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# Advancing haemostasis automation – successful implementation of robotic centrifugation and sample processing in a tertiary service hospital

## Abstract

**Background:** Laboratories today face increasing pressure to automate operations due to increasing workloads and the need to reduce expenditure. Few studies to date have focussed on the laboratory automation of preanalytical coagulation specimen processing. In the present study, we examined whether a clinical chemistry automation protocol meets the preanalytical requirements for the analyses of coagulation.

**Methods:** During the implementation of laboratory automation, we began to operate a pre- and postanalytical automation system. The preanalytical unit processes blood specimens for chemistry, immunology and coagulation by automated specimen processing. As the production of platelet-poor plasma is highly dependent on optimal centrifugation, we examined specimen handling under different centrifugation conditions in order to produce optimal platelet deficient plasma specimens. To this end, manually processed models centrifuged at 1500 *g* for 5 and 20 min were compared to an automated centrifugation model at 3000 *g* for 7 min.

**Results:** For analytical assays that are performed frequently enough to be targets for full automation, Passing-Bablok regression analysis showed close agreement between different centrifugation methods, with a correlation coefficient between 0.98 and 0.99 and a bias between –5% and +6%. For seldom performed assays that do not mandate full automation, the Passing-Bablok regression analysis showed acceptable to poor agreement between different centrifugation methods.

**Conclusions:** A full automation solution is suitable and can be recommended for frequent haemostasis testing.

**Keywords:** automation; centrifugation; coagulation; manually processed.

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## Introduction

Clinical laboratory automation has traditionally focused on analytical operation. Specimens requiring separation by centrifugation have represented a bottleneck in laboratory specimen throughput because of the time required to manually balance tubes, loading into the centrifuge and removal after completion of the centrifugation step [1–3].

After the first installation of total laboratory automation (TLA) in the early 1990s, many laboratories were convinced that TLA was the optimal solution for handling increasing workloads, labour shortages and high laboratory labour budgets. However, for many smaller laboratories, TLA was too costly or too large to install without expensive renovation costs. As a result, modular, task-orientated automation was developed to reduce the high labour costs associated with specimen-processing in clinical laboratories [1, 3].

Preanalytical processing units can be categorised into two distinct types. Modular preanalytical processors are assembled from individual modules, such as a sample stockyard, conveyor belt transporter, centrifuge, decapper, barcode reader, aliquoter or sorter whereas a stand-alone independent unit is deployed in the laboratory in the same fashion as a stand-alone chemistry analyser [3].

Preanalytical specimen processing units dedicated to selected tasks, such as decapping and sorting, have also been shown to provide substantial improvements in laboratory efficiency [4]. Furthermore, automated specimen processors and transportation systems have been successfully implemented in several large clinical laboratories as part of TLA [5].

The benefits of automation are well-documented [3, 6–10]. Specimen preparation, including centrifugation, aliquoting, sorting and consumable costs make

up approximately 20% of the total costs and 37% of the time required for testing. In contrast, disposal and result reporting represent 10% of the total costs and 18% of the time required for testing [11].

Most errors occur in the preanalytical phase [12, 13]. Haemolysis has been recognised as one of the most prevalent preanalytical errors and the most prevalent interference in clinical laboratory testing [14, 15]. In contrast to manual sample processing, the ability to visually detect preanalytical issues such as haemolysis, icteric or lipaemic plasma and clotted tubes is not possible in all automation modules.

Only a few studies related to coagulation have provided information on laboratory automation that focuses on preanalytical specimen processing [3, 16–19].

In this study, we evaluated whether an automated solution in clinical chemistry meets the preanalytical requirements for coagulation. We examined the handling of specimens under different centrifugation durations to produce satisfactory platelet deficient plasma specimens.

## Materials and methods

We established an AutoMate 800 preanalytical sample processor [20] (Beckman Coulter, Brea, CA, USA), linked by a transportation belt to two serially linked ACL Top coagulation analysers (IL, Milan, Italy) followed by two DXI immunochemistry analysers, three DXC chemistry analysers and a 3000-tube refrigerated stockyard (all Beckman Coulter, Brea, CA, USA) serially placed along the belt.

