


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

Steve Kitchen¹  | Ulrich Geisen² | Janos Kappelmayer³ | Peter Quehenberger⁴ | Julia Drieß² | Anna Lowe¹ | Robert Jones¹ | Jana G. Boehm⁵ | Gabrielle Miles⁶ | Gergely Rozsnyai⁷

¹Sheffield Haemostasis and Thrombosis Centre, Sheffield, UK

²Institute for Clinical Chemistry and Laboratory Medicine, Medical Centre – University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany

³Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

⁴Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria

⁵Roche Diagnostics GmbH, Mannheim, Germany

⁶Roche Diagnostics Inc., Indianapolis, Indiana

⁷Roche Diagnostics International Ltd, Rotkreuz, Switzerland

Correspondence: Steve Kitchen, Sheffield Haemostasis and Thrombosis Centre, Royal Hallamshire Hospital, Glossop Road, Sheffield, S11 8RN, UK (Steve.Kitchen@sth.nhs.uk).

Funding information

This study was funded by Roche Diagnostics International Ltd (Rotkreuz, Switzerland). Medical writing assistance was provided by David Evans, PhD, and Louise Kelly (Gardiner-Caldwell Communications, Macclesfield, UK), which was funded by Roche Diagnostics International Ltd (Rotkreuz, Switzerland).

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 $\leq 1.3\%$ for within-run precision and $\leq 6.3\%$ 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 $r \geq 0.995$). Lot-to-lot consistency was high for all assays and comparisons (Pearson's $r \geq 0.998$). 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 23.9–33.2 (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.¹⁻⁶ 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.^{3,7-11} 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 antithrombotic therapies.^{5,8-10,12-19} In particular, assessments of prothrombin time (PT) and activated partial thromboplastin time (aPTT), relating to the activity of the extrinsic/common and intrinsic/common pathways of coagulation, respectively, are widely used to detect hypocoagulation.^{4,5,8,15-17,20-25}

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.^{26,27}

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 equivalency 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-spun (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. (Nümbrecht, 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.²³ 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.²⁸ 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.^{18,20,25,29} 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) EP05-A3 guidelines.³⁰ 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 guidelines³¹: (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 ($N = 200$; $n \approx 67$ 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 Pathromtin 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 ^a (%)	Within-run precision, range of % CV		Total reproducibility acceptance criteria ^a (%)	Total reproducibility, range of % CV	
		cobas t 711	cobas t 511		cobas t 711	cobas t 511
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.³² 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.³³

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

TABLE 2 Method comparison: cobas t 711 vs comparator device

Comparison	Evaluation	Acceptance criteria ^d	Freiburg	Sheffield	Debrecen	Vienna	Vienna
			Lot 1	Lot 2	Lot 3	Lot 2	Lot 3
PT Rec vs Innovin ^a	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 <i>r</i>	≥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 ^a	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 <i>r</i>	≥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 ^b	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 <i>r</i>	≥0.850	0.986	0.982	0.980	0.980	
aPTT Lupus vs Actin FSL ^b	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 <i>r</i>	≥0.850	0.967	0.983	0.987	0.982	
aPTT Screen vs Pathromtin SL ^b	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 <i>r</i>	≥0.850	0.980	0.985	0.985	0.964	
aPTT vs STA Cephascreen ^{b,c}	n		175				
	Pearson's <i>r</i>	NA ^e	0.819				
aPTT Lupus vs STA Cephascreen ^{b,c}	n		99				
	Pearson's <i>r</i>	NA ^e	0.943				
aPTT Lupus vs STA-LA ^{b,c}	n		128				
	Slope (Deming)	NA ^e	0.591				
	Intercept	NA ^e	10.21				
	Pearson's <i>r</i>	NA ^e	0.958				
aPTT Screen vs aPTT ^{b,c}	n		153				
	Pearson's <i>r</i>	NA ^e	0.833				

aPTT, activated partial thromboplastin time; INR, international normalized ratio; LA, lupus anticoagulant; NA, not applicable; PT, prothrombin time.

