Evaluation of a High Throughput Multi-wavelength Blood Coagulation Analyser - Sysmex CS-5100

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We assessed the performance of a prototype high throughput coagulation system (CS-5100) from Sysmex Corporation Japan, in parallel with their intermediate throughput CS-2000i. The new analyser has the advantage of 20 multi-wavelength reaction detector positions as opposed to 10 on the CS-2000i, thereby approximately halving the processing time of most batched assays. In our evaluation we assessed performance in clotting (prothrombin time [PT], activated partial thromboplastin time [APTT], Clauss fibrinogen [Fbg], one-stage factor VIII [FVIII]) chromogenic (antithrombin [AT], factor XIII [FXIII]), immuno-turbidometric (D-dimer [DDi], von Willebrand factor antigen [VWF:Ag]) and platelet agglutination based ristocetin co-factor (VWF:RCo) test systems. For the FVIII and VWF:RCo assays, all samples were tested using the multi-dilution analysis (MDA) utility, to assess linearity and parallelism of the dose response curve to detect possible false results due to inhibitors or sample activation. For all other assays a single point determination was made with automatic redilution if the relative potency was outside the range of the standard curve. The CS-5100 methods showed good linearity and reproducible standard curves, and gave low inter-assay imprecision using commercial normal (CV = 0.73-6.3%) and pathological (CV = 0.04-9.9%) control plasmas (preparations tested 10 times on each of 5 days). Good correlations were observed between CS-2000i and CS-5100 using clinical samples in each of the test systems (PT, APTT, Fbg, FVIII, FXIII, VWF:Ag, VWF:RCo, AT and D-Dimer. R^2 = 0.96-0.99), with no clinically significant misclassification, and data points scattered closely around the line of identity. Our results demonstrated that using the CS-5100 analyser, routine coagulation testing and specialised assays can be performed with satisfactory imprecision and show good correlation with the CS-2000i.

Key Words Coagulation Analyser, High Throughput, Multi-wavelength Blood Coagulation Analyser

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INTRODUCTION

Centralisation of diagnostic laboratory testing, with an ever growing number of urgent requests increases the need for short turn-around times and high-throughput coagulation analysers. Sysmex Corporation has recently introduced the CS series of coagulation analysers[1,2] as their next generation replacement for the CA range[3-6]. In addition to standard sample analysis in clotting, chromogenic and immunoturbidometric test systems, the new analysers have the facility to measure factor XIII by ammonia release assay[7] and von Willebrand factor ristocetin co-factor activity (VWF:RCo) in a platelet agglutination technique[8,9]. The new series of analysers also has the ability to undertake pre-analytical checks to determine the quality of samples being analysed. Primary sample tube volume can be assessed for inappropriate sample collection. The instrument then flags sample results based upon an incorrect tube fill volume according to user-programmable configuration settings. The system can also perform a pre-analytical scan at three wavelengths (405nm, 575nm, and 660nm) to check plasma samples for haemolysis, icterus, and lipemia (HIL Check)[2].

The new analyser, CS-5100 (***Fig. 1***), has the advantage of 20 multi-wavelength reaction detector positions as opposed to 10 on the intermediate throughput CS-2000*i*, thereby approximately halving the processing time of most batched assays. Additionally the new analyser has the capability to either aspirate samples from a primary tube using a cap piercing probe or a non-piercing probe. The non-piercing probe is employed in micro-mode, which as its name suggests, was designed minimise the sample volume requirement by taking the sample directly from the primary sample tube to the reaction tube as opposed to taking a daughter aliquot and sub-sampling from that.

- Size of Main Unit: 1030mm × 1150mm × 1270mm (W × D × H)
- Weight: 284Kg
- Sampling Modes: Cap Piercing or Micro Mode
- Reaction Types: Clotting, Chromogenic, Immunoturbidometric, Agglutination
- Number of Reaction Detectors: 20 Multi-wavelength, 8 Stirred
- Capacity of Sampler Unit: 10 × 10 samples racks

Fig. 1 *Physical Description of CS-5100*

In the current study we assessed the performance of a prototype high throughput coagulation system (CS-5100), in parallel with a CS-2000*i* production analyser. The study was undertaken in two phases, initially CS-5100 assay imprecision was assessed using commercial lyophilised control plasmas. The second phase examined comparability of results from the CS-5100 and CS2000*i* using a range of normal and clinical samples.