The Center for Laboratory Medicine, St. Gallen, Switzerland routinely processes up to 2000 requests with up to 8000 analyses per day.

The coagulation samples are centrifuged in the centrifuge unit, after which the decapper removes the caps from the sample tubes. The AutoMate 800 exchanges barcode information with the laboratory information system and then generates a work list that initiates specimen processing. Individual samples are then directed to the analysers.

After analysis, all samples for coagulation are collected in a specific area and undergo visual inspection for preanalytical errors such as clotted tubes and haemolysis. This information is communicated to the clinicians in written form. The samples are then archived for 3 days, however, after 2–3 h (due to instability) further analyses are not recommended. For additional analyses such as the measurement of coagulation factors, samples can be stored at −80°C.

Blood samples were collected into 2.7 mL vacutainer tubes containing sodium citrate (Becton Dickinson, ORT, STAAT). Informed consent of the patients for this study was not necessary because all samples were processed solely for routine analyses.

The analytical test systems for coagulation parameters were measured on ACL Top coagulation analysers (IL, Milan, Italy), using RecombiPlasTin for Quick, APTT RGT, fibrinogen Clauss XL, D-dimer HS, and immunodepleted plasmas (Factor-deficient plasma II, V, VII, VIII, IX, X, XI and XII) (all HemosiL; AxonLab, Baden-Daettwill, Switzerland). Internal quality controls were routinely performed with normal and abnormal control plasma, respectively. The day-to-day variation coefficients for the normal and abnormal control plasma were 4.5% and 4.2% for Quick, 2.7% and 5.1% for PTT, 7.7% and 5.0% for fibrinogen, 8.7% and 7.6% for D-dimer, 2.6% and 2.8% for FII, 4.9% and 10.5% for FV, 1.7% and 10.7% for FVII, 8.9% and 13.1% for VIII, 10.6% and 7.9% for FIX, 5.8% and 7.1% for FX, 3.1% and 10.1% for FXI and 8.5% and 8.2% for FXII.

All analyses, reagents, standards and controls were used according to the manufacturer's recommendations (Axon Lab). The results for each analysis are shown in Table 1. High throughput analysis included Quick, INR, PTT, fibrinogen, and D-dimer. Special coagulation analysis included the analysis of coagulation factors.

Production of platelet-poor plasma required for blood coagulation testing systems is dependent on an optimal centrifuge speed to ensure that the majority of platelets are removed from the plasma before coagulation tests are performed [21].

As the AutoMate 800 centrifuge rotor speed is fixed at 3000 *g*, a centrifugation time of 7 min was compared with manually processed samples which were routinely centrifuged in the laboratory for 5 and 20 min at 1500 *g* to determine the protocol producing the lowest residual platelet count values.

The processing of samples was divided into three different centrifugation models:

1. Manually processed at 1500 *g* for 5 min (all assays evaluated).
2. Manually processed at 1500 *g* for 20 min (only coagulation factors and D-dimer).
3. Processed by AutoMate at 3000 *g* for 7 min (all assays evaluated).

The platelet count was determined on a XE-5000 haematology analyser (Sysmex Europe, Hamburg, Germany) in 10 samples for each centrifugation model. Turnaround time (TAT) was defined as the time from sample receipt to report dispatch.

### Statistics

The results were analysed by Passing-Bablok regression, Spearman's coefficient of rank correlation and Bland-Altman plots. We tested the assumption of linearity by using the cumulative sum linearity test. A $p<0.05$ indicated significant deviation from linearity. We performed all calculations using Medcalc version 5.00.010.

