ORIGINAL ARTICLE

Stago Start Max analyser validation and early reaction errors (ERE) in haemostasis testing at Wellington SCL

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ABSTRACT

Background: At the Southern Community Laboratories (SCL) Wellington laboratory, the Sysmex CS2100i analyser (Siemens) is responsible for the testing of all samples submitted for haemostasis evaluation. One of the limitations of this equipment is a tendency to produce invalid international normalised ratio (INR) and activated partial thromboplastin (APTT) tests on some samples due to early reaction errors (ERE). This requires additional sample processing and sometimes a patient re-bleed. The STart Max (Stago) semi-automated benchtop analyser showed promise as a suitable alternative method since it used mechanical clot detection, rather than an optical method, and potentially the ability to eliminate problematic ERE's. This in turn might reduce delays in reporting results and sample recollection.

Methods: The STart Max analyser underwent validation using the methodology outlined in the IANZ specific criteria for accreditation (Medical Testing 7). Validation included the development of reference ranges for the prothrombin time/international normalised ratio (PT/INR) and the APTT. Accuracy & precision characteristics were assessed using patient samples, external quality control samples and samples that had previously produced ERE results on the CS2100i. All results were statistically evaluated using Analyse-it software.

Results: Results for the PT/INR and the APTT showed good correlation with the Sysmex CS2100i analyser (*r*-value >0.95) and external QC samples. However, for the APTT, there was a significant difference between the two methods (1-11 secs). The reference ranges for the STart Max were found to be similar to those in use for the INR on the Sysmex CS2100i. For APTT, the reference ranges did not show uniform similarity between the two methods. Tests for precision produced a coefficient of variation (CV) of < 4% in all tests except for the elevated range of the APTT where this was 4.96%. The STart Max analyser was able to generate reportable results for all samples that generated ERE results on the Sysmex CS2100i analyser.

Conclusions : The generation of patient sample results affected by unresolvable ERE results with the Sysmex CS2100i analyser highlighted the need for an alternative method in the laboratory. This study has shown that the STart Max analyser produced comparable results to those from the CS2100i. With the exception of the APTT, a regional biological reference range can be used for reporting results from the STart Max analyser. The STart Max analyser also showed that it was able to generate reportable APTT results on samples rejected for ERE using the Sysmex CS2100i analyser. The results of this study has allowed the validation of the STart Max analyser for use at Wellington SCL.

Key words: haemostasis, international normalised ratio, activated partial thromboplastin, early reaction errors.

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INTRODUCTION

Early reaction errors (ERE) are encountered on patient plasma samples using the photo-optical clot detection method used on the Sysmex CS2100i analyser (Siemens) currently installed at Wellington SCL. Early reaction errors are abnormal reactions that occur on some samples at the initial stages of the APTT coagulation reaction (1). This finding leads to additional sample preparation steps to resolve the issue or may lead to a sample recollect. To resolve this problem, the STart Max semi-automated analyser (Stago) was assessed for use as it uses mechanical clot detection and was considered to be cost effective as an alternative method.

The literature reports conflicting information about the advantages of photo-optical and mechanical clot detection systems for coagulation testing. Discrepancy between the two methodologies has been demonstrated for some samples linked to the turbidity, colour, haemolysis, and other sample-related factors; while others report that the two methods are equivalent (2-7). This study looked to determine if the STart Max analyser would provide a solution to ERE produced by some samples on the high throughput Sysmex CS2100i analyser used at Wellington SCL.

MATERIALS AND METHODS

The equipment used in the study included the Sysmex CS2100i and the Stago STart Max coagulation analysers. The validation procedure used for the STart Max analyser was taken from the IANZ specific criteria for accreditation (Medical Testing 7) (8). Plasma samples used in the study were separated from citrate anticoagulated whole blood collected from community and hospital patients in the greater Wellington region served by the Wellington SCL laboratory. For all samples, testing was performed in duplicate and the mean for each pair of tests was derived. If there was more than a 10% difference in the clotting times of duplicate samples, tests were either repeated, or if insufficient, excluded from data sets.

