510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:

K172286

B. Purpose for Submission:

To expand the use of previously cleared assay reagents for Factor V Leiden, Coagulation Factor VIII Deficient Plasma, Coagulation Factor IX Deficient Plasma, LA 1 Screening Reagent and LA 2 Confirmation Reagent to the Sysmex® Automated Blood Coagulation Analyzer CS-2500.

C. Measurand:

Factor V Leiden Assay Ratio
Factor VIII Activity (% of norm)
Factor IX Activity (% of norm)
LA 1 Reagent Clotting Time (seconds)
LA 2 Confirmation Reagent Clotting Time (seconds)
LA Ratio (LA 1/LA 2)

D. Type of Test:

The Factor V Leiden with Factor V Leiden Assay, Coagulation Factor VIII with Dade Actin FSL, Coagulation Factor IX with Dade Actin FSL, Lupus Anticoagulant with LA 1 Screening Reagent, Lupus Anticoagulant with LA 2 Confirmation Reagent, and Lupus Anticoagulant with LA 1/LA 2 Ratio are quantitative clot-based applications.

E. Applicant:

Siemens Healthcare Diagnostics Product GmbH

F. Proprietary and Established Names:

Sysmex® Automated Blood Coagulation Analyzer CS-2500
Factor V Leiden Assay
Coagulation Factor VIII Deficient Plasma
Coagulation Factor IX Deficient Plasma
LA 1 Screening Reagent
LA 2 Confirmation Reagent

G. Regulatory Information:

1. Regulation section:

Device	Regulation Number
Sysmex CS-2500	21 CFR 864.5425, Multipurpose system for in vitro coagulation studies
Factor V Leiden Assay	21 CFR 864.7925, Partial thromboplastin time tests
Coagulation Factor VIII Deficient Plasma	21 CFR 864.7290, Factor deficiency test
Coagulation Factor IX Deficient Plasma	
LA 1 Screening Reagent	21 CFR 864.8950, Russell viper venom reagent
LA 2 Confirmation Reagent	
LA 1/ LA 2 Ratio	

2. Classification:

   Class II

3. Product code:

Device	Product Code
Sysmex CS-2500	JPA, System, Multipurpose For In Vitro Coagulation Studies
Factor V Leiden Assay	GGW, Test, Time, Partial Thromboplastin
Coagulation Factor VIII Deficient Plasma	GJT, Plasma, Coagulation Factor Deficient
Coagulation Factor IX Deficient Plasma	
LA 1 Screening Reagent	GIR, Reagent, Russell Viper Venom
LA 2 Confirmation Reagent	

4. Panel:

   Hematology (81)

H. Intended Use:

1. Intended use(s):

   Sysmex CS-2500

   The Sysmex® Automated Blood Coagulation Analyzer CS-2500 is a fully automated blood coagulation analyzer intended for in vitro diagnostic use using plasma collected from venous blood samples in 3.2% sodium citrate tubes to analyze clotting, chromogenic and immunoassay methods in the clinical laboratory.

   The performance of this device has not been established in neonate and pediatric patient

populations.

2. Indication(s) for use:

   Same as Intended Use(s) above

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Sysmex® Automated Blood Coagulation Analyzer CS-2500

I. Device Description:

Sysmex CS-2500
The Sysmex® Automated Blood Coagulation Analyzer CS-2500 (hereafter Sysmex CS-2500) is an automated blood coagulation instrument which analyzes samples collected in 3.2% sodium citrate using clotting, chromogenic and immunoassay methods. Results are displayed on the Information Processing Unit (IPU) screen and can be printed on external printers or transmitted to a host computer. The instrument is capable of analyzing samples in a normal mode and micro-sample mode.

Factor V Leiden with Factor V Leiden Assay

The Factor V Leiden Assay is based on the activation of endogenous Protein C by incubation of plasma with *Agkistrodon contortrix contortrix* (Southern Copperhead) venom. The kit contains the venom activator in stabilizing-buffer solution and the PR3V Reagent: dilute phospholipid rich Vipera Russelli venom, calcium and heparin inhibitor. Both reagents contain sodium azide as a preservative.

Coagulation Factor VIII and IX Deficient Plasmas

Coagulation Factor Deficient Plasmas are lyophilized human plasmas with a residual Factor VIII, IX, XI or XII activity of ≤ 1 %. The deficient plasmas are manufactured by immunoadsorption from normal plasma and are free from antigen of the respective factor. Fibrinogen is present in a quantity of at least 1 g/L, and the remaining coagulation factors are present in an activity greater than 40% of the norm. The plasmas contain mannitol (20 g/L) as a stabilizer.

LA 1 Screening and LA 2 Confirmation Reagents

LA 1 Screening Reagent and LA 2 Confirmation Reagent both contain Russell's viper venom, phospholipids, antiheparin agents, calcium, buffers, stabilizers, sodium azide and dyes.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Sysmex® CA-1500

2. Predicate 510(k) number(s):

   K011235 (Sysmex CA-1500)

   The performance of the Factor V Leiden Assay on the Sysmex CA-1500 was evaluated in K992456. The performance of Coagulation Factor VIII Deficient Plasma and Coagulation Factor IX Deficient Plasma in combination on the Sysmex CA-1500 was evaluated in K924396. The performance of LA 1 Screening Reagent, LA 2 Confirmation Reagent, and LA 1 /LA 2 Ratio was evaluated on the Sysmex CA-1500 in K993299.

3. Comparison with predicate:

Similarities		
Item	Device Sysmex CS-2500	Predicate Sysmex CA-1500
Intended Use	The Sysmex® Automated Blood Coagulation Analyzer CS-2500 is a fully automated blood coagulation analyzer intended for in vitro diagnostic use using plasma collected from venous blood samples in 3.2% sodium citrate tubes to analyze clotting, chromogenic and immunoassay methods in the clinical laboratory. The performance of this device has not been established in neonate and pediatric patient populations.	The intended use of the Sysmex® CA-1500 is as a fully automated, computerized blood plasma coagulation analyzer for in vitro diagnostic use in clinical laboratories. The instrument uses citrated human plasma to perform the following parameters and calculated parameters: Clotting Analysis Parameters: Prothrombin Time (PT); Activated Partial Thromboplastin Time (APTT); Fibrinogen (Clauss); Batroxobin Time; Extrinsic Factors (II, V, VII, X); Intrinsic Factors (VIII, IX, XI, XII); Protein C. Chromogenic Analysis Parameters: Antithrombin III; Factor VIII; Plasminogen; Heparin; Protein C; α2-Antiplasmin. Immunologic Analysis Parameters: D-dimer. Calculated Parameters: PT Ratio; PT INR; PT %; Derived Fibrinogen; Factor Assays % Activity.
Sample Type	Human plasma, 3.2% sodium citrate	Same
Application type	Clotting Applications: Prothrombin Time (PT) with Dade Innovin	Same

Similarities		
Item	Device Sysmex CS-2500	Predicate Sysmex CA-1500
	Activated Partial Thromboplastin Time (APTT) with Dade Actin FSL Fibrinogen (Clauss) with Dade Thrombin Reagent Coagulation Factor V with Dade Innovin Coagulation Factor VII with Dade Innovin Coagulation Factor VIII with Dade Actin FSL Coagulation Factor IX with Dade Actin FSL Lupus Anticoagulant with LA 1 Screening and LA 2 Confirmation Reagents Factor V Leiden with Factor V Leiden Assay Protein C with Protein C Reagent	
	Calculated Application: PT INR with Dade Innovin	Same
	Immuno-Chemical Application: D-dimer with INNOVANCE D-Dimer	Same
	Chromogenic Applications: Antithrombin with INNOVANCE Antithrombin Protein C with Berichrom® Protein C	Same
Clinical Reportable Range	Coagulation Factor VIII with Dade Actin FSL: 3.0–182.0.0% of norm Coagulation Factor IX with Dade Actin FSL: 3.0–145.5% of norm Factor V Leiden with Factor V Leiden Assay: 0.72–5.91 ratio LA 1 with LA 1 Screening Reagent: 24.9–158.8 sec.	Same
Specimen Processing	Automatic pipetting and dilution	Same
Random Access	Yes	Same
Liquid Level Sensing	Yes – reagent and sample	Same
Barcode Reader	Sample and reagent	Same
STAT Testing	Yes	Same
Sampling Capabilities	Normal and Micro Mode	Same
Sample Volumes	PT with Dade Innovin (50 µL)	Same