## Results

Passing-Bablok regression analysis showed close agreement between different centrifugation methods. In terms of high throughput analysis the results for the manually processed samples (1500 *g* for 5 min) showed agreement with samples processed by AutoMate (3000 *g* for 7 min). Results showed a correlation coefficient between 0.98 and 0.99 and a bias between −3% and +2%, as indicated by the Bland-Altman plot (Table 1A).

| Parameters | Passing-Bablok regression analysis | | | | Rank correlation analysis | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Intercept | 95% CI | Slope | 95% CI | Correlation coefficient | 95% CI | $r^2$ | p-Value | Number |
| A | | | | | | | | | |
| Fibrinogen | −0.2 | −0.34 to 0.04 | 1.06 | 1.01–1.1 | 0.987 | 0.972 –0.993 | 0.974 | <0.0001 | 31 |
| PTT | 0.5 | −0.87 to 2.17 | 0.97 | 0.9242–1.0126 | 0.978 | 0.954–0.993 | 0.956 | <0.0001 | 29 |
| INR | 0.0 | 0.00 to 0.05 | 1.00 | 0.9444–1.0 | 0.986 | 0.972–0.990 | 0.972 | <0.0001 | 31 |
| Quick | 3.4 | 1.14 to 7.28 | 0.97 | 0.9375–1.0075 | 0.980 | 0.959–0.990 | 0.960 | <0.0001 | 31 |
| D-dimer | −0.0 | −0.01 to 0.00 | 1.00 | 0.9976–1.0244 | 0.980 | 0.958–0.991 | 0.960 | <0.0001 | 29 |
| B | | | | | | | | | |
| Factor V | 18.4 | −37.45 to 65.08 | 0.87 | 0.61–1.28 | 0.888 | 0.640–0.968 | 0.788 | 0.003 | 12 |
| Factor VII | 15.2 | −14.37 to 36.13 | 0.82 | 0.64–1.13 | 0.586 | 0.105–0.845 | 0.343 | 0.028 | 16 |
| Factor VIII | 0.0 | −24.65 to 17.28 | 0.96 | 0.83–1.13 | 0.967 | 0.912–0.987 | 0.935 | <0.0001 | 20 |
| Factor IX | 12.9 | 1.66 to 24.31 | 0.87 | 0.76–0.98 | 0.988 | 0.972–0.995 | 0.976 | <0.0001 | 23 |
| Factor X | 8.5 | −46.32 to 47.99 | 0.95 | 0.63–1.51 | 0.685 | 0.287–0.881 | 0.469 | 0.008 | 16 |
| Factor XI | 12.5 | 4.72 to 19.58 | 0.82 | 0.75–0.91 | 0.969 | 0.923–0.987 | 0.939 | <0.0001 | 21 |
| Factor XII | 7.6 | −2.22 to 19.97 | 0.92 | 0.79–1.03 | 0.964 | 0.911–0.985 | 0.929 | <0.0001 | 21 |
| C | | | | | | | | | |
| Factor V | 12.81 | −10.86 to 37.33 | 0.91 | 0.75–1.10 | 0.916 | 0.751–0.974 | 0.839 | <0.0001 | 14 |
| Factor VII | −1.29 | −35.23 to 18.03 | 0.99 | 0.83–1.32 | 0.777 | 0.487–0.913 | 0.604 | <0.0001 | 18 |
| Factor VIII | 11.54 | −11.58 to 27.73 | 0.88 | 0.74–1.05 | 0.960 | 0.905–0.984 | 0.922 | <0.0001 | 22 |
| Factor IX | 10.3 | 0.30 to 16.43 | 0.86 | 0.89–0.96 | 0.980 | 0.954–0.991 | 0.960 | <0.0001 | 25 |
| Factor X | 2.49 | −31.47 to 26.82 | 1.01 | 0.78–1.33 | 0.822 | 0.577–0.932 | 0.676 | <0.0001 | 18 |
| Factor XI | 12.07 | 1.41 to 19.20 | 0.82 | 0.75–0.94 | 0.977 | 0.946–0.991 | 0.955 | <0.0001 | 23 |
| Factor XII | 1.74 | −7.09 to 16.95 | 0.95 | 0.80–1.07 | 0.967 | 0.923–0.986 | 0.935 | <0.0001 | 23 |
| D | | | | | | | | | |
| Factor V | −7.06 | −39.92 to 16.82 | 0.99 | 0.83–1.23 | 0.917 | 0.779–0.970 | 0.841 | <0.0001 | 17 |
| Factor VII | −15.68 | −31.46 to −2.93 | 1.18 | 1.04–1.27 | 0.948 | 0.864–0.981 | 0.899 | <0.0001 | 18 |
| Factor VIII | 3.73 | −7.77 to 21.63 | 0.98 | 0.85–1.07 | 0.986 | 0.965–0.995 | 0.972 | <0.0001 | 20 |
| Factor IX | −2.98 | −15.33 to 3.03 | 0.99 | 0.91–1.11 | 0.984 | 0.962–0.993 | 0.968 | <0.0001 | 23 |
| Factor X | −2.85 | −27.37 to 18.88 | 1.02 | 0.83–1.24 | 0.792 | 0.501–0.925 | 0.627 | 0.002 | 17 |
| Factor XI | 0.35 | −9.26 to 8.55 | 0.99 | 0.90–1.09 | 0.975 | 0.942–0.989 | 0.951 | <0.0001 | 23 |
| Factor XII | −5.72 | −14.44 to 1.88 | 1.04 | 0.96–1.14 | 0.959 | 0.905–0.983 | 0.920 | <0.0001 | 23 |