^aAnalyses based on INR.

^bAnalyses based on seconds.

^cPerformed at one site only (Mannheim, Germany).

^dUnits for acceptance criteria are INR for PT Rec and PT Owren, and seconds for aPTT, aPTT Lupus and aPTT Screen.

^eTest and report.

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 33.2 (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 (21.7-24.6) to 39.8 (36.5-57.7).

Assay	Evaluation	Acceptance criteria ^c	Freiburg	Sheffield
			Lot 1	Lot 2
PT Rec ^a	n		129	135
	Slope (Passing-Bablok)	1.00 ± 0.10	1.006	0.984
	Intercept	NA	0.006	0.033
	Pearson's <i>r</i>	≥0.900	1.000	1.000
	Bias at 1.0 INR	NA	0.0119	0.0175
PT Owren ^a	n		145	
	Slope (Passing-Bablok)	1.00 ± 0.10	0.982	
	Intercept	NA	0.00947	
	Pearson's <i>r</i>	≥0.900	1.000	
	Bias at 1.0 INR	1 ≤ 0.10	−0.008	
aPTT ^b	n		139	129
	Slope (Passing-Bablok)	1.00 ± 0.10	1.000	1.000
	Intercept	NA	0	0.100
	Pearson's <i>r</i>	≥0.900	0.998	0.995
	Difference in median of normal range samples	NA	0.15	0.05
aPTT Lupus ^b	n		145	125
	Slope (Passing-Bablok)	1.00 ± 0.10	0.996	1.009
	Intercept	NA	0.139	−0.130
	Pearson's <i>r</i>	≥0.900	0.999	0.999
	Difference in median of normal range samples	NA	0.20	0.20
aPTT Screen ^b	n		131	128
	Slope (Passing-Bablok)	1.00 ± 0.10	0.996	0.980
	Intercept	NA	0.507	0.604
	Pearson's <i>r</i>	≥0.900	0.999	0.998
	Difference in median of normal range samples	NA	0.35	0.10

aPTT, activated partial thromboplastin time; INR, international normalized ratio; NA, not applicable; PT, prothrombin time.

^aAnalyses based on INR.

^bAnalyses based on seconds.

^cUnits for acceptance criteria are INR for PT Rec and PT Owren, and seconds for aPTT, aPTT Lupus and aPTT Screen.

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 within 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

TABLE 3 Method comparison between cobas t 711 and cobas t 511 analysers

TABLE 4 Lot-to-lot comparison on the **cobas t 711** analyser

Assay	Evaluation	Acceptance criteria ^c	Freiburg	Sheffield	Debrecen
			Lot 2 vs 1	Lot 3 vs 2	Lot 1 vs 3
PT Rec ^a	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 <i>r</i>	≥0.975	1.000	1.000	1.000
	Bias at 1.0 INR	NA	0.01	0.01	−0.00476
PT Owren ^a	n		144	132 ^d	135
	Slope (Passing–Bablok)	1.00 ± 0.10	1.002	0.989	1.000
	Intercept	NA	−0.00243	0.0219	−0.0070
	Pearson's <i>r</i>	≥0.950	1.000	1.000	1.000
	Bias at 1.0 INR	NA	0.000	0.011	−0.007
aPTT ^b	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 <i>r</i>	≥0.975	0.999	0.999	0.999
	Difference in median of normal range samples	NA	0.30	0.40	0.30
aPTT Lupus ^b	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 <i>r</i>	≥0.975	0.999	1.000	1.000
	Difference in median of normal range samples	NA	0.8	0.4	0.4
aPTT Screen ^b	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 <i>r</i>	≥0.975	0.998	0.999	0.999
	Difference in median of normal range samples	NA	0.45	0.30	0.15

aPTT, activated partial thromboplastin time; INR, international normalized ratio; NA, not applicable; PT, prothrombin time.

^aAnalyses based on INR.

^bAnalyses based on seconds.