Similarities		
Item	Device Sysmex CS-2500	Predicate Sysmex CA-1500
(Plasma)	APTT with Dade Actin FSL (50 µL) Fibrinogen with Dade Thrombin Reagent (10 µL) Coagulation Factor V with Dade Innovin (5 µL) Coagulation Factor VII with Dade Innovin (5 µL) Protein C with Protein C Reagent (5 µL) Protein C with Berichrom Protein C (15 µL) Coagulation Factor VIII with Dade Actin FSL (2 µL) Coagulation Factor IX with Dade Actin FSL (2 µL) Lupus Anticoagulant with LA 1 Screening Reagent (100 µL) Lupus Anticoagulant with LA 2 Confirmation Reagent (100 µL) Factor V Leiden with Factor V Leiden Assay (50 µL)	
Sample Volumes in Micro Mode (Plasma)	PT with Dade Innovin (50 µL) APTT with Dade Actin FSL (50 µL) Fibrinogen with Dade Thrombin Reagent (10 µL) Coagulation Factor V with Dade Innovin (5 µL) Coagulation Factor VII with Dade Innovin (5 µL) Protein C with Protein C Reagent (5 µL) Protein C with Berichrom Protein C (15 µL) Coagulation Factor VIII with Dade Actin FSL (2 µL) Coagulation Factor IX with Dade Actin FSL (2 µL) Lupus Anticoagulant with LA 1 Screening Reagent (100 µL) Lupus Anticoagulant with LA 2	Same

Similarities		
Item	Device Sysmex CS-2500	Predicate Sysmex CA-1500
	Confirmation Reagent (100 µL) Factor V Leiden with Factor V Leiden Assay (50 µL)	
Rinse and Buffer Solutions On-board External	CA-CLEAN I CA-CLEAN II Dade Owren's Buffer Water	Same
Light Source: Chromogenic	Halogen Lamp	Same
Light Source: Immuno-chemical	Halogen Lamp	Same
Probes	1 Sample probe 1 Reagent probe	Same
Wavelengths used in Analysis	Coagulation Factor VIII with Dade Actin FSL (Default = 660 nm; Sub-wavelength = none) Coagulation Factor IX with Dade Actin FSL (Default = 660 nm; Sub-wavelength = none) Lupus Anticoagulant with LA 1 Screening Reagent (Default = 660 nm; Sub-wavelength = none) Lupus Anticoagulant with LA 2 Confirmation Reagent (Default = 660 nm; Sub-wavelength = none) Factor V Leiden with Factor V Leiden Assay (Default = 660 nm; Sub-wavelength=none)	Same
Temperature Control	Sample incubation well: 37 °C ± 1.0 °C	Same

Differences		
Item	Device	Predicate
Operating Principle	Clotting: Transmitted Light Detection (Absorbance) at 340, 405, 575, 660 or 800 nm. Wavelengths 340, 405 and 575 are technically available but not validated in combination with the intended applications.	Scattered Light Detection at 660 nm

Differences		
Item	Device	Predicate
	Chromogenic: Transmitted Light Detection (Absorbance) at 340, 405, 575, 660, 800 nm. Wavelengths 340, 575, 660, and 800 are technically available but not validated in combination with the intended applications.	Transmitted Light Detection (Absorbance) at 405, 575, 800 nm
	Immunochemical: Transmitted light detection (absorbance) at 340, 405, 575, and 660 nm	Transmitted Light Detection (Absorbance) at 405, 575, or 800 nm
Light Source Clotting	Halogen Lamp	Light Emitting Diode
Cap Piercing	Cap Piercer only	Both Cap Piercer model and Non-Cap Piercer models are available
Temperature Control	Detector: 37 ± 0.5ºC Reagent probe: 37.5 ± 0.5ºC	Detector: 37 ± 1.0°C Reagent probe: 37 ± 1.0°C
Reagent Cooling	10 ± 2°C, when ambient temperature is 20–28°C. During operation 4–15°C, when ambient temperature is 15–30°C	15 ± 2°C, when ambient temperature is 15–30°C
Pipetting Capabilities	Reagent probe: 20–200 µL Sample probe: 4–270 µL	Reagent probe: 4–200 µL Sample probe: 5–450 µL
Clinical Reportable Range	LA 2 with LA 2 Confirmation Reagent: 32.2–80.0 sec. LA Ratio with LA 1 /LA 2 reagent: 0.71–2.60 ratio	LA 2 with LA 2 Confirmation Reagent: 32.2–111.2 sec. LA Ratio with LA 1 /LA 2 reagent: 0.71–2.98 ratio
Sample Volumes (Plasma)	Antithrombin with INNOVANCE Antithrombin (14 µL) D-dimer with INNOVANCE D-Dimer (15 µL)	Antithrombin with INNOVANCE Antithrombin (10 µL) D-dimer with INNOVANCE D-Dimer (13 µL)

K. Standard/Guidance Document Referenced (if applicable):

CLSI EP05-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition

CLSI EP06-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline

CLSI EP07-A2:. Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition

CLSI EP09-A3. Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline-Third Edition

CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition

CLSI EP25-A. Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline

CLSI EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline-Third Edition

CLSI AUTO11-A2: Information Technology Security of In Vitro Diagnostic Instruments and Software Systems; Approved Standard-Second Edition

L. Test Principle:

Factor V Leiden Assay

The Factor V Leiden Assay is based on the activation of endogenous Protein C. A point mutation in the Factor V gene (Factor V (Leiden) slows the inactivation of factor Va by activated Protein C (APC) causing a hypercoagulable state. The presence of a mutation is detected by measuring APC resistance in plasma upon activation of a clotting cascade with Agkistrodon contortrix contortrix (Southern Copperhead) venom. A dilute Russell's Viper Venom Time (DRVVT) is then performed on the plasma. The DRVVT is sensitive to prolongation in the presence of APC. Results are reported as a ratio of clotting times obtained with and without protein C activation which indicates presence or absence of APC resistance. Generally, ratios less than or equal to the claimed cut-off value of 1.8 suggest the Leiden variant of Factor V.

Coagulation Factor VIII and Coagulation IX Deficient Plasmas

A plasma deficient in coagulation Factor VIII or Factor IX results in a prolonged partial thromboplastin time (APTT). Results are reported as percent of norm.

Lupus Anticoagulant with LA 1 Screening and LA 2 Confirmation Reagents:

LA 1 Screening Reagent and LA 2 Confirmation Reagent are Simplified Dilute Russell's Viper Venom Tests (DRVVT) for detection of Lupus Anticoagulants. The Russell's viper venom present in LA 1 Screening Reagent initiate plasma clotting by directly activating factor X. LA antibodies prolong the LA 1 Screening Reagent clotting time. LA 2 Confirmation Reagent is similar to LA 1 Screening Reagent but contains a high phospholipid concentration. The extra phospholipid counteracts the LA antibody and largely corrects the clot time. Results are reported as a ratio.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*

      Precision:

The precision study was performed to establish instrument-to-instrument precision data for the Sysmex CS-2500 analyzer. Two instruments were tested for within-run, between-run, between-day and total precision, using one lot of reagent for 5 days, with two runs per day and four replicates of each sample per run. The total precision (within-site combined instruments) values of each application fulfilled the pre-defined acceptance criteria.

