this SNP. Furthermore, in more than 50 nondeletional Hb H disease patients of Thai origin, we have not encountered any allele dropout of the α-globin gene when performing the 7-deletion multiplex PCR assay. It is likely that this is a rare SNP among these 2 ethnic populations.

The present report underscores the importance of always correlating clinical and hematologic data, and family study if available, with DNA-based diagnostic results to arrive at the correct globin genotypes. Although the discrepancy is obvious when a molecular diagnostic result is incompatible with life, such as when a molecular diagnostic result is assigned to a patient with a nondeletional α-thalassemia.

References


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Influence of Centrifuge Temperature on Routine Coagulation Testing

To the Editor:

A standardized procedure for specimen collection is essential to achieve accurate and reliable coagulation measurements (1). Efforts have been made to identify the most suitable procedures for collecting, handling, and storing specimens for coagulation testing (2–6), including a recommendation that samples be centrifuged at room temperature (7). There is no definitive evidence, however, that centrifugation at alternative temperatures might significantly influence results of coagulation testing.

A single practiced phlebotomist collected blood from 12 healthy volunteers and 13 consecutive patients on oral anticoagulant therapy directly into three 4.5-mL siliconized evacuated tubes containing 0.5 mL of 0.105 mol/L buffered trisodium citrate (Becton Dickinson), using a 20-gauge, 0.80 × 19 mm Venoject multiscanple straight needle (Terumo Europe NV). Venipunctures were performed in the morning of the same day on fasting patients, who had given explicit informed consent. Tourniquet placement was <20 s, and all tubes were from the same lot. Venous accesses were straightforward in all cases, and no hemolyzed or lipemic specimens were encountered.

The tubes from each patient were gently mixed, pooled, and finally divided into 3 identical 4-mL aliquots. The 3 aliquots were centrifuged at 1500 g for 10 min at 4 °C, 12 °C, or room temperature (21 °C). We performed the 3 centrifugation procedures sequentially on the same centrifuge (Varifuge 3.2RS; Heraeus Instruments) and verified the effective correspondence between theoretical and operative temperatures.

After centrifugation, plasma was separated and immediately analyzed. Activated partial thromboplastin time (aPTT), prothrombin time (PT), and fibrinogen measurements were performed on the Behring Coagulation System (Dade-Behring) with proprietary reagents (Thromborel S for PT, Pathromtin for aPTT, and Multifibren U for fibrinogen determinations, respectively). We measured plasma D-dimer with Vidas DD, an ELISA with fluorescent detection, on the Mini Vidas Immunoanalyzer (bioMerieux). We performed all measurements in duplicate within a single analytical session and calculated the mean value of all results. Analytical imprecision, expressed in terms of mean interassay CV, was given by the manufacturers as 2%-5%.

The significance of differences between samples was assessed by paired Student t-test, and statistical significance was set at P <0.05. We used Bland–Altman plots to evaluate differences between results obtained by independent analyses of samples centrifuged at either 4 or 12 °C and results obtained for the 21 °C sample (8). We observed no statistically significant differences for PT, aPTT, fibrinogen, or D-dimer (Table 1). Linear regression analysis yielded satisfactory regression parameters and high correlation coefficients. Mean percentage variations from the
21 °C specimen were 0% for PT (both 4 and 12 °C samples) and ranged from 0.5% (4 °C sample) to −0.3% (12 °C sample) for aPTT, from −0.6% (4 °C sample) to −0.3% (12 °C sample) for fibrinogen, and from 0.1% (4 °C sample) to −2.5% (12 °C sample) for D-dimer; thus, the differences were always within the current analytical quality specifications for desirable bias derived from biological variation (9).

Pitfalls related to cold activation of blood samples in coagulation testing have been reported, in particular for assays of activated factor VII, factor VIII, and von Willebrand factor (4, 10). Although current Clinical and Laboratory Standards Institute guidelines recommend room temperature centrifugation for whole-blood specimens designated for coagulation testing, insignificant PT variations were observed for refrigerated centrifuged blood or samples with no temperature control during centrifugation (3, 5). No definitive data are currently available, however, on the effect of different centrifuge temperatures on results of aPTT, fibrinogen, and D-dimer testing. The present investigation suggests that centrifugation of whole-blood specimens at temperatures different from those currently recommended is not likely to generate significant analytical or clinical biases. We did not evaluate the influence of centrifuge temperature on second-line coagulation testing, such as von Willebrand factor and clotting factors VII and VIII. We therefore cannot rule out that cold activation of these proteins might occur, particularly at very low centrifuge temperatures, as observed previously (4, 10).

References


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