QUALITY OF COLLECTED BIOLOGICAL SPECIMENS – THE INVISIBLE PART OF THE ERROR SOURCES IN HEMOSTASIS FOR THE CLINICIANS

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QUALITY OF COLLECTED BIOLOGICAL SPECIMENS – THE INVISIBLE PART OF THE ERROR SOURCES IN HEMOSTASIS FOR THE CLINICIANS (Abstract): The introduction of modern analyzers in the laboratories that carry out hemostasis tests has led to the devolution of the sources of error from the preanalytical phase, ensuring results with a high level of reliability. The identification of the non-compliant coagulation specimens due to events related to the biological product collection from the clinic are reported by the laboratories that make these determinations. Aim: To inform clinicians about these problems, so that through preventive actions we can ensure optimal clinical management of patients. Material and methods: Selective research were conducted in the national and international specialized literature. Inclusion criteria were studies presenting the prevalence and impact of preanalytical errors with the determination of hemostasis tests, as well as recommendations regarding the identification and rejection of the non-compliant samples. Results: In the study were introduced works that reported the sources of error which may influence the quality of the laboratory results. The errors associated with blood sampling were related to the samples with hemolyzed plasma, insufficient volume taken and the accidental presence of the clot in the erythrocyte sediment. Available literature data suggests that, out of the total of 75% of the errors identified in the laboratory, 26% can have harmful effects on patient’s care, which contribute to unnecessary investigations or to the inadequate treatment and implicitly, extension of hospitalization. Conclusions: Applying corrective measures can improve the quality indicators of the coagulation specimens and implicitly the quality of the patient’s health care by informing clinicians and using educational activities. Keywords: HEMOSTASIS, HEMOLYSIS, VOLUME, CLOT, EDUCATION.

Available literature data suggest that errors once appeared with the sampling of the biological product can have very serious consequences on the patients care, since the medical decisions are made in a proportion of 60 to 80% based on the laboratory tests results (1, 2, 3). Because the technique of sampling the biological product is an invasive and complex procedure, it is performed in the clinic and is considered a pre-analytical phase. This aspect represents a major error source in the testing process (4, 5, 6, 7).

In order to minimize possible errors on
the determinations, improvement of the analytical phase in the laboratory was desired by modernizing and automating the methods of coagulation tests determination, being able to provide very precise results (3, 8). In 2005, van Geest-Daalderop et al. in their study mentioned that oral administration of the anticoagulants with coumarin derivatives depends on PT/INR (prothrombin time/ international normalized ratio) results, evaluating the effect of preanalytical variables on test determination, including those related to the sampling and transport of samples (9).

In the national literature, in agreement with the international specialized literature, among the causes of rejection of the samples taken for the determination of PT and APTT (activated partial thromboplastin time) tests, are mentioned: insufficient volume taken, less than 90% (0.5 mL blood less) of the recommended volume (4.5 mL blood), intensely hemolyzed plasma and the whole coagulated sample, as described in (fig. 1) (10).

**Fig. 1.** Representation of the causes of rejection of the coagulation specimens (personal collection)

**OBJECTIVES**

The purpose of this study is to inform clinicians about the negative interference of error sources from the pre-analytical phase, with the determination of coagulation tests, to better understand the possible discrepancies between the results released by the laboratory during dosing and the monitoring of anticoagulant therapies. Emphasizing the need for educational activities in order to reduce the incidence of preanalytical errors that generate non-compliant blood samples.

**MATERIAL AND METHODS**

In order to investigate the topics of interest, it was chosen as a means of using existing data to review the literature. It was desired to use primary sources based on evidence, which used different methodologies and theoretical comments, on the effect of preanalytical factors on the samples taken for the determination of hemostasis tests.

To make a comprehensive approach regarding the negative impact of the sources of error from the pre-analytical phase on the determination of coagulation tests PT and APTT we performed a search on PubMed and Google Scholar.

**INCLUSION CRITERIA**

To select this information, we looked for original studies, reviewed by colleagues that describe the pre-preanalytical and post-postanalytical phases in the testing process. We considered the study protocols, conclusions, as well as the reported cases. This study has some limitations represented by the impossibility of approaching all articles dealing with preanalytical factors and the consequences on hemostasis tests. The level of the works number approached is quite small, but despite this fact, based on the selected studies, as well as the presentation of the conduct in our laboratory, we want to draw
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attention to the sources of error regarding the quality of the sample taken.

RESULTS
In our research, we were able to introduce studies that analyzed the changes occurred during the test process to determine PT test and APTT in the hemolysate samples, with insufficient volume taken and the accidental presence of the clot in erythrocyte sediment.

The selection of the most recent national and international studies (2014-2019) was abstracted using a table, starting from the design mode, objectives, methods of evaluation, sample size and conclusions; implicitly based on the keywords: pre-analytical, post-analytical, coagulation, errors, hemolysis, volume, clot, procedure, false, rejected, education, patient, clinician, nurse, laboratory medicine, trust; as described in first table.