Reagents used for testing included Siemens Thromborel® S for the PT/INR, Dade® Actin FS and $CaCl_2$ for the APTT. Some samples (for the reference range and ERE) were aliquoted and stored frozen at -20°C until testing was performed. Frozen samples were thawed in a 37°C water bath and all testing was completed within two hours, post-thaw. All statistical calculations were performed using Analyse-it™ software.

International Sensitivity Index and Local Mean Normal Prothrombin Time

The thromboplastin used by Wellington SCL was Thromborel S and was calibrated against a reference thromboplastin (Siemens PT Multicalibrator) to derive the International Sensitivity Index (ISI). To establish the local mean normal prothrombin time (MNPT), 20 "normal" citrated plasma samples were analysed and the geometric mean calculated. The ISI and local MNPT were then used to calculate the International Normalised Ratio (INR) for patient samples.

Accuracy

Twenty randomly selected patient samples that were representative of the measuring range for each of the two tests (PT/INR, APTT) were run in parallel on the STart Max and CS2100i analysers. In addition, 16 lyophilised plasma samples were provided by the Royal College of Pathologists of Australasia, Quality Assurance Programme (RCPAQAP). These samples were provided with the PT, INR and APTT results from 31 laboratories that had tested the samples using the Stago STart 4 (previous model to STart Max). Scatter plots and difference plots were used to analyse the paired samples.

Precision

The reproducibility of each test was assessed by 10 repeated measurements of the same patient plasma. Samples with normal and elevated results were chosen for the PT/INR & APTT assays. Precision was assessed using the coefficient of variation (CV) calculated for each of the tests.

Reference interval

To establish the reference ranges for the PT/INR and APTT for the STart Max analyser, 120 patient samples were selected using the laboratory IT3000 middleware. Patients were included if they were >16 years of age and had a normal coagulation screen performed within 4 hours post collection. Patients were excluded if they had a history of bruising, bleeding or thrombosis; were post-operative; had clinical data that suggested either drug therapy; an active deteriorating or resolving disease process; or had other concurrent abnormal results or results associated with a recognised disease process (e.g. abnormal renal or liver function tests, abnormal cardiac markers).

Internal QC and Measurement of Uncertainty (MU)

Internal QC limits for the STart Max analyser were established using Siemens Ci-Trol 1 and 2. The mean and standard deviations of the ten replicates of testing were used to establish a target and to set allowable limits for the internal QC of the analyser. Measurement of uncertainty (MU=2(CV)) was calculated using the CV of the normal QC replicates to show the dispersal of results from the estimated value.

ERE's

Fourteen samples that had shown an ERE on the Sysmex CS2100i analyser had been collected from the 1st February to the 30st April 2017 and stored frozen. All samples were thawed and rerun on the STart Max analyser.