MATERIALS AND METHODS

For all haemostatic tests, reagents were from Siemens Healthcare Diagnostics (Marburg, Germany) and assays were performed on a CS-5100 automated coagulation analyser (Sysmex Corporation, Kobe, Japan) with a CS-2000*i* being used as the reference analyser. Innovin and Actin FS were used in prothrombin time (PT) and activated partial thromboplastin time (APTT) respectively; fibrinogen (Fbg) was measured by the Clauss technique. Antithrombin (Innovance Antithrombin), and factor XIII (BC Factor XIII) were assayed using chromogenic techniques; D-Dimer (Innovance D-Dimer kit) was assayed using an Immunoturbidometric method. Factor VIII was assayed using a one-stage clotting technique (FVIII:C) and VWF:RCo by ristocetin dependant platelet agglutination (BC von Willebrand reagent), with samples in both assays being tested at three dilutions prepared using the multi-dilution analysis utility (MDA). Levels of clotting factors and physiological inhibitors of coagulation were determined relative to the same commercial reference plasma, Standard Human Plasma (Siemens Healthcare Diagnostics). Local assignment of thromboplastin international sensitivity index (ISI) was undertaken with AK Calibrant (Technoclone GmbH. Vienna, Austria).

Imprecision studies were performed using commercial lyophilised normal and pathological plasmas from Siemens Healthcare Diagnostics. For PT and APTT these were CiTrol 1, 2 and 3; for Fbg, FVIII, FXIII, VWF:RCo, and AT assays, Control Plasma N (CPN) and Control Plasma P (CPP) were used; and for D-Dimer Innovance normal and pathological controls, Control 1 and 2 were employed. For PT, APTT, Fbg, FXIII, AT, D-Dimer, each preparation was assayed with ten replicates for five days, while for FVIII and VWF:RCo three dilution MDA assays with 10 replicates for 5 days were used. For comparability studies samples were obtained from healthy normal subjects (n = 30) and various clinical groups which are detailed in ***Table 1***; the samples from patients with VWD were taken from various sub-types (Type 1, n = 14; Type 2A, n = 2; Type 2B, n = 8; Type 2M, n = 9; Type 2N, n = 2; Type 3, n = 3; Acquired, n = 1).

Table 1 *Sample groups tested to compare results from CS-5100 and CS-2000i*

Normal	n = 30			
Haemolysed	n = 10 (Plasma Hb 0.5–5.7 g/L)			
Icteric	n = 20 (Total Bilirubin up to 350 µmol/L)			
Lipaemic	n = 40 (Cholesterol up to 8.6 mmol/L (Triglycerides up to 9.4 mmol/L)			
D-Dimers Positive	n = 40 (> 0.5 mg/L FEU)			
High Fibrinogen	n = 10 (> 4.0 g/L)			
low Fibrinogen	n = 10 (< 1.5 g/L)			
Warfarin	n = 20 (INR > 1.5)			
Heparin (UF)	n = 10 (0.17–0.80 iu/mL)			
Heparin (LMWH)	n = 10 (0.42–0.97 iu/mL)			
LA positive	n = 20			
VWD	n = 39			
Biochemical Reference Ranges	Plasma Hb (g/L)	Total Bilirubin (µmol/L)	Cholesterol (mmol/L)	Triglycerides (mmol/L)
	< 0.5	< 17	3.60–5.20	0.46–1.88

The biochemical analytes: Total bilirubin (Bil), Cholesterol (Chol), and Triglycerides (Trig) were quantified using a COBAS MIRA analyser (Horiba ABX-UK, Northampton, UK) with reagents from Horiba ABX. Plasma Haemoglobin (Plasma Hb) was measured with a HemoCue, Plasma/Low Hb Photometer (HemoCue Ltd, Dronfield, Derbyshire, UK).