TABLE I
Relevant studies included

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<tr>
<td>8.</td>
<td>Delianu C, Foia L, Dobreaunu M (43)</td>
<td>2017</td>
<td>The importance of medical personnel training in reduction of pre-analytical phase laboratory.</td>
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**DISCUSSION**

Favaloro *et al.* in their study mentioned that between 9 and 15% of errors are the result of a misdiagnosis, with a significant impact on the patients care, and the probability of inadequate care represents between 2-7% of such cases, agreeing with Plebani’s study (8, 11). Errors resulting from inadequate sampling, associated with improper handling and processing of samples, will reflect the status of the sample taken and will not accurately reflect the actual clinical status of the investigated patient, being considered pre-analytical variables (8).

In 2016, Magnette *et al.*, point out that the ratio of blood taken and Na citrate of (1:9) for coagulation tests, (12) must be respected, corresponding to 0.5 mL anticoagulant and 4.5 mL blood, quantities recommended in the national literature since 1959 (13). A volume of blood taken less than 89% of the recommended amount (0.5 mL blood missing) interferes with the determination of the APTT test, which is clinically significant, and a volume of 78% (3.5 mL blood taken) affects fibrinogen determination, and less than 67% (lacking 1.5 mL of blood) from the recommended volume affects factor VIII (Antihemophilic A) determination, being in agreement with the subsequent studies (7, 12, 14, 15). In our laboratory, in cases where the sample has a lack of 0.5 mL blood, in order to inform the clinician, the non-compliance is explained textually, once the result is released, with the following comment: “Sample with insufficient volume taken, lack 0.5 mL of blood; possible interference with the determination”. Leblanc, Ellouze and Guermazi recommend rejecting the sample, if the vacutainer has a volume of less than 80% (1 mL of blood is missing) (16, 17). In accordance with these recommendations, we reject the determinations made in these samples, explaining to clinician the non-compliance: “Repeat the sampling. Sample with insufficient volume taken, 1 mL of blood is missing”.

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<td>15.</td>
<td>Freitas F (18)</td>
<td>2015</td>
<td>What’s new about sample quality in routine coagulation testing?</td>
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In medical laboratories, the receipt of samples with plasma hemolysis is a common phenomenon, representing a reason for refusing the determinations (8, 18, 19). Some authors believe that this rejection creates discomfort for the patient, delayed outcomes and additional laboratory costs (19, 20). Most of the time, in the laboratories carrying out hemostasis tests the interpretation of the in vitro hemolysis, is performed post-centrifugation, by visual examination (21). From a technical point of view, plasma hemolysis can be classified according to the hemoglobin concentration in the supernatant:

- a) non-hemolysis (0.05 g / L, yellow)
- b) slightly hemolyzed (from 0.05 to 0.3 g / L, toned yellow to light pink)
- c) medium hemolyzed (0.3 to 0.6 g / L, pink to slightly red)
- d) hemolysis (0.6 to 2.0 g / L, slightly red)
- e) intensely hemolysis (2.0 g / L, red to brown), as exemplified in (fig. 2) (21, 22).

As the assessment is done by the laboratory assistant who prepares the samples for the analysis itself, it would be ideal to use color diagrams for the correct interpretation of the degree of hemolysis (22).

![Color diagram for in vitro hemolysis detection.](image)

The numbers indicate the degree of hemolysis expressed in mg/dL (22).

Favaloro et al. draws attention to cell lysis products that include tissue factors which activate coagulation, and, as hemolysis increases, fibrinogen levels may decrease, D-dimers increase, while APTT may increase or decrease. Through the activation effect, PT test values may decrease depending on the fibrinogen decrease (8). Laga et al. and Arora et al. concludes on the basis of the research carried out on the samples of the healthy volunteers, that the differences of the results were insignificant, and the statistical difference found does not have clinical significance. This hypothesis is safe if the result falls within the normal reference range, with no certainty for the results at the lower or upper limit (20, 23).

Using different reagents for test determination, Woolley et al. observed no correlation between variations in results and level of hemolysis (24). Wan Azman et al. recommends standardizing the interpretation of hemolyzed plasma samples and drawing up a laboratory guide, thus providing essential information to clinicians (25). However, the results being controversial, further studies on larger groups are needed to determine the effect of hemostasis (19). Rejecting the samples with hemolyzed plasma being considered inadequate, it is recommended to add a brief comment that suggests doubt about the interpretation of the results and their benefits for the patients care (26, 27,
In accordance with these recommendations, in the case of hemolyzed plasma samples, we inform the clinician, releasing the result with the following comment: “Hemolyzed plasma sample, possible interference with the determination”.

Current analyzers for coagulation tests determination can detect in the pre-analytical phase fibrin, foam, hemolysis plasma, as well as insufficient blood volume is taken (29), without being able to detect the accidental presence of the clot in the erythrocyte sediment, as described in (fig. 3).

