

Confidence level in venipuncture and knowledge on causes of *in vitro* hemolysis among healthcare professionals

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Abstract

Introduction: This study aimed to assess confidence level of healthcare professionals in venipuncture and their knowledge on the possible causes of *in vitro* hemolysis.

Materials and methods: A sample of 94 healthcare professionals (nurses and laboratory technicians) participated in this survey study. A four-section questionnaire was used as a research instrument comprising general information for research participants, knowledge on possible causes of *in vitro* hemolysis due to type of material used and venipuncture technique and specimen handling, as well as assessment of healthcare professionals' confidence level in their own ability to perform first and last venipuncture.

Results: The average score on the knowledge test was higher in nurses' than in laboratory technicians (8.11 ± 1.7 , and 7.4 ± 1.5 , respectively). The difference in average scores was statistically significant ($P = 0.035$) and Cohen's *d* in the range of 0.4 indicates that there is a moderate difference on the knowledge test among the health care workers. Only 11/94 of healthcare professionals recognized that blood sample collection from cannula and evacuated tube is method which contributes most to the occurrence of *in vitro* hemolysis, whereas most risk factors affecting occurrence of *in vitro* hemolysis during venipuncture were recognized. There were no significant differences in mean score on the knowledge test in relation to the confidence level in venipuncture ($P = 0.551$).

Conclusion: Confidence level at last venipuncture among both profiles of healthcare staff was very high, but they showed insufficient knowledge about possible factors affecting hemolysis due to materials used in venipuncture compared with factors due to venipuncture technique and handling of blood sample.

Key words: hemolysis; patient safety; phlebotomy; medical staff; questionnaire; preanalytical phase

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Introduction

Blood sampling by venipuncture for various laboratory analyses is one of the most invasive common procedures, yet at the same time most underrated procedures in a hospital setting (1,2). Although venipuncture is considered as safe and foolproof procedure for patients, several studies show that it carries inherent risks (3,4). Namely, despite the well-trained staff with the most sophisticated instruments in clinical laboratories, accurate analyses cannot be performed, unless biological

materials are adequately collected (2). When performing venipuncture without adhering to the recommendations of good phlebotomy practice, a number of complications can occur, with *in vitro* hemolysis being particularly common (3).

In vitro hemolysis affects the quality of analyte and can lead to incorrect interpretation of obtained results (5-8). Besides, it also requires re-sampling of blood, which both increases health care costs and causes unnecessary pain for patients (9-11).

Hemolysis in blood samples is usually a consequence of a number of factors, which can be classified as those occurring during venipuncture itself, during specimen transportation and while preparing the blood sample (3,9). Causes of hemolysis during venipuncture may include: extraction methods, materials used for venous access, needle size, arm position, vein selection, handling blood specimen, skills and abilities of those sampling biological materials, particularities of blood vessels in a patient and others. *In vitro* hemolysis can occur from errors in specimen transport associated with the position of the tube (horizontal or vertical), mode and time of transport, as well as a temperature (extremely high or low), while the characteristics of centrifugation and separation are the factors affecting occurrence during the preparation of the blood sample for laboratory analysis (3,7,9).

Since laboratory blood test results are crucial for 60-80% of medical decision-making, any error in the phlebotomy process could have serious consequences (12). Therefore, it is the phlebotomist's responsibility to ensure proper blood specimen collection and handling, which are essential to obtaining valid analyte for laboratory testing. (2,5). In member countries of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), nurses and laboratory technicians perform most of the phlebotomy procedures in primary health institutes, as well as in secondary and tertiary health institutions (13). For this reason it is important that they are familiar with the latest recommendations for blood sampling, since laboratory methods, and associated phlebotomy instructions are continually changing (13). It is also vital that they acquire the knowledge about possible causes of pre-analytical errors, such as *in vitro* hemolysis, as this can reduce error rates (14).

Phlebotomy is a complex procedure that requires theoretical knowledge and manual skills, as well as accuracy, responsibility and ability of the individual performing the procedure (2,15). Unfortunately, there is a limited number of studies into the quality of blood sampling by venipuncture and the level of phlebotomists' knowledge of the potential risks of this procedure (4,16-18). Therefore, it was

the aim of this study to assess the level of confidence of nurses and laboratory technicians with respect to performing venipuncture, as one of the indications of the acquisition of this skill, as well as evaluating their knowledge of the possible causes affecting *in vitro* hemolysis.