Factor V Leiden with Factor V Leiden Assay

Sample	Mean (Ratio)	Within-run		Between-run		Between-day		Between-instrument		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma Pool Low	0.879	0.008	0.88	0.005	0.53	0.000	0.00	0.003	0.29	0.009	1.07
ProC Control	1.17	0.05	3.94	0.01	1.17	0.01	0.63	0.00	0.00	0.05	4.15
Control Plasma N	3.545	0.031	0.87	0.035	0.99	0.030	0.85	0.031	0.87	0.063	1.79
Plasma Pool MDP	1.761	0.013	0.73	0.009	0.51	0.013	0.72	0.012	0.67	0.023	1.33
Plasma Pool High	5.423	0.067	1.23	0.169	3.12	0.000	0.00	0.028	0.51	0.184	3.40

Coagulation Factor VIII with Dade Actin FSL

Sample	Mean (% of norm)	Within-run		Between-run		Between-day		Between-instrument		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma Pool MDP 3	74.65	2.75	3.69	0.78	1.04	0.65	0.87	4.31	5.77	5.21	6.98
Plasma Pool Low	14.87	0.63	4.27	0.12	0.78	0.28	1.85	1.06	7.13	1.27	8.55
Control Plasma P	27.84	1.38	4.97	0.00	0.00	0.25	0.91	2.16	7.78	2.58	9.27
Plasma Pool MDP 2	45.93	1.59	3.47	0.66	1.43	0.00	0.00	3.69	8.03	4.07	8.86
Plasma Pool MDP 1	6.45	0.24	3.77	0.03	0.51	0.14	2.21	0.59	9.09	0.65	10.10
Control Plasma N	86.47	2.38	2.76	1.30	1.50	0.00	0.00	5.71	6.60	6.32	7.31
Plasma Pool AMR	110.04	3.71	3.37	0.00	0.00	0.70	0.64	5.42	4.92	6.60	6.00
Plasma Pool High	74.65	6.57	4.18	2.23	1.42	1.51	0.96	11.54	7.35	13.55	8.62

Coagulation Factor IX with Dade Actin FSL

Sample	Mean (% of norm)	Within-run		Between-run		Between-day		Between-instrument		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma Pool MDP 3	78.77	1.67	2.11	0.18	0.23	1.18	1.50	5.27	6.69	5.65	7.18
Control Plasma P	36.44	1.05	2.87	1.05	2.88	0.00	0.00	0.33	0.89	1.52	4.16
Plasma Pool MDP 2	40.08	0.97	2.42	0.32	0.80	0.69	1.73	3.76	9.39	3.96	9.88
Plasma Pool MDP 1	7.85	0.34	4.28	0.00	0.00	0.17	2.10	1.12	14.30	1.18	15.07
Control Plasma N	104.45	2.24	2.15	0.84	0.81	0.97	0.93	4.93	4.72	5.57	5.33
Plasma Pool AMR	113.02	2.29	2.02	1.02	0.91	1.44	1.27	3.18	2.82	4.30	3.80
Plasma Pool High	144.71	3.54	2.44	2.15	1.49	0.00	0.00	N/A‡	N/A‡	6.97	4.82

Lupus Anticoagulant with LA 1 Screening Reagent

Sample	Mean (seconds)	Within-run		Between-run		Between-day		Between-instrument		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma Pool Low	31.91	0.60	1.89	0.00	0.00	0.53	1.65	0.00	0.00	0.80	2.51
Control Plasma N	39.34	0.25	0.64	0.25	0.64	0.00	0.00	0.19	0.47	0.40	1.02
Plasma Pool MDP	52.65	0.91	1.72	0.92	1.75	0.00	0.00	0.00	0.00	1.30	2.46
LA Control 1	69.74	0.56	0.80	0.77	1.10	0.00	0.00	0.16	0.22	0.96	1.38
LA Control 2	94.41	0.88	0.93	0.56	0.60	0.00	0.00	0.20	0.22	1.06	1.12
Plasma Pool High	145.21	0.88	0.61	1.15	0.79	0.69	0.48	0.00	0.00	1.61	1.11

Lupus Anticoagulant with LA 2 Confirmation Reagent

Sample	Mean (seconds)	Within-run		Between-run		Between-day		Between-instrument		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma Pool Low	37.06	0.29	0.78	0.25	0.69	0.00	0.00	0.19	0.50	0.43	1.15
Control Plasma N	42.68	0.11	0.26	0.15	0.34	0.00	0.00	0.17	0.39	0.25	0.58
Plasma Pool MDP	42.54	0.50	1.17	0.00	0.00	0.18	0.41	0.22	0.52	0.57	1.34
LA Control 1	45.88	0.19	0.41	0.09	0.19	0.04	0.08	0.21	0.46	0.30	0.65
LA Control 2	47.31	0.18	0.38	0.16	0.33	0.00	0.00	0.17	0.36	0.29	0.62
Plasma Pool High	74.63	0.37	0.50	0.42	0.56	0.25	0.34	0.23	0.30	0.65	0.88

Lupus Anticoagulant with LA 1/LA 2 Ratio

Sample	Mean (Ratio)	Within-run		Between-run		Between-day		Between-instrument		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma Pool Low	0.889	0.005	0.56	0.003	0.38	0.003	0.36	0.000	0.00	0.007	0.77
Control Plasma N	0.921	0.006	0.65	0.008	0.86	0.000	0.00	0.000	0.00	0.010	1.08
Plasma Pool MDP	1.238	0.012	0.98	0.021	1.71	0.000	0.00	0.000	0.00	0.024	1.97
LA Control 1	1.521	0.014	0.94	0.016	1.04	0.000	0.00	0.000	0.00	0.021	1.40
LA Control 2	1.996	0.017	0.87	0.010	0.51	0.000	0.00	0.002	0.11	0.020	1.01
Plasma Pool High	2.575	0.019	0.73	0.000	0.00	0.014	0.54	0.000	0.00	0.023	0.91

Reproducibility:

The reproducibility study was performed in the Normal Mode of the Sysmex CS-2500 analyzer at three external sites. At one site, additional testing was performed in the Micro Mode. For each test application, the reproducibility study was carried out using one Sysmex CS-2500 analyzer following the 20 x 2 x 2 study design (20 days with 2 runs/day and 2 replicates/run). The same reagent lot, control lot, calibrator lot and plasma pools were used at each site. For each application, plasma pools and quality control materials were used to cover each assay measuring interval. The site specific data were analyzed separately and combined to achieve standard deviation and percent CV for within-run, between-run, between-day, between-site, and total imprecision components for each panel member and control. Data analysis was performed using ANOVA and the truncated analysis method.

Factor V Leiden with Factor V Leiden Assay

Sample	Mean (Ratio)	Within-run		Between-run		Between-day		Between-Site		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma Pool 1	0.883	0.008	0.93	0.007	0.80	0.001	0.06	0.007	0.81	0.013	1.47
ProC Control	1.125	0.014	1.28	0.012	1.05	0.006	0.49	0.015	1.32	0.025	2.18
Control Plasma N	1.650	0.021	1.25	0.012	0.71	0.017	1.03	0.050	3.03	0.058	3.51
Plasma Pool 2 (MDP)	3.159	0.053	1.66	0.062	1.95	0.000	0.00	0.124	3.91	0.148	4.68
Plasma Pool 3	4.944	0.163	3.30	0.108	2.19	0.000	0.00	0.119	2.40	0.229	4.63

Coagulation Factor VIII with Dade Actin FSL

Sample	Mean (% of norm)	Within-run		Between-run		Between-day		Between-Site		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma Pool 1 (MDP 1)	7.00	0.33	4.73	0.25	3.62	0.00	0.00	0.00	0.00	0.42	5.96
Control Plasma P	24.89	1.00	4.00	0.45	1.80	0.40	1.61	0.36	1.45	1.22	4.89
Plasma Pool2 (MDP 2)	44.73	2.28	5.09	0.84	1.89	0.00	0.00	0.90	2.01	2.59	5.79
Plasma Pool 3 (MDP 3)	74.38	3.25	4.36	2.50	3.36	0.00	0.00	0.79	1.06	4.17	5.61
Control Plasma N	88.79	3.04	3.42	1.75	1.97	0.73	0.82	0.93	1.04	3.70	4.16
Plasma Pool 4	143.85	7.19	5.00	2.84	1.98	1.10	0.76	3.51	2.44	8.56	5.95

Coagulation Factor IX with Dade Actin FSL

Sample	Mean (% of norm)	Within-run		Between-run		Between-day		Between-Site		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma Pool 1 (MDP 1)	8.63	0.57	6.61	0.54	6.21	0.00	0.00	0.46	5.37	0.91	10.54
Control Plasma P	33.81	1.47	4.36	1.13	3.34	0.00	0.00	0.84	2.48	2.04	6.02
Plasma Pool2 (MDP 2)	40.37	1.50	3.72	1.12	2.77	0.00	0.00	1.30	3.21	2.28	5.64
Plasma Pool 3 (MDP 3)	79.99	2.71	3.39	2.50	3.13	0.00	0.00	2.77	3.46	4.61	5.76
Control Plasma N	107.81	3.70	3.43	3.81	3.54	0.00	0.00	2.56	2.38	5.90	5.47
Plasma Pool 4	129.31	5.35	4.14	5.02	3.88	0.00	0.00	2.72	2.10	7.83	6.05