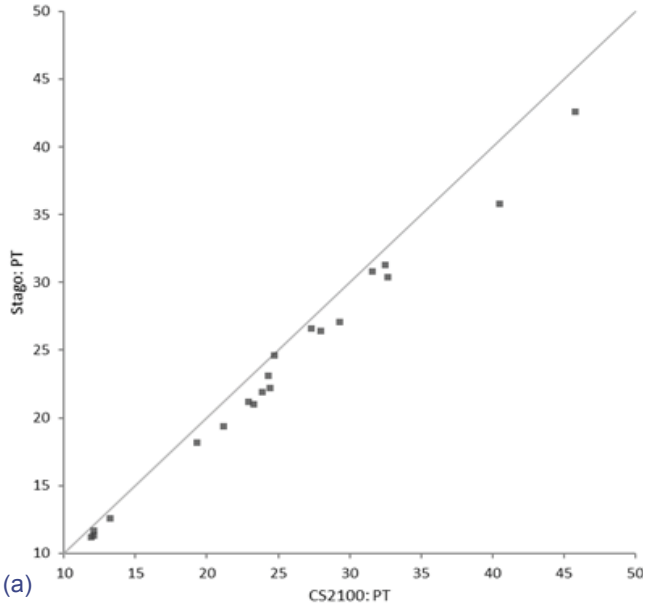
RESULTS

ISI and local MNPT determination

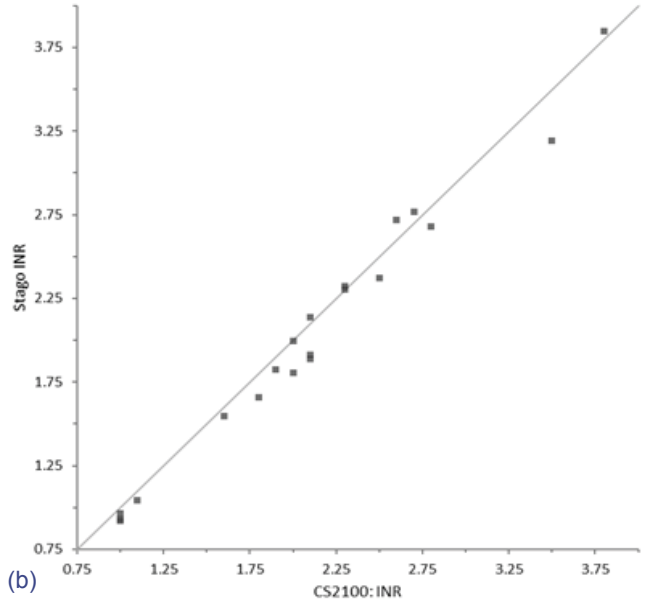
Results for the MNPT from the STart Max analyser provided a geometric mean of 12.1 seconds. An ISI value of 1.05 was determined from the PT multi-calibrator. The MNPT and ISI values were programmed into the STart Max software and used for subsequent PT/INR testing.

Accuracy

The results produced by the STart Max analyser for of the PT/INR and the APTT for 20 randomly selected patient samples were compared with the results for the same samples produced by the CS2100i (Figures 2 a-c). The r values (a) PT 0.996 (b) INR 0.990, (c) APTT 0.979 showed strong correlations. Difference plots were prepared for each of the tests and are presented in Figures 1 a-c.



(a)



