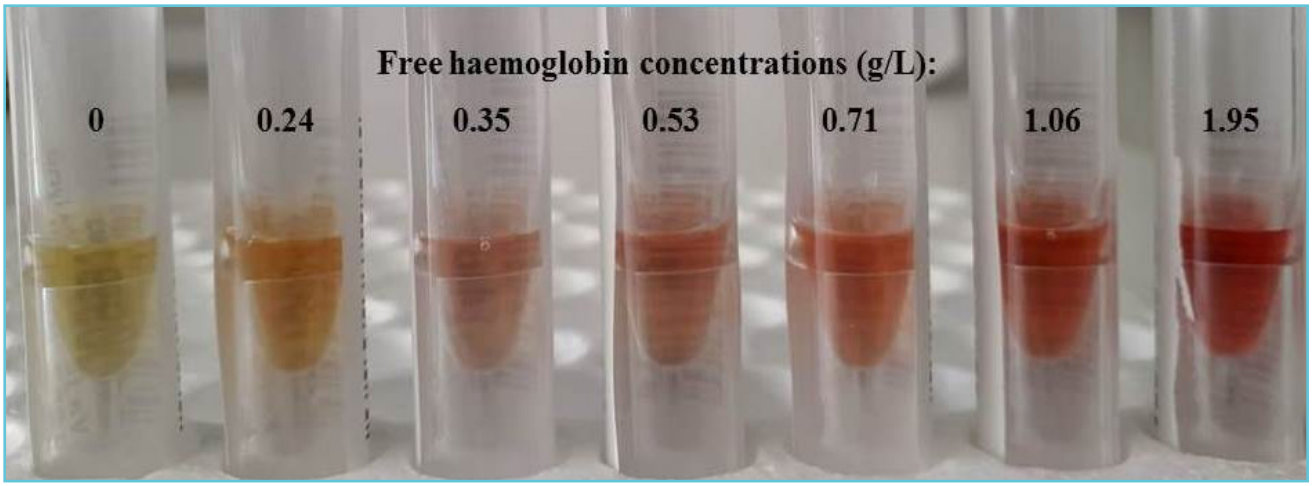
The prevalence of haemolysis can be as high as 3.3% of all routine samples, accounting for up to 40-70% of all unsuitable specimens identified, nearly five times higher than other causes, such as incorrect and clotted samples [6]. Only less than 2% of samples with detectable haemolysis are due to *in vivo* haemolysis [7].

In vitro haemolysis mostly occurs during blood collection and transportation, and it generates biological and analytical interferences [5, 8]. The cut-off of clinically significant interference was defined as 0.5 g/L for cell-free haemoglobin [9]. Although the assessment of sample quality was historically based on visual inspection of the specimen before and after centrifugation, visual detection of haemolysis is inaccurate [5]. After separation of the serum, haemolysis can be detected visually, when the free haemoglobin concentration is 0.2-0.3 g/L (Figure 1) [6, 10].

Clinical chemistry analysers are capable of automated assessment of serum indices including haemolysis index and they provide quantitative measurement with high reproducibility. Presently systematic automated measurement of haemolysis index is strongly recommended [8].

Preanalytical problems can be more frequent in case of preterm and term newborns, and analysis of their samples has special aspects compared to the general patient population. Every year, approximately 15 million babies are born preterm globally and the number is rising [11].

Figure 1 **Comparison of visual and automated detection of haemolysis in serum samples**



Haemolysis indices were measured spectrophotometrically by Roche COBAS analysers. According to the literature, haemolysis can be visible as a pink to red colour of the serum, when the free haemoglobin concentration is 0.2-0.3 g/L.

The World Health Organisation (WHO) defines preterm birth as any birth before 37 completed weeks of gestation, or fewer than 259 days since the first day of the woman's last menstrual period. This is further subdivided on the basis of gestational age: extremely preterm (<28 weeks), very preterm (28-32 weeks) and moderate or late preterm (32-37 completed weeks of gestation).

Our aim was to detect and analyse the leading preanalytical errors in our laboratory; and to monitor the prevalence of haemolysis and its impact on laboratory test results in case of all samples and in preterm and term newborns. We also present here the efforts to improve the diagnostic workup of newborns' samples.