Statistical analysis was performed using Analyse-It® software version 2.03 (Analyse-It Software Ltd, www.analyse-it.com). After assessing the data for normality of distribution, summary results were presented as mean and standard deviation; comparisons of data were made using Pearson correlation. For all analyses a 2-tailed test was used and $p < 0.05$ was considered significant.

RESULTS

Assay imprecision was assessed using ten replicate tests of commercial lyophilised normal and pathological plasmas (Siemens Healthcare Diagnostics) on each of five days. The coefficient of variation (CV) was calculated for each test on the CS-5100 (***Table 2***). Acceptable levels of imprecision were obtained for all tests. The highest levels of imprecision, as assessed by CV, were observed in those assays using MDA, FVIII (CPN 5.6%, CPP 7.1%) and VWF:RCo (CPN 6.3%, CPP 9.9%) (***Table 2***).

Results from the CS-5100 compared well with those from the CS-2000*i* reference analyser exhibiting good correlation (Pearson correlation) for all assays, with a close relationship to the line of identity, as seen in ***Figs 2 to 3***. Prothrombin time correlation was very good at $R^2 = 0.999$ ($p \leq 0.0001$). Similarly, INR values obtained for warfarinsed samples produced an R^2 value of 0.995 ($p \leq 0.0001$), with normal samples appearing to continue in this trend (***Fig. 2B***). APTT using Actin FS reagent correlated well between the analysers ($R^2 = 0.983$, $p \leq 0.0001$). All samples investigated for VWF Antigen (***Fig. 4A***) and VWF:RCo (***Fig. 4B***) correlated well, with R^2 values of 0.995 and 0.990, respectively ($p \leq 0.0001$), and VWF:Ag to VWF:RCo ratios (***Fig. 5***) were commensurate with their VWD sub type on both analysers.

In samples containing interfering substances (haemolysis; icterus; and lipaemia. n = 70. ***Table 1***), there was no apparent effect on the correlation between analysers for PT, APTT, Fbg, AT, FXIII or DDi (***Figs 2 and 3***). At higher FVIII potency, icteric samples appeared to deviate from the line of identity with a possible bias towards the CS-2000*i*, however the correlation still remained good ($R^2 = 0.967$, $p \leq 0.0001$; ***Fig. 3A***).

Throughput rates for sample analysis were calculated from the start of an analytical run (i.e. including initialisation of the analyser) and were extrapolated from processing 50 samples. The CS-5100 was able to process over 150 samples per hour for APTT (Actin FS) and PT (Innovin) compared to 51 samples per hour for the CS-

Table 2 *CS-5100 inter-assay imprecision*

	Control	Min	Max	Mean	SD	CV (%)
PT (s)	Citrol 1	11.4	11.7	11.5	0.08	0.73
	Citrol 2	30.9	31.9	31.4	0.24	0.76
	Citrol 3	53.4	55.5	54.5	0.44	0.81
APTT (s)	Citrol 1	27.4	27.9	27.6	0.10	0.38
	Citrol 2	50.2	51.6	50.8	0.30	0.58
	Citrol 3	71.1	72.9	72.2	0.51	0.70
Fbg (g/L)	CPN	2.22	2.60	2.38	0.09	3.96
	CPP	0.70	0.82	0.75	0.03	0.04
FVIII (%)	CPN	77.8	98.4	87.3	4.89	5.60
	CPP	26.4	35.4	30.9	2.18	7.08
VWF: RCo (%)	CPN	76.8	108.0	92.2	5.83	6.32
	CPP	21.3	34.0	26.3	2.61	9.92
FXIII (%)	CPN	77.2	91.4	853	2.83	3.32
	CPP	23.7	32.3	27.6	1.64	5.94
AT (%)	CPN	95.9	105.8	99.7	2.14	2.14
	CPP	33.6	36.8	35.2	0.87	2.46
DDi (mg/L FEU)	Control 1	0.29	0.36	0.32	0.02	5.47
	Control 2	2.54	2.92	2.71	0.09	3.48