Lupus Anticoagulant with LA 1 Screening Reagent

Sample	Mean (sec)	Within-run		Between-run		Between-day		Between-Site		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma Pool 1	33.76	1.14	3.38	0.33	0.97	0.47	1.39	0.12	0.34	1.28	3.80
Control Plasma N	39.82	0.23	0.59	0.40	1.00	0.19	0.47	0.40	1.00	0.64	1.60
Plasma Pool 2 (MDP)	60.72	1.01	1.66	0.93	1.54	1.08	1.78	0.58	0.96	1.84	3.04
LA Control Low	72.44	0.69	0.95	0.76	1.05	0.86	1.19	0.70	0.96	1.51	2.08
LA Control High	97.03	0.95	0.97	0.84	0.87	1.54	1.59	1.14	1.18	2.30	2.37
Plasma Pool 3	157.77	0.96	0.61	1.08	0.68	2.36	1.50	0.60	0.38	2.83	1.79

Lupus Anticoagulant with LA 2 Confirmation Reagent

Sample	Mean (seconds)	Within-run		Between-run		Between-day		Between-Site		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma Pool 1	37.41	0.52	1.38	0.29	0.78	0.00	0.00	0.38	1.03	0.71	1.89
Control Plasma N	41.59	0.13	0.32	0.11	0.27	0.14	0.35	0.20	0.49	0.30	0.73
Plasma Pool 2 (MDP)	42.56	0.49	1.14	0.00	0.00	0.18	0.41	0.17	0.40	0.54	1.28

Sample	Mean (seconds)	Within-run		Between-run		Between-day		Between-Site		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
LA Control Low	46.08	0.21	0.46	0.14	0.31	0.13	0.28	0.16	0.36	0.33	0.72
LA Control High	47.16	0.23	0.48	0.16	0.34	0.04	0.08	0.22	0.47	0.36	0.76
Plasma Pool 3	74.39	0.78	1.04	0.12	0.16	0.25	0.34	0.60	0.81	1.02	1.37

Lupus Anticoagulant with LA 1 / LA 2 Ratio

Sample	Mean (Ratio)	Within-run		Between-run		Between-day		Between-Site		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma Pool 1	0.909	0.008	0.87	0.007	0.80	0.004	0.47	0.003	0.37	0.012	1.32
Control Plasma N	0.957	0.006	0.65	0.011	1.11	0.005	0.49	0.008	0.86	0.016	1.63
Plasma Pool 2 (MDP)	1.427	0.014	0.99	0.024	1.66	0.025	1.72	0.012	0.82	0.039	2.71
LA Control Low	1.572	0.018	1.12	0.018	1.15	0.018	1.14	0.014	0.89	0.034	2.16
LA Control High	2.058	0.024	1.15	0.015	0.71	0.033	1.63	0.024	1.18	0.050	2.42
Plasma Pool 3*	2.598	0.028	1.09	0.022	0.83	0.061	2.36	0.048	1.84	0.085	3.29

* Fraction of results with Ratio >CRR: 41.6%

b. Linearity/assay reportable range:

Linearity was evaluated for two calibrated coagulation assay applications: Coagulation Factor VIII with Dade Actin FSL and Coagulation Factor IX with Dade Actin FSL. The study was performed using one Sysmex CS-2500 analyzer and one lot of reagent (Coagulation Factor VIII/Factor IX). Testing was performed with a high concentration sample pool (high pool) serially diluted to various concentrations using a diluent pool (low pool). Low pools were prepared by dilution with respective factor deficient plasma; high pools were prepared by adding respective factor concentrate. Thirteen different dilutions were prepared; each analyte concentration was measured in replicates of four. For each sample, deviation between the linear regression model (predicted value from 1st order regression) and the best fitting polynomial regression model was calculated.

Sysmex CS-2500: Summary of Linearity and Measuring Range

Application	Linear Range (% of Norm)	Clinically Reportable Range (% of Norm)
Coagulation Factor VIII with Dade Actin FSL Reagent	2.12–246.41	3.0–182.0
Coagulation Factor IX with Dade Actin FSL Reagent	2.38–193.79	3.0–145.5

Linearity is not applicable for the following applications:

- Factor V Leiden with Factor V Leiden Assay
- Lupus Anticoagulant with LA 1 Screening Reagent
- Lupus Anticoagulant with LA 2 Confirmation Reagent
- Lupus Anticoagulant with LA 1/LA 2 Ratio

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Commercial Standard Human Plasma (SHP) is used for the calibration of the following applications: Coagulation Factor VIII with Dade Actin FSL Reagent and Coagulation Factor IX with Dade Actin FSL Reagent.

Control Plasma N (CPN), and Control Plasma P (CPP) are assayed controls used to monitor assay performance on the Sysmex CS-2500. SHP, CPN, and CPP are traceable to the following WHO Standard reference materials: 6th WHO Standard 07/316 and 4th WHO Standard 09/172.

On-Board Stability Testing for Calibrators and Controls

Real-time on-board stability testing for Control Plasma N, Control Plasma P, Pro C Control Plasma, LA Control Low and LA Control High was conducted internally. The on-board stability of the calibrators (Standard Human Plasma) were not determined since calibrators are intended to be used immediately. Three lots of each of the controls were tested over multiple time points distributed over the expected stability claim. Stability was assessed in terms of measurand drift for each control level and lot and found to be acceptable to support the following on-board stability: 25 hours for control Plasma N and Pro C Control Plasma for Factor V Leiden with Factor V Leiden Assay; 11 and 7 hours for Control N and P, respectively for Coagulation Factor VIII with Dade Actin FSL; 25 hours for Control N and P for Coagulation Factor IX with Dade Actin FSL; 25 hours for Control Plasma N and 24 hours for LA Control Low and High with Lupus Anticoagulant with LA 1 Screening, LA 2 Confirmation Reagents and LA 1/LA 2 Ratio.

On-Board Stability Testing – Reagents

Real-time on-board stability testing for Factor V Leiden with Factor V Leiden Assay, Coagulation Factor VIII with Dade Actin FSL, Coagulation Factor IX with Dade Actin FSL, and Lupus Anticoagulant with LA 1 Screening and LA 2 Confirmation Reagents was conducted internally using pooled plasma and control materials as test samples. The test samples were selected to cover the clinically reportable range and medical decision points for each assay. For each reagent, multiple testing time points were pre-defined and distributed over the expected on-board stability claims. For each application, one lot of reagent was tested and 4–6 replicates were measured at each time point. Stability was assessed in terms of measurand drift for each test sample and found to be acceptable to support the following claims.

On-Board Stability for Factor V Leiden with Factor V Leiden Assay

Reagent: Bottle Size/with (-out) cap	On-Board Stability Claim (hours)
FVLAct/FVLReag: Screw top glass vial 5mL /without cap	44
OVB: Screw top glass vial 15mL without cap	24

On-Board Stability for Coagulation Factor VIII with Dade Actin FSL

Reagent: Bottle Size/with (-out) cap	On-Board Stability Claim (hours)
APTT FSL: Screw top glass vial 15mL /without cap	69
Factor VIII Deficient Plasma: 4 mL sample cup / without cap	8
CaCl2: Screw top glass vial 15mL /without cap	91
OVB: Screw top glass vial 15mL /without cap	26

On-Board Stability for Coagulation Factor IX with Dade Actin FSL

Reagent: Bottle Size/with (-out) cap	On-Board Stability Claim (hours)
APTT FSL: Screw top glass vial 15mL /without cap	52
Factor IX Deficient Plasma: 4 mL sample cup / without cap	25
CaCl2: Screw top glass vial 15mL /without cap	98
OVB: Screw top glass vial 15mL /without cap	26

On-Board Stability for Lupus Anticoagulant with LA 1 Screening Reagent, LA 2 Confirmation Reagent and for the LA 1/LA 2 Ratio

Reagent: Bottle Size/with (-out) cap	On-Board Stability Claim (hours)
LA 1: Screw top glass vial 5mL / without cap	49
LA 2: Screw top glass vial 5mL / without cap	
LA 1/LA 2: Screw top glass vial 5mL / without cap	

Shelf-life and Open-Vial Stability Testing:

Shelf-life and open-vial stability were reviewed in the following premarket notifications: K992456 (Factor V Leiden Assay), K924396 (Coagulation Factor VIII and Coagulation Factor IX Deficient Plasma), K922326 (LA 1 Screening Reagent), and K922156 (LA 2 Confirmation Reagent and LA Ratio). Therefore, additional stability studies were not required to support substantial equivalence in this premarket notification.

Expected values:

Not applicable

d. Detection limit:

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ)

The following assays are non-calibrated clotting-based assays for which there is no detection limit. Therefore, LoB and LoD are not applicable.