MATERIALS AND METHODS

Preanalytical quality indicators (e.g. misidentified samples and requests, test transcription errors, clotted samples, incorrect sample type or fill level, inappropriate time and temperature of transport and storage) were analysed in all samples analyzed at the Department of Laboratory Medicine, University of Debrecen in the period of January 2018 to December 2018. Most of these indicators were recorded in the General Laboratory Information Management System (GLIMS, Medical Information for Professional Systems, Gent, Belgium) by the laboratory staff. Unidentifiable samples or samples without test requests were noted in a printed register. Serum indices (haemolysis, icterus, lipaemia) were detected automatically and they were stored in the laboratory information system.

In all serum samples that arrived for routine and STAT clinical chemistry and immunochemical assays between the period of January 2012 to December 2018, haemolysis index was measured spectrophotometrically by COBAS-8000, -6000 and 501 analysers (Roche Ltd, Basel, Switzerland), and the ratio of haemolysed samples and the test rejection rates were analysed. A sample was identified as haemolysed when the free haemoglobin concentration of the sample was higher than 0.5 g/L (the haemolysis index was higher than 31 mmol/L). The test rejection ratio was calculated as the number of tests rejected due to haemolysis divided by the total number of tests requested. Data of newborns and other patients were analysed separately.

In order to study whether preterm and term newborns have dominantly *in vitro* or *in vivo* haemolysis, their haemolysis indices were compared to the haemolysis indices of those patients who had severe intravascular haemolysis (serum haptoglobin concentration <0.1 g/L). Haptoglobin concentration was measured by an immunoturbidimetric assay on COBAS-8000, 6000 and 501 analysers (Roche Ltd, Basel, Switzerland). The CRP concentration of the newborns – as a marker of perinatal infections – was measured by a latex-sensitized immunoturbidimetric assay (Roche COBAS-501 analyser).

RESULTS

Among the 868 441 samples that were received by our laboratory in 2018, 11 379 preanalytical errors were registered by the laboratory staff in the laboratory information system. In that year the haemolysis index was measured in 295 130 serum samples.

The most frequent preanalytical error was haemolysis: 4.34% of the serum samples were haemolysed. Sample identification error was the second most frequent cause of preanalytical problems: 0.81% of all samples had some kind of identification error, most of them had less than two identifiers.

The third most frequent preanalytical error was the presence of fibrin clot in anticoagulated samples: the ratio of clotted samples was 0.36%. During the tested year, 9 017 samples were collected from preterm and term newborns, among these the ratio of haemolysed serum was 53.4%. Overall, 8.28% of all samples from newborns had identification errors (93.4% of sample identification problems were caused by less than two identifiers) and among their anticoagulated blood samples, 6.67% were clotted.

In case of all requests, the ratio of test transcription errors (e.g., absent or erroneous barcode is assigned to the request, inadequate test is requested, one or more test request(s) is/are missed) was 0.257 %. Incorrect fill level of evacuated tubes was found in case of 0.066 % of all samples, most of them had inappropriate sample-anticoagulant volume ratio. Inappropriate time and temperature of transport and storage were registered in case of 0.005 % of all samples, the main problem was excessive transportation time. Incorrect sample type and the incidence of unidentifiable samples or samples without test requests were infrequent (0.004 %).

In a second series of studies we checked for haemolysed serum samples that arrived for routine and STAT clinical chemistry and immunochemical

Table 1 Ratio of haemolysed samples and test rejection ratio due to haemolysis in general patient population and in preterm and term newborns in a seven-year period (2012-2018)