PT: Prothrombin Time, APTT: Activated Partial Thromboplastin Time, Fbg: Clauss Fibrinogen,
FVIII: Factor VIII, VWF: RCo: von Willebrand factor ristocetin co-factor activity, FXIII: Factor XIII,
AT: Antithrombin; DDi: D-Dimer CPN = Control Plasma N CPP = Control Plasma P

2000*i*. Greater than 300 samples per hour could be processed for PT (Innovin) on the CS-5100, almost twice the throughput of the CS-2000*i*. Approximately 169 samples could be processed for VWF Antigen on the CS-5100 compared with 72 on the CS-2000*i*. VWF:RCo throughput was comparable on both instruments (26 and 19 samples per hour on the CS-5100 and CS-2000*i*, respectively).

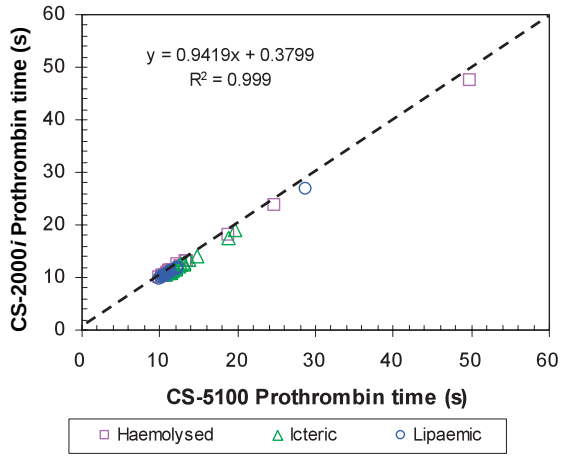
DISCUSSION

In the present study a prototype CS-5100 fully automated coagulation analyser was evaluated against the CS-2000*i* (intermediate throughput). The CS-5100 was assessed for imprecision and method correlation for the parameters PT, APTT, Fbg, FVIII, FXIII, VWF:RCo, AT, and D-Dimer. Low levels of imprecision were seen for normal and abnormal plasma preparations in each of the assay types. Within-run (data not shown) and between day imprecision remained at low levels for most assays; however higher coefficients of variation were observed for FVIII and VWF:RCo assays, although these may be considered acceptable levels of variance for assays performed at multiple dilutions[8]. Additionally a new calibration curve was processed prior to analysis of the quality control material which may also account for higher variation between assays.

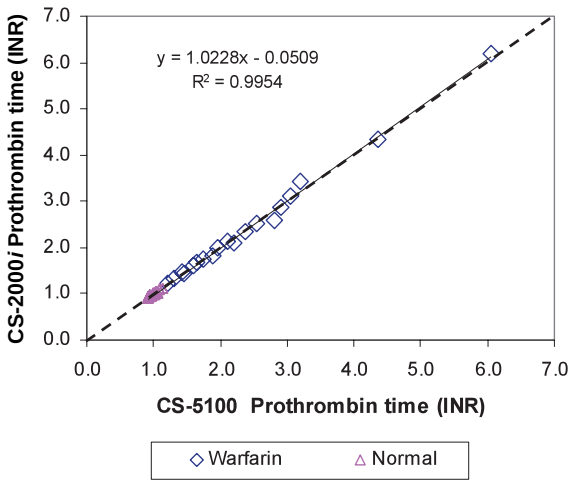
Method comparison was performed against the current Sysmex reference analyser CS-2000*i*, using clinical samples from patients with various pathologies (***Table 1***). Statistically significant correlation was observed for all parameters measured with both instruments. Little bias was seen in most assays, with the bulk of results closely surrounding the line of identity (***Figs 2-4***). Warfarinsed samples' INR values were calculated using locally determined ISI values generated from AK Calibrants (Technoclone). For INR in the range 1.5 to 6.0 good correlation was seen between analysers (R^2 = 0.995, p = < 0.0001) with all data on or closely associated with the line of identity as were INR values determined from normal healthy donor samples (***Fig. 2B***). VWFAg and VWF:RCo assays demonstrated excellent correlation between analysers (***Fig. 4***) with VWF:RCo/VWF:Ag ratio (< 0.7) detecting dysfunctional VWF in those samples from type 2 VWD[10-12] (***Fig. 5***).