^cUnits for acceptance criteria are INR for PT Rec and PT Owren, and seconds for aPTT, aPTT Lupus and aPTT Screen.

^dAnalysis performed at Vienna site.

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

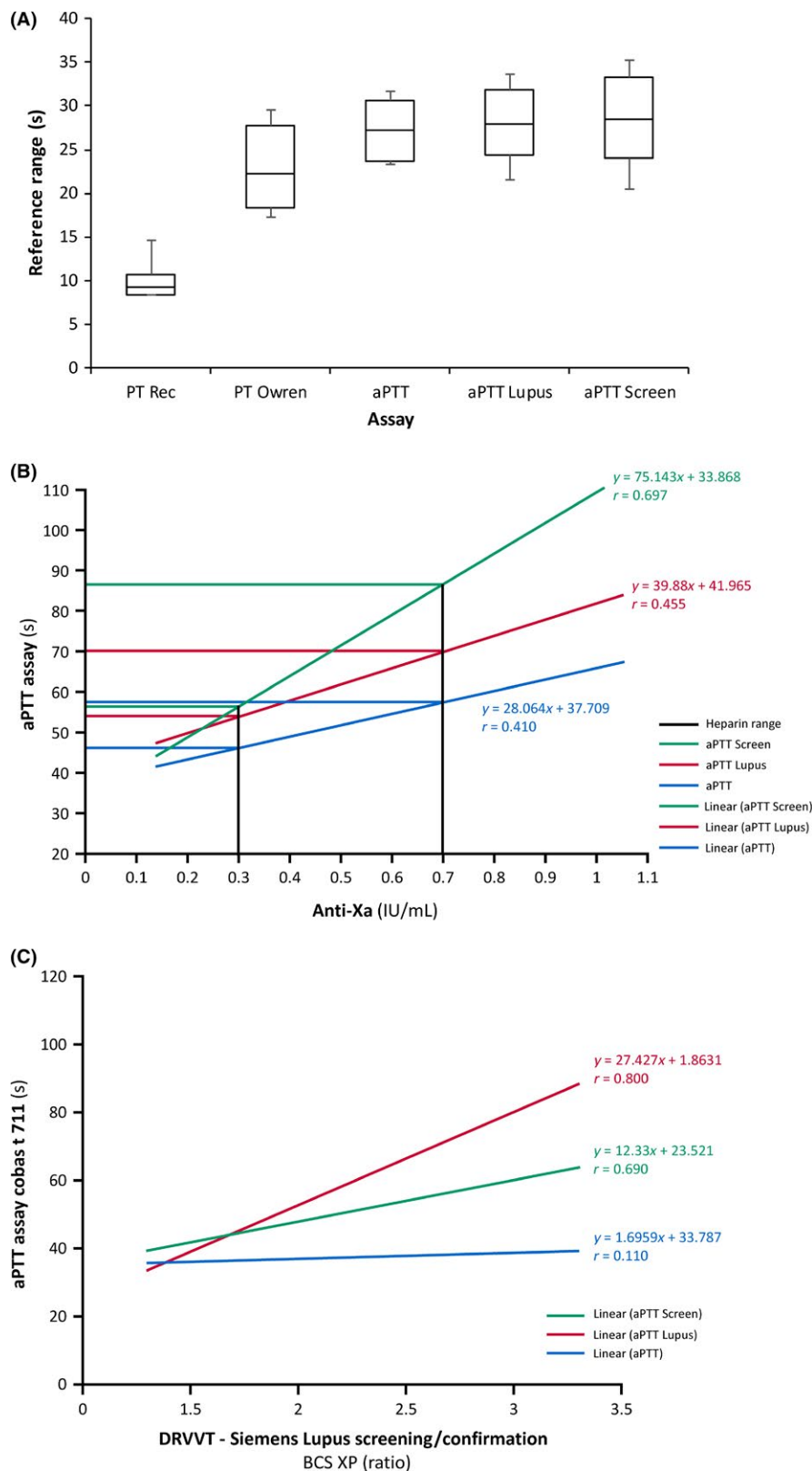


FIGURE 1 Reference Ranges for the Five Assays^a (A) and Evaluation of aPTT Assay Sensitivity to Unfractionated Heparin (UFH) and Lupus Anticoagulant (LA), Shown as Therapeutic Ranges of UFH (B) and Comparative LA Sensitivity of the Roche aPTT Assays on the cobas t 711 Analyser (C). ^aFor each assay, 200 fresh samples (0.109M/3.2% citrate in Sarstedt tubes) were measured using three reagent lots. Boxes depict the median value and reference range (2.5th and 97.5th percentiles); whiskers represent minimum and maximum values. aPTT, activated partial thromboplastin time; PT, prothrombin time

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.^{17,23,28,38} 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).²⁸ 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.^{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).^{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, ~10% 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.

ACKNOWLEDGEMENTS

The authors wish to thank Regina Perez (Roche Diagnostics) and David Caley (Icon Docs, employed as a CRA for Roche Diagnostics)

for support with additional data analysis and recalculation, as well as John Burden and Juan Wu (Roche Diagnostics) for their contributions to the aPTT sensitivity analyses.

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.

ORCID

Steve Kitchen  <http://orcid.org/0000-0002-6826-8519>

REFERENCES

1. Strauss R, Wehler M, Kreutzer 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. Maegele 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-liver and a hard place. *Ann Pharmacother*. 2016;50(5):402-409.

