510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k111822

B. Purpose for Submission:
Clearance of a new device

C. Measurand:
Heparin

D. Type of Test:
Chromogenic assay

E. Applicant:
Diagnsotica Stago Inc.

F. Proprietary and Established Names:
STA®-Liquid Anti-Xa
STA®-Multi Hep Calibrator
STA®-Quality NHF/UFH
STA®-Quality HBPM/LMWH

G. Regulatory Information:

1. Regulation section:
   21 CFR §864.7525, Heparin Assay
   21 CFR §864.5425, Multipurpose system for in vitro coagulation studies
   21 CFR §862.1150, Calibrator
2. Classification:
   Class II
3. Product code:
   KFF, Assay, Heparin
   GGN, Plasma, Coagulation Control
   JIS, Calibrator, Primary
4. Panel:
   81 Hematology

H. Intended Use:

1. Intended use(s):
   The STA® Liquid Anti-Xa kits are intended for use with STA-R®, STA Compact® and STA Satellite® analyzers, for the quantitative determination of the plasma levels of unfractionated (UFH) and low molecular weight (LMWH) heparins by measuring their anti-Xa activity on antithrombin in a competitive assay using a synthetic chromogenic substrate.

   The STA® Multi Hep Calibrator is a set of calibrator plasmas intended for use with STA-R®, STA Compact® and STA Satellite® analyzers, for the calibration of heparin (UFH and LMWH) activity assay by measuring the anti-Xa activity.

   The STA® Quality HNF/UFH kit is a set of two plasmas intended for the quality control of unfractionated heparin (UFH) activity assay by measuring the anti-Xa activity performed on STA-R®, STA Compact® and STA Satellite® analyzers.

The STA® Quality HBPM/LMWH kit is a set of two plasmas intended for the quality control of low molecular weight heparin (LMWH) activity assay by measuring the anti-Xa activity performed on STA-R®, STA Compact® and STA Satellite® analyzers.

2 Indication(s) for use:
Same as above

3. Special conditions for use statement(s):
For prescription use only.

4. Special instrument requirements:
STA® line of analyzers: STA Satellite®, STA-R® and STA Compact®

I. Device Description:

The STA® Liquid Anti-Xa kit is available in two sizes. A 4 mL kit consists of 6x4 mL vials of liquid chromogenic substrate and 6x4 mL vials of bovine factor Xa, buffers and preservative. The 8 mL kit has the same reagents as the 4 mL kit.

The STA® Multi Hep Calibrator kit consists of 5 levels of lyophilized human plasma containing a defined quantity of both UFH and LMWH. Calibrator 1 has no heparin, calibrators 2 and 4 contain different concentrations of UFH and calibrators 3 and 5 contain different concentrations of LMWH.

The STA® Quality HBPM/LMWH Controls come as reagent 1 and reagent 2 sets, each consisting of six (6) 1 mL vials. Reagent sets 1 and 2 contain different known concentrations of LMWH.

The STA® Quality HNF/UFH Controls come as reagent 1 and reagent 2 sets, each consisting of six (6) 1 mL vials. Reagent sets 1 and 2 contain different known concentrations of UFH.

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) number(s):
STA®-Rotachrom® Heparin, k010455
STA®-Calibrator HBPM/LMWH, k010350
STA®-Hepanorm H Calibrator Plasma Set, k854762
STA®-Heparin Control k943520
STA®-Quality HBPM/LMWH, k010324

2. Comparison with predicate:

Similarities		
Item	Device STA®-Liquid Anti-Xa	Predicate STA®-Rotachrom® Heparin (k010455)
Intended Use	Quantitative determination of unfractionated heparin (UFH), low molecular weight heparin (LMWH) in citrated human plasma.	Assay of UFH and LMWH on STA analyzers
Assay Method	Chromogenic method (measurement of the chromogenic substrate at 405 nm)	Same
Test Principle	Measurement of anti-Xa activity in terms of antithrombin-heparin complex. The activity of the complex is a function of the level of heparin	Same
Sample type	Citrated human plasma	Same
Analyzers	STA® Satellite, STA-R® and STA®-Compact	Same

Differences		
Item	Device STA®-Liquid Anti-Xa	Predicate STA®-Rotachrom® Heparin (k010455)
Assay Form	Liquid Reagents	Lyophilized (reagents 1 & 2) Liquid (reagent 3)
Packaging Content	Two reagents Reagent 1 is the chromogenic substrate Reagent 2 contains bovine Factor Xa	Three reagents Reagent 1 is the chromogenic substrate. Reagent 2 and 3 consist of bovine Factor Xa and its solvent, respectively.

