Evaluating the analytical performance of five new coagulation assays for the measurement of prothrombin time and activated thromboplastin time

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Abstract

Introduction: New methods for coagulation tests require careful assessment before routine use. We evaluated the analytical performance of five new coagulation assays for measuring prothrombin time (PT) and activated partial thromboplastin time (aPTT).

Methods: PT Rec, PT Owren, aPTT, aPTT Lupus and aPTT Screen assays (Roche Diagnostics) were evaluated on cobas t 711 and cobas t 511 analysers (Roche Diagnostics) at four European centres. Analytical performance and method comparisons with relevant commercially available assays were performed to Clinical Laboratory Standards Institute guidelines using residual anonymized samples. Lot-to-lot comparison and equivalency of the cobas t analysers were also assessed; reference ranges were determined using samples from apparently healthy volunteers.

Results: Overall, coefficients of variation were ≤1.3% for within-run precision and ≤6.3% for total reproducibility across all sites. Deming regression analyses showed good agreement between each assay (cobas t 711) and respective comparator method (Pearson’s r: 0.964-0.999, n > 120 samples/assay/site). Passing–Bablok regression analyses demonstrated equivalence of the two cobas t platforms for use with each assay (Pearson’s r ≥ 0.995). Lot-to-lot consistency was high for all assays and comparisons (Pearson’s r ≥ 0.998). Reference ranges (2.5th-97.5th percentiles; n = 200 samples/assay) in seconds were 8.4-10.6 (PT Rec), 18.2-27.2 (PT Owren), 23.6-30.6 (aPTT), 24.1-31.7 (aPTT Lupus) and 23.8-33.2 (aPTT Screen).

Conclusion: Based on the excellent analytical performance and good agreement with relevant comparator methods, the five coagulation assays on the novel cobas t 711 and cobas t 511 analysers are suitable for routine use in core laboratories.

Keywords: activated partial thromboplastin time, coagulation analyser, prothrombin time
1 | INTRODUCTION

Coagulopathies occur in patients across all healthcare settings, often resulting in clinically significant bleeding or thrombosis, depending on the nature of the defect. A number of possible aetiologies exist, including trauma, disease (eg, liver disease, sepsis, cancer), surgery, pharmacological treatment (eg, unfractionated heparin [UFH], vitamin K antagonists, direct factor Xa- and direct thrombin inhibitors) and genetic or acquired deficiencies in coagulation factors. The ability to accurately, reliably and quickly measure indicators of haemostatic function, and to subsequently implement corrective measures, is thus important for patient health.

Coagulation tests are used widely for the screening, diagnosis and assessment of coagulopathies, and for the monitoring of anticoagulant and anti-thrombotic therapies. In particular, assessment of coagulopathies, and for the monitoring of anticoagulation. Laboratories, as well as for potential expansion of the range of tests offered. Five new commercially available assays (PT Rec, PT Owren, aPTT, aPTT Lupus and aPTT Screen) have recently become available for use on cobas t coagulation analysers, and may offer potential advantages over existing assays/platforms in terms of handling, precision and throughput. Prior to their adoption, it is important that new assays are evaluated for precision, compared with existing methods and that relevant reference ranges are established, thus ensuring that the assays are suitable for routine use in core laboratories and able to reliably inform clinical decisions.

The current generation of automated, high-throughput coagulation analysers provides scope for improved turnaround times in core laboratories, as well as for potential expansion of the range of tests offered. Five new commercially available assays (PT Rec, PT Owren, aPTT, aPTT Lupus and aPTT Screen) have recently become available for use on cobas t coagulation analysers, and may offer potential advantages over existing assays/platforms in terms of handling, precision and throughput. Prior to their adoption, it is important that new assays are evaluated for precision, compared with existing methods and that relevant reference ranges are established, thus ensuring that the assays are suitable for routine use in core laboratories and able to reliably inform clinical decisions.

This multicentre study evaluated the performance of the five new coagulation assays on the cobas t analysers. For each assay, the analytical performance was evaluated and a method comparison with existing, commercially available assays/platforms was performed; reference ranges for each assay were also determined using samples from apparently healthy volunteers.

2 | MATERIALS AND METHODS

2.1 | Study design

The study was performed between June 2016 and March 2017 in core laboratories at four centres in Europe (University of Freiburg, Germany; Royal Hallamshire Hospital, Sheffield, UK; University of Debrecen, Hungary; AKH Wien, Austria).