Materials and methods

Subjects and methods

This survey study was carried out in two tertiary health institutions in Serbia, in November 2014 and convenience sampling was used. The research included N = 94 healthcare professionals, of which N = 44 (0.47) were nurses and N = 50 (0.53) laboratory technicians who work in clinical departments and clinical laboratories. Healthcare staff who were unwilling to participate or had not performed venipuncture one month prior to the study were excluded.

Data collection in each medical institution took a period of three weeks. During the data collection period, the researchers personally collected completed questionnaires in sealed envelopes (provided by the researchers) from respondents. Of the total 105 questionnaires distributed, 94 (0.89) were fully and correctly completed and available for statistical analysis, whereas 11 (0.11) questionnaires were found uncompleted.

The implementation of this study was approved by the Ethics Committee of the Medical Faculty of the University of Novi Sad, and the administration of health institutions where the study was conducted.

Questionnaire

The instrument used for data collection was a questionnaire used by Makhumula-Nkhoma *et al.* (17). The questionnaire contains 18 questions and includes sets of questions divided into four sections.

The first section of the questionnaire refers to general information on research participants.

The second section of the questionnaire assessed confidence levels of healthcare professionals at

the first and the last performed venipuncture in the career. A five-point Likert scale from 1 to 5 was used for level of confidence evaluation, ranging from 1 - unconfident, 2 - less confident, 3 - neither confident nor unconfident, 4 - confident, and 5 - more confident. In answering the questionnaire respondents were able to select only one answer.

The third and the fourth section of the questionnaire refer to knowledge of the possible causes of *in vitro* hemolysis due to the materials used in venipuncture, venipuncture technique and handling blood specimens. As the questionnaire was copyrighted, permission was obtained from author and also from publisher (John Wiley and Sons Inc; License no. 3679291167626) in order to use and modify some of the items for our study based on the guidelines set by the World Health Organization relevant to the procedure for sampling blood: Best practices in phlebotomy from 2010 (19).

The questionnaire was translated from English and adapted to Serbian speaking area, with modifications made in the section relating to the knowledge of risk factors affecting *in vitro* hemolysis due to venipuncture technique. Two questions were added: one relating to the potential influence of 70% isopropyl alcohol or ethyl alcohol and the other relating to the impact of hematoma on the occurrence of *in vitro* hemolysis (Appendix 1).

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences for Windows, program version 19.0 (SPSS, Inc., Chicago, IL, USA). Data processing included descriptive and inferential statistics. Numerical characteristics were obtained by using the average values (arithmetic mean and median) and measures of variability (standard deviation and range), whereas attributive features were obtained using frequencies and ratios.

Knowledge of the possible causes of *in vitro* hemolysis was calculated and added up to a total score of 11.

The Kolmogorov-Smirnov test was used to assess the normality of distribution of all variables and Levene test for homogeneity of variance. Differences in frequency of attributive features were assessed using χ^2 test. Comparison of numerical val-

ues between the two groups was performed *via* independent-samples t-test, while a single factor analysis of variance (ANOVA) was used to compare means of three or more groups. The Tukey's *post hoc* test was applied for multiple comparisons. The P values less than 0.05 were considered statistically significant.

Results

Sample characteristics

Of the 94 healthcare professionals who participated in the study, N = 84 (0.89) were female, while N = 10 (0.11) were male. Most of the healthcare professionals N = 85 (0.90) completed secondary school, compared to N = 9 (0.10) with higher or university education. The average length of service was 12.6 ± 9.5 years, whereas the range in years of service was from one to thirty-nine years.

More than half of healthcare professionals, or N = 58 (0.62) reported that performing venipuncture was part of their daily routine and only N = 15 (0.16) reported they were performing it several times a week, while less than a quarter of healthcare professionals N = 21 (0.22) performed venipuncture several times a month. The difference of frequency in conducting venipuncture compared to the profile of health professionals was statistically significant ($\chi^2 = 23.979$, $P < 0.001$), whereby nurses were more frequently carrying out this procedure.

In total, N = 50 (0.53) reported that they completely mastered venipuncture technique and handling blood samples during their formal education, i.e. at school or college, prior to performing intervention themselves, while N = 44 (0.47) stated the opposite.

Despite the continuing legal education requirements, the majority of surveyed nurses and laboratory technicians N = 56 (0.60) did not receive an additional practical training in venipuncture technique and specimen handling as part of continuing education compared to N = 38 (0.40) of those who received an additional practical training in venipuncture technique.

Healthcare staff confidence level in venipuncture

Only three N = 3 (0.03) nurses and laboratory technicians reported being very confident when first ever performing venipuncture, while N = 78 (0.83) reported having high level of confidence at the last or most recently performed venipuncture (Figure 1).