• Factor V Leiden with Factor V Leiden Assay

• Lupus Anticoagulant with LA 1 Screening Reagent

• Lupus Anticoagulant with LA 2 Confirmation Reagent

• Lupus Anticoagulant with LA 1/LA 2 Ratio

Limit of Quantitation

The limit of quantitation (LoQ) was established for the following calibrated assays: Coagulation Factor VIII with Dade Actin FSL Reagent and Coagulation Factor IX with Dade Actin FSL Reagent. The LoQ studies were performed internally using plasma pools prepared by dilution of normal plasma with the respective application-specific deficient plasma. Each study was conducted over three testing days with four replicates per run. Testing was performed using one Sysmex CS-2500 analyzer, two reagent lots (factor VIII/factor IX), one calibrator lot, and five independent low analyte samples. Each application met the pre-defined acceptance criteria to support the lower limit of the clinically reportable range (CRR).

Sysmex CS-2500: Summary of LoQ

Application	Lower Limit of Clinical Reportable Range (% of norm)	Measured Limit of Quantitation (% of norm)
Coagulation Factor VIII with Dade Actin FSL Reagent	3.0% of norm	0.51% of norm
Coagulation Factor IX with Dade Actin FSL Reagent	3.0% of norm	1.01% of norm

e. Analytical specificity:

Interference Study

Interference with hemoglobin, conjugated bilirubin, unconjugated bilirubin, and triglycerides was tested for each assay. The study was performed with one reagent lot, one calibrator lot, and one trained operator. A dose-response experiment was carried out to determine the degree of interference as a function of the interferent concentration. For each of the 3rd wave applications at least two plasma pools, normal and abnormal, were prepared, representing the low pools of interference testing. The summarized data is provided in table form below. No interference was observed up to the following interferent concentrations as per the pre-defined acceptance criteria.

Application	Highest Level without Interference (mg/dL)			
	Hemoglobin	Conjugated Bilirubin	Unconjugated Bilirubin	Triglycerides
Factor V Leiden with FactorV Leiden Assay	391	40	60	773
Coagulation Factor VIII with Dade Actin FSL	1000	40	60	1052
Coagulation Factor IX with Dade Actin FSL	1000	40	60	1052
Lupus Anticoagulant with LA 1 Screening Reagent	1000	22	30.5	689.5
Lupus Anticoagulant with LA 2 Confirmation Reagent	1000	40	26.6	1113.5
Lupus Anticoagulant With LA1/LA2 Ratio	1000	38.3	13.8	554.5

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was conducted at four clinical sites including three external sites in the United States. Subjects over 18 years of age were enrolled. At each site, a minimum of 300 samples were included for a total of 2500 samples across all four sites. Samples were tested on both the predicate device (Sysmex CA-1500) and the Sysmex CS-2500 in random order. Remnant citrated plasma samples from routine laboratory testing were selected according to inclusion and exclusion criteria. The percentage of frozen and contrived samples was limited to approximately 10% of the number of total samples. Samples included in the method comparison study covered the clinical reportable ranges and respective medical deicision points. Results of the study met the defined acceptance criteria.

Application	N	Sample range (% of norm)	r	Slope (95% CI)	Intercept (95% CI)
Factor V Leiden with Factor V Leiden Assay	494	0.76–5.86	0.978	0.919 (0.907, 0.931)	0.098 (0.066, 0.124)
Coagulation Factor VIII with Dade Actin FSL	408	4.6–181.8	0.958	1.037 (1.015, 1.057)	-1.051 (-2.682, 0.616)
Coagulation Factor IX with Dade Actin FSL	459	3.2–145.2	0.984	1.000 (0.986, 1.013)	-1.200 (-2.060, -0.553)
Lupus Anticoagulant with LA 1 Screening Reagent	402	29.8–155.5	0.995	0.961 (0.950, 0.971)	1.767 (1.262, 2.243)

Application	N	Sample range (% of norm)	r	Slope (95% CI)	Intercept (95% CI)
Lupus Anticoagulant with LA 2 Confirmation Reagent	390	34.1–79.6	0.988	0.962 (0.946, 0.977)	2.044 (1.367, 2.724)
Lupus Anticoagulant with LA 1/LA 2 Ratio	347	0.81–2.51	0.989	0.956 (0.933, 0.980)	0.035 (0.010, 0.059)

b. Matrix comparison:

For the purpose of including frozen samples in the method comparison studies, a fresh/frozen comparison study was performed to demonstrate that the assays on the Sysmex CS-2500 analyzer are not affected by freezing and thawing specimens. The study results demonstrated comparability between frozen and fresh samples for the following assays: Factor V Leiden with Factor V Leiden Assay, Coagulation Factor IX with Dade Actin FSL, Lupus Anticoagulant with LA 1 Screening Reagent, LA 2 Confirmation Reagent and LA 1/LA 2 Ratio. Samples were not tested with the Coagulation Factor VIII with Dade Actin FSL assay as no frozen samples were included in the method comparison study. The study results demonstrated comparability between fresh and frozen samples and met the defined acceptance criteria for all applicatons.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off:

FV Leiden Cut-off Validation Study: Blood samples from the intended use population were collected at three external clinical sites including one U.S. site. The samples were tested with the Factor V Leiden Assay on the Sysmex CS-2500 analyzer and using the FDA cleared FV Leiden PCR method. The results of the FV Leiden assay on the Sysmex CS-2500 analyzer were subsequently compared to the FV Leiden genotype to calculate positive percent agreement (PPA) and negative percent agreement (NPA). A ratio of ≤ 1.8 obtained with the FV Leiden assay is considered as suggestive for the FV Leiden variant (single point mutation G1691A). The Siemens FV Leiden Assay does not support

a distinction between hetero- or homozygote variant carriers. A ratio > 1.8 is considered as negative for the FV Leiden variant.

All Sites Combined (U.S. and OUS) Factor V Leiden Assay		**Reference (Factor V Leiden PCR method)**		
		Negative	Positive	Total
Factor V Leiden Assay on Sysmex CS-2500 analyzer	Negative	152	0	152
	Positive	01	220	221
	Total	153	220	373

Positive Percent Agreement= 100.0% 97.5% Confidence Interval= 98.3% – 100%
Negative Percent Agreement= 99.3% 97.5% Confidence Interval= 96.4% – 100%

U.S. Site Factor V Leiden Assay		**Reference (Factor V Leiden PCR method)**		
		Negative	Positive	Total
Factor V Leiden Assay on Sysmex CS-2500 analyzer	Negative	50	0	50
	Positive	01	76	77
	Total	51	76	127

Positive Percent Agreement= 100.0% 97.5% Confidence Interval= 95.3% – 100%
Negative Percent Agreement= 98.0% 97.5% Confidence Interval= 89.6% – 100%

5. Expected Values/Reference Range:

The reference interval studies were conducted at three clinical sites in the U.S. at different geographic locations to reflect the U.S. population. Citrated plasma samples obtained from apparently healthy individuals between ages 18 and 80 years old with no current or recent history of coagulation disease (e.g. thrombosis or bleeding), use of anticoagulation medication, apparent infection or acute phase reaction, known pregnancy, hospitalization within four weeks, or use of oral contraceptives or hormone replacement therapy (exclusive to protein C testing). Separate reference intervals were established using fresh and frozen samples for the LA 1/LA 2 Assay. Reference intervals for the Factor V Leiden Assay and the Lupus Anticoagulant applications were calculated as two-sided 95 % central interval with 2.5^{th}/ 97.5^{th} percentiles. For Coagulation Factors VIII and IX, the reference intervals were calculated as one-sided 97.5 % interval (2.5th percentile) and one-sided 95 % interval (5th percentile).

