Editorial

Primary blood tubes mixing: time for updated recommendations

Giuseppe Lippi and Mario Plebani

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Due to spasmodic efforts made by international organizations (1, 2), national and international working groups on quality in laboratory medicine (3, 4) as well as from the activities and publications of several independent research teams (5–7), the quality throughout the total testing process has dramatically increased over the past decades, especially in the manually intensive activities of the preanalytical phase that still represent the most vulnerable steps in laboratory diagnostics (2, 5–7). Reliable data attest that the errors in the pre-preanalytical phase (8) are still prevalent and most of these involve unsuitable or mishandled procedures for collection of the specimens. Although major focus is increasingly placed on accurate patient (and tube) identification and use of appropriate blood collection tubes (9), the most suitable procedures for handling of blood specimens have been somehow underestimated or even completely overlooked. Essentially, two major sources can be searched to gather reliable information on this topic, i.e., the consensus documents of the Clinical Laboratory Standards Institute (CLSI, former NCCLS) and the specific manufacturer’s recommendations. In both cases, it is typically recommended that any blood sample should be gently mixed immediately after collection, preferably by 4- to 6-time inversion. This has long been considered the most conservative procedure for appropriate mixing of blood and additives (either pro- or anti-coagulants), and thereby preventing spurious test results on otherwise unsuitable specimens.

Over the past few years some papers have however assessed whether this general recommendation is suitable for any category of specimen (i.e., serum, whole blood EDTA, heparin or citrate plasma), as well as for any type of test. As specifically concerns hemostasis testing, Lippi et al. previously showed that the mixing between blood and buffered sodium citrate that occurs during blood collection, may be still adequate to allow reliable results of activated partial thromboplastin time (APTT), prothrombin time (PT), fibrinogen and D-dimer, provided the tubes are adequately filled (10). An identical conclusion was reached for PT/International Normalized Ratio (INR) in another independent study by Chuang et al. (11). Lippi et al. also investigated the influence of primary tube mixing on hematological testing and in particular, the minimal amount of inversions required for the primary specimens immediately after collection. Interestingly, results on unmixed specimens revealed a significant decrease of red blood cell count (RBC), hemoglobin, hematocrit and platelets count, whereas the mean platelet volume (MPV) was significantly increased when compared with a reference specimen, inverted 6 times (12). However, in no case, these differences exceeded the analytical quality specifications, so that it was again concluded that the degree of mixing that occurs during sample collection would still be sufficient. Along with this findings, it has been reliably shown that no production of thrombin occurs in blood tubes containing anticoagulants and left unmixed for up to 2 min after drawing, whereas a modest thrombin generation may occur in less than one-third of the samples when these are left unmixed for 3 min or more (13). Although the modest amount of thrombin generated may still cause a modest increase of some blood coagulation markers (e.g., thrombin-antithrombin complex), most clotting and platelet function tests are negligibly biased. It can hence be concluded that only samples destined to more sensitive tests (e.g., ADP-induced platelet aggregation) should be collected and mixed with the anticoagulant within 2 min after venipuncture.

In a previous issue of Clinical Chemistry and Laboratory Medicine, we published an interesting article by Anna Parenmark and Eva Landberg (14), who convincingly confirmed that: (a) mixing blood samples immediately after collection may be not mandatory for all types of tubes; and (b) instant mixing may produce spurious hemolysis and thereby introduce a bias for those parameters that are most susceptible to RBC injury. This latter aspect is particularly relevant for at least three main reasons. First, in vitro hemolysis is the most prevalent preanalytical error across countries, healthcare facilities and categories of clinical laboratory (15–20). Several causes have been traditionally associated with an increased rate of spurious hemolysis, including difficult venipuncture, use of inappropriate devices, underfilling of blood tubes, exposure to extreme temperatures and centrifugation at a too high speed of partially coagulated specimens. Among these causes, excessive shaking or mixing of blood after collection (i.e., for times longer than recommended or with great forces) has also been acknowledged as a leading source of RBC injury, but no previous evidence has been provided that instant and gentle mixing may yet increase hemolysis in venous blood samples. The second take-home message from the article of Parenmark and Landberg is more practical. The prevalence of hemolytic specimens is increasingly considered a reliable index for assessing preanalytical quality (21–23). The evidence that the
standard procedures for sample handling may still generate hemolysis, calls for an urgent revision of this policy, in that a certain number of unsuitable specimens may still be produced, while strictly following what is currently considered the “best practice”. Finally, the data provided by Parenmark and Landberg, as well as that provided in previous studies (9–12), demonstrate that large, well-designed studies, based on the most possible types of tubes or anticoagulants and performing the largest possible number of tests, are required for updating the current recommendations for primary blood tubes handling immediately after collection.

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**References**


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Giuseppe Lippi*
 Mario Plebani**

*Clinical Chemistry and Hematology Laboratory, Department of Pathology and Laboratory Medicine, Academic Hospital of Parma, Parma, Italy
**Department of Laboratory Medicine, University-Hospital of Padova, Padova, Italy

*Corresponding author: Prof. Mario Plebani, CCLM Editor-in-Chief, Department of Laboratory Medicine, University-Hospital of Padova, Via Giustiniani 2, 35128 Padova, Italy
Phone: +39 0498212792, Fax: +39 049663240, E-mail: mario.plebani@unipd.it