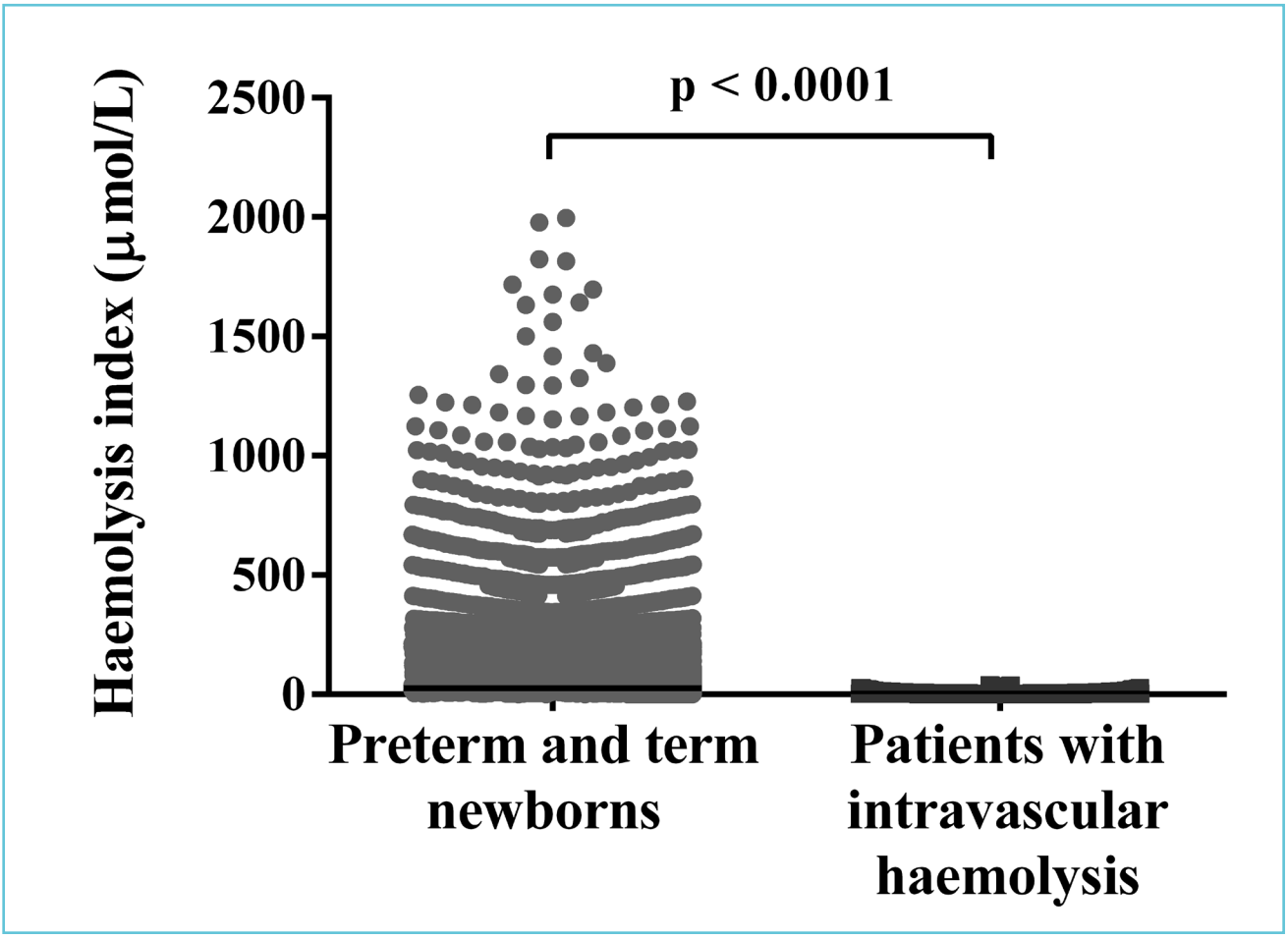
Serum samples	Ratio of haemolysed samples	Ratio of rejected tests due to haemolysis
All patients	4.00%	0.57%
General patients	3.33%	0.56%
Newborns	46.4%	3.71%

assays during a seven-year period (from January 2012 to December 2018). Haemolysis index was measured in case of 1 882 721 serum samples. 98.4% of the samples was collected from general patient population and 1.60% from preterm and term newborns. The ratio of haemolysed samples was 14 times higher in preterm and term newborns compared to the general patient population, while their test rejection ratio due to haemolysis was 7 times higher, respectively (Table 1). 7.88% of the serum samples arrived at the laboratory from intensive care units and 8.05% from the adult emergency department.

In case of these departments, haemolysis was more frequent compared to the other blood collection sites of the general patient population. The ratio of haemolysed samples was 5.93% in case of intensive care units and 6.81% in emergency department, while the test rejection rates due to haemolysis were 0.97% and 1.73%, respectively.