**Table 1** Passing-Bablok and rank correlation analysis.
Part A: High throughput analysis, processed by AutoMate (centrifuged by 3000 *g*) for 7 min vs. manually processed (1500 *g* for 5 min). Part B: Special analysis, processed by AutoMate (centrifuged by 3000 *g*) for 7 min vs. manually processed (1500 *g* for 5 min). Part C: Special analysis, processed by AutoMate (centrifuged by 3000 *g*) for 7 min vs. manually processed (1500 *g* for 20 min). Part D: Special analysis, manually processed (1500 *g* for 20 min) vs. manually processed (1500 *g* for 5 min). Data for Factor II are not shown because of low number of samples.

For special coagulation analysis the Passing-Bablok regression analysis showed good to acceptable agreement between different centrifugation methods. Manually processed samples (1500 *g* for 5 min) compared to samples processed by AutoMate showed a correlation coefficient between 0.60 and 0.99 and a bias between −4.9% and +5.1% (Table 1B). The results indicated that some factors such as VII and X showed significant variation after manual processing compared to those processed by AutoMate.

Similar results were obtained from samples by manually processed (1500 *g* for 20 min) centrifugation and those processed by AutoMate 800; results showed a correlation coefficient between 0.78 and 0.98 and a bias between −4.7% and +3.6% (Table 1C, Figure 1).

Significant deviation of the line of identity was found for factor IX and XI. Actual slopes β and intercepts α were not identical to the identity line with $\beta \equiv 1.0$ and $\alpha \equiv 0$.

A Cusum test for linearity showed no significant deviation from linearity ($p>0.05$ for high throughput analysis and $p>0.10$ for special coagulation analysis) (Table 1). The confidence intervals for the rank correlation coefficient are summarised in Table 1.

Table 1D shows the Passing-Bablok regression lines obtained for coagulation factor analysis when comparing the two different manual centrifugation methods. Spearman's coefficients of rank correlation between 0.79 and 0.98 and bias between –1% and +6% from Bland-Altman plots were obtained.