Samples containing haemolysis, icterus and lipaemia were selected for analysis in each of the haemostatic tests to look for interference with the optical detection principle. Plasma haemoglobin, total bilirubin, triglycerides and cholesterol concentrations in most of these samples exceeded the normal plasma range;

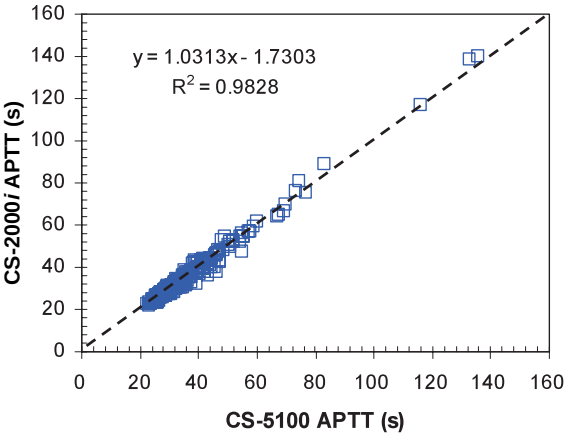
(A) PT HIL Samples Only



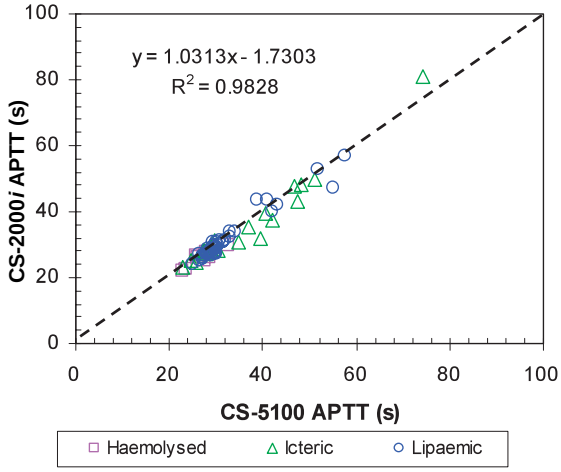
(B) PT Warfarin and Normal Samples Only



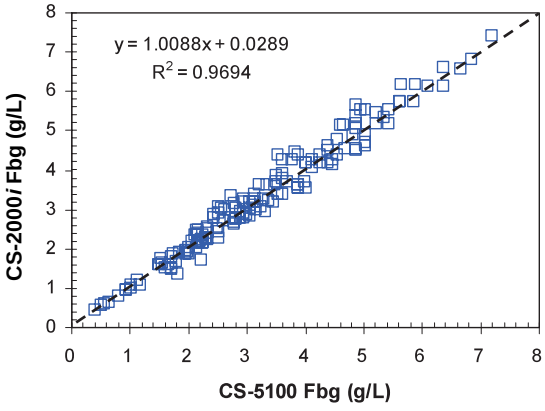
(C) APTT All samples



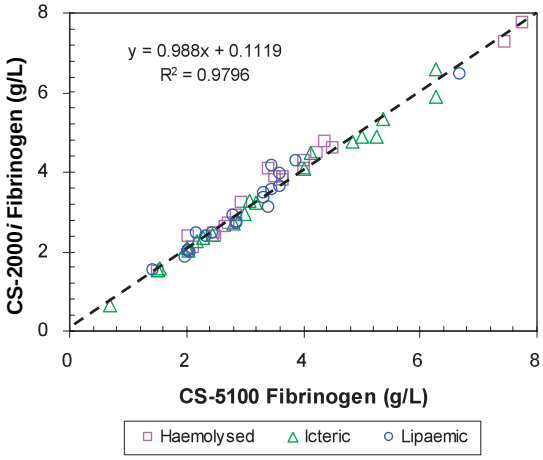
(D) APTT HIL Samples Only



(E) Fbg All samples



(F) Fbg HIL Samples Only



Broken line = Line of Identity

Fig. 2 *Comparison of CS-5100 and CS-2000i Prothrombin time, APTT and Clauss Fibrinogen results*

