EDITORIAL

What's New About Sample Quality in Routine Coagulation Testing?

Francisco Freitas

Serviço de Pat. Clínica, Centro Hospit. Tondela-Viseu, Escola Sup. de Saúde Dr. Lopes Dias, Inst. Politécnico de Castelo Branco

Today, laboratories are very much different than they were decades ago. They have improved instruments to work with, multiple off-site collection points, more competent personnel, a greater analyte panel and quality requirements for those analytes. Nevertheless, sample quality has to be assured along the total testing process (TTP) in order to guarantee reliable results to the patient. In routine coagulation, prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (Fbg) and D-dimer are the essential part of the test panel, for which new evidences about sample quality requirements have been presented in recent years.

What's new about patient preparation?

To fast or not to fast is a much discussed topic in laboratory medicine these days (1). Apart from those discussions, we must take in consideration that reference intervals and biological variation data are derived from fasting individuals (2), and so patient test results should in theory be compared in the same manner.

In hemostasis, the most recent evidence (3), have shown that a standardized morning light meal has no clinical significant influence on test results (PT, APTT, Fbg, AT, PC and PS) as compared with reference change value (RCV). Despite that, significant statistical lipemia interference was observed after one hour for several analytes (APTT, PT, AT and PC), even though it wasn't detected by visual inspection. As laboratories have no control over what people eat in the morning, and the great majority of test requests also include other tests with fasting requirement, the wisest move is to instruct patients to fast.

In clinical settings such as emergency department or oral anticoagulant therapy (with blood collection after lunch), clearly lipemic samples are more prone to occur and leading to erroneous results (4). What to do in such occasions? To postpone the analysis and collect a novel sample later or in the fasting state is always the best option. Where this is not feasible, interference removal can be attempted, either by ultracentrifugation (5), dilution or extraction, each one having its drawbacks (4,6).

What's new about sample collection?

Phlebotomy is a highly variable procedure, because it depends on many factors such as the patient condition, the more or less controlled setting of execution (outpatient *vs* inpatient *vs* emergency departments), the skill of the healthcare professional, and the material choice (evacuated *vs* non-evacuated tubes; straight needle *vs* butterfly devices *vs* IV starts).

One of the main questions frequently arising in the moment of phlebotomy is the tube order of draw. Currently, CLSI GP41-A6 - *Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture* (7), recommends that coagulation tubes should be drawn after blood culture bottles (if they are requested) and before non-additive tubes and clot activators (for serum), sodium heparin, lithium heparine, EDTA, acid-citrate-dextrose and oxalate/fluoride tubes (for whole blood). This so called "order of draw" has been established to prevent test result errors due to cross-contamination from tube additives.

However, Indevuyst *et al* (8) have found no significant influence at least for PT/INR and APTT values (measured in Sarstedt S-monovette tubes), if the citrate tube is obtained after a heparin, EDTA or a serum tube with clot activator. In a similar line of investigation, Salvagno *et al* (9) demonstrated

that chemistry parameters (potassium, sodium, calcium, magnesium, and Phosphorus) that are more likely to be biased by cross-contamination of ion-chelating additives (EDTA and sodium citrate), are instead not affected by the order of draw of serum tubes (Venosafe, Terumo Europe). Both of these observations add to the evidence that the order of draw is no longer a requirement in coagulation testing.

Another issue in phlebotomy is the tube filling, which ideally is made up to the indicated mark. Though, underfilled tubes are a frequent finding (10, 11) in routine coagulation testing, and laboratories should have procedures to deal with these kind of samples. CLSI recommends a minimum of 90% tube filling if APTT is to be tested (12), and recent evidence by Lippi *et al* (13) and Ver Elst *et al* (14) still support this recommendation. For PT and Fbg Lippi *et al* (15) found the minimum allowed threshold to be >61% and >71% filling respectively, while Ver Elst *et al* (14) determined a 73% and 63% filling threshold. In a similar study, Pretorius *et al* (16) recommended as safe in their setting an 80% fill volume for complete routine testing. Different analytical platforms and tubes might explain the slight differences encountered, which puts in evidence the need for blood tubes validation by laboratories, as highlighted by Lima-Oliveira *et al* (17).

For specialized coagulation tests, the available evidence adressing the influence of different blood volumes is scarce. In one study, Lippi *et al* (13) found that for activated protein C resistance (APCR) a blood volume as low as 67% has no clinical significance on test results, while for FVIII it is advisable to obtain at least 80%.

What's new about sample stability – time to analysis?

With the consolidation era in laboratory medicine, more samples are collected in outside facilities, leading to possible delays in sample testing, so being aware of sample stability is key in coagulation testing. The issued recommendation by CLSI (H21-A5) on sample stability states that the assays should be completed, mostly within 4 hours of collection, except for PT (24h maximum), either on uncentrifuged or centrifuged samples kept at room temperature. Although this is a more conservative and safer approach, accumulated evidence in recent years suggests that proper measurements (without clinical significant difference) can still be made within a 24h frame not only for PT, but also for Fbg, D-dimers and thrombin time (TT) in samples transported/stored at room temperature and interestingly also at 4ºC (18-23). For APTT the evidence also suggests that storage can be extended, in this case up to 6-8 hours before testing (18-21, 23-25), both at room temperature and 4ºC.

What's new about testing interference in hemostasis?

Hemolysis interference is the leading cause of specimen rejection in coagulation laboratories (6), which is in agreement with CLSI recommendations (H21-A5) not to test samples with visible hemolysis. However, the evidence on the effects of spurious hemolysis on routine coagulation testing not only is scarce, but currently conflicting, because two recent studies (with updated measurement instruments) by Arora *et al* (26) and Lippi *et al* (27) concluded that the results obtained from hemolyzed samples for PT/INR, APTT and Fbg were reliable and would not change the clinical interpretation when compared with non-hemolyzed samples, which adds to the observations of Laga *et al* back in 2006 (28). D-dimer assays seem not to be affected by hemolysis even in the presence of high concentrations of cell-free haemoglobin (29, 30)

From the current state of the art we can conclude that sample quality requirements for routine coagulation testing are evolving, and it seems essential that laboratories have updated and validated criteria on specimen acceptance/rejection, in order to avoid rejecting good specimens, reduce costs and delays, and improve patient safety.

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