Report on behalf of the Working Group on Haemostasis

Influence of time on PT and aPTT results determined on reconstituted lyophilized plasma samples

Marjan Van Blerk[1], Iona Culea[2] and Piet Meijer[3]

1. Department of Quality of Medical Laboratories, Institute of Public Health, Belgium; 2. RoEQALM, Romania; 3. ECAT Foundation, The Netherlands

Aim of the study

For practical reasons, external quality assessment schemes (EQAS) for blood coagulation use lyophilized plasma samples which have to be reconstituted by participating laboratories. The aim of the present study was to investigate the influence of time between reconstitution and analysis on prothrombin time (PT) and activated partial thromboplastin time (aPTT) results to determine the time interval wherein reliable results can be obtained. For this purpose, a study was carried out by the Belgian and Romanian EQAS for blood coagulation.

Materials and methods

For the Belgian investigation, 4 types of samples were used: 1 normal plasma, 1 plasma containing 0.72 IU/mL nadroparin (Fraxodi®), 1 artificially depleted plasma with an INR of 2.3 and 1 plasma pool from patients on oral anticoagulants with an INR of 3.9. The first 3 samples were prepared as described earlier (1) by the department of Quality of Medical Laboratories of the Belgian Scientific Institute of Public Health, which organizes the Belgian EQAS for blood coagulation. The plasma pool from patients treated with oral anticoagulants was purchased from Technoclone Gmbh (Coagulation Control AK lot number 2B520RV, Vienna, Austria).

The lyophilized plasmas were reconstituted with 1.0 mL distilled water at room temperature and PT and aPTT were determined 15', 30', 45', 60', 75' and 90' after reconstitution. PT and aPTT analyses were performed on a Start 4 with Neoplastin CI Plus (rabbit brain thromboplastin) and STA-PTT A (cephalin from rabbit brain and silica as activator), respectively (all Diagnostica Stago, Asnières, France).

For the Romanian investigation, 4 commercial samples were used (all Siemens Healthcare Diagnostics, Marburg, Germany): Control Plasma N (CN), Control Plasma P (P1) with an INR of 2.3, Ci-Trol 2 (P2) with an INR of 3.64, and Ci-Trol 3 (P3) with an INR of 6.35.

The lyophilized plasmas were reconstituted with 1.0 mL distilled water at room temperature and PT and aPTT were determined 15', 30', 45', 60', 75', 90', 105' and 120' after reconstitution. PT and aPTT analyses were performed on a BCS-XP with Thromborel S (human placental thromboplastin) and Pathromtin SL (aPTT1, vegetable phospholipids and silicon dioxide as activator) and Actin FS (aPTT2, soya bean phospholipids and ellagic acid as activator) (all Siemens Healthcare Diagnostics), respectively.

Changes were considered important if they exceeded the expanded uncertainty (2 standard deviations) of the PT and aPTT analyses.

Results

The profiles of changes (% difference) related to coagulation time at 15' after reconstitution are presented in Figures 1, 2 (Belgian results), 3, 4 and 5 (Romanian results).
Except for the PT result of the Ci-Trol 3 sample with an INR of 6.35 determined 120' after reconstitution in the Romanian investigation, all changes were below the limit of the expanded uncertainty.

Figure 1. Difference (%) against time after reconstitution for PT (Belgian results).

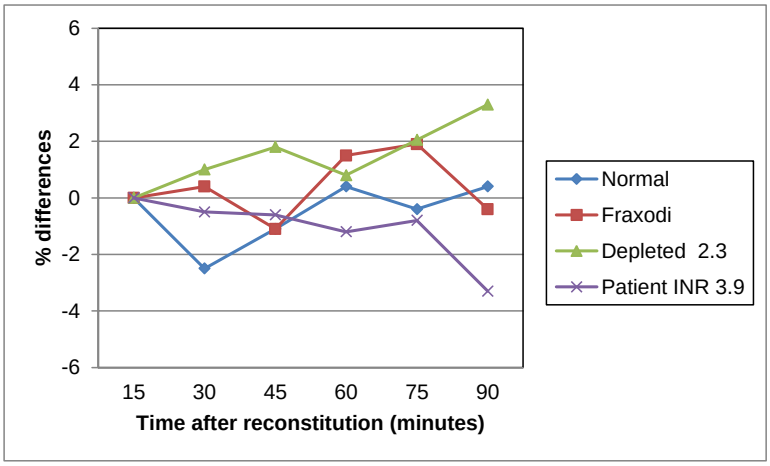


Figure 2. Difference (%) against time after reconstitution for aPTT (Belgian results).

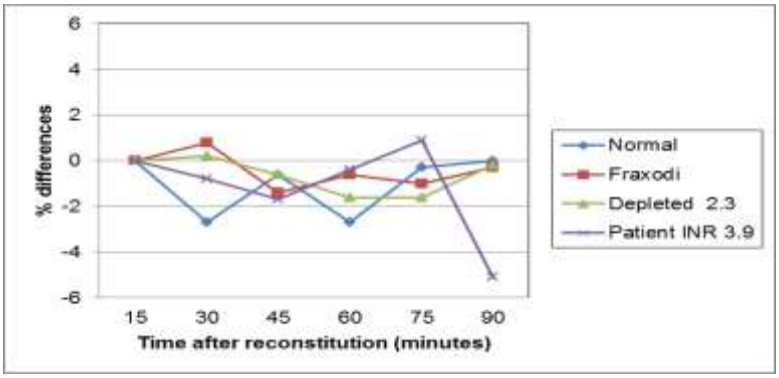


Figure 3. Difference (%) against time after reconstitution for PT (Romanian results).