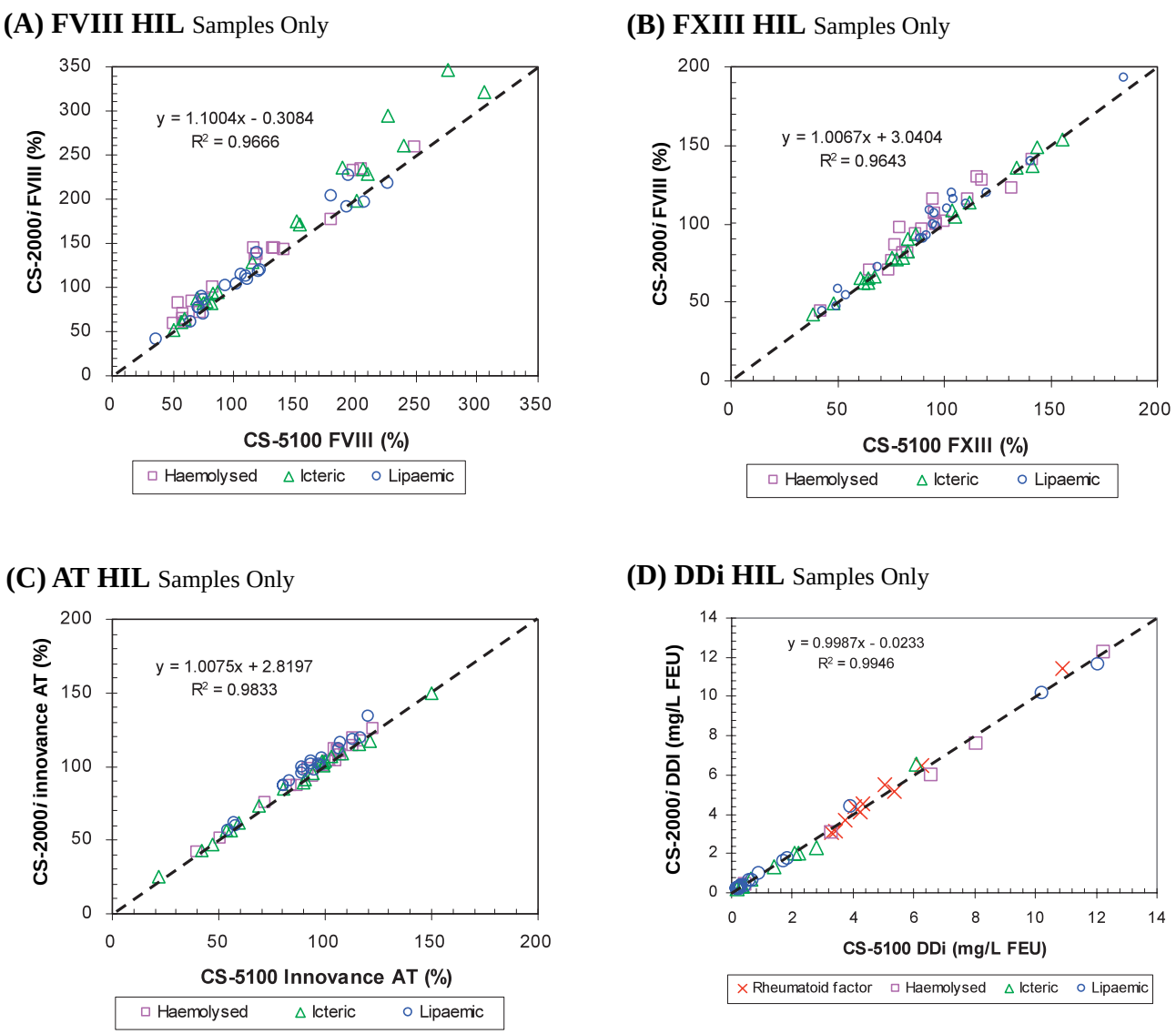


Fig. 3 *Comparison of CS-5100 and CS-2000i Factor VIII, Factor