Similarities		
Item	Device STA®-Multi Hep Calibrator	Predicate STA®-Calibrator HBPM/LMWH (k010350)
Intended Use	The STA® - Multi Hep Calibrator is a set of calibrator plasmas intended for use with STA-R®, STA Compact® and STA Satellite® analyzers for the calibration of heparin (UFH and LMWH) activity assay by measuring the anti-Xa activity using the chromogenic method, STA® - Liquid Anti-Xa.	The STA® - Calibrator HBPM/LMWH kit provides a set of calibrator plasmas intended for use by analyzers of the STA® line suitable with these reagents, for the calibration of low molecular weight heparin (LMWH) assays based on the anti-Xa principle, STA® - Rotachrom® Heparin.
Design	Lyophilized human plasmas reconstituted with distilled water, used in IVD analyzers. Stored at 2-8°C	Same

Differences		
Item	Device STA®-Multi Hep Calibrator	Predicate STA®-Calibrator HBPM/LMWH (k010350)
Traceability	The UFH levels are determined against the 07/328 International Standard for UFH established in 2009. The LMWH levels are determined against the 01/608 International Standard for LMWH established in 2003.	The LMWH levels are determined against a secondary standard of the 01/608 International Standard for LMWH established in 2003.
Kit Contents	Five reagents Reagent 1 is free of heparin. Reagents 2 and 4 contain different quantities of UFH. Reagents 3 and 5 contain different quantities of LMWH.	Three reagents Reagent 1 is free of heparin. Reagents 2 and 3 contain different quantities of LMWH.

Similarities		
Item	Device STA®-Multi Hep Calibrator	Predicate STA®-Hepanorm H (k854762)
Intended Use	The STA®-Multi Hep Calibrator is a set of calibrator plasma intended for use with the STA-R®, STA Compact® and STA Satellite® analyzers for the calibration of heparin (UFH and LMWH) activity assay by measuring the anti-XA activity using the chromogenic method, STA® Liquid Anti-Xa.	The STA® Hepanorm H kit provides a set of calibrator plasma intended for use by analyzers of the STA® line suitable with these reagents, for the calibration of unfractionated heparin (UFH) assays based on the anti-Xa principle (STA Rotachrom® Heparin, STA Staclot® Heparin, Stachrom® Heparin.
Design	Lyophilized human plasmas reconstituted with distilled water, used in IVD analyzers. Stored at 2-8°C.	Same

Differences		
Item	Device STA®-Multi Hep Calibrator	Predicate STA®-Hepanorm H (k854762)
Principles of Operation	Chromogenic	Chromogenic and clotting methods
Kit Contents	Five reagents Reagent 1 is free of heparin. Reagents 2 and 4 contain different quantities of UFH. Reagents 3 and 5 contain different quantities of LMWH.	Three reagents Reagent 1 is free of heparin. Reagents 2 and 3 contain different quantities of UFH.
Traceability	The UFH levels are determined against the 07/328 International Standard for UFH established in 2009. The LMWH levels are determined against the 01/608 International Standard for LMWH established in 2003.	The LMWH levels are determined against a secondary standard of the 07/328 International Standard for UFH established in 2009.

Similarities		
Item	Device STA®-Quality NHF/UFH	Predicate STA®-Heparin Control (k943520)
Intended Use	The STA® - Quality HNF/UFH is a set of two plasmas intended for the quality control of unfractionated heparin (UFH) activity assay by measuring the anti-Xa activity using the chromogenic method, STA® - Liquid Anti-Xa.	The STA® - Heparin Control kit provides a set of two plasmas intended for the quality control of unfractionated heparin (UFH) activity assay by measuring the anti-Xa activity using clotting assay (STA® - Staclot® Heparin) and chromogenic assays (STA® - Rotachrom® Heparin, Stachrom® Heparin).
Design	Lyophilized human plasmas reconstituted with distilled water, used in IVD analyzers. Stored at 2-8°C.	Same

Differences		
Item	Device STA®-Quality NHF/UFH	Predicate STA®-Heparin Control (k943520)
Analyzers	STA-R®, STA Compact® and STA Satellite® analyzers	STA-R®, STA Compact® and STA Satellite® analyzers (STA® - Rotachrom® Heparin, STA® - Staclot® Heparin). Water bath (STA® - Staclot® Heparin and Stachrom® Heparin)

Similarities		
Item	Device STA®-Quality HBPM/LMWH	Predicate STA®-HBPM/LMWH (k010324)
Intended Use	The STA® - Quality HBPM/LMWH is a set of two plasmas intended for the quality control of low molecular weight heparin (LMWH) activity assay by measuring the anti-Xa activity using the chromogenic method, STA® - Liquid Anti-Xa.	The STA® - Quality HBPM/LMWH kit is a set of two plasmas intended for the quality control of low molecular weight heparin (LMWH) activity assay by measuring the anti-Xa activity performed on analyzers of the STA® line suitable with these reagents.
Design	Lyophilized human plasmas reconstituted with distilled water, used in IVD analyzers. Stored at 2-8°C.	Same