Application		N	Reference Interval
Factor V Leiden with Factor V Leiden Assay (ratio, no unit)		187	1.38–9.82 (2.5^{th} to 97.5^{th} percentile)
Coagulation Factor VIII with Dade Actin FSL (% norm)		191	78.7% (2.5^{th} percentile)
Coagulation Factor IX with Dade Actin FSL (% norm)		190	81.4% (2.5^{th} percentile)
Lupus Anticoagulant with LA 1 Screening Reagent (seconds)	Fresh samples	192	32.7–51.1 (2.5^{th} to 97.5^{th} percentile)
	Frozen samples	193	33.4–51.5 (2.5^{th} to 97.5^{th} percentile)
Lupus Anticoagulant with LA 2 Confirmation Reagent (seconds)	Fresh samples	192	35.8–43.5 (2.5^{th} to 97.5^{th} percentile)
	Frozen samples	193	36.3–44.5 (2.5^{th} to 97.5^{th} percentile)
Lupus Anticoagulant with LA 1 / LA 2 Ratio (no units)	Fresh samples	192	0.88–1.25 (2.5^{th} to 97.5^{th} percentile)
	Frozen samples	193	0.90–1.26 (2.5^{th} to 97.5^{th} percentile)

N. Instrument Name:

Sysmex® Automated Blood Coagulation Analyzer CS-2500

O. System Descriptions:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes ___X_____ or No _____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

   Yes _____ or No ___X_____

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes ___X_____ or No _____

3. Specimen Identification:

   Manual entry and barcode reader

4. Specimen Sampling and Handling:

   The Sysmex CS-2500 supports two different analysis modes; normal mode for capped (closed) and uncapped (open) sampling from collection tubes, and the micro-sample mode for open (uncapped) sampling. In the normal mode, capped and uncapped samples may be loaded into the same sample rack for analysis. Automatic reanalysis is also an exclusive function of the normal mode. In the micro-sample mode, uncapped samples may be loaded in the sampler or STAT holder.

5. Calibration:

   Calibration is performed using Standard Human Plasma (SHP) as an automated function of the Sysmex CS-2500. SHP is obtained from pooled citrated plasma collected from healthy blood donors, and is stabilized with HEPES buffer solution. A new standard curve must be established when changing a reagent lot, post-major maintenance or service, if indicated by quality control results, and when required according to laboratory control procedures and government regulations.

6. Quality Control:

   Quality control testing using Control Plasma N, Control Plasma P, ProC Control Plasma, and LA Control Low and High should all be performed at least every 8 hours during intervals of patient testing. Controls should be run after a new standard curve is established and after each change of reagent. Patient test results should not be reported if controls are out of range.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

1. Dilution Study

The Sysmex CS-2500 analyzer offers additional dilution options for each application carried out by an auto-dilution mode when measurement results are observed outside the analytical measuring range (AMR): auto-dilution mode 1:4 for Factor VIII with Dade Actin FSL, for the results above 120% of norm and the auto-dilution mode 1:2 for Factor IX with Dade Actin FSL for the results above 120% of norm. The Sysmex CS-2500 analyzer also provides the 2:1 processing mode for results below the AMR.

In addition to automatic dilution settings, the Sysmex CS-2500 analyzer also provides an

on-demand ‘dilution analysis.’ The application is programmed with a list of pre-defined dilutions that may be selected for such purposes. The following options are provided by the Sysmex CS-2500 analyzer to the customer for ‘dilution analysis’: 1/2, 1/4, 1/8 dilution and processing modes 3/2, 2/1 on-demand. The dilutions that may be selected are application-specific and are listed in the application sheets.

The auto-dilution study was carried out with one analyzer and one reagent lot (deficient plasma) with three different plasma samples covering the range outside the calibration curve between the end of the analytical measurement range (AMR) and the clinically reportable range (CRR), for each of the applicable reagent applications. Three test samples were analyzed for each dilution, in five replicates. The same samples, but undiluted, were measured upon auto-dilution by the instrument. All observed maximum deviations in the dilution analysis study were within the pre-defined acceptance criteria.

2. *Normal Mode versus Micro Mode*

The Sysmex CS-2500 has two analysis modes: normal and micro. In the normal-sample mode, a capped sample tube analysis can be performed for all the analyses and re-analyses. Automatic re-analysis can also be performed. In the micro-sample mode, the sample volume from samples set in the sampler or STAT holder is taken into the instrument through a secondary dispensing sample probe and analyzed. This analysis mode can also be performed with less sample volume than normal mode. However, in the micro-sample mode, capped sample tube analysis and automatic re-analysis cannot be performed.

The comparison study between micro versus normal mode was conducted for each application using samples (84-Factor V Leiden, 69-Factor VIII/IX, 82-LA 1, 83-LA 2, and 76-LA 1/LA 2 ratio) covering clinical reportable ranges, measured with one reagent lot on one Sysmex CS-2500 analyzer and the predicate instrument. The study results met pre-defined acceptance criteria and demonstrated acceptable comparability between micro versus normal mode.

3. *Carryover Studies*

<u>Reagent Carryover</u>

The reagent carryover study investigated whether a reagent or a test component of a donor application may affect an acceptor application. A combination of donor and acceptor applications was tested in a defined sequence. One normal plasma pool and one pathological plasma pool were used as test samples. The reagent carryover studies data showed no cross-contamination between applications.

<u>Sample Carryover</u>

A donor, was defined as a test sample with the highest possible analyte concentration that might interfere in the subsequent assay. Whereas an acceptor, was defined as a low analyte concentration test sample measured after the measurements of the donor assay. If

sample carryover occurs, the results of the acceptor assay demonstrates interference. Samples were prepared by dilution or spiking of a normal plasma pool with the respective application-specific deficient plasma or respective analyte concentrate. The donor and acceptor assay measurements were performed in a defined sequence. The study data demonstrated that there is no cross-contamination caused by high carryover test samples.

4. *Ambient Temperature Testing*

   The study investigated the influence of environmental temperatures on test results. Each study was carried out with one Sysmex CS-2500 analyzer, one reagent lot, and one calibrator lot. Samples were selected to represent the respective clinical reportable range (CRR) and medical decision points (MDPs). In addition to pooled plasma, ProC Global Control, Control Plasma N and P were tested in a minimum of five replicates on three different days, each day representing a different ambient temperature (15°C, 22°C, and 30°C). The mean values were calculated for each sample and the relative (percent) or absolute differences were calculated for each application and found to be within the pre-defined acceptance criteria. All observed maximum deviations in the ambient temperature study were below the pre-defined acceptance criteria.

5. *Bridging Study*: *CS-2100i to CS-2500 Instrument comparison within instrument family*

   The aim of the study was to show equivalent performance in a method comparison study between the Sysmex CS-2500 analyzer (new device) and the Sysmex CS-2100 (K151259, K162688) with the representative applications. The study was performed according to CLSI EP09-A3 "Measurement Procedure Comparison and Bias Estimation Using Patient Samples"; Approved Guideline-Third Edition. The same samples and reagents lots were used. The comparison between instruments comprise of the evaluation of analytical studies for the detection limit, linearity, precision and method comparison. The following assays were evaluated:

Assay	Methodology
Prothrombin Time (PT) seconds and PT INR with Dade Innovin Activated Partial Thromboplastin Time (APTT) with Dade Actin FSL Fibrinogen (Fbg) with Dade Thrombin Reagent Dade Innovin with Coagulation V Deficient Plasma Dade Innovin with Coagulation VII Deficient Plasma Protein C with Protein C Reagent	Clotting
Antithrombin (AT) with INNOVANCE Antithrombin Protein C with Berichrom Protein C Reagent	Chromogenic
D-dimer with INNOVANCE D-Dimer	Immunochemical

a. Method Comparison

The purpose of the study is to demonstrate equivalency between the instrument family member, Sysmex CS-2500 analyzer and the parent instrument, Sysmex CS-2100i analyzer (K151259), with each reagent application. Samples were measured on both analyzers (Sysmex CS-2500 and Sysmex CS-2100i) in random order as to eliminate any inherent bias. Measurements were performed according to the IFU for each cleared application. Samples were analyzed on the Sysmex CS-2500 and the Sysmex CS-2100i analyzer within 2 hours or less in single determination. If a sufficient number of samples that adequately span the clinical reportable range (CRR) was not available, diluted and/or spiked samples (contrived samples) were used, but not more than 10% overall.