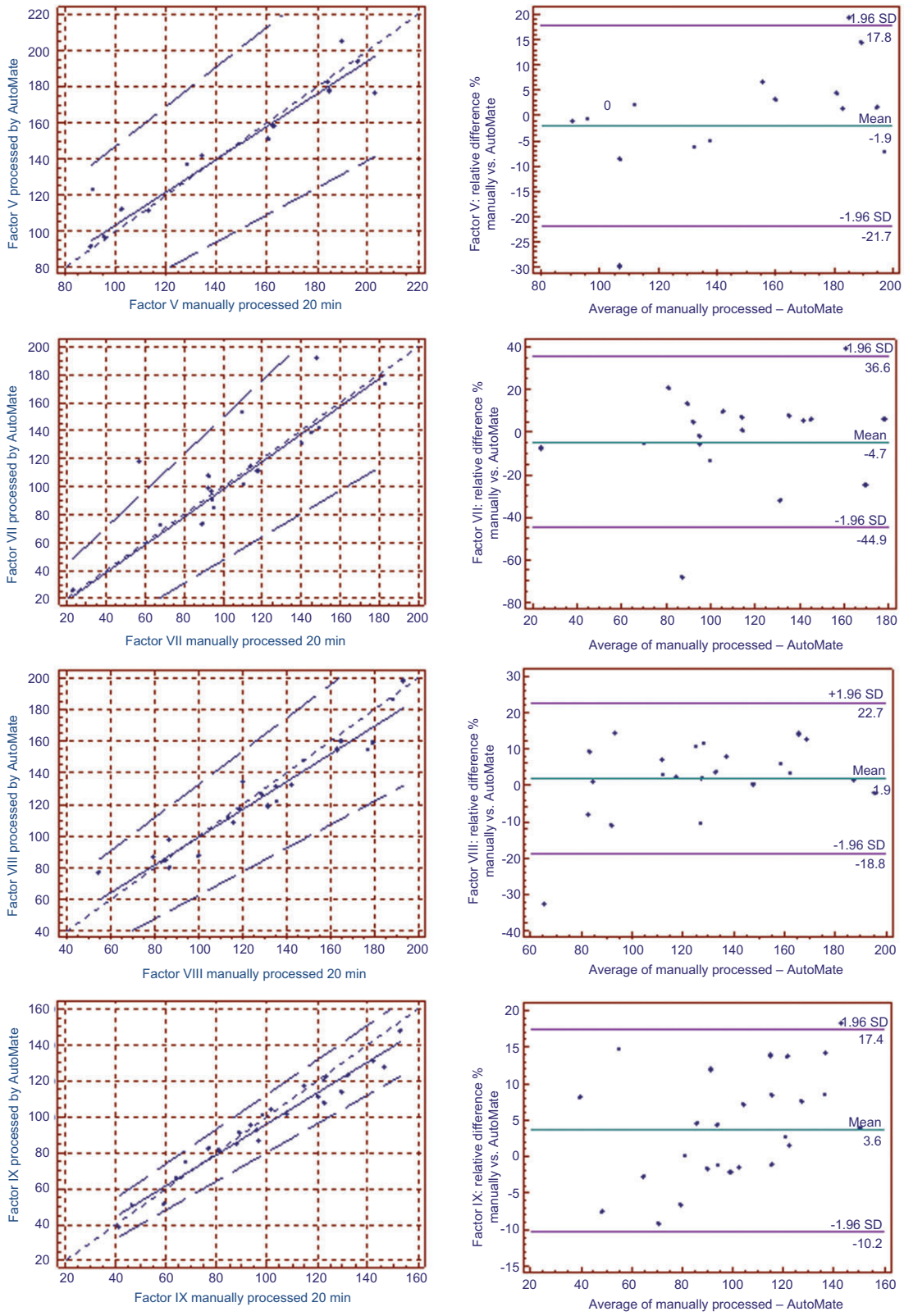


**Figure 1** Coagulation factor analysis.

Passing-Bablok regression lines (left panel) and Bland-Altman plots (right panel) comparing analytical data obtained from samples manually processed (1500 *g*) for 20 min centrifugation with those processed by AutoMate (3000 *g*) for 7 min. The regression line is indicated by a solid dark line with confidence limits (dashed lines) which were obtained from the limits of the slope β and the intercept α. The line of identity is depicted by the dotted line.

The manual preparation (1500 *g* for 5 min) of platelet-poor plasma contained 17,000–125,000 platelets/µL. However, concentrations of manually processed samples at 1500 *g* for 20 min were 1000–6000 platelets/µL. Results from the AutoMate 800 showed an acceptable platelet-poor preparation. Residual platelet concentrations were 3000–9000 platelets/µL (Figure 2).