Five assays (PT Rec, PT Owren, aPTT, aPTT Lupus and aPTT Screen; Roche Diagnostics GmbH, Mannheim, Germany) were each evaluated for their analytical performance, and compared with existing methodologies/technologies (see below) in independent method comparison experiments; lot-to-lot variability, the equivalence of each assay on two cobas t platforms and reference ranges in apparently healthy volunteers were also assessed. All assays and instruments were used according to their respective manufacturers’ instructions and quality control measurements were performed at least twice every day, to monitor the appropriate function of each analyser. Anonymized human residual (3.2% [0.109M]) sodium citrate plasma samples were used for all experiments; samples for reference range evaluation were sourced from a blood bank (Freiburg, Germany). Samples were double-spin (10 minutes at 3300 g followed by 10 minutes at 2700 g) before freezing at −80°C and shipment on dry ice. Samples were subsequently stored below −60°C, and storage conditions were similar between different sample sources. Sample tubes were sourced from Becton-Dickinson and Company (Wokingham, UK), Sarstedt AG & Co. (Nürnbrecht, Germany), or Greiner Bio One International GmbH (Kremsmünster, Austria).

Independent ethics committee approval or waiver was obtained where necessary before study initiation according to local laws and guidelines. The study was performed according to the principles of the Declaration of Helsinki and International Conference on Harmonisation Good Clinical Practice guidelines.

2.2 | Assay principles

The PT Rec assay contains thromboplastin (recombinant human thromboplastin reagent containing a heparin-neutralizing substance) and calcium, which initiate the activation of the extrinsic coagulation cascade when added to citrated human plasma. The PT Owren assay reagent contains rabbit brain thromboplastin plus bovine plasma (containing factor V and fibrinogen and lacking factors II, VII and X) and a heparin neutralizer. For both assays, the time between the addition of the reagent to the plasma and the formation of the fibrin clot was measured and reported in seconds, international normalized ratio (INR), or percentage of normal. Due to the inclusion of a heparin neutralizer, presence of UFH does not affect the PT clotting time of either assay. The assays are intended as an aid for management of vitamin K antagonist therapy, and the PT Rec and aPTT assays can also assist diagnosis of fibrinogen deficiency.

The aPTT assay reagent consists of ellagic acid as surface activator and purified soy phosphatides with added buffer, stabilizer and preservative. The aPTT Lupus assay reagent consists of ellagic acid as surface activator and a blend of purified soy and rabbit brain phosphatides with added buffer, stabilizer and preservative. The aPTT Screen assay reagent consists of silicon dioxide particles as activator and a blend of purified soy phosphatides with added buffer, stabilizers and preservative. For each aPTT assay, the addition of calcium chloride prompts the initiation of the intrinsic coagulation cascade and the time from the addition of the calcium chloride until clot formation is measured. The aPTT assay is designed to have reduced sensitivity to lupus anticoagulant (LA), while the aPTT Lupus assay has increased sensitivity to LA. The aPTT Screen assay is designed to have the highest sensitivity towards UFH.
2.3 Analytical performance

Following a familiarization phase, within-run precision of each assay was evaluated: reproducibility was also evaluated according to Clinical Laboratory Standards Institute (CLSI) EP09-A3 guidelines. Each assay was independently evaluated on the cobas t 711 analyser (high-throughput: 390 tests/h; evaluated at all four sites) and cobas t 511 analyser (mid-throughput: 195 tests/h; evaluated at two sites [UK; Germany]). Within-run precision for each assay was evaluated in one run using two (PT Rec, PT Owren) or three controls (aPTT, aPTT Lupus, aPTT Screen) and five human plasma samples (n = 21 replicates per sample), covering the measuring range; each site performed their experiments with an individual reagent and control lot, which varied by site.

Reproducibility was evaluated over 5 days using two or three controls and five human plasma pools (one aliquot of each measured five times daily), covering the measuring range; three different reagent lots (one per site) and the same control lots were used across all sites. Results were evaluated across the study sites.