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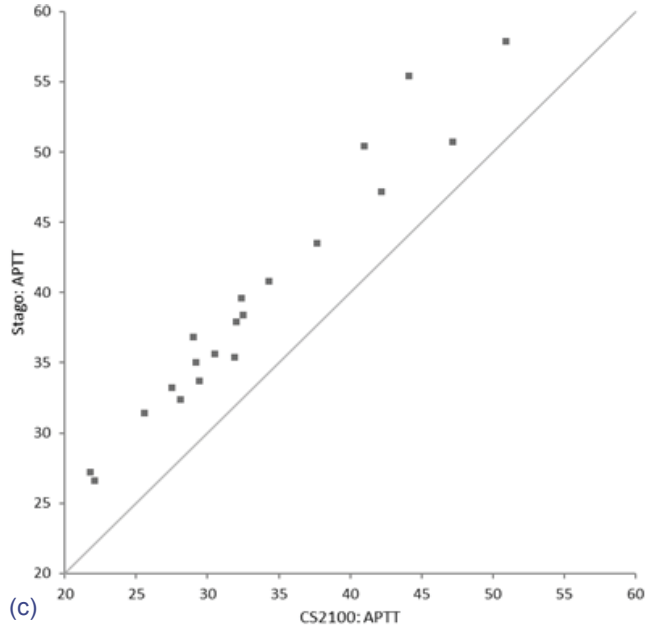
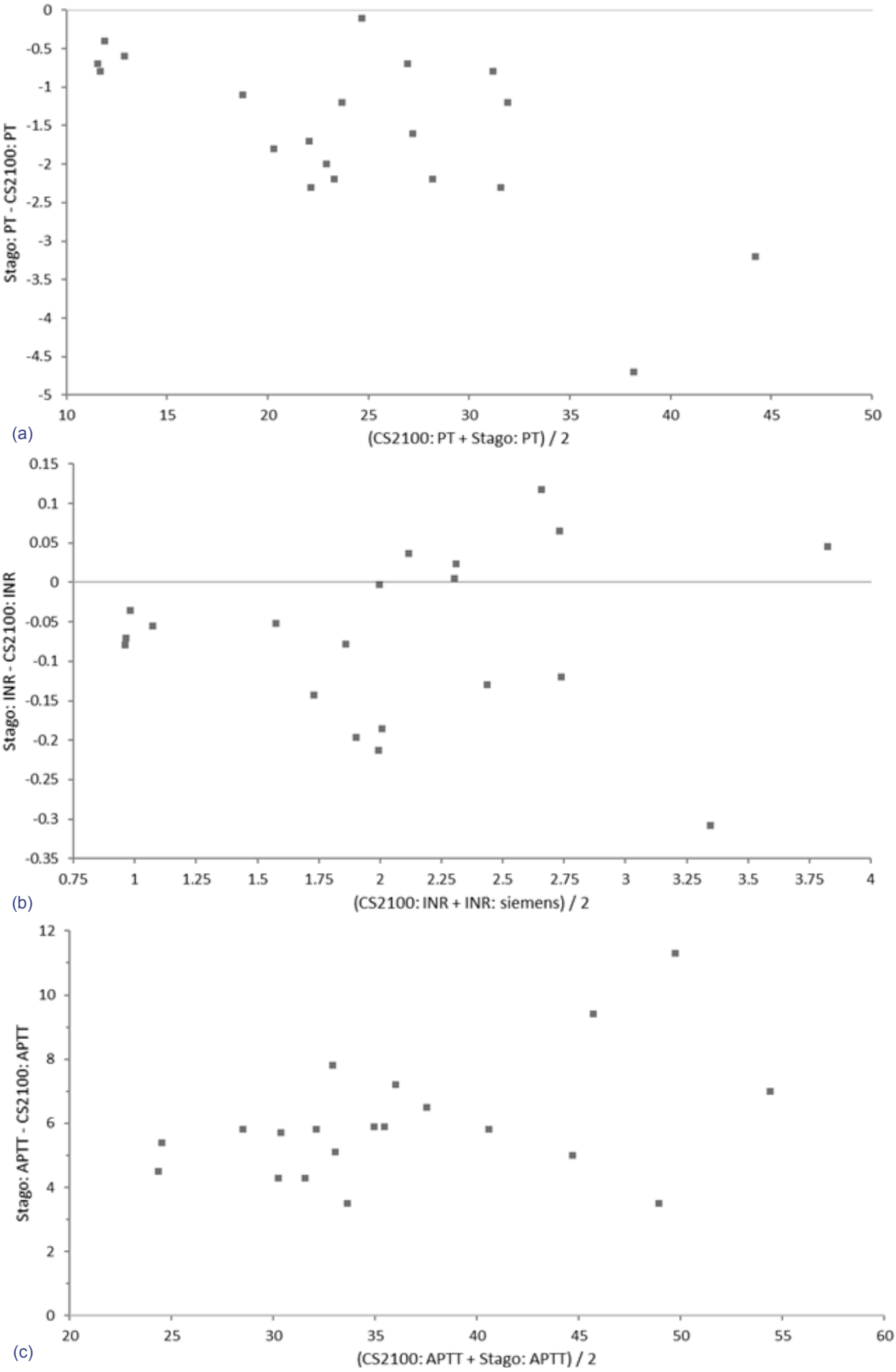