XIII, Antithrombin, and D-dimer results*

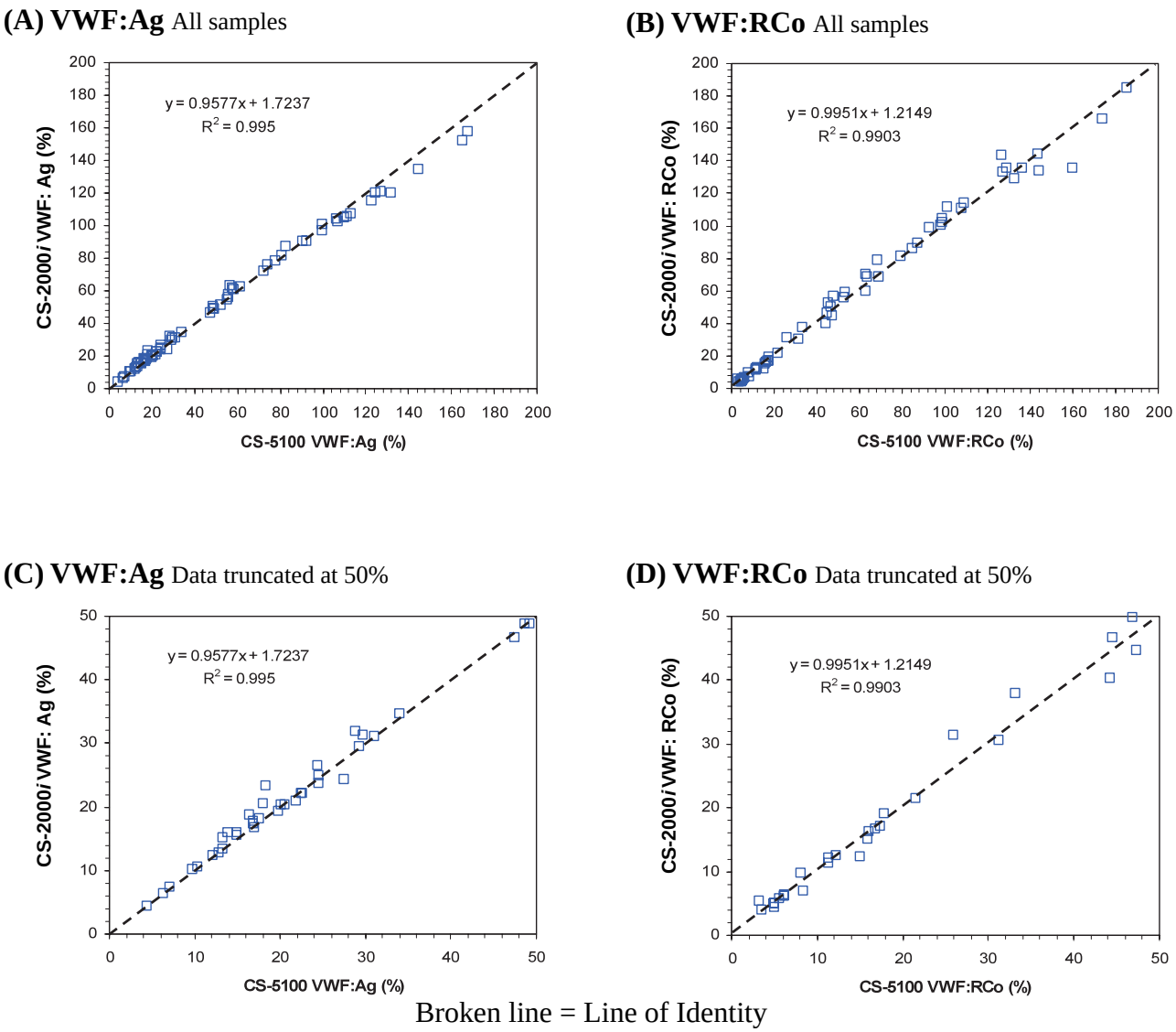
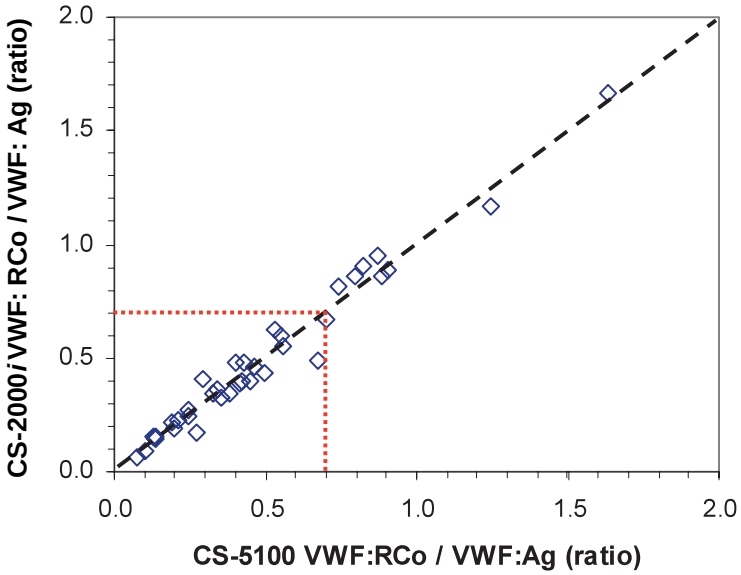