2.4 Method comparison

Method comparison was performed for each assay (cobas t 711 analyser) vs the following comparator methods, according to CLSI EP09-A3 guidelines: (a) PT Rec vs Innovin (Siemens Healthcare, Marburg, Germany) on Sysmex CS-5100 or CS-2000i (Sysmex, Kobe, Japan); (b) PT Owren vs Hepato-Prest (Diagnostica Stago SAS, Asnières-sur-Seine, France) on Stago STA-R Evolution (Diagnostica Stago SAS); (c) aPTT vs Actin FS (Siemens Healthcare) on Sysmex CS-5100 or CS-2000i, and aPTT vs STA Cephascreen (Diagnostica Stago SAS) on Stago STA-R Evolution (performed in Mannheim only); (d) aPTT Lupus vs Actin FSL (Siemens Healthcare) on Sysmex CS-5100 or CS-2000i, and aPTT Lupus vs STA Cephascreen/STA-LA (Diagnostica Stago SAS) on Stago STA-R Evolution (performed in Mannheim only); and (e) aPTT Screen vs Pathromtin SL (Siemens Healthcare) on Sysmex CS-5100 or CS-2000i. Except for the aPTT method comparison vs comparator assays on Stago STA-R Evolution, each comparison was performed at three or four sites (one reagent lot per site), using a minimum of 120 residual anonymized human plasma samples per assay, per site (representing the appropriate measuring range). The measuring ranges tested for each assay were as follows: PT Rec, up to INR 5.5 or approximately 60 seconds; PT Owren, up to INR 5.5 or approximately 150 seconds; aPTT, aPTT Screen and aPTT Lupus, up to 175 seconds for each assay.

2.5 Equivalency of the cobas t analysers

Equivalency of the cobas t 511 and cobas t 711 platforms was evaluated by method comparison, using each of the five assays (PT Rec, PT Owren, aPTT, aPTT Lupus and aPTT Screen). A minimum of 120 anonymized human plasma samples were tested on each platform at each of the two sites (Freiburg and Sheffield; one reagent lot per site).

2.6 Lot-to-lot comparison

Lot-to-lot comparability was assessed for each assay on the cobas t 711 analyser. Each assay was tested using three reagent lots (two lots per site) and a minimum of 120 anonymized human plasma samples per comparison.

2.7 Reference range studies

Reference ranges for each assay were determined using anonymized residual samples from apparently healthy adult donors (0.109M/3.2% citrate) sourced from a blood bank; samples were measured fresh at the sampling site in Freiburg, Germany. Inclusion criteria were apparently healthy adults (aged 18-50 years and originating from Europe or the US) and able to provide written informed consent; exclusion criteria were self-declared pregnancy or breast-feeding, and use of anticoagulation medication including but not limited to acetyl salicylic acid, direct oral anticoagulants, phenprocoumon and warfarin. Experiments were performed with three different reagent lots (N = 200; n = 67 samples per lot). Reference ranges for each assay were also determined using frozen 0.109M/3.2% citrated samples (BIOMEX GmbH, Heidelberg, Germany; plasma frozen within 1 hour of sampling) purchased in Becton-Dickinson tubes (San Jose, CA, USA) and in aliquots of the anonymized residual samples from healthy adult donors, collected in Sarstedt tubes. The time between sampling and measurement for reference range samples was typically 1-3 hours (Sarstedt tubes). To obtain frozen ranges (Sarstedt tubes), these samples were immediately frozen after measurement of the Sarstedt fresh ranges. Both frozen sample types (Sarstedt and Becton-Dickinson) were measured at three different sites after thawing (one reagent lot per site).

2.8 Sensitivity analysis of aPTT, aPTT Lupus and aPTT Screen assays to heparin and lupus anticoagulant

Sensitivity of the three new aPTT assays towards UFH and LA was analysed. The presence of warfarin (UFH analyses) or any anticoagulant (LA analyses) therapy was excluded by testing with PT (Thromborel S reagent), TT (BC Thrombin) and anti-Xa (Hyphen Biophen UFH Liquid Reagent Technology). Therapeutic ranges for the three aPTT assays were also determined according to CLSI H47-A2 guidelines, using linear regression.

Platelet-poor samples (n = 117) were sourced from patients receiving UFH, and each sample was measured with three Roche aPTT reagents on a cobas t 711 analyser. Results were compared against measurements from three Siemens aPTT reagents (Actin FS, Actin FSL and Pathrombin SL) on a BCS XP system.