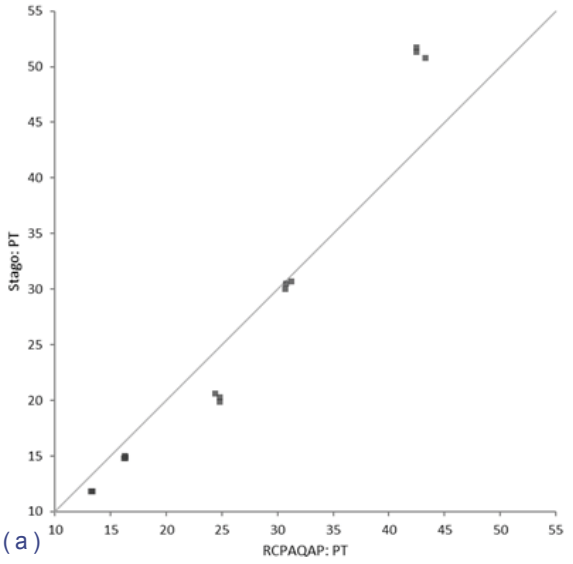
(c)

Figures 1 a-c. Scatter plots for 20 patient samples tested for PT (a) and INR (b) and APTT (c) on both the STart Max and CS2100i analysers.

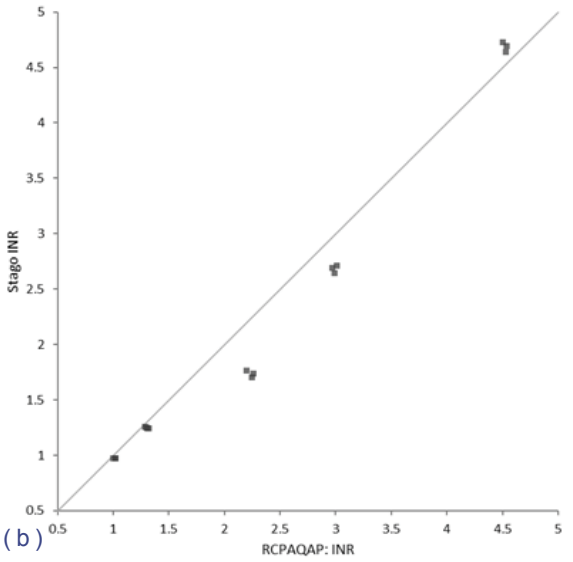


Figures 2 a-c. Difference plots of 20 patient samples tested for PT (a) and INR (b), and APTT (c) on the STart Max and CS2100i analysers.

The results for 16 RCPAQAP external quality control samples were compared with the mean values provided from the 31 users of equivalent STart 4 coagulation analysers. Results are presented in Figures 3 a-c with *r*-values of 0.981 for the PT, 0.986 for the INR and 0.978 for the APTT.



(a)



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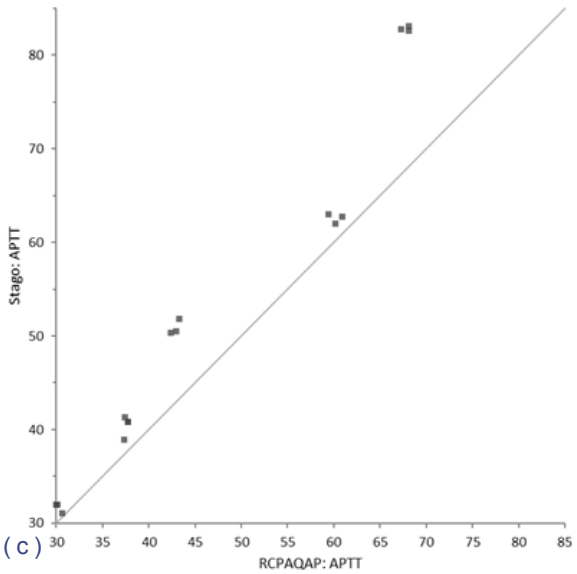
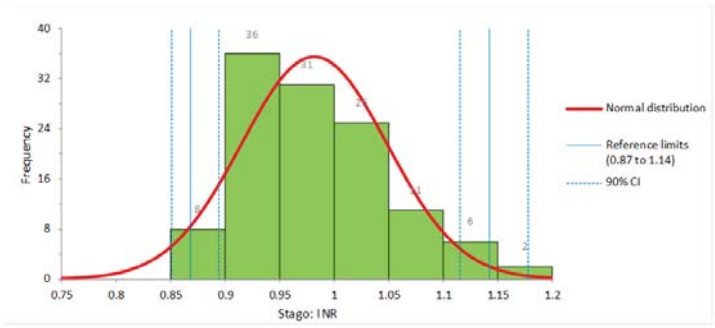


(c)

Figures 3 a-c. Scatter plots for RCPAQAP samples tested for PT (a) and INR (b) and APTT (c) using the STart Max and CS2100i analyser.