Differences		
Item	Device STA®-Quality HBPM/LMWH	Predicate STA®-HBPM/LMWH (k010324)
Assay Reagent	STA®- Liquid Anti-Xa and STA® - Rotachrom® Heparin.	STA® - Rotachrom® Heparin

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

- CLSI EP05-A2: Evaluation of Precision Performance of Quantitative Measurement Methods, 2nd Edition, 08/20/2004.
- CLSI EP06-A2: Evaluation of Linearity of Quantitative Measurement Procedures: A Statistical Approach, Approved Guidelines, 01/01/2003
- CLSI EP07-A2: Interference Testing in Clinical Chemistry; Approved Guideline. 2nd Edition, 01/01/2005
- CLSI EP09-A2-1R: Method Comparison and Bias Estimation Using Patient Samples: Approved Guideline. 2nd Edition (Interim Edition), 07/01/2010
- CLSI EP17-A: Protocols for the Limit of Detection ad Limit of Quantitation; Approved Guideline, 01/01/2004
- CLSI EP25-A: Evaluation of Stability of In Vitro Diagnostic Reagents: Approved Guideline, 01/01/2009

L. Test Principle:

The normal function of a molecule of factor Xa, when present in plasma, is to cleave its natural substrate, prothrombin, to generate thrombin. Thrombin is the enzyme responsible for the formation of the fibrin clot. In the presence of heparin, competition occurs between this mechanism and the inhibitory mechanism exerted by the heparin-antithrombin complex. This inhibitory mechanism is largely responsible for the anticoagulant action of heparin.

The proposed method is a one-step reaction based on a similar principle: as soon as factor Xa is added to the plasma-substrate mixture, two reactions take place simultaneously, namely,

– Hydrolysis of the substrate by factor Xa

– Inhibition of factor Xa by the heparin-antithrombin complex*.

After the necessary period of time for the competitive reaction to reach equilibrium, the quantity of paranitroaniline that is released is inversely proportional to the concentration of heparin present in the test medium.

* The heparin-antithrombin complex is made up from the heparin and the antithrombin (AT) specific to the patient.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility

   Reproducibility and repeatability studies were performed using six human plasma samples spiked with various known concentrations of UF and LMW heparin. All samples are lyophilized human plasmas reconstituted. Samples 1, 2 and 3 were spiked with UFH and samples 4, 5 and 6 were spiked with LMWH. Within each run, the raw data results obtained were processed using one of two different calibrators (3 or 5 point). Testing included using 2 different lots of the assay on alternating days at two sites. Each sample was tested for 22 operating days, twice a day with one operator at each of the two laboratory sites.

   For repeatability (within-day precision), each sample was run twice a day for 22 days with one operator per site. The overall mean, SD and %CV were calculated for each sample tested.

   For reproducibility (between-run and total-run precision), for each day, two separate runs were performed alternating two reagent lots of STA Liquid Anti Xa

on two analyzers (STA-R and STA Compact) with one operator at each site over 22 days.
The within-run, between-run and total-run performance was assessed. The mean SD, %CV were calculated for each of the 6 samples tested. The overall %CV for repeatability was <7 % and reproducibility was <10%. These results were within the acceptance criteria of ≤12% for repeatability and ≤14% for reproducibility.

Sample/ Calibrator	Repeatability %CV		Total-Run Precision %CV	
	UHF	LMWH	UHF	LMWH
Sample 1-5pt	6.1		8.7	
Sample 1-3pt	5.7		8.0	
Sample 2-5pt	3.4		6.0	
Sample 2-3pt	3.2		5.6	
Sample 3-5pt	3.2		5.5	
Sample 3-3pt	3.2		4.9	
Sample 4-5pt		3.0		4.6
Sample 4-3pt		3.0		5.0
Sample 5-5pt		2.6		4.6
Sample 5-3pt		2.6		4.0
Sample 6-5pt		2.9		5.0
Sample 6-3pt		2.4		5.1

b. *Linearity/assay reportable range:*
Assay linearity was determined using separate WHO International LMW and UF Heparin standards diluted with normal pooled plasma. The WHO standards and normal pooled plasma were mixed and diluted to 5 levels of concentration ranging from 0 to 1.10 IU/mL for UFH and 0 to 2.0 IU/mL for LMWH. Each dilution level was analyzed 10 times on representative STA® analyzers (STA-Compact®, STA-R® and STA Satellite®) with the STA-Liquid Anti-Xa assay and the mean value was plotted against the expected values. Results demonstrated acceptable linearity up to 1.10 IU/mL for UHF and up to 2.0 IU/mL for LMWH on all the analyzers in the study.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Traceability:
Each lot of calibrator and control are prepared from citrated plasma, and are traceable to the 2nd International WHO Standards 07/328 (established in 2009) and 01/608 (established in 2003) for UFH and LMWH respectively.
Stability
A real-time stability study for STA-Liquid Anti-Xa heparin reagent was performed using three lots of heparin reagent with four different control samples and calibrators. Heparin reagents were tested at time zero and stored at 2-8°C. Stability testing was conducted at 6, 12, 15 and 16 months with LMWH and UFH controls and analyzed 10 times on STA-R® analyzer using the stored reagents and calibrators. The mean results were compared to the mean result at time zero with a maximum shift of <0.07 IU/mL which met the acceptance criteria of <0.15 IU/mL. Real-time stability data are available for up to 16 months and the study is ongoing to support a 24 month claim.