Analyzing the confidence level at the first and at the last venipuncture in relation to the profile, it was found that there was no statistically significant difference ($\chi^2 = 7.636, P = 0.106$). Generally, confidence level among both profiles of healthcare staff was higher at the last performance of this procedure (Table 1).

Knowledge of possible factors affecting hemolysis in vitro due to materials used in venipuncture

Assessment of knowledge of possible factors affecting hemolysis *in vitro* due to materials used during venipuncture included four questions related to the use of: an evacuated tube, syringe, needle and a tourniquet.

Most respondents, N = 91 (0.97), answered positively that the tourniquet application for more than a minute contributes most to the occurrence of hemolysis, whereas N = 75 (0.80) considered forcing the blood into the tube by pushing the plunger in order to fill the tube as most frequent cause of hemolysis. Answers concerning the relationship of the needle gauge size to the appearance of hemolysis varied widely. Less than one-half of nurses and laboratory technicians N = 39 (0.42) indicated that the needle gauge size less than 18G is most closely associated with the occur-

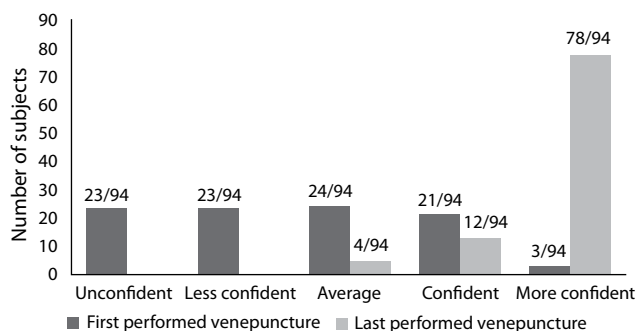


FIGURE 1. Healthcare staff confidence level at first and last performed venipuncture.

rence of hemolysis, while N = 27 (0.29) of them reported the needle gauge size > 22G. However, N = 26 (0.28) of respondents believe that the needle size has no impact on the occurrence of hemolysis. The use of evacuated system in blood sampling from the cannula as a possible risk factor for the appearance of *in vitro* hemolysis was reported by N = 11 (0.12) of respondents (Table 2).

Knowledge of potential factors affecting hemolysis in vitro due to venepuncture technique and handling of blood sample

Assessment of knowledge of the potential factors affecting hemolysis *in vitro* due to venipuncture technique and handling of blood sample included seven questions. Most of the respondents answered 6 of the questions correctly. However, respondents were divided in their opinion on whether 70% isopropyl or ethyl alcohol, which disinfects the skin, should be allowed to dry completely for up to 30 seconds. Namely, N = 55 (0.59) of respondents stated that *in vitro* hemolysis can-

TABLE 1. Confidence level at first and last venipuncture in relation to profile of healthcare staff.

Profile of healthcare staff	Number	Mean ± Standard deviation	
		First venipuncture	Last venipuncture
Nurses	44/94	2.4 ± 1.3	4.9 ± 0.3
Laboratory technicians	50/94	2.7 ± 1.1	4.7 ± 0.6

A five-point Likert scale from 1 to 5 was used for level of confidence evaluation, ranging from 1 - unconfident, 2 - less confident, 3 - neither confident nor unconfident, 4 - confident, and 5 - more confident.

TABLE 2. Knowledge of possible factors affecting hemolysis in vitro due to materials used in venipuncture.

Possible factors affecting hemolysis	The response rate	
	Number	Ratio
Sampling methods and hemolysis		
• Syringe and needle	46	0.49
• Cannula and syringe	30	0.32
• Cannula and evacuated tube*	11	0.11*
• Evacuated tube and needle	7	0.8
Tourniquet application and hemolysis		
• Less than or equal to one minute	3	0.03
• More than a minute *	91	0.97*
Needle size and hemolysis		
• Less than 18G	39	0.41
• 20–22G	2	0.02
• More than 22G*	27	0.29*
• None of the proposed	26	0.28
Syringe and needle and hemolysis		
• Forcing blood through a needle on a syringe*	75	0.80*
• Withdrawing the needle and pouring the blood from the syringe into the into the test tube	14	0.15
• A blood sample flows freely into the tube	5	0.05

*Correct answers.

not occur as a result of alcohol not being allowed to dry completely for up to 30 seconds, compared with N = 39 (0.42) of respondents who reported that this could be the cause of hemolysis. It was also noted that N = 71 (0.76) of health professionals stated that a venipuncture should be performed above a hematoma as compared to N = 23 (0.25) of those who stated that it should be punctured at the site below hematoma (Table 3).