Reference intervals

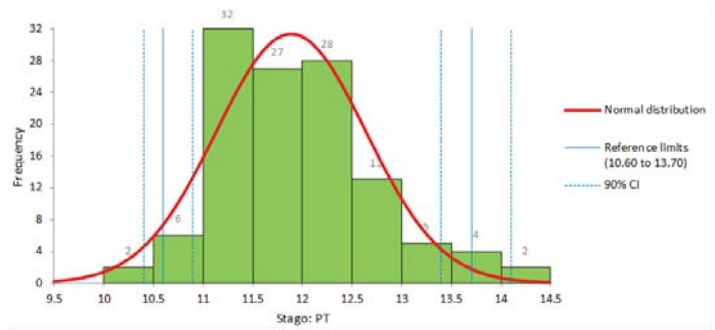
Of the patient samples selected for the reference interval, 119 were included in the validation series. A 95% confidence interval was determined based on the standard deviation of the population mean for the INR 0.9-1.1 (Figure 4), PT 11-14 secs (Figure 5) and the APTT 24 -36 secs (Figure 6). The difference between these and the biological regional reference ranges (used in reporting Sysmex CS2100 results) are shown in Table 1.



	N	Mean	SD	Skewness	Kurtosis
Stago: INR	119	0.98	0.07	0.7	0.34

	Minimum	1st quartile	Median	3rd quartile	Maximum	IQR
Stago: INR	0.9	0.93	0.97	1.02	1.2	0.09

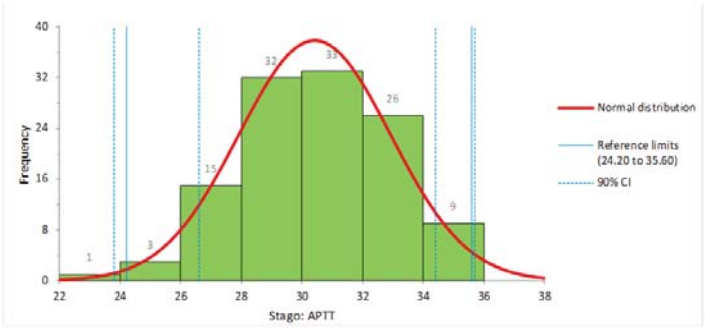
Figure 4. INR reference interval.



	N	Mean	SD	Skewness	Kurtosis
Stago: PT	119	11.89	0.76	0.7	0.32

	Minimum	1st quartile	Median	3rd quartile	Maximum	IQR
Stago: PT	10.4	11.30	11.80	12.30	14.1	1.00

Figure 5. PT reference interval.



	N	Mean	SD	Skewness	Kurtosis
Stago: APTT	119	30.42	2.51	-0.1	-0.07

	Minimum	1st quartile	Median	3rd quartile	Maximum	IQR
Stago: APTT	23.8	28.70	30.40	32.28	35.7	3.58

Figure 6. APTT reference interval.

Table 1. Summary of the reference range values for the STart Max vs CS2100i analysers.

Test	STart Max	CS2100i
PT (sec)	11 – 14 secs	10 – 14 secs
INR	0.9 – 1.1	0.9 – 1.2
APTT (sec)	24 – 36 secs	22-30 secs

Precision

The precision evaluation results for the STart Max analyser are presented in Table 2. The CV's for PT normal and elevated results were 1.33% and 2.28% respectively. The CV's for the APTT for normal and elevated results were 1.15% and 4.96% respectively.

Table 2. Precision evaluation testing for the STart Max analyser.