We hypothesized that in the case of preterm and term newborns – although they might also have *in vivo* haemolysis – the major reason of elevated haemolysis indices was the *in vitro* haemolysis. In order to prove our hypothesis, their

Figure 2 **Haemolysis index of preterm and term newborns and patients with documented severe intravascular haemolysis**



Haemolysis indices were significantly higher in case of preterm and term newborns (median: 27 mmol/L, 25 percentile: 11 mmol/L, 75 percentile: 75 mmol/L, maximum: 1997 mmol/L) than in patients with documented severe intravascular haemolysis (median: 4 mmol/L, 25 percentile: 2 mmol/L, 75 percentile: 7 mmol/L, maximum: 39 mmol/L).

haemolysis indices were compared to the haemolysis indices of those patients who had documented severe intravascular haemolysis (serum haptoglobin concentration <0.1 g/L).

Haptoglobin concentration was measured in case of 2530 serum samples during the seven-year period, and in 557 samples haptoglobin was lower than 0.1 g/L, which refers *in vivo* hemolysis. In patients with documented severe intravascular haemolysis, haemolysis indices were significantly lower compared to the group of preterm and term newborns (Figure 2).

The measurement of specific proteins is less sensitive to haemolysis than several frequent clinical chemistry tests, but severe *in vitro* haemolysis may interfere with some methods. For example, haemolysis index >622 mmol/L may cause underestimation of CRP levels.

In case of preterm and term newborns, CRP measurement is very important to monitor perinatal infections. In 199 samples of newborn patients, the CRP test was rejected because of severe haemolysis, the highest haemolysis index was 1997 mmol/L.

Repeating the blood sampling, the haemolysis index decreased significantly and all CRP results became reliable and were reportable (Table 2).

DISCUSSION

In our laboratory the leading preanalytical errors are haemolysis in serum samples, inadequate sample identification and clotting of anticoagulated blood regarding all samples or from newborns. Preanalytical quality indicator data collected from all participating laboratories of the IFCC „Laboratory Errors and Patient Safety" project on Quality Indicators between 2009 and 2013 showed that the ratio of haemolysed samples was maximum 3% [2].

Howanitz et al found a haemolysis rate less than 3% in 71% of the studied 772 laboratories, and a rate between 3-6% in 15% of the laboratories [12]. Their haemolysis rates were the highest in case of samples from emergency departments.

Heireman et al also found that haemolysis was more often observed in samples received from the emergency department, affecting as much as 10-30% of emergency department samples [13].

Our ratio of haemolysed samples (4% for all samples) is in the higher range of the published values. One possible reason can be the presence of Perinatal Intensive Center where a lot of extremely and very preterm babies are treated and the ratio of haemolysed samples was 46.4% due to the complicated blood collection.

Table 2 Representative cases of severe in vitro haemolysis in newborn patients

Cases	First blood sampling (1-2 days after birth)		Repeated blood sampling (2-5 hours later)	
	Haemolysis index (µmol/L)	CRP (mg/L)	Haemolysis index (µmol/L)	CRP (mg/L)
Case 1	1997	haemolysed	118	0.61
Case 2	1677	haemolysed	23	1.36
Case 3	875	haemolysed	16	4.02

The reference range of CRP is <2.2 mg/L (1-2 days after birth).

Furthermore at the University of Debrecen there is a busy emergency department with a significant sample number, and there are several intensive care units. The higher ratio of haemolysis at the departments where patients are in critical condition suggests the need for education and blood collection trainings. In the literature limited information is available for haemolysis rates detected in neonatal units. Another problem is that many laboratories do not use automated, objective assessment of haemolysis, lipaemia and icterus. In neonatal samples where elevated bilirubin concentration is common, the ability to detect haemolysis by visual inspection may be further biased by underestimation of haemolysis [14]. Khedr et al published that in 2012 the haemolysis rate of the Neonatal Unit in Baystate Medical Center was near or over 40%, and as a consequence of changes (e.g., use of heel warmers to get a more consistent warming prior to drawing, increased education of correct blood withdrawal techniques) it was reduced to 28.6% within a few months [15]. This study also highlights the importance of monitoring and feedback to collecting personnel in improving and maintaining correct blood sampling method.