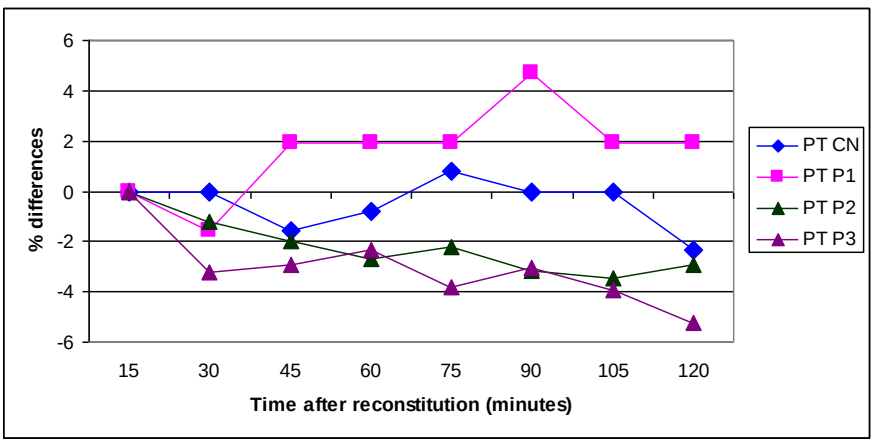


Figure 4. Difference (%) against time after reconstitution for aPTT1 (Romanian results).

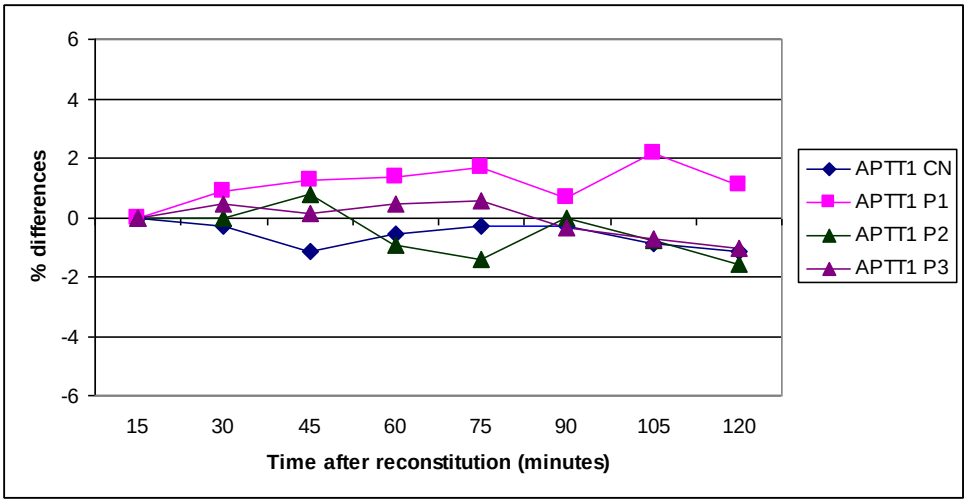
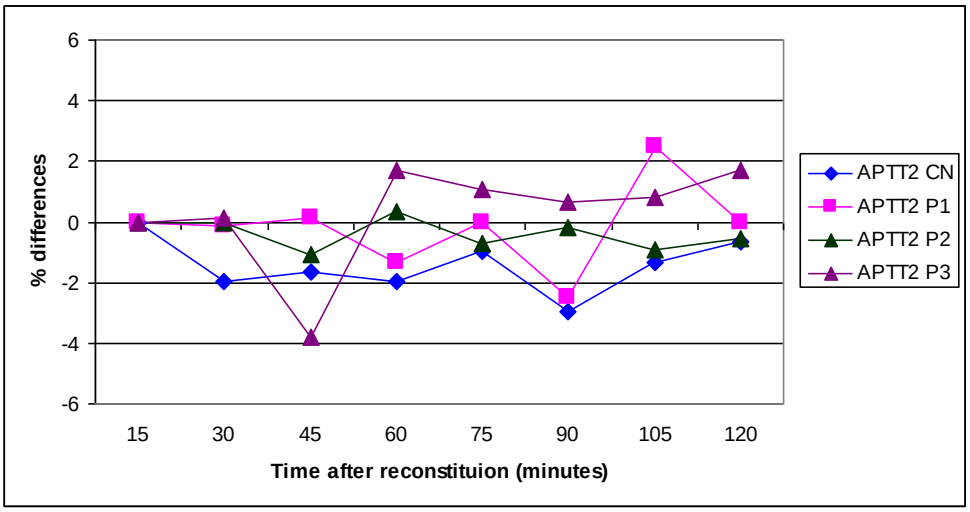


Figure 5. Difference (%) against time after reconstitution for aPTT2 (Romanian results).



Conclusions

The PT and aPTT results in this study remained valid when analysis was performed within 1 hour after reconstitution. Specifically for samples with high INR values, stability seemed to decrease when time between reconstitution and testing was extended. The findings of this study underline that it is important that EQA organizers provide their participants with clear instructions on reconstitution and testing.

References

1. Preparation of Belgian control materials for external quality assessment of coagulation tests. Marjan Van Blerk, Tania Crucitti, Nicole Hamers, Louis Simonet, Jean-Claude Libeer. EQAnews, 10: 73-4, 1999