The manual preparation at 1500 *g* for 20 min provided the lowest residual platelet concentration compared to the other two methods. However, the automated preparation was also sufficient to produce platelet-poor plasma below the Clinical and Laboratory Standards Institute (CLSI) – recommended threshold of 10,000 platelets/µL [21].

The average TAT for the high throughput tests was 0.64 h (processed by AutoMate).

The previous TAT by manually processed samples was 0.62 h. These data show that the average TAT did not increase after implementing full haemostasis automation.

## Discussion

The coagulation factor data show good agreement between plasma samples obtained either by manual or automated centrifugation. The variability between the different results obtained for individual coagulation factors is a reflection of the precision of the analytical method used and the measurement range of the tested plasma samples.

Optimal correlations with $r>0.95$ and $p<0.0001$ were obtained for the high throughput analysis of most of the coagulation factors. The poorer correlation results for FV, FVII and FX might relate to the low number of samples tested and the data range because <20% were in the clinical relevant range of <100% activity.

Thus, we would recommend using automated sample processing for high throughput analysis reserving manual procedures for specific coagulation analysis.

As the number of samples analysed daily for special coagulation factor testing is low and the stability of reagents on the ACL system have limited stability, automated procedures for special coagulation analyses cannot be recommended due to the significant cost of these tests.

In terms of high throughput tests our results indicate that a centrifuge speed of 3000 *g* for 7 min by AutoMate is sufficient to produce platelet-poor plasma below the CLSI-suggested threshold [21].

Our data show that the average TAT is not increased after implementing full haemostasis automation. The TAT for manually processed samples depends on the number of samples involved. In manual processing, the analytical cycle from sample requisition, centrifugation and loading to validation of results might be extended in the event of increasing workload and limited resources, such as centrifugation capacity.

Although the average TAT is unchanged, the introduction of automated sample processing leads to significant reductions in selected manual processing steps including loading tubes into centrifuges, manually balancing tubes and removal of tubes after completion of centrifugation as well as decapping the tubes before loading onto the analyser.

This time-saving advantage of automation for a mean sample number of 300 per day enables the laboratory technicians to produce analytically more accurate results and focus on procedures of higher value, such as validation of results, interpretation, test development and translation of innovative tests into clinical practice.

It is interesting to note that although technological developments have considerably improved the productivity

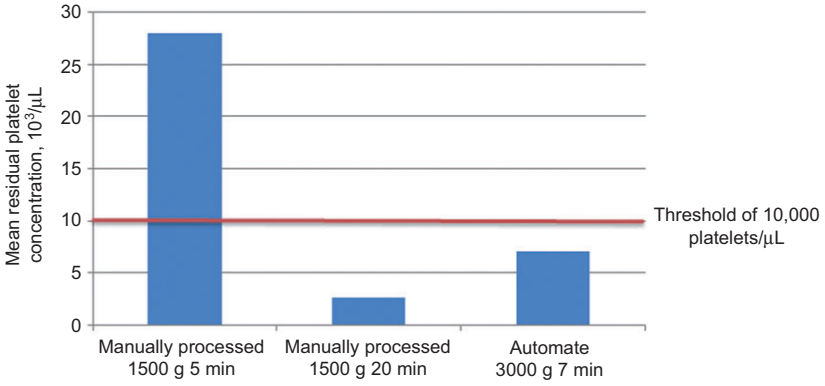


**Figure 2** Efficiency of platelet depletion.
Data are shown as mean±SD of residual platelet concentrations $10^3$/µL: manually processed (1500 *g*) for 5 min: 27.8±12.8, manually processed (1500 *g*) for 20 min: 2.6±1.8 and processed by AutoMate (3000 *g*) for 7 min: 7±2.1.

of clinical laboratories, a recent study by the College of American Pathologists showed that the quality and reliability of results represent the most important concern for 32% of physicians, more than twice the number of physicians choosing TAT (15%) [22].

The data presented here clearly show that an automation solution in clinical chemistry meets the preanalytical requirements for quality and reliability in coagulation testing. For medium and large laboratories, full haemostasis automation is suitable for high throughput analysis and can be recommended.

## Conflict of interest statement

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