Lupus anticoagulant-positive plasma samples (n = 96) were sourced commercially (Clinisys) and the LA ratio determined for each sample on a BCS XP system with LA1 and LA2 reagents (Siemens). The sensitivity of the three aPTT assays on a cobas t 711 analyser was assessed by comparison with the LA ratio.
TABLE 1 Within-run precision and total reproducibility (across all four sites) of the five coagulation assays on the cobas t 711 and cobas t 511 analysers, based on human plasma pools (range for the five human plasma pools is presented)

| Assay (s) | Within-run precision acceptance criteria(% CV) | Within-run precision, range of % CV | Total reproducibility acceptance criteria(% CV) | Total reproducibility, range of % CV |
|-----------|--------------------------------------------|-----------------------------------|-----------------------------------------------|-------------------------------------|
| PT Rec    | CV ≤ 3.0                                   | 0.2-0.7; 0.1-0.4                  | CV ≤ 25.0                                     | 1.9-3.2; 2.2-3.7                    |
| PT Owren  | CV ≤ 3.0                                   | 0.3-0.8; 0.2-0.6                  | CV ≤ 25.0                                     | 1.5-3.4; 0.7-2.2                    |
| aPTT      | CV ≤ 4.0                                   | 0.2-1.1; 0.1-0.8                  | CV ≤ 25.0                                     | 0.9-2.9; 0.8-3.8                    |
| aPTT Lupus| CV ≤ 4.0                                   | 0.2-1.3; 0.3-0.7                  | CV ≤ 25.0                                     | 0.8-3.1; 0.3-2.2                    |
| aPTT Screen| CV ≤ 4.0  | 0.3-1.2; 0.2-0.8                   | CV ≤ 25.0                                     | 1.4-3.4; 0.8-6.3                    |

aPTT, activated partial thromboplastin time; CV, coefficient of variation; PT, prothrombin time.

*aUnit for acceptance criteria is PT in seconds.

2.9 | Data analysis

Assay output was captured directly using WinCAEv, a CFR 21 Part 11 compliant electronic data capture software developed and validated for Roche-sponsored studies. The coefficient of variation (CV) was calculated for within-run precision and reproducibility and compared against prespecified acceptance ranges (within-run precision: ≤3.0% [PT Rec, PT Owren] or ≤4.0% [aPTT, aPTT Lupus, aPTT Screen]; total reproducibility [all assays] ≤25.0%), which were consistent with published guidelines.

For method comparisons (comparator method, cobas t platform and lot-to-lot), slope and intercept were calculated according to Passing–Bablok or Deming, and Pearson's r correlation coefficient was estimated; analyses were based on INR for the PT Rec and PT Owren assays, and on seconds for the aPTT, aPTT Lupus and aPTT Screen assays, as acceptance criteria were defined in these units. Bias was examined using Bland-Altman analysis.

Reference ranges (seconds) were calculated based on the 2.5th and 97.5th percentile values of the series of measurements for each assay with 90% confidence intervals (CI); median values were also estimated.

3 | RESULTS

3.1 | Analytical performance

For each assay, the CVs for within-run precision and total reproducibility are presented in Table 1; all values were within the prespecified acceptance criteria. Across all five assays, CVs for within-run precision of the human sample pools, across all four sites, ranged from 0.2% to 1.3% on the cobas t 711 analyser, and from 0.1% to 0.8% on the cobas t 511 analyser; CVs for total reproducibility across all four sites ranged from 0.8% to 3.4% on the cobas t 711 analyser, and from 0.3% to 6.3% on the cobas t 511 analyser.

3.2 | Method comparison

Method comparison experiments demonstrated good agreement for each assay (cobas t 711) vs their respective comparator method according to prespecified criteria (specified in Product Specifications Document) based on Deming regression analyses (Table 2). For each comparison, Pearson's correlation coefficients (presented as a range across sites) confirmed an excellent correlation between methods: PT Rec vs Innovin, r = 0.988-0.999; PT Owren vs Hepato-Prest, r = 0.990-0.994; aPTT vs Actin FS, r = 0.980-0.986; aPTT vs STA Cephascreen, r = 0.819; aPTT Lupus vs Actin FSL, r = 0.967-0.987; aPTT Lupus vs STA Cephascreen, r = 0.943; aPTT Lupus vs STA LA, r = 0.958; aPTT Screen vs Pathromtin SL, r = 0.964-0.985; aPTT Screen vs aPTT on Stago STA-R Evolution, r = 0.833. Bias within the data was analysed and is shown in the Bland-Altman analyses for cobas t 711 vs comparator methods (Figures S1-S5).

3.3 | Equivalency of cobas t 711 and cobas t 511 analysers

For each of the five assays evaluated, the cobas t 711 and cobas t 511 platforms demonstrated equivalence, according to prespecified acceptance criteria, based on Passing–Bablok regression analyses (Table 3). Across all assays (n = 5) and sites (n = 2 sites per assay, except PT Owren where n = 1 site), Pearson's correlation coefficient was ≥0.995. Bland-Altman plots are presented (Figures S6-S10) and demonstrate constant bias for the five assays, with consistent results for the two sites.

