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SHORT COMMUNICATION

Blood Clot Identification Procedures in Clinical Laboratory Sampling

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SUMMARY

Background: **Monitoring of anticoagulation therapy is based on screening tests: prothrombin time (PT) and activated partial thromboplastin time (APTT). The accidental presence of a clot in the coagulation samples determines a false prolongation of PT by fibrinogen (FI) consumption and the false or delayed prolongation of APTT, depending on FI consumption or activation. The purpose of this study is to document from the present data regarding procedures used to exclude the accidental presence of clot in the sample.**
Methods: **For a more efficient approach, we conducted a study based on research from the main databases that included original and peer-reviewed studies.**
Results: **We have reported studies in which pre-analytical procedures have been recommended and studies that have also presented post-analytical protocols. A correlation between the efficiency of the procedures in terms of additional laboratory costs has been performed, as well.**
Conclusions: **Focusing on patient safety, it remains a continuous challenge for each laboratory to be able to establish its own pre-analytical and post-analytical procedure for highlighting accidental clot presence, thus ensuring provision of results with maximum confidence to the clinicians.**
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INTRODUCTION

Hemostasis is one of the disciplines of biology that is totally dependent on the pre-analytical phase in the clinic, starting with patient preparation, biological product collection, manipulation and, last but not least, specimen transport [1]. This phase is considered to be the most vulnerable part of the testing process [2], as it contains many manual activities, sources of non-interchangeable or inaccurate results [1] which in turn, could be reduced by standardizing the pre-analytical stage [3-5].
Quality management in laboratory medicine is valuable due to its contribution to reducing diagnostic errors [6]. Although sustained efforts are being made in this re-

spect, nonconformities continue to occur as frequently as in the past [7], accounting for 70% of laboratory errors, thus hampering the appropriate patient care and increasing their morbidity and mortality [8]. Lima-Oliveira and colleagues, referring to previous studies, mentioned the association of the extra-analytical phase with the "dark side of the moon" in laboratory medicine, which is still unknown and often overlooked by quality assurance specialists [9-12].

The results released by medical laboratories play an important role in making therapeutic decisions and establishing a diagnosis, based in particular on specialized tests [9], which must provide efficient care, being correlated with the patient's clinical condition [6].

Monitoring of oral anticoagulant therapy is based on PT (prothrombin time) and APTT (*activated partial thromboplastin time*) test results, which should provide patient safety [13]. One of the essential conditions in the laboratories performing coagulation tests is the quality of the collected sample, which is proved by the lack of fibrin in the supernatant, the clot in the sediment, and a non-hemolyzed plasma [1]. Referring also to previous research, Hernaningsih and Akualing in their study concluded that the policy of rejecting samples of hemolyzed plasma should be reconsidered, suggesting the need for additional research on a larger sample scale and taking into account coagulation factors that decrease as a result of hemolysis [14]. Regardless of the period of collected data, our findings showed that whether a clot is suspected or identified, the rejection recommendation was maintained [15], indicating sampling repeat.

Each laboratory has to establish its own policy in relation to the procedure used in order to document the presence of the clot in the sample. The purpose of this study is to present the procedures used to highlight the accidental presence of the clot. The objectives envisage identification of the recommendations from the literature, based on the preclinical studies in which both pre-analytical and post-analytical procedures followed.

MATERIALS AND METHODS

We conducted a search on PubMed, Google Scholar, and Cochrane to adjust a comprehensive approach to pre-analytical and post-analytical procedures recommended and used in laboratories performing PT and APTT coagulation tests. To illustrate identification procedures, we used word-drawn pictures and pictures that capture platelet aggregates, fibrin and clot when examining a venous blood smear. We explored the most recent studies describing the incidence of clot specimens, the manner it is formed, and the procedures used to identify the clot. We took study protocols, reported cases, and opinion polls into consideration.

RESULTS AND DISCUSSION

During our research we have been able to find articles and laboratory guides that explain how to prevent clotting *in vitro* [16-20]. We have also taken into account the studies that mention the negative impact of fibrin on the results and implicitly on the analyzers [21]. There have been studies in which the deficiencies in the pre-analytical use of verification procedures were reported [18].

The accidental presence of clot or fibrin represents an argument for specimen rejection [7,15,17,22] due to subsequent clinical impact on patient care. Clot removal is not recommended [17] because performing determinations from these samples generates errors in the results.