**Fig. 3.** Detection of fibrin, foam, hemolysis in the supernatant, the insufficient volume is taken and clot in the erythrocyte sediment (personal collection)

Total blood coagulation in vitro (fig. 1) is caused by the loss of cellular elements, as well as the factors such as: fibrinogen, prothrombin, proaccelerin, anti-hemophilic A, and differentiated loss of von Willebrand factor, leading to the serum formation (8). Determination of hemostasis tests from partially coagulated samples, as a consequence of the clot presence in vitro (fig. 3) may result in false shortening or prolongation of coagulation tests, because the differentiation between plasma and serum is difficult to perform post-centrifugation by visual inspection (8, 28).

In our laboratory, starting with 2014, in order to exclude or confirm the presence of the clot in the erythrocyte sediment during the test, we also re-verify post-analytically by transvasation (fig. 4) (30, 31) the samples with hemolyzed plasma, in agreement with Favaloro et al. as well as the samples from which false or possibly false tests are obtained (8). In the case of clotting, the results are canceled and recommended sampling repeat, textually explaining the non-conformity: “Sample with clot. Repeat the sampling”.

**Fig. 4.** Detection of the clot by transvasation (personal collection) (30, 31)
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Clinical departments ensure the selection and tests recommending, sampling, handling, transport and preparation of samples for analysis, a process that begins and ends with the patient, that’s why we can consider that patients are also part of this team (32). It is important to maintain total control of the testing process in a systematic and transparent manner, encouraging and promoting errors identification, because, if the team fails, patients will suffer the most (32, 33). For patient’s safety, nurses must be aware of the influence and possible consequences of preanalytical errors on laboratory results (32).

The recognition of these aspects is a challenge for the nurses who have an important role in the handling and sampling, an activity that should be considered as a necessary competence, and not just a technical trick (35). Being in agreement with previous studies (8, 17, 20, 34), from 2015, we pay special attention in the identification and monitoring of non-compliant samples (35), which represents error source for determining coagulation tests: insufficient volume has taken (lack of 0.5 mL blood), plasma hemolysis, as well as the presence of the clot identified in erythrocyte sediment post-analytical. In this way we can minimize the effects of biological factors that might have a significant impact on patient care.

It is necessary to train the nurses during the service, as well as to introduce in the syllabus the basic theoretical aspects regarding correct sampling technique of the biological product (32). Makitalo and Liikanen state that, through multi-professional cooperation, involving laboratory staff, the number of errors in the pre-analytical phase could be reduced (32). Guder in his article (36) mentions that the term preanalytical phase was included in textbooks in 1983 (37), and in the teaching books of Laboratory Medicine in 1987 and 2005 (38, 39). The lack of a standardized training program and periodic skills assessment has an impact on the quality of the results (40, 41, 42). Crous and Armstrong believe that errors during sampling can produce negative outcomes for the patient, which can vary from financial implications to death, according to the study of Al-Ghaithi et al. (41, 42).

In 2017 we presented the importance of the laboratory specialists involvement in errors reducing from pre-analytical phase through a training based management and acquired knowledge verification of the medical personnel (43), being in accordance with Al-Ghaithi’s study (44), continuing in 2018 with a study, by which we drew attention to the false hypercoagulability in vitro, as a result of highlighting the error sources regarding the quality of the sample taken (45).

The focus of patient care, accepted on internationally level, changes the responsibility and delivery of healthcare (45). Medicine laboratory through a tripartite collaboration with clinicians and patients who are the beneficiaries of these services should include this change (46). The involvement key is enhanced by better communication, knowledge management, information disseminations, and educational support (46, 47). In the paper presented by Wu et al., one of the 10 topics addressed refers to communication with clinicians and to laboratory role in educating clinicians about preanalytical and analytical influences on outcomes (48). Highlighting the importance of these errors is necessary for the clinician, who decides which tests to recommend in the pre-preanalytical phase, following by post post-analytical to decide the
appropriate treatment based on the laboratory results received (49). These aspects are supported by available literature data regarding incorrect diagnosis associated with laboratory test errors (50, 51).

CONCLUSIONS
We wanted to emphasize through this work, that evaluation of medical services quality in laboratories has become more and more important, both for the pressures exerted to reduce the additional costs due to the repetition of the determinations, but also for the evidence of the wrong diagnosis associated with the error sources tests.

The impact in the case of false results, generated by the interference of the error sources with the determination of the coagulation tests, can be especially serious because, in the case of specialized hemostasis tests, these are often considered “diagnostic”. That’s why we consider being very important identifying error sources before the results are released to the clinician and before to became a real harm for the patient's life.

Establishing a permanent link between clinicians and laboratory specialists would be useful by combining the unique talent of knowing the physiological reason behind the tests and conducting quality laboratory analyzes.

In order to ensure the quality of hemostasis exploration, we strongly support the creation of an initiative for developing the continuous educational programs for health professionals, representing also a priority for medical institutions.

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