3.4 | Lot-to-lot comparison

A high level of consistency between lots was observed for all five assays (cobas t 711 analyser); the specified equivalence criteria based on Passing–Bablok analyses were met (Table 4). For all assays (n = 5) and comparisons (Lot 2 vs 1, Lot 3 vs 2 and Lot 1 vs 3), Pearson's correlation coefficient was ≥0.998. Bias analysed by Bland-Altman plots are presented (Figures S10-S15), showing constant bias for the different lots and consistent results between sites.

3.5 | Reference range

Based on fresh samples in Sarstedt tubes, reference ranges (2.5th to 97.5th percentiles [90% CI]; n = 200 fresh samples per assay) in seconds
were 8.40 (8.26-8.48) to 10.6 (10.3-11.8; PT Rec), 18.2 (17.2-18.7) to 27.2 (26.7-29.5; PT Owren), 23.6 (23.3-24.0) to 30.6 (30.2-31.0; aPTT), 24.1 (21.6-24.7) to 31.7 (31.1-32.4; aPTT Lupus) and 23.9 (20.5-24.3) to 32.2 (32.9-33.7; aPTT Screen; Figure 1A). Comparable reference ranges (90% CI) were obtained using frozen samples stored in Sarstedt tubes (n = 200 samples per assay): PT Rec, 8.47 (8.35-8.52) to 10.6 (10.4-11.9; PT Owren, 18.2 (17.1-18.8) to 27.1 (26.7-30.1; aPTT, 23.6 (23.1-24.1) to 30.8 (30.4-31.5); aPTT Lupus, 24.6 (21.8-24.9) to 32.4 (31.5-32.8); aPTT Screen, 24.0 (20.3-24.6) to 34.3 (33.6-35.9). Similar reference ranges (90% CI) were also obtained using frozen samples stored in Becton-Dickinson tubes (n ≥ 150 samples per assay): PT Rec, 8.11 (7.84-8.21) to 12.3 (11.4-12.6; PT Owren, 18.8 (18.1-18.9) to 28.5 (27.1-36.3; aPTT, 24.3 (23.1-24.5) to 32.1 (31.0-34.6; aPTT Lupus, 24.3 (19.5-24.7) to 33.4 (32.4-40.5; aPTT Screen, 23.5 (21.7-26.4) to 39.8 (36.5-57.7).

**TABLE 2  Method comparison: cobas 711 vs comparator device**

| Comparison                      | Evaluation | Acceptance criteria<sup>d</sup> | Freiburg | Sheffield | Debrecen | Vienna | Vienna |
|---------------------------------|------------|---------------------------------|----------|-----------|----------|--------|--------|
|                                 |            |                                 | Lot 1    | Lot 2     | Lot 3    | Lot 2  | Lot 3  |
| PT Rec vs Innovin<sup>a</sup>   | n          |                                 | 131      | 135       | 130      |        |        |
|                                 | Slope (Deming) | 1.00 ± 0.10                  | 1.008    | 1.036     | 0.900    |        |        |
|                                 | Intercept  | NA                              | 0.090    | 0.045     | 0.238    |        |        |
|                                 | Pearson’s r  | ≥0.900                          | 0.999    | 0.997     | 0.988    |        |        |
|                                 | Bias at 1.0 INR | 1 ± 0.15                      | 0.097    | 0.081     | 0.138    |        |        |
| PT Owren vs Hepato-Prest<sup>b</sup> | n          |                                 | 144      | 129       | 139      |        |        |
|                                 | Slope (Deming) | 1.00 ± 0.10                  | 0.853    | 0.845     | 0.833    |        |        |
|                                 | Intercept  | NA                              | 0.132    | 0.149     | 0.196    |        |        |
|                                 | Pearson’s r  | ≥0.900                          | 0.994    | 0.993     | 0.990    |        |        |
|                                 | Bias at 1.0 INR | 1 ± 0.15                      | -0.015   | -0.006    | 0.029    |        |        |
| aPTT vs Actin FS<sup>b</sup>    | n          |                                 | 136      | 123       | 142      | 193    |        |
|                                 | Slope (Deming) | 0.65-1.35                   | 1.037    | 1.061     | 1.040    | 0.953  |        |
|                                 | Intercept  | NA                              | 2.27     | 4.38      | 1.99     | 4.72   |        |
|                                 | Pearson’s r  | ≥0.850                          | 0.986    | 0.982     | 0.980    | 0.980  |        |
| aPTT Lupus vs Actin FSL<sup>b</sup> | n          |                                 | 144      | 122       | 150      | 204    |        |
|                                 | Slope (Deming) | 0.65-1.35                   | 1.041    | 1.286     | 1.126    | 1.098  |        |
|                                 | Intercept  | NA                              | 1.37     | -5.86     | -2.47    | 1.29   |        |
|                                 | Pearson’s r  | ≥0.850                          | 0.967    | 0.983     | 0.987    | 0.982  |        |
| aPTT Screen vs Pathromtin SL<sup>b</sup> | n          |                                 | 132      | 125       | 139      | 183    |        |
|                                 | Slope (Deming) | 0.65-1.35                   | 0.849    | 0.982     | 1.002    | 0.909  |        |
|                                 | Intercept  | NA                              | 4.27     | -2.47     | -2.37    | 3.32   |        |
|                                 | Pearson’s r  | ≥0.850                          | 0.980    | 0.985     | 0.985    | 0.964  |        |
| aPTT vs STA Cephascreen<sup>b,c</sup> | n          |                                 | 175      |           |          |        |        |
|                                 | Pearson’s r  | NA<sup>e</sup>                 | 0.819    |           |          |        |        |
| aPTT Lupus vs STA Cephascreen<sup>b,c</sup> | n          |                                 | 99       |           |          |        |        |
|                                 | Pearson’s r  | NA<sup>e</sup>                 | 0.943    |           |          |        |        |
| aPTT Lupus vs STA-LA<sup>b,c</sup> | n          |                                 | 128      |           |          |        |        |
|                                 | Slope (Deming) | NA<sup>e</sup>               | 0.591    |           |          |        |        |
|                                 | Intercept  | NA<sup>e</sup>                 | 10.21    |           |          |        |        |
|                                 | Pearson’s r  | NA<sup>e</sup>                 | 0.958    |           |          |        |        |
| aPTT Screen vs aPTT<sup>b,c</sup> | n          |                                 | 153      |           |          |        |        |
|                                 | Pearson’s r  | NA<sup>e</sup>                 | 0.833    |           |          |        |        |