Among the most frequent clinical chemistry tests, lactate dehydrogenase (LDH), creatine kinase (CK), MB isoenzyme of creatine kinase (CK-MB), potassium, conjugated bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and iron are the most sensitive analytes to haemolysis. Even the measurement of specific proteins e.g. CRP, haptoglobin may have significant interference with free haemoglobin in serum. The interference may be optical, chemical or haemoglobin may interact with antigen or antibody in immunoassays. For this reason, the upper limit of haemoglobin in a serum or plasma sample is test- and method-dependent. The method description of reagents should refer to limitations such as serum indices (haemolysis, icterus, lipaemia).

The most frequent reason of test rejection is haemolysis. It is critical in case of premature babies receiving oxygen supplementary respiratory treatment who are monitored by laboratory tests (Blood gas analysis, LDH). LDH is a basic monitoring test and very sensitive to haemolysis, therefore decreasing *in vitro* haemolysis is essential especially in premature babies. As the haemolysis is often observed in preterm and term newborns [16], we may be more permissive in these cases: we can sligthly elevate the borderline of haemolysis and report the approximate LDH result, with a note that the result is affected by moderate haemolysis. This is in accordance with the result of a survey [10] in which neonatologists preferred to receive the test results with a qualitative comment according to the interference of haemolysis; rather than the rejection of LDH result at moderate haemolysis.

The second most frequent preanalytical error in our laboratory was the identification error, 0.81% of the total samples had less than two identifiers and this ratio was 10 times higher in newborns, simply because there is not enough place to write two identifiers on the label of these special small tubes. Plebani et al published that during their project the frequency of misidentification errors was not more than 0.3% [2], while in a Canadian study the majority of reported errors concerned patient or sample misidentification [17, 18].

A recent survey of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), carried out in 12 European countries, reported that compliance of phlebotomy procedures with the Clinical and Laboratory Standards Institute (CLSI) H3-A6 guidelines was unacceptably low, accurate patient identification and tube labelling were the most vulnerable steps in blood sampling [19]. Although correct use of barcode may reduce identification errors.

Among anticoagulated blood samples, 0.36% were clotted in our laboratory, while in case of preterm and term newborns this ratio was 6.67% according to blood collection difficulties. Clotting in blood tubes containing anticoagulants mostly occurs for challenging/prolonged venipuncture or failing to appropriately mix the tube after collection [20]. Plebani et al found that the median values for the ratio of clotted samples were between 0.05% and 0.21% in their study [2].

Twenty-six percent of laboratory errors can affect outcome of patient's care, errors result in further inappropriate investigations, patient discomfort, increased costs and/or modification of the therapy [17, 21]. Monitoring of preanalytical quality indicators is a valuable tool to guarantee and improve the quality of the preanalytical phase. Automated assessment of serum indices characterizes the quality of sera, therefore it is recommended for all laboratories - it provides more objective evaluation of the sample and assists to obtain reliable results. Application of serum indices described in each test method should be applied in the analysers when a new test is introduced, and checking the test-specific serum index cut-off values for the most sensitive tests is recommended.

In conclusion, we determined the leading preanalytical errors and their frequency both in case of general patients and newborns. We have to reduce them and therefore the implementation of the joint EFLM-COLABIOCLI recommendation for venous blood sampling is needed [22]. This recommendation covers all steps of the venous blood collection procedure using closed system in case of in- and outpatients except for children and unconscious patients. Implementation of the guidelines, systematic theoretical education and practical trainings of the medical staff, periodical audits of phlebotomy, continuous monitoring of the preanalytical quality indicators and automated assessment of serum indices are recommended to improve the quality of the total testing process.

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REFERENCES

1. Aita A, Sciacovelli L, Plebani M. Extra-analytical quality indicators - where to now? Clin Chem Lab Med 2018;57(1): 127-133.

2. Plebani M, Sciacovelli L, Aita A, Pelloso M, Chiozza ML. Performance criteria and quality indicators for the preanalytical phase. Clin Chem Lab Med 2015;53(6):943-948.

3. Aita A, Sciacovelli L, Plebani M. The silk road to total quality in Laboratory Medicine. Clin Chem Lab Med 2019; 57(6):769-772.