The accelerated stability study for STA-Quality NHF/UHF control and Multi Hep Calibrator was performed on 3 lots at elevated temperature of 30°C for 21 days for estimating 24 month stability at 2-8°C. Overall change compared to time zero at 3 weeks was <0.04 IU/mL which met the acceptance criteria of < 0.10 IU/mL for the control and calibrators. Data supported a stability claim of 24 months.

d. Detection limit:

Detection limit was determined by spiking each of 6 human plasma samples with a known concentration (0.03-0.16 IU/mL) of UFH and LMWH. Testing was performed on four replicates over 3 runs on 3 different STA® analyzers (2-STA-R® and 1-STA Compact®) using 2 lots of Liquid Anti-Xa assay. The detection limit was determined for each test lot using the calculation LoD = LoB +cß SDs. The UFH detection threshold is 0.07 IU/mL and the LMWH detection threshold is 0.10 IU/mL.

e. Analytical specificity:

Interference studies were determined on the STA® instrument line (STA-R®, STA-Satellite® and STA-Compact®) for STA-Liquid-Xa, STA-Multi Hep, STA-Quality HNF/UFH and STA Quality HBPM/LMWH. Normal pooled plasma was spiked with two levels of UFH and LMWH. Interferents were spiked into the 2 levels of spiked UFH and LMWH plasma resulting in 5 concentrations and each concentration was tested five times. Acceptance criterion was <0.25 IU/mL when compared to the un-spiked sample results. The maximum tolerated concentrations were:

Interferents	**Max tolerated concentration**
Hemoglobin	1.5 g/L
Unconjugated bilirubin	236 µmol/L or 138 mg/L
Conjugated bilirubin	342 µmol/L or 288 mg/L
Triglyceride	8 mmol/L or 6.9 g/L

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was conducted at 5 sites (1-internal, 1-US and 3 European), comparing STA-Liquid Anti Xa assay, controls and calibrator with the predicate device (STA-Rotachrom Heparin). A total of 1017 patients (623 on UFH and 394 on LMWH) participated in the study. Patients used the following LMWH in the study as part of their therapy: Lovenox, Fragmin, and Innohep. Acceptance criteria were established as the following:

UHF-Linear Regression	LMWH-Linear Regression
0.85 ≤ Slope ≤1.10 Y-intercept ≤0.010 IU/mL r ≤ 0.95	0.90 ≤ Slope ≤1.10 Y-intercept ≤0.010 IU anti-Xa/mL r ≤ 0.95

The results of the studies are as follows:

Sites	**N**	**Slope 95% confidence interval**		**Intercept 95% confidence interval**		**Correlation Coefficient**	
	LMWH/UFH	LMWH	UFH	LMWH	UFH	LMWH	UFH
Internal	110/62	0.988 (0.974-1.001)	0.905 (0.835-.976)	-0.008 (-0.015-(-0.001))	-0.051 (-0.077-(-0.024))	0.997	.0957
Site 1	41/150	1.032 (0.993-1.071)	1.026 (0.994-1.059	-0.015 (-0.043–(-0.012))	-0.074 (-0.089-(-0.058))	0.993	0.981
Site 2	134/124	0.998 (0.983-1.013)	1.059 (1.018-1.099)	0.024 (0.013-0.035)	-0.068 (-0.085-(-0.051))	0.996	.0978
Site 3	109/148	0.985 (0.966-1.004)	0.995 (0.962-1.028)	0.027 (0.018-0.035)	-0.029 (-0.041-(-0.018))	0.995	0.980
Site 4	0/139	Not applicable	1.014 (0.977-1.051)	Not applicable	-0.047 (-0.061- (-0.034))	Not applicable	0.977
Total	394/623	1.004 (0.995-1.014)	0.993 (0.975-1.011)	0.008 (0.002-0.014)	-0.046 (-0.054-(-0.039))	0.995	0.974

Based on the above results from the study sites, the acceptance criteria were met.

b. Matrix comparison:
Not applicable

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**:**
   Not applicable
5. Expected values/Reference range:
   Not applicable

N. Proposed Labeling:

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

O. Conclusion:

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