<sup>a</sup>Analyses based on INR.

<sup>b</sup>Analyses based on seconds.

<sup>c</sup>Performed at one site only (Mannheim, Germany).

<sup>d</sup>Units for acceptance criteria are INR for PT Rec and PT Owren, and seconds for aPTT, aPTT Lupus and aPTT Screen.

<sup>e</sup>Test and report.
3.6 | Sensitivity of the aPTT, aPTT Lupus and aPTT Screen assays towards heparin and lupus anticoagulant

The therapeutic range for the aPTT assays was shorter than for the comparator agents: lower (0.3 IU/mL) and upper (0.7 IU/mL) limits for the coagulation time (seconds) were 46.1-57.4 (aPTT) vs 55.2-74.2 (Actin FS); 53.9-69.9 (aPTT Lupus) vs 55.7-74.7 (Actin FSL); and 56.4-86.5 (aPTT Screen) vs 57.6-79.8 (Pathromtin SL). aPTT Screen showed the steepest response to UFH in UFH-treated plasma samples, demonstrating the greatest sensitivity towards this anticoagulant, while aPTT demonstrated the lowest sensitivity (Figure 1B). Using either aPTT Lupus or aPTT Screen, more than 80% of samples with an anti-Xa activity range of 0.3-0.7 IU/mL resulted in a measurable clotting time. The relative differences in clotting time ratio were 0.97 (aPTT vs Actin FS), 0.95 (aPTT Lupus vs Actin FSL) and 0.94 (aPTT Screen vs Pathromtin SL).

Activated partial thromboplastin time Lupus demonstrated the greatest sensitivity towards LA antibodies, with the steepest response to LA in LA-positive plasma samples (Figure 1C). As with
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UFH, aPTT showed the lowest sensitivity to LA. The relative differences in clotting time ratio were 1.00 (aPTT vs Actin FS), 1.04 (aPTT Lupus vs Actin FSL) and 0.96 (aPTT Screen vs Pathromtin SL).

4 | DISCUSSION

The five assays demonstrated excellent analytical performance on both the cobas t 711 and cobas t 511 analysers. Overall, CVs for within-run precision were ≤1.3% and those for total reproducibility were ≤6.3%; lot-to-lot comparisons with each assay showed a high level of consistency across all sites. Furthermore, each of the five assays performed on the cobas t 711 analyser showed good agreement with the commercially available assays/platforms used as comparator methods, which have previously demonstrated favourable performance for the measurement of their respective analytes.34-36

The reference range in apparently healthy volunteers measured with the aPTT assay was consistent with those previously reported for other assays/platforms,20,34,35 and the reference ranges for the aPTT Lupus and aPTT Screen assays were in line with those of the aPTT assay. The reference range for the PT Rec assay (8.4-10.6 seconds) was slightly lower than that previously reported for similar assays.34,37 Reference ranges for each assay were consistent whether fresh or frozen samples were used, and with two different types of sample tube.