Fig. 4 *Comparison of CS-5100 and CS-2000i VWF antigen and Ristocetin Co-factor results*



Black Broken line = Line of Identity
Red Dotted line = cut-off for normality

Fig. 5 *Comparison of CS-5100 and CS-2000i VWF:RCo / VWF:Ag Ratios from VWD patients' samples*

however little influence was observed on any of the parameters measured. The HIL check system worked satisfactorily for these samples, detecting the presence of multiple interfering substances simultaneously in a single sample, however this feature is only available when analysis is performed in the standard cap-piercing mode. HIL flags returned by the CS-5100 were the same as the CS-2000*i* for most samples. No significant interference in results was seen for the majority of assays at high levels of haemolysis, icterus or lipaemia. The apparent bias in icteric samples with high FVIII levels was further investigated by examination of the clot reaction curve, but did not indicate that high concentrations of bilirubin or other optical interfering substances were a definitive cause for this bias.

Analyser throughput was measured during a number of runs and in general, the CS-5100 was found to be at least twice as fast as the CS-2000*i*. Software upgrades to the CS-5100 allowed the addition of stirrer bar (SB) cuvettes whilst the instrument was running a VWF:RCo assay (by MDA). This increased throughput to approximately 26 samples per hour, which negated the effect of four less SB cuvette positions on the prototype analyser compared with the CS-2000*i*. However, more intensive testing of analyser throughput would be beneficial to confirm rates of analysis in multiple test combinations.

Evaluation of the CS-5100 analyser returned encouraging results. The CS-5100 demonstrated that routine haemostasis testing as well as more specialised and specific assays of haemostatic parameters using clotting, chromogenic, immuno-turbidometric and platelet agglutination techniques can be performed with satisfactory imprecision. All assay results correlated well with the reference analyser, and little interference was seen in samples with high levels of total bilirubin, cholesterol, triglycerides or haemoglobin. We conclude that the CS-5100 is well suited for coagulation laboratories with a requirement for high sample throughput and a high number of both capped and open vial samples.

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