The quality of the testing process depends entirely on the pre-analytical stage that can be improved by continuing education activities for the medical staff [23]. Prolonged tourniquet applied under difficult conditions, traumatic maneuvers with the intravenous needle may predispose to clot or fibrin formation *in vitro*, or partial or total coagulation of the sample [7]. For blood collection 21G needles are recommended [24] and for special patients from oncology, geriatrics or pediatrics departments, 22G and 23G needles are recommended [25]. In order to avoid clot formation, a complete distribution of the anticoagulant is provided by 3 - 6 tube inversion [1, 26-29] maneuver to be done very gently. Incomplete homogenization between the collected blood and the anticoagulant can lead to partially coagulated samples [7]. The insufficiently sampled blood volume, less than 90%, is a cause of rejection of the sample, considered as a cause of fibrin or clot formation [16], in the case of fibrin, being visually identified post-centrifugation. Studying the effect of pre-analytical variables on PT assay, van Dongen-Lases and colleagues recommended the transport of the samples vertically, considering that horizontal transport, overstimulates coagulation, by mechanical agitation, a phenomenon whose mechanism could not be explained at that time [5]. This recommendation was also taken up by adding the importance of transport in the shortest possible time [1], since any delay may induce plasma protein degradation *in vitro* [2]. In critical situations that are strictly related to the condition of the patient, if samples do not meet the quality criteria and the determinations required by the clinician, the report results of the analyses must be accompanied by a textual explanation describing the noncompliance and possible interference with the determination, indicating that the result cannot be validated.

In 2006, American pathologists recommended visualization of the clot by the slight inversion of the specimen (Figure 1), a procedure used today by most laboratories [30].

The second recommended procedure of the same period refers to the use of two wood applicators inserted into the sample before or after centrifugation (Figure 2) in the case of unexpected results [7,18,22,27,30]. Howev-

Figure 1. Clot identification before centrifugation by inversion (personal collections).

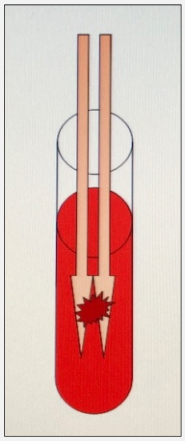

Figure 2. Wood applicators for clot identification (personal collections).

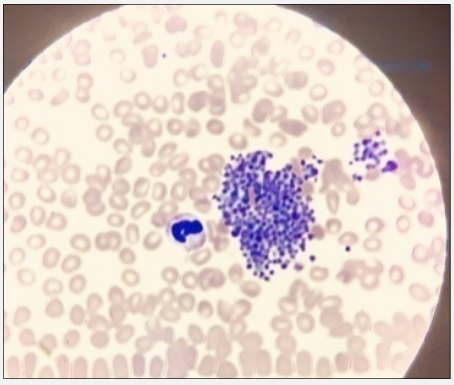

Figure 3. Platelet aggregates on venous blood smear (personal collections).

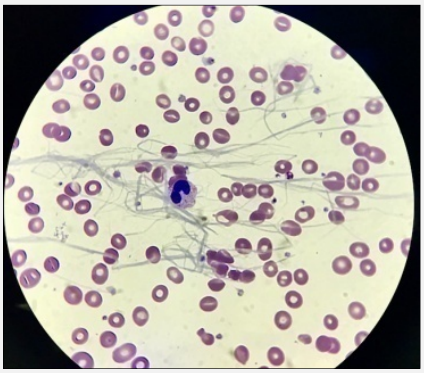

Figure 4. Highlighting fibrin under the microscope (personal collections).

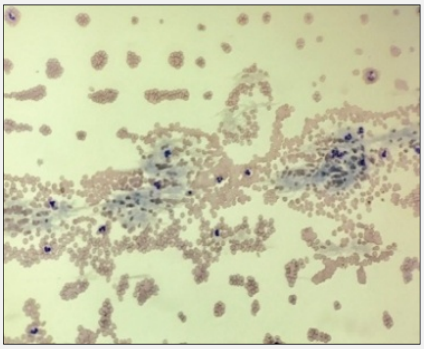

Figure 5. Highlighting the clot under the microscope (personal collections).

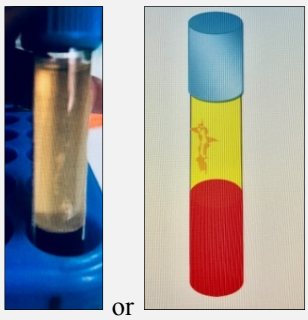

Figure 6. Fibrin in the supernatant identified post-centrifugation (personal collections).

Figure 7. Post-analytical transvasation procedure (personal collections).