Statistical analysis of response on the knowledge section was performed to score each of correct answers on the questionnaire with 1 point, so that the obtained scores fall within the maximum total score of 11. The participants obtained scores in the range of 3 to 11, where nurses' average score on

TABLE 3. Knowledge of factors that can potentially affect hemolysis in vitro including venipuncture technique and blood specimen handling.

Possible factors affecting hemolysis	The response rate	
	Number	Ratio
Blood flow and hemolysis		
• Turbulent*	78	0.83*
• Laminar	16	0.17
Blood collection site		
• Antecubital fossa (elbow pit)	13	0.14
• Distal to the antecubital fossa (elbow pit)*	81	0.86*
The number of attempts for venipuncture and hemolysis		
• One attempt	5	0.05
• More than one attempt*	89	0.95*
Alcohol and hemolysis		
• Yes	39	0.42
• No*	55	0.59*
Hematoma and hemolysis		
• In the area above the hematoma	71	0.76
• In the area below the hematoma*	23	0.25*
The rate of hemolysis in samples		
• Full-draw tube	15	0.16
• Half-filled greater than or equal half-full tube	3	0.03
• Less than half filled tube*	76	0.81*
Sample preparation and hemolysis		
• Specimen should not be mixed with anticoagulants	16	0.17
• Specimen should be inverted 5-10 times for proper mixing of the anticoagulants	5	0.05
• Specimen should be mixed with anticoagulants by strongly shaking the tube holding it in horizontal position*	73	0.78*

*Correct answers.

the knowledge test was higher and equaled 8.11 ± 1.7 , while lab technicians' was 7.4 ± 1.5 . The difference in average score was statistically significant ($P = 0.035$). Cohen's d (standard deviation units) was 0.4 indicating a difference in mean score at

medium level on the knowledge test among the observed healthcare professionals.

The association between level of knowledge and other variables

A statistically significant difference in mean score on the knowledge test has not been established in relation to the work experience; P = 0.581) and the frequency in conducting venepuncture; P = 0.095. Similarly, there were no statistically significant differences in mean score on the knowledge test in relation to the level of confidence in their own abilities at the last performed venipuncture; P = 0.551 (Table 4).

TABLE 4. Average score values of knowledge test compared to the length of service, frequency in conducting venipuncture and confidence level at the last venipuncture.

Variables	N	Score (mean ± SD)	P (ANOVA)
Length of service			
• up to 5 years	24	7.3 ± 2.1	0.581
• from 5 to 10 years	29	7.8 ± 1.6	
• from 10 to 19 years	22	7.9 ± 1.4	
• more than 19 years	19	7.8 ± 1.3	
Frequency in conducting venipuncture procedures			
• On daily basis	58	7.9 ± 1.6	0.095
• Several times a week	15	7.9 ± 1.3	
• Several times a month	21	7.1 ± 1.7	
Confidence levels			
• Unconfident	--	--	0.551
• Neither confident nor unconfident	4	7.3 ± 0.9	
• Confident	90	7.8 ± 1.6	

N - Number of participants

Discussion

There are many opportunities for *in vitro* hemolysis to occur in the preanalytical phase (7). Nurses and laboratory technicians must be aware of the possibility of complications, should they fail to fol-

low an accurate and standardized venipuncture procedure (13).

There are no significant differences in confidence level during venipuncture among nurses and laboratory technicians, whereas a significant confidence increase is noted at last performed venipuncture in both profiles, compared to confidence level at first ever performed procedure. Nearly identical results were obtained by Makhumula-Nkhoma *et al.* in a study that was conducted in the UK on a sample of 290 healthcare professionals (17). This increase could have a positive impact on reducing the rate of hemolysis of blood samples in clinical laboratories (19).

In our study, nurses had a higher level of knowledge on causes of *in vitro* hemolysis compared to laboratory technicians, and these results of the knowledge test may be explained by the fact that nurses who participated in the survey conducted venipuncture procedure more frequently than laboratory technicians. However, a statistically significant difference in mean score on the knowledge test has been determined by neither frequency in conducting venipuncture, nor work experience.