Test	PT (secs)		APTT (secs)	
	Normal	**Elevated**	**Normal**	**Elevated**
1	11.7	31.2	28.9	59.7
2	11.7	29.5	28.3	54.4
3	11.7	29.5	29.2	55.6
4	12.0	30.4	28.8	61.4
5	12.1	30.5	29.2	64.1
6	12.0	29.6	28.6	615
7	11.7	29.2	28.5	59.8
8	11.8	29.3	29.1	60.5
9	12.0	29.7	28.4	61.0
10	11.8	29.1	28.6	62.6
Mean	11.85	29.80	28.76	60.06
2 SD	0.32	1.36	0.66	5.96
CV	1.33%	2.28%	1.15%	4.96%

Internal QC and Measurement of Uncertainty (MU)

Internal QC results from the STart Max analyser are presented in Table 3. For Ci-Trol 1, the CV's were; PT (1.46%) and APTT (1.27%). For Ci-Trol 2 the CV's were; PT (1.70%) and APTT (1.64%). Measurement of uncertainty (MU=2(CV)) was estimated based on Ci-Trol 1 results for the PT (2.92%) and the APTT (2.54%).

Table 3. CV's for Ci-Trol 1 & 2 (CT) using the STart Max analyser.

	PT		APTT	
Replicate	**CT1**	**CT2**	**CT1**	**CT2**
1	12.2	40.3	30.8	55.8
2	11.9	40	30.1	54.8
3	12.2	40.5	30.3	55.9
4	12.1	39	29.8	54.6
5	12.1	41	30.3	57
6	12.1	40.1	29.7	54.7
7	12.1	39.9	30.4	55
8	12.2	39.6	30	55.1
9	12.6	40.8	29.9	57.1
10	12.1	41.3	29.5	55.8
Mean	12.16	40.25	30.08	55.58
2 SD	0.36	1.37	0.76	1.82
CV	1.46%	1.70%	1.27%	1.64%

ERE's

The 14 stored patient samples that had previously flagged as an ERE on the CS2100i were retested using the STart Max (Table 4). The APTT had been affected in all cases and in one sample the PT/INR was also affected. When reanalysed on the STart Max analyser all 14 patients produced reportable results for the APTT, PT and the INR. In Table 4 the ERE codes are presented in the column on the left: Slow Reaction (0008.0128.0001), Start Angle 1 (0008.0128.0002), Start Angle 2 (0008.0128.0004), Early % (0008.0128.0016).

Table 4. Early Reaction Error (ERE) samples from the CS2100i rerun on the STart Max analyser.

ERE samples	CS2100i			STart Max		
	PT (secs)	INR	APTT (secs)	PT (secs)	INR	APTT (secs)
0008.0128.0001 0008.0128.0016	14.4	1.2	***	14.6	1.2	51.3
0008.0128.0016	16.2	1.4	***	12.9	1.1	27.8
0008.0128.0016	20.5	1.8	***	18.3	1.6	55.6
0008.0128.0016	13.1	1.1	***	12	1	44.5
0008.0128.0016	14.1	1.2	***	13.6	1.1	28.2
0008.0128.0016	14.4	1.2	***	12.9	1.1	42.6
0008.0128.0016	12.6	1.1	***	11.6	1	41.7
0008.0128.0001 0008.0128.0002 0008.0128.0016	***	***	***	106	11.88	211
0008.0128.0004	13.4	1.2	*21.9	13.1	1.1	25.5
0008.0128.0004	20.5	1.8	*28.4	15.7	1.3	37.8
0008.0128.0004	16.8	1.4	*45.7	14	1.2	55.6
0008.0128.0004	14.1	1.2	*34.5	13.3	1.1	37.7
0008.0128.0004	20.1	1.7	*32.2	17	1.4	41.8
0008.0128.0016	15	1.3	***	13.8	1.2	21.8

* and *** = ERE

DISCUSSION

Haemostasis testing is subject to inter-laboratory distortion due to pre-analytical and analytical variables, including differences in method and endpoint detection technologies such as photo-optical vs. mechanical clot detection. In addition, fully automated vs. semi-automated equipment and reagent variables can influence the results (9). This study was undertaken to validate a backup system to the Sysmex CS2100i analyser at Wellington SCL. The analyser selected was the Stago STart Max machine and one of the drivers for an alternative to the CS2100i was to enable the reporting of results affected by the ERE seen on this analyser.