4. Sciacovelli L, Panteghini M, Lippi G, Sumarac Z, Cadamuro J, Galoro CAO, et al. Defining a roadmap for harmonizing quality indicators in Laboratory Medicine: a consensus statement on behalf of the IFCC Working Group "Laboratory Error and Patient Safety" and EFLM Task and Finish Group "Performance specifications for the extra-analytical phases". Clin Chem Lab Med 2017;55(10): 1478-1488.

5. Wan Azman WN, Omar J, Koon TS, Tuan Ismail TS. Hemolyzed Specimens: Major Challenge for Identifying and Rejecting Specimens in Clinical Laboratories. Oman Med J 2019;34(2):94-98.

6. Lippi G, Blanckaert N, Bonini P, Green S, Kitchen S, Palicka V, et al. Haemolysis: an overview of the leading cause of unsuitable specimens in clinical laboratories. Clin Chem Lab Med 2008;46(6):764-772.

7. Carraro P, Servidio G, Plebani M. Hemolyzed specimens: a reason for rejection or a clinical challenge? Clin Chem 2000; 46(2):306-307.

8. Lippi G, Cadamuro J, von Meyer A, Simundic AM, European Federation of Clinical C, Laboratory Medicine Working Group for Preanalytical P. Practical recommendations for managing hemolyzed samples in clinical chemistry testing. Clin Chem Lab Med 2018;56(5):718-727.

9. Lippi G. Systematic Assessment of the Hemolysis Index: Pros and Cons. Adv Clin Chem 2015;71:157-170.

10. Joshi S, Vaitkute R, Jeffery J, Ayling RM. Haemolysis in neonatal blood samples: a survey of practice. Ann Clin Biochem 2007;44(Pt 2):178-181.

11. Quinn JA, Munoz FM, Gonik B, Frau L, Cutland C, Mallett-Moore T, et al. Preterm birth: Case definition & guidelines for data collection, analysis, and presentation of immunisation safety data. Vaccine 2016;34(49):6047-6056.

12. Howanitz PJ, Lehman CM, Jones BA, Meier FA, Horowitz GL. Practices for Identifying and Rejecting Hemolyzed Specimens Are Highly Variable in Clinical Laboratories. Arch Pathol Lab Med 2015;139(8):1014-1019.

13. 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(18):1317-1322.

14. Jeffery J, Sharma A, Ayling RM. Detection of haemolysis and reporting of potassium results in samples from neonates. Ann Clin Biochem 2009;46(Pt 3):222-225.

15. Khedr S, Blake V, Erdogan E. Neonatal Unit Hemolysis Rates From an Academic Medical Center: A Quality Improvement Project. Arch Pathol Lab Med 2016;140(6): 502-503.

16. Christensen RD, Yaish HM. Hemolysis in Preterm Neonates. Clin Perinatol 2016;43(2):233-240.

17. Aita A, Sciacovelli L, Plebani M. Laboratory-related errors: you cannot manage what you don't measure. You manage what you know and measure. Diagnosis (Berl) 2017;4(4):193-195.

18. Restelli V, Taylor A, Cochrane D, Noble MA. Medical laboratory associated errors: the 33-month experience of an on-line volunteer Canadian province wide error reporting system. Diagnosis (Berl) 2017;4(2):79-86.

19. Simundic AM, Church S, Cornes MP, Grankvist K, Lippi G, Nybo M, et al. Compliance of blood sampling procedures with the CLSI H3-A6 guidelines: An observational study by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) working group for the preanalytical phase (WG-PRE). Clin Chem Lab Med 2015;53(9):1321-1331.

20. Giavarina D, Lippi G. Blood venous sample collection: Recommendations overview and a checklist to improve quality. Clin Biochem 2017;50(10-11):568-573.

21. Plebani M, Carraro P. Mistakes in a stat laboratory: types and frequency. Clin Chem 1997;43(8 Pt 1): 1348-1351.

22. Simundic AM, Bolenius K, Cadamuro J, Church S, Cornes MP, van Dongen-Lases EC, et al. Joint EFLM-COLABIOCLI Recommendation for venous blood sampling. Clin Chem Lab Med 2018;56(12):2015-2038.