Application (Clinical Reportable Range)	N	Sample range	r	Slope (95% CI)	Intercept (95% CI)
Prothrombin Time with Dade Innovin (8.7 – 90.0 seconds)	302	9.80–81.45 seconds	1.000	1.000 (1.000, 1.000)	-0.100 (-0.100, -0.100)
Prothrombin Time (INR) with Dade Innovin (0.93 – 8.00 INR)	300	0.940–7.665 INR	1.000	1.000 (1.000, 1.000)	-0.010 (-0.010, -0.010)
Activated Partial Thromboplastin Time with Dade Actin FSL (20.0 – 139.0 seconds)	304	23.15–136.25 seconds	1.000	0.990 (0.986, 0.993)	0.106 (-0.015, 0.236)
Fibrinogen with Dade Thrombin Reagent (50 – 860 mg/dL)	300	52.0–835.5 mg/dL	0.998	1.026 (1.020, 1.031)	- 8.651 (-10.902, -7.261)
Antithrombin with INNOVANCE Antithrombin (9.0 – 128.0% of norm)	301	9.90–126.80 % of norm	0.999	1.011 (1.006, 1.016)	- 0.237 (-0.561, 0.129)
D-dimer with INNOVANCE D-Dimer (0.19 – 35.20 mg/L FEU)	302	0.190–32.565 mg/L FEU	0.998	1.025 (1.015, 1.034)	-0.005 (-0.013, 0.006)
Factor V with Dade Innovin (6.0 - 149.0 % of norm)	308	7.4-143.3	0.991	1.056 (1.042, 1.071)	-0.218 (-0.766, 0.298)
Factor VII with Dade Innovin (6.0 - 149.0 % of norm)	353	6.1-146.5	0.996	0.973 (0.965, 0.980)	1.691 (1.277, 2.056)
Protein C with Protein C Reagent (10.1 – 131.0 % of norm)	456	11.3-131.0	0.994	0.986 (0.975, 0.996)	1.481 (0.697, 2.141)
Protein C with Berichrom Protein C (10.0 – 138.0 % of norm)	462	13.3-137.9	0.995	1.022 (1.016, 1.028)	-1.511 (-1.988, -1.096)

b. Precision

The study was performed on three Sysmex CS-2500 analyzers with one lot of reagent over 20 days, with two runs per day and two replicates per run at one site. For each application, several samples (plasma pools) as well as different control materials were investigated as samples. The samples were chosen to represent the entire CRR and

medical decision points. Statistical analyses were conducted for each instrument separately as well as on the data combined over the three instruments. For the precision evaluation of the combined instruments, a three-factorial random effects ANOVA was conducted with the factors instrument, day (nested within instrument) and run (nested within day) for each sample concentration. The SDs and CVs were calculated for the following variance components: within-run, between-run, between-day, between-instrument and total (combined instruments).

Prothrombin Time (PT) seconds and PT INR with Dade Innovin

Sample	Mean (Seconds)	Within-run		Between-run		Between-day		Between-instrument		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma Pool 1	9.47	0.16	1.71	0.00	0.00	0.06	0.59	0.02	0.22	0.17	1.82
Plasma Pool 2	10.57	0.11	1.01	0.05	0.47	0.02	0.23	0.04	0.37	0.13	1.19
Plasma Pool MDP1	12.47	0.20	1.57	0.00	0.00	0.07	0.56	0.05	0.38	0.21	1.71
Plasma Pool MDP2	15.55	0.23	1.45	0.00	0.00	0.12	0.75	0.11	0.69	0.28	1.78
Plasma Pool MDP3	18.63	0.66	3.57	0.00	0.00	0.07	0.39	0.12	0.64	0.68	3.64
Ci-Trol 2	26.95	0.19	0.72	0.11	0.41	0.15	0.55	0.28	1.05	0.39	1.44
Ci-Trol 3	42.98	0.36	0.84	0.00	0.00	0.28	0.65	0.48	1.13	0.66	1.55
Plasma Pool 3	73.91	3.63	4.91	1.34	1.81	1.14	1.54	0.55	0.74	4.07	5.51
Plasma Pool 4	88.21	0.79	0.90	0.83	0.94	1.54	1.75	0.08	0.09	1.92	2.18

Prothrombin Time PT INR with Dade Innovin

Sample	Mean (INR)	Within-run		Between-run		Between-day		Between-instrument		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Ci-Trol 1	1.011	0.007	0.66	0.003	0.30	0.002	0.20	0.006	0.61	0.010	0.97
Plasma Pool 1	1.028	0.014	1.35	0.008	0.83	0.000	0.00	0.003	0.32	0.017	1.61
Plasma Pool MDP1	1.947	0.074	3.78	0.020	1.03	0.020	1.04	0.000	0.00	0.079	4.05
Ci-Trol 2	2.594	0.018	0.71	0.010	0.38	0.014	0.56	0.027	1.03	0.037	1.42
Plasma Pool MDP2	2.899	0.041	1.43	0.017	0.58	0.016	0.54	0.015	0.53	0.050	1.72
Ci-Trol 3	4.134	0.034	0.82	0.000	0.00	0.026	0.64	0.045	1.08	0.062	1.50
Plasma Pool MDP3	4.664	0.211	4.53	0.044	0.94	0.034	0.73	0.021	0.45	0.219	4.70
Plasma Pool 2	6.450	0.308	4.78	0.085	1.32	0.107	1.66	0.000	0.00	0.337	5.23
Plasma Pool 3†	7.092	0.288	4.05	0.201	2.83	0.000	0.00	0.068	0.96	0.357	5.04
Plasma Pool MDP3 PTI40	4.628	0.016	0.34	0.028	0.60	0.015	0.32	0.020	0.44	0.041	0.88
Plasma Pool MDP3 PTI40a	4.726	0.019	0.40	0.019	0.41	0.027	0.57	0.025	0.52	0.045	0.96

APTT with Dade Actin FSL

Sample	Mean (seconds)	Within-run		Between-run		Between-day		Between-instrument		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma Pool 1	22.98	0.75	3.25	0.00	0.00	0.14	0.60	0.23	0.98	0.79	3.45
Ci-Trol 3	59.33	0.45	0.75	0.08	0.13	0.22	0.38	0.59	0.99	0.77	1.30
Plasma Pool 3	100.44	3.02	3.01	0.00	0.00	0.73	0.73	2.07	2.06	3.73	3.72
Plasma Pool 4	119.82	3.96	3.30	0.00	0.00	1.39	1.16	2.65	0.22	4.96	4.14
Plasma Pool MDP2	65.90	3.52	*5.33*	0.00	0.00	1.16	1.77	0.40	0.61	3.72	5.65
Plasma Pool MDP1 FSL20	33.02	0.28	0.84	0.03	0.08	0.19	0.59	0.35	1.07	0.49	1.49

Sample	Mean (seconds)	Within-run		Between-run		Between-day		Between-instrument		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma Pool MDP1 FSL20	32.60	0.18	0.56	0.07	0.21	0.04	0.13	0.40	1.22	0.45	1.37
Plasma Pool 2 FSL30	52.63	0.34	0.65	0.31	0.59	0.22	0.42	0.69	1.32	0.86	1.64
Plasma Pool 2 FSL30a	53.07	0.30	0.57	0.15	0.28	0.16	0.31	0.67	1.26	0.77	1.44
Plasma Pool MDP2 FSL50	68.32	0.41	0.60	0.00	0.00	0.22	0.32	0.95	1.38	1.05	1.54
Plasma Pool MDP2 FSL50a	68.02	0.47	0.69	0.26	0.38	0.26	0.39	0.99	1.46	1.16	1.71

AT with INOVANCE Antithrombin

Sample	N	Mean (mg/dL)	Within-run		Between-run		Between-day		Between-instrument		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Control Plasma P	240	32.25	0.80	2.48	0.68	2.11	0.00	0.00	0.31	0.95	1.10	3.39
Plasma Pool 1	240	11.97	0.61	5.08	0.49	4.13	0.00	0.00	0.77	6.46	1.10	9.20
Plasma Pool 2	240	14.59	0.60	4.11	0.53	3.61	0.00	0.00	0.93	6.35	1.22	8.38
Control Plasma N	240	95.51	1.23	1.28	1.41	1.47	0.00	0.00	1.21	1.26	2.23	2.32
Plasma Pool MDP1	240	54.85	0.87	1.59	0.94	1.72	0.10	0.18	0.82	1.49	1.53	2.78
Plasma Pool MDP2	240	82.55	1.15	1.40	1.39	1.69	0.00	0.00	0.65	0.79	1.92	2.33
Plasma Pool 3	240	122.73	1.47	1.20	1.37	1.12	0.00	0.00	0.54	0.44	2.08	1.70
Plasma Pool 4	Samples were consistently found above the upper limit of measuring (>128% of norm)											