This work evaluated the accuracy of the STart Max analyser compared to both the Sysmex CS2100i analyser and STart 4 analyser users in Australasia for the PT/INR and APTT tests. The *r*-values of >0.95 for each of the tests indicated a linear correlation between the STart Max and the other analysers. There was, however, poor agreement between the two data sets for the APTT, with the difference plot showing a positive bias and a clinically significant results difference (up to 11 secs). Difference plots for the PT and the INR showed only marginal differences and were not considered to be clinically significant.

Reference intervals for the STart Max analyser were established for the PT/INR and the APTT. Since the STart Max used a different reaction principle, it was expected that the results would differ significantly using regional reference ranges. This proved not to be the case for the PT/INR allowing the use of the existing reference range for these tests on both analysers at Wellington SCL. The finding that the APTT results from the STart Max showed a considerable shift from those from the CS2100i meant that an independent reference interval for STart Max APTT would need to be used.

Precision evaluation of the STart Max analyser for the PT/INR and the APTT against normal and prolonged ranges, showed a CV of approximately 2% for most tests. The exception was in the elevated range of the APTT where the STart Max showed a CV of 4.96% for the 10 replicates of the same prolonged sample.

Internal QC targets and allowable limits were established based on the mean and standard deviation of 10 replicates for two QC levels. The CV was used to calculate the MU, which was <5% for each test. Since the STart Max was a semi-automated method, there was likely to be some degree of intra-user variability attributable to the manual pipetting required. As such, the targets, allowable limits and the MU established during this commissioning exercise may not be reflective of true values. A bigger data set will be required to provide a more accurate evaluation once the analyser goes into regular use.

Finally, the STart Max analyser produced reportable results in all of the samples that had produced an ERE on the Sysmex CS2100i machine showing an advantage for mechanical clot detection ahead of the photo-optical technology for these samples in this study. A number of theories have been proposed to describe why ERE are encountered using the Sysmex CS2100i. A review of the clinical records of the patients included in the study showed some commonalities.

Some patients had been treated with unfractionated heparin and some were on dialysis. For others there was a history of calcium antagonist, ACE inhibitors and beta-blockers (Metoprolol, Amlodipine, and Cilazapril) medications. In others, records showed a history of propofol usage, something previously reported as a possible cause of coagulation testing error (10).

CONCLUSIONS

In this study the Stago STart Max analyser produced precise and accurate results for each of the method validation stages. The PT, INR and APTT test results were statistically comparable to those obtained from the Sysmex CS2100i analyser. With the exception of the APTT, the existing biological reference ranges for the population served by Wellington SCL could be used to report the STart Max results. For the APTT, a new reference interval was established. Since the scatter plot for the APTT indicated a constant proportional bias, future work to perform regression analysis on a larger validation series would be required. The use of the regression equation ($y = mx + c$) might uncover a closer correlation between the two methods may yet enable the future reporting of the APTT using a single reference range for both machines.

In this study the Stago STart Max analyser demonstrated its suitability as a tool for use in routine coagulation testing and that it could be used interchangeably with the Sysmex CS2100i analyser. When samples affected by ERE using the Sysmex CS2100i machine were retested on the STart Max analyser, all samples generated a valid reportable result. Early reaction errors result in delays in reporting and/or unnecessary sample re-collections. This could elevate clinical risk with the inability to report a reliable result, particularly when the ERE cannot be resolved. This study has shown the Stago STart Max to be a robust analyser that offers a cost-effective alternative to the elimination of clinical risks associated with ERE affected sample results in the haemostasis laboratory. Its introduction at Wellington SCL is a quality improvement measure which will have a positive impact on future patient care.

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