Together, these assays provide core laboratories with a method for monitoring anticoagulant therapy and for screening for specific coagulation abnormalities. For example, the PT Rec and PT

| TABLE 4 | Lot-to-lot comparison on the cobas t 711 analyser |
|---|---|---|---|
| Assay | Evaluation | Freiburg Lot 2 vs 1 | Sheffield Lot 3 vs 2 | Debrecen Lot 1 vs 3 |
| | | | | |
| PT Rec<sup>a</sup> | n | 129 | 135 | 129 |
| | Slope (Passing–Bablok) | 1.00 ± 0.10 | 1.000 | 1.000 | 1.008 |
| | Intercept | NA | 0.010 | 0.010 | −0.013 |
| | Pearson’s r | ≥0.975 | 1.000 | 1.000 | 1.000 |
| | Bias at 1.0 INR | NA | 0.01 | 0.01 | −0.00476 |
| PT Owren<sup>a</sup> | n | 144 | 132<sup>d</sup> | 135 |
| | Slope (Passing–Bablok) | 1.00 ± 0.10 | 1.002 | 0.989 | 1.000 |
| | Intercept | NA | −0.00243 | 0.0219 | −0.0070 |
| | Pearson’s r | ≥0.950 | 1.000 | 1.000 | 1.000 |
| | Bias at 1.0 INR | NA | 0.000 | 0.011 | −0.007 |
| aPTT<sup>b</sup> | n | 139 | 128 | 145 |
| | Slope (Passing–Bablok) | 1.00 ± 0.10 | 0.979 | 1.009 | 1.013 |
| | Intercept | NA | 0.704 | 0.156 | −0.595 |
| | Pearson’s r | ≥0.975 | 0.999 | 0.999 | 0.999 |
| | Difference in median of normal range samples | NA | 0.30 | 0.40 | 0.30 |
| aPTT Lupus<sup>b</sup> | n | 147 | 125 | 151 |
| | Slope (Passing–Bablok) | 1.00 ± 0.10 | 0.943 | 1.014 | 1.031 |
| | Intercept | NA | 1.38 | 0.174 | −1.04 |
| | Pearson’s r | ≥0.975 | 0.999 | 1.000 | 1.000 |
| | Difference in median of normal range samples | NA | 0.8 | 0.4 | 0.4 |
| aPTT Screen<sup>b</sup> | n | 131 | 129 | 144 |
| | Slope (Passing–Bablok) | 1.00 ± 0.10 | 1.023 | 0.987 | 0.992 |
| | Intercept | NA | −0.707 | 0.357 | 0.222 |
| | Pearson’s r | ≥0.975 | 0.998 | 0.999 | 0.999 |
| | Difference in median of normal range samples | NA | 0.45 | 0.30 | 0.15 |

<sup>a</sup>aPTT, activated partial thromboplastin time; INR, international normalized ratio; NA, not applicable; PT, prothrombin time.

<sup>b</sup>Analyses based on INR.

<sup>c</sup>Analyses based on seconds.

<sup>d</sup>Units for acceptance criteria are INR for PT Rec and PT Owren, and seconds for aPTT, aPTT Lupus and aPTT Screen.

<sup>e</sup>Analysis performed at Vienna site.
Owren assays are intended to aid in the management of vitamin K antagonist therapy and PT Rec may also be used as a diagnostic tool for liver function testing. Compared with PT Rec, the PT Owren assay is designed to be less sensitive to interference by lupus antibodies and is insensitive to variations in factor V and fibrinogen; the PT clotting time measured with both assays is not influenced by therapeutic plasma concentrations of UFH or low-molecular-weight heparin (data not shown). The aPTT assays provide a means of screening for abnormalities in intrinsic coagulation pathway factors VIII, IX, XI, XII and common pathway factors II, V,
X and fibrinogen.\textsuperscript{18,20,25,29} An aPTT test may be required as part of pre-surgical evaluation for bleeding tendencies, for monitoring UFH therapy, or following treatment with thrombin inhibitors (eg, bivalirudin, argatroban, or dabigatran).\textsuperscript{10,12,14} Additionally, compared with the aPTT Screen assay, the aPTT assay has reduced lupus sensitivity, while the aPTT Lupus assay has increased lupus sensitivity. The aPTT Screen assay has the highest sensitivity towards UFH, compared with the aPTT and aPTT Lupus assays.