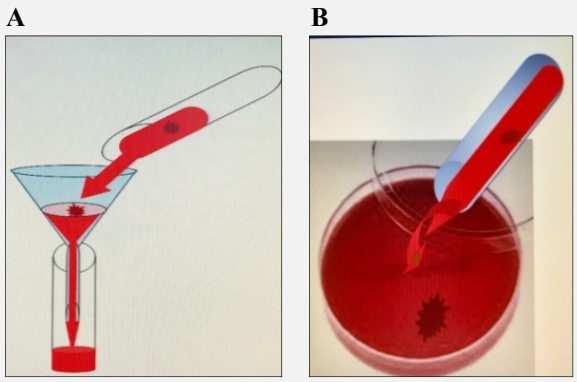

Figure 8. (A) Blood transvasation from the primary sample through a plastic funnel B) Use of Petri dishes to evidence the clot in the sample sediment (personal collections).

er, used before centrifugation it determines cell lysis [18], and through circular motion in order "to search for the clot", it accelerates coagulation factors and, implicitly the platelets, similar to the negative effect recorded through vigorous agitation or excessive vibration [1,4, 7,28,31-33].

The American College recommends highlighting the platelet aggregation through the microscope (Figure 3), the fibrin (Figure 4) or clot (Figure 5), by displaying a venous blood smear [30], stained May-Grunwald-Giemsa.

Starting from this remodeling, we believe that the procedure is really effective, but it involves additional costs for the lab and inevitably for the hospital with regard to the purchase of the lamellae and related dyes, and most importantly, the delay in the results delivery to the clinicians, especially in biopsies or surgical emergencies, due to the time spent on displaying, coloring, drying, and last but not least, examining the microscope smear. It is also possible that the drop of blood in the sample used to show the smear does not contain fibrin (Figure 4) or, most importantly, the clot generated *in vitro* to highlight coagulation plasma cellular elements (Figure 5).

The first step in the macroscopic identification of fibrin in the sample supernatant is the one after centrifugation (Figure 6), an important point as its presence in the plasma can cause a block of the analyzer capillaries in the aspiration area [17,21] and, last but not least, interfere with test determinations, affecting the results [21]. Favaloro et al. recommended the use of electromagnetic or mechanical methods for clot detection in hemolyzed

plasma samples [22]. Although modern analyzers for complete blood count (CBC) and coagulation tests are provided with additional volume and clotting sensors [7,22], or use graphical representation on the histogram [30], there are situations where fibrin or clots are not aspired together with the blood to be analyzed and therefore, cannot be identified, thus pointing out the need for an upgrade of the highlighting methods.

Paying particular attention to the reliability of the results delivered to the clinicians, starting with 2014, we implemented post-analytical transvasation procedure in order to reveal the clot in the sample, when the results obtained do not correlate with the evolution of the patient's paraclinical profile (Figure 7).

For this maneuver, plastic tubes without anticoagulant or gel, called "control" are required, in which the blood of the specimen suspected to be non-conforming is poured out, through gentle inclination. On the same principle, Petri dishes (Figure 8A) or small plastic funnels provided with a paper filter or gauze (Figure 8B) may be used as control, instead of the tubes, in order to retain the clot from the transvasate blood specimen.

The main disadvantage of using Petri dishes is given by the aspect of blood recovery after verification, in the primary specimen, a maneuver involving possible contamination with the biological product, the blood. When using the paper filter or gauze funnel, it will be too long to apply the filter, transvasation and blood recuperation from the test tube to the primary vacutainer. The use of plastic objects has some advantage also, as they are disposable and can be discarded in boxes for the retrieval and neutralization of contaminated waste.

Not to be neglected are the additional costs that involve the purchase of test tubes, Petri dishes and plastic funnels for the above-mentioned procedures.

However, performing determinations from partially coagulated samples leads to the release of false results in case of anemia, bicytopenia or pancytopenia depending on the consumption degree of the cell elements for the determination of the CBC [7,22,34,35]. In the case of coagulation determinations from samples containing fibrin or clots, depending on plasma factor consumption, false prolonged time can be recorded.

These costs should be considered unequivocally insignificant in relation to the patient's life and care, given the efforts made at national and international levels to identify and reduce errors in the above-mentioned determinations, as well as the clinically negative implications on the recovery of the subject's health status. Nonetheless, the additional costs allocated to ensure the proper functioning of the analyzers are also not to be neglected.

CONCLUSION

It remains challenging for each laboratory to be able to settle upon its own pre-analytical and post-analytical identification of the presence of an accidental clot in the sample used for coagulation tests, complete blood count, and erythrocyte sedimentation rate (ESR) determinations. In this context, the presence of a clot in the sediment of the sample may produce false results of hypercoagulability or hypocoagulability depending on the partial or total consumption of coagulation factors (I, II, V, and VIII), leucopenia, anemia, thrombocytopenia depending on the consumption of cellular elements, such as leukocytes, erythrocytes or platelets. Results related to a false inflammatory process in the case of a false elevated ESR test can arise as well, as they cannot be correlated with the real clinical condition of the patient.

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All authors contributed equally to this work.

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Declaration of Interest:
The authors declare no conflicts of interest.

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