Also, the results obtained show that only 11/94 of health workers reported which method of blood sampling most likely contributes to occurrence of *in vitro* hemolysis. In fact, nearly one-half of them associated using a syringe and a needle, but not cannula and evacuated tube with higher rates of *in vitro* hemolysis. These results may be, on the one hand, explained by the fact that the majority of blood samples are today drawn using vacuum system in order to reduce rates of blood-borne infections, despite its negative characteristics (20). On the other hand, by the fact that taking blood samples through an intravenous catheter or cannula is applied mainly at the intensive care units or emergency departments (21). However, despite this fact, every nurse should know that combination of evacuated tubes and intravenous catheter or cannula is associated with a significantly higher rate of hemolysis than blood sampling performed by manual vacuum aspiration syringe (22). Also, the same misconceptions about the modes of

blood sampling were found among health care staff in the study conducted by Makhumula-Nkhoma *et al.* (17).

Majority of respondents knew the correct answer to almost every question on the potential factors of *in vitro* hemolysis due to venipuncture technique and specimen handling. Our respondents gave correct answers regarding turbulent blood flow into the test tube, blood collection below the antecubital fossa, more than one venipuncture attempt, and identified a small amount of blood in a test tube, as well as vigorous mixing or shaking of a specimen and anticoagulant as factors which affect appearance of hemolysis (9).

However, dilemma still remains whether failing to let disinfectant 70% isopropyl alcohol or ethyl alcohol dry completely for up to 30 seconds is a cause of hemolysis. The study results indicate that 39/94 respondents considered alcohol as a significant risk factor for the development of *in vitro* hemolysis. Their attitude could be explained by current recommendations for allowing 70% disinfectant alcohol to dry completely for up to 30 seconds prior to venipuncture, substantially prolonging the time of tourniquet placing, a well-known cause of hemoconcentration and consequently of hemolysis. According to Salvagno *et al.* (23), the amount of alcohol that could possibly be aspirated during venipuncture is not sufficient to cause hemolysis. Subject to further discussion on this issue remains whether the presence of alcohol at the site of venipuncture may be a cause of discomfort for the patient, assuming that the potential painful sensations (tingling) are caused by impaired skin integrity regardless of whether the alcohol is removed from the venipuncture site.

It was observed that as many as 71/94 healthcare professionals reported that puncture should be performed at the site above a hematoma which is not based on guidelines established by World Health Organization. In patients with a hematoma a venipuncture should not be performed in the area of a hematoma but distal to the hematoma. Hematoma contains decomposed red blood cells and if the puncture is performed on a hematoma or above, it can result in contamination of cellular blood components (19).

In order to minimize the possibility of obtaining unsuitable samples, phlebotomy procedures should be standardized, well-documented and written instructions should be available at every workplace. Two key documents are used as phlebotomy guidelines worldwide: CLSI (Clinical Laboratory Standards Institute) H3–A6 Procedures for the Collection of Diagnostic Blood Specimens by Venepuncture (CLSI, 2007); and WHO (World Health Organization) Guidelines on Drawing Blood: Best Practices in Phlebotomy (WHO, 2010) (12,19). Some EFLM member countries have designed national guidelines on venous blood sampling, unfortunately, Serbia is not one of them.

In the present study, despite the continuing legal education requirements, most surveyed nurses and laboratory technicians did not have additional practical training in venipuncture technique and specimen handling through continuing education training. This suggests the need for additional education and practical training through accredited seminars and conferences, in order to improve adherence to and compliance with phlebotomy guidelines (15), which are, according to the study conducted in 12 European countries by Simundic *et al.*, unacceptably low (18).

Results from this study add information to the body of knowledge on healthcare professionals' understanding of causes of *in vitro* hemolysis and could be important in evaluating the available service and preventing errors during phlebotomy. However, some limitations should be noted. The use of convenience sampling, drawn only from two hospitals in Serbia may limit the generalizability of the findings due to diversity in blood sampling practice and education among different countries.

Conclusion

Assessment of perceptions of potential risk factors affecting *in vitro* hemolysis and confidence level in conducting venipuncture is an efficient method to assess critical steps in phlebotomy. Periodical check of nurses' perceptions about risk factors for the development of *in vitro* hemolysis, using a questionnaire that was applied in this study, can

help laboratory managers to determine in which part of the phlebotomy process healthcare staff should receive education or training courses, which can lead to long-term improvements in patient safety.

Our study shows that most risk factors affecting occurrence of *in vitro* hemolysis during venipuncture were recognized by healthcare professionals. However, most of them did not recognize which method for blood sampling contributes most to the occurrence of *in vitro* hemolysis.

It is interesting to note that nurses showed a higher level of knowledge compared with laboratory technicians, although in clinical practice nurses regularly perform other interventions on a daily basis. These issues highlight the need for immediate consideration and improvement.

Potential conflict of interest

None declared.

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