Importantly, measurements with each assay showed good agreement when performed on the cobas t 711 and cobas t 511 analysers, thus demonstrating equivalence between the two platforms. Both systems are built from functionally identical components and implement identical assay processes using identical reagents and disposables. The systems provide automatic reagent reconstitution, continuous loading of all samples, reagents and cuvettes, and a high reagent storage capacity and are thus aligned with the need for efficient workflow in core laboratories; the main difference between platforms is the level of throughput, with the cobas t 711 platform being classed as high-throughput (390 tests/h) and the cobas t 511 platform as medium throughput (195 tests/h). Laboratory coagulation testing accounts for around one-fifth of the total sample workload of typical core laboratories. For example, in the core laboratory at Debrecen, \textasciitilde10\% of the 4.5 million tests per year are coagulation tests; however, the actual workload is much higher due to the requirement for further tests such as those for LA, factor deficiencies and von Willebrand disease. Therefore, improvements in throughput and efficiency are highly desirable and can result in significant capacity gains and cost savings.

Strengths of the study include the multicentre design (including four core laboratories in different European countries) and adherence to CLSI guidelines. Furthermore, method comparisons were performed against relevant commercially available assays/platforms and reference ranges were established for each assay using samples from 200 apparently healthy volunteers. Although not a limitation of the study design, which aimed to assess the analytical performance of the assays and their interchangeability with existing commercially available methods, the authors note that this study did not aim to assess the clinical performance of the assays on the cobas t analysers in terms of their ability to detect abnormalities in coagulation factors, or assess therapeutic drug levels; nevertheless, checks of factor-deficient samples included in the study showed that the assays were able to detect the deficiencies adequately (data not shown).

In summary, based on the excellent analytical performance and good agreement with relevant comparator methods, the five coagulation assays on the novel cobas t 711 and cobas t 511 analysers are suitable for routine use in core laboratories.

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CONFLICT OF INTEREST

S. Kitchen has received speaker fees from Roche Diagnostics. U. Geisen’s organization has received research grants from Roche Diagnostics International AG, Rotkreuz, Switzerland; he personally has received a consulting fee from Roche Pharma AG, Grenzach-Wyhlen, Germany. J. Kappelmayer has received speaker fees from Roche Diagnostics. P. Quehenberger has no competing interests. A. Lowe has no competing interests. R. Jones has no competing interests. J.G. Boehm was employed by Roche Diagnostics International Ltd as a consultant and study manager at the time of the study. G. Miles is an employee of Roche Diagnostics Inc., Indianapolis. G. Rozsnyai is an employee of Roche Diagnostics International Ltd.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to the conception or design of the work, or the acquisition, analysis, or interpretation of data for the work; drafted or revised the manuscript critically for important intellectual content; approved the version to be published; and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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REFERENCES

1. Strauss R, Wehler M, Kreuter D, et al. Incidence and implications of coagulopathy in medical intensive care patients. Crit Care. 2001;5(Suppl 1):P105.
2. Jairath V, Kahan BC, Stanworth SJ, et al. Prevalence, management, and outcomes of patients with coagulopathy after acute nonvariceal upper gastrointestinal bleeding in the United Kingdom. Transfusion. 2013;53(5):1069-1076.
3. Maegle M. Acute traumatic coagulopathy: incidence, risk stratification and therapeutic options. World J Emerg Med. 2010;1(1):12-21.
4. Tripodi A, Chantarangkul V, Martinelli I, Bucciarelli P, Mannucci PM. A shortened activated partial thromboplastin time is associated with the risk of venous thromboembolism. Blood. 2004;104(12):3631-3634.
5. Lin CH, Kuo YW, Kuo CY, et al. Shortened activated partial thromboplastin time is associated with acute ischemic stroke, stroke severity, and neurological worsening. J Stroke Cerebrovasc Dis. 2015;24(10):2270-2276.
6. Boral BM, Williams DJ, Boral LI. Disseminated intravascular coagulation. Am J Clin Pathol. 2016;146(6):670–680.
7. Ha NB, Regal RE. Anticoagulation in patients with cirrhosis: caught between a rock-and-a-hard place. Ann Pharmacother. 2016;50(5):402-409.