8. Mehta TP, Smythe MA, Mattson JC. Strategies for managing heparin therapy in patients with antiphospholipid antibody syndrome. *Pharmacotherapy*. 2011;31(12):1221-1231.
9. Van Cott EM, Roberts AJ, Dager WE. Laboratory monitoring of parenteral direct thrombin inhibitors. *Semin Thromb Hemost*. 2017;43(3):270-276.
10. Vandiver JW, Vondracek TG. Antifactor Xa levels versus activated partial thromboplastin time for monitoring unfractionated heparin. *Pharmacotherapy*. 2012;32(6):546-558.
11. Falanga A, Russo L, Milesi V. The coagulopathy of cancer. *Curr Opin Hematol*. 2014;21(5):423-429.
12. Aarab R, van Es J, de Pont AC, Vroom MB, Middeldorp S. Monitoring of unfractionated heparin in critically ill patients. *Neth J Med*. 2013;71(9):466-471.
13. Adcock DM, Gosselin R. Direct oral anticoagulants (DOACs) in the laboratory: 2015 review. *Thromb Res*. 2015;136(1):7-12.
14. Faust AC, Kanyer D, Wittkowsky AK. Managing transitions from oral factor Xa inhibitors to unfractionated heparin infusions. *Am J Health Syst Pharm*. 2016;73(24):2037-2041.
15. Kamal AH, Tefferi A, Pruthi RK. How to interpret and pursue an abnormal prothrombin time, activated partial thromboplastin time, and bleeding time in adults. *Mayo Clin Proc*. 2007;82(7):864-873.
16. Samuelson BT, Cuker A, Siegal DM, Crowther M, Garcia DA. Laboratory assessment of the anticoagulant activity of direct oral anticoagulants: a systematic review. *Chest*. 2017;151(1):127-138.
17. Hirsh J, Fuster V, Ansell J, Halperin JL, American Heart Association/American College of Cardiology Foundation. American Heart Association/American College of Cardiology Foundation guide to warfarin therapy. *Circulation*. 2003;107(12):1692-1711.
18. Hayward CP, Moffat KA, Liu Y. Laboratory investigations for bleeding disorders. *Semin Thromb Hemost*. 2012;38(7):742-752.
19. Sorensen B, Ingerslev J. Dynamic APTT parameters: applications in thrombophilia. *J Thromb Haemost*. 2012;10(2):244-250.
20. Bowyer A, Kitchen S, Makris M. The responsiveness of different APTT reagents to mild factor VIII, IX and XI deficiencies. *Int J Lab Hematol*. 2011;33(2):154-158.
21. Hron G, Eichinger S, Weltermann A, Quehenberger P, Halbmayer WM, Kyrle PA. Prediction of recurrent venous thromboembolism by the activated partial thromboplastin time. *J Thromb Haemost*. 2006;4(4):752-756.
22. Ten Boekel E, Bartels P. Abnormally short activated partial thromboplastin times are related to elevated plasma levels of TAT, F1+2, D-dimer and FVIII:C. *Pathophysiol Haemost Thromb*. 2002;32(3):137-142.
23. Quick J, Stanley-Brow M, Bancroft F. A study of the coagulation defect in hemophilia and in jaundice. *Am J Med Sci*. 1935;190:501-511.
24. Green D. Interpreting coagulation assays. *Blood Coagul Fibrinolysis*. 2010;21(Suppl 1):S3-S6.