Fibrinogen (Fbg) with Dade Thrombin Reagent

Sample	N	Mean (mg/dL)	Within-run		Between-run		Between-day		Between-instrument		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Control Plasma P	240	82.6	2.4	2.93	0.8	0.91	0.0	0.00	1.4	1.69	2.9	3.50
Plasma Pool MDP1	240	60.0	2.8	4.63	0.7	1.19	0.0	0.00	0.4	0.71	2.9	4.83
Plasma Pool MDP2	240	101.2	2.2	2.21	0.0	0.00	0.9	0.85	0.9	0.86	2.5	2.52
Control Plasma N	240	263.4	8.9	3.38	1.5	0.57	4.7	1.78	7.7	2.93	12.8	4.85
Plasma Pool MDP3	240	194.2	4.7	2.44	0.0	0.00	0.8	0.42	1.2	0.63	5.0	2.56
Plasma Pool MDP4	240	431.0	9.7	2.44	0.0	0.00	0.0	0.00	8.7	2.02	13.0	3.02
Plasma Pool 1	240	787.9	12.7	1.61	0.0	0.00	3.1	0.39	16.8	2.13	21.2	2.69
Plasma Pool 2	240	831.5	12.7	1.52	4.6	0.56	0.0	0.00	16.6	2.00	21.4	2.57

D-dimer with INNOVANCE D-Dimer

Sample	Mean (mg/LFEU)	Within-run		Between-run		Between-day		Between-instrument		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
INNOVANCE D-Dimer Control 1	0.351	0.011	3.01	0.003	0.78	0.003	0.79	0.013	3.81	0.017	4.98
Plasma Pool 3	28.715	1.602	5.58	0.000	0.00	0.849	2.96	1.086	3.78	2.114	7.36
Plasma Pool 2	11.046	0.401	3.63	0.160	1.45	0.145	1.31	0.515	4.67	0.688	6.23
INNOVANCE D-Dimer Control 2	2.514	0.082	3.27	0.000	0.00	0.045	1.80	0.058	2.30	0.110	4.39
Plasma Pool 1 DD10	0.266	0.010	3.60	0.003	1.09	0.004	1.58	0.004	1.65	0.012	4.40
Plasma Pool 1 DD10a	0.242	0.009	3.66	0.004	1.53	0.005	1.97	0.004	1.63	0.011	4.72
Plasma Pool MDP1 DD20	0.467	0.014	3.05	0.003	0.63	0.009	1.98	0.011	2.36	0.020	4.38
Plasma Pool MDP1 DD20a	0.506	0.015	3.04	0.005	1.07	0.011	2.08	0.012	2.36	0.023	4.50

Coagulation Factor V with Dade Innovin

Sample	Mean (% of norm)	Within-run		Between-run		Between-day		Between-instrument		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma Pool Low	7.20	0.18	2.55	0.11	1.49	0.06	0.88	0.20	2.79	0.30	4.16
Control Plasma P	29.74	0.80	2.70	0.63	2.13	0.39	1.31	0.63	2.13	1.26	4.25
Plasma Pool Medium	46.36	1.27	2.74	0.57	1.24	0.89	1.91	0.95	2.05	1.90	4.11
Plasma Pool MDP	71.10	1.93	2.72	0.40	0.56	1.03	1.45	2.98	4.19	3.72	5.23
Control Plasma N	108.50	4.21	3.88	1.88	1.72	1.41	1.30	4.96	4.58	6.92	6.38
Plasma Pool High*	136.76	4.13	3.02	2.62	1.91	1.43	1.04	4.30	3.14	6.67	4.87
Plasma Pool High†	137.06	4.31	3.15	3.05	2.23	0.00	0.00	4.60	3.36	7.01	5.11

Coagulation Factor VII with Dade Innovin

Sample	Mean (% of norm)	Within-run		Between-run		Between-day		Between-instrument		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma Pool Low	10.78	0.26	2.39	0.12	1.10	0.26	2.45	0.31	2.88	0.50	4.60
Control Plasma P	41.32	1.11	2.69	0.33	0.79	090	2.18	10.7	2.59	1.82	4.40
Plasma Pool Medium	53.44	1.09	2.04	0.46	0.87	0.84	1.56	0.72	1.34	1.62	3.02
Plasma Pool MDP	65.22	1.48	2.24	0.14	0.21	1.39	2.14	0.79	1.22	2.19	3.35
Control Plasma N	104.75	3.10	2.92	1.64	1.57	2.06	1.97	0.49	0.46	4.09	3.91
Plasma Pool High	137.65	3.29	2.39	0.66	0.48	2.65	1.93	1.10	0.80	4.41	3.21

Protein C with Protein C Reagent

Sample	Mean (% of norm)	Within-run		Between-run		Between-day		Between-instrument		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma Pool Low	16.97	0.56	3.30	0.00	0.00	0.16	0.95	0.61	3.58	0.84	4.96
Control Plasma P	40.01	1.10	2.74	0.00	0.00	0.43	1.07	1.00	2.51	1.55	3.86
Plasma Pool MDP	72.93	1.72	2.35	0.19	0.26	0.47	0.65	1.93	2.64	2.63	3.61
Control Plasma N	99.19	2.54	2.56	0.45	0.45	1.20	3.28	3.25	3.28	4.32	4.35
Plasma Pool High	120.77	3.07	2.54	2.27	1.88	0.00	0.74	0.89	0.74	3.92	3.25

Protein C with Berichrom Protein C

Sample	Mean (% of norm)	Within-run		Between-run		Between-day		Between-instrument		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma Pool Low	16.17	0.50	3.11	0.33	2.06	0.00	0.00	0.48	2.95	0.77	4.75
Control Plasma P	32.11	1.22	3.79	0.00	0.00	0.00	0.00	0.32	0.99	1.26	3.92
Plasma Pool MDP	76.17	1.63	2.14	0.73	0.95	0.00	0.00	0.20	0.27	1.80	2.36
Control Plasma N	102.85	2.58	2.51	0.73	0.71	0.00	0.00	1.52	1.48	3.09	3.00
Plasma Pool High	130.2	1.15	0.88	0.80	0.61	0.42	0.33	1.46	1.12	2.06	1.59

c. Limit of Quantitation (LoQ)

The LoQ studies were performed internally. Plasma pools were prepared by dilution of normal plasma with the respective application-specific deficient plasma. For each reagent application the LoQ-verification was performed with five independent low-analyte plasma pools.

Each study was conducted over three testing days, one run per day, with one Sysmex CS-2500 analyzer, using two different reagent lots, one calibrator lot, five low analyte samples, and four replicates per run. Each application met the pre-defined acceptance criteria to support the lower limit of the CRR.

Application	Measured Limit of Quantitation
Fibrinogen (Fbg) with Dade Thrombin Reagent	45.2 mg/mL
Antithrombin (AT) with INNOVANCE Antithrombin	8.22 % of norm
D-dimer with INNOVANCE D-Dimer	0.155 mg/L FEU
Coagulation Factor V with Dade Innovin	4.80 % of norm
Coagulation Factor VII with Dade Innovin	3.39 % of norm
Protein C with Protein C Reagent	9.35 % of norm
Protein C with Berichrom Protein C	8.32 % of norm

d. Linearity

Linearity was evaluated for four calibrated coagulation assay applications: Fibrinogen (Fbg) with Dade Thrombin Reagent; Antithrombin (AT) with INNOVANCE Antithrombin; D-dimer with INNOVANCE D-Dimer; and Coagulation Factor VIII with Dade Actin FSL.

Testing was performed with a high concentration sample pools (high pool) mixed with low concentration pools, to obtain samples covering the measuring range. Low pools were prepared by dilution with factor deficient plasma, high pools were

prepared by adding factor concentrate. At least 11 different dilutions were prepared; each analyte concentration was measured in replicates of four. The study was performed on one Sysmex CS-2500 analyzer with one lot of reagent on one day, with one run, four replicates per sample. For each sample, deviation between the linear regression model (predicted value from 1st order regression) and the best fitting polynomial regression model was calculated.

Application	Linear Range
Fibrinogen (Fbg) with Dade Thrombin Reagent	36–1177 mg/mL
Antithrombin (AT) with INNOVANCE Antithrombin	6.68–172% of norm
D-dimer with INNOVANCE D-Dimer	0.11–45.36 mg/L FEU
Coagulation Factor V with Dade Innovin	3.4–180.7% of norm
Coagulation Factor VII with Dade Innovin	4.3–179.5% of norm
Protein C Reagent	7.0–187.7% of norm
Berichrom Protein C	7.1–181.3% of norm

All observed maximum deviations in the linearity studies at the dilution points were below the pre-defined acceptance criteria. In particular, the linearity at the medical decision point for each application showed acceptable performance.

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.