25. Ignjatovic V. Activated partial thromboplastin time. *Methods Mol Biol*. 2013;992:111-120.
26. Monagle P, Barnes C, Ignjatovic V, et al. Developmental haemostasis. Impact for clinical haemostasis laboratories. *Thromb Haemost*. 2006;95(2):362-372.
27. Gardiner C, Kitchen S, Dauer RJ, Kottke-Marchant K, Adcock DM. Recommendations for evaluation of coagulation analyzers. *Lab Hematol*. 2006;12(1):32-38.
28. Owren PA. Thrombotest. A new method for controlling anticoagulant therapy. *Lancet*. 1959;2(7106):754-758.
29. Langdell RD, Wagner RH, Brinkhous KM. Effect of antihemophilic factor on one-stage clotting tests; a presumptive test for hemophilia and a simple one-stage antihemophilic factor assay procedure. *J Lab Clin Med*. 1953;41(4):637-647.
30. Clinical and Laboratory Standards Institute (CLSI). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline*, 3rd edn. CLSI document EP05-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
31. Clinical and Laboratory Standards Institute (CLSI). *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline*, 3rd edn. CLSI document EP09-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2013.
32. Kunst A, Busse-Grawitz A, Engeldinger W, Koch W, Luthe H, Stockmann W. WinCAEv – a new program supporting evaluations of reagents and analysers. *Clin Chim Acta*. 2005;355:S361.
33. Clinical and Laboratory Standards Institute (CLSI). *One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test; Approved Guideline*. CLSI document H48-A. Wayne, PA: Clinical and Laboratory Standards Institute; 1996.
34. Geens T, Vertessen F, Malfait R, Deiteren K, Maes MB. Validation of the Sysmex CS5100 coagulation analyzer and comparison to the Stago STA-R analyzer for routine coagulation parameters. *Int J Lab Hematol*. 2015;37(3):372-381.
35. Toulon P, Fischer F, Appert-Flory A, Jambou D. Evaluation and performance characteristics of the Q Hemostasis Analyzer, an automated coagulation analyzer. *Thromb Res*. 2014;133(5):927-935.
36. Milos M, Herak D, Kuric L, Horvat I, Zadro R. Evaluation and performance characteristics of the coagulation system: ACL TOP analyzer - HemosIL reagents. *Int J Lab Hematol*. 2009;31(1):26-35.
37. Flieder T, Gripp T, Knabbe C, Birschmann I. The Sysmex CS-5100 coagulation analyzer offers comparable analytical performance and excellent throughput capabilities. *Pract Lab Med*. 2016;6(Supplement C):38-47.
38. Limdi JK, Hyde GM. Evaluation of abnormal liver function tests. *Postgrad Med J*. 2003;79(932):307-312.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Kitchen S, Geisen U, Kappelmayer J, et al. Evaluating the analytical performance of five new coagulation assays for the measurement of prothrombin time and activated thromboplastin time. *Int J Lab Hem*. 2018;00:1-10. <https://doi.org/10.1111/ijlh.12897>