**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION**
**DECISION MEMORANDUM**

**A. 510(k) Number:**

K162540

**B. Purpose for Submission:**

New device

**C. Measurand:**

Heparin (IU/mL)

**D. Type of Test:**

Quantitative chromogenic assay for heparin activity in citrated human plasma

**E. Applicant:**

Siemens Healthcare Diagnostics Products GmbH

**F. Proprietary and Established Names:**

INNOVANCE® Heparin Assay
INNOVANCE® Heparin Calibrator
INNOVANCE® Heparin UF and LMW Controls

**G. Regulatory Information:**

1. Regulation section:

   21 CFR 864.7525, Heparin assay
   21 CFR 862.1150, Calibrator
   21 CFR 864.5425, Multipurpose System for in vitro coagulation studies

2. Classification:

   Class II

3. Product code:

   KFF: Assay, Heparin
   JIS: Calibrator, Primary

GGN: Plasma, Coagulation Control

4. Panel:

   Hematology (81)

## H. Intended Use:

1. Intended use (IU):

   INNOVANCE® Heparin Assay

   *In vitro* diagnostic automated chromogenic assay for the quantitative determination of unfractionated heparin (UFH) and low molecular weight heparin (LMWH) activity in human plasma collected from venous blood samples in 3.2% sodium citrate tubes on the BCS® XP System in the clinical laboratory. For use with plasma from patients undergoing heparin anticoagulant therapy with either UFH or LMWH. The performance of this device has not been established in neonate and pediatric patient populations.

   INNOVANCE® Heparin Calibrator

   For calibration of the INNOVANCE® Heparin assay for the quantitative determination of the activity of unfractionated heparin (UFH) and low molecular weight heparin (LMWH) in citrated human plasma.

   INNOVANCE® Heparin UF and LMW Controls

   For quality control of the INNOVANCE® Heparin assay for the quantitative determination of the activity of unfractionated heparin (UFH) and low molecular weight heparin (LMWH) in citrated human plasma.

2. Special conditions for use statement(s):

   For prescription use only.

3. Special instrument requirements:

   BCS® XP System

## I. Device Description:

1. INNOVANCE® Heparin Assay

   The INNOVANCE Heparin assay is an one-stage chromogenic assay. The reagent kit consists of two components: the INNOVANCE Heparin Reagent which contains Coagulation Factor Xa (FXa) and the INNOVANCE Heparin Substrate, specific for FXa.

2. INNOVANCE® Heparin Calibrator

   The INNOVANCE Heparin Calibrator consists of five calibrator levels. INNOVANCE Heparin Calibrator 1 represents plasma containing no heparin. INNOVANCE Heparin Calibrators 2, 3, 4 and 5 are plasmas with defined activities of LMWH and are calibrated against the WHO (World Health Organization) International Standards for UFH and LMWH.

3. INNOVANCE® Heparin UF and LMW Controls

   The INNOVANCE Heparin Control consists of plasmas with defined activities of either UFH (control 1 and 2) or LMWH (control 1 and 2).

## J. Substantial Equivalence Information:

1. Predicate device name(s):

   Coamatic Heparin Assay

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

   K983178

4. Comparison with predicate:

a. Assay

| Similarities | | |
|---|---|---|
| **Item** | **Device (K162540) INNOVANCE Heparin Assay** | **Predicate (K983178) Coamatic Heparin Assay** |
| Intended Use | *In vitro* diagnostic automated chromogenic assay for the quantitative determination of unfractionated heparin (UFH) and low molecular weight heparin (LMWH) activity in human plasma collected from venous blood samples in 3.2% sodium citrate tubes on the BCS® XP System in the clinical laboratory. For use with plasma from patients undergoing heparin anticoagulant therapy with either UFH or LMWH. The | For the quantitative determination of unfractionated heparin (UF Heparin) or low molecular weight heparin (LMW Heparin) in human citrated plasma using automated and microplate methods. |
| Heparin Type | UFH and LMWH | Same |
| Test Principle | Chromogenic | Same |
| SampleType | 3.2% citrated human plasma | Same |

| | | |
|---|---|---|
| Antithrombin | None | Same |
| Calibration Curve | Single calibration curve for LMWH and UFH | Same |
| Control Level | 2 levels for LMWH<br>2 levels for UFH | Same |
| Units | IU/mL | Same |

| **Differences** | | |
|---|---|---|
| **Item** | **Device (K162540)<br>INNOVANCE Heparin Assay** | **Predicate (K983178)<br>Coamatic Heparin Assay** |
| Measuring Range | 0.10 to 1.50 IU/mL | 0 to 1.5 IU/mL |
| Composition | Liquid<br>(Factor Xa and chromogenic substrate) | Lyophilized<br>(Factor Xa and chromogenic substrate) |
| Stability | Open-vial: 8 weeks at 2–8ºC | Open-vial: 3 months at 2–8ºC |
| | On-board: 24 hours | Not available |
| | Shelf-life: 24 months at 2–8ºC | Not available |

b. Calibrator:

The Coamatic Heparin Assay does not contain calibrator and the UF and LMW heparin international standards were used in the Method Comparison studies. Since the UF and LMW heparin international standards are not same as the INNOVANCE Heparin Calibrator in IU, matrix and stability, therefore only the difference were listed in the table below.

| **Differences** | | |
|---|---|---|
| **Item** | **Device (K162540)<br>INNOVANCE Heparin Calibrator** | **Predicate<br>3rd International Standard for LMWH<br>6th International Standard for UFH** |
| Intended Use | For quality control of the INNOVANCE® Heparin assay for the quantitative determination of the activity of unfractionated heparin (UFH) and low molecular weight heparin (LMWH) in citrated human plasma. | International Standard for LMWH<br>International Standard for UFH |
| Matrix | Human Plasma (Lyophilized) | Porcine Mucosa |
| Components | LMWH | UFH and LMWH |
| Traceable to WHO Standard | Yes | WHO Standard |
| Number of Calibrator Levels | 5 Levels | 1 Level |
| On-Board Stability | 4 hours (BCS XP) | Not Available |

| Differences | | |
|---|---|---|
| **Item** | **Device (K162540)<br>INNOVANCE Heparin Calibrator** | **Predicate<br>3rd International Standard for LMWH<br>6th International Standard for UFH** |
| Shelf-life | 24 months at 2–8ºC | LMWH: 6 months at 4ºC or below<br>UFH: Not available |
| Reconstitution Stability | 48 hours at 2–8ºC<br>24 hours at 15–25ºC | Not available |

c. Controls

The Coamatic Heparin Assay does not contain heparin controls. The items listed in the table below is for comparison purpose only.

| Differences | | |
|---|---|---|
| **Item** | **Device (K162540)<br>INNOVANCE Heparin UF and LMWControls** | **Coamatic Heparin Control** |
| Intended Use | For quality control of the INNOVANCE® Heparin assay for the quantitative determination of the activity of unfractionated heparin (UFH) and low molecular weight heparin (LMWH) in citrated human | Not available |
| Matrix | Human Plasma (Lyophilized) | Not available |
| Traceability to WHO | Yes | Not available |
| Number of Controls | 2 levels for LMWH<br>2 levels for UFH | Not available |
| Reconstitution Stability | 24 hours at 15–25ºC<br>48 hours at 2–8ºC<br>4 weeks at < – 18ºC | Not available |
| Stability | On-Board: 4 hours (BCS® XP) | Not available |
| | Shelf-life: 24 months at 2–8ºC | Not available |

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

EP05-A2; Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline; 2014

EP06-A; Evaluation of the Linearity of Quantitative Measurement Procedures; a Statistical Approach; Approved Guideline; 2003

EP07-A2; Interference Testing in Clinical Chemistry; Approved Guideline; 2005

EP09-A3; Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline; 2013

EP17-A2; Evaluation of Detection Capability For Clinical Laboratory Measurement Procedures; Approved Guideline; 2012

EP14-A5; Collection, Transport, and Processing for Testing Plasma-Based Coagulation Assay and Molecular Hemostasis Assays; Approved Guideline; 2008

EP25-A3; Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline; 2009

## L. Test Principle:

1. INNOVANCE® Heparin Assay

   Upon mixing of INNOVANCE Heparin Reagent and INNOVANCE Heparin Substrate, FXa converts the chromogenic substrate into two products, one of them is paranitroaniline. The formation of paranitroaniline can be quantified by the coagulation analyzer employing light absorption at a specific wavelength (405 nm). In the presence of a heparin containing sample the formation of paranitroaniline will be reduced in a time dependent manner. This is due to inhibition of FXa by the heparin/antithrombin (AT) complex. This complex is formed in the patient's plasma and competes with the substrate conversion by FXa. The concentration of the complex is not only dependent on the concentration of heparin but also on the availability of the patient's endogenous antithrombin. By comparison to a reference curve the heparin activity of the sample can be quantified. To reduce the influence from heparin antagonists, such as platelet factor 4 (PF4), dextran sulfate is included in the reaction mixture.

2. INNOVANCE® Heparin Calibrator

   The calibrator levels are used to establish a reference curve (calibration curve) which then can be employed to quantify the heparin activity of UFH and LMWH containing plasmas.

3. INNOVANCE® Heparin UF and LMW Controls

   Recovery of these controls within their assigned ranges indicates proper functionality of the assay system.

## M. Performance Characteristics (if/when applicable):

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

   *a. Precision:*

      i. INNOVANCE Heparin Assay

Repeatability

Three reagent lots were tested at one site with three calibrator lots on the BCS XP system. One lot of LMWH and UFH controls (levels 1 and 2) and three heparin (LMWH and UFH) plasma pool samples were tested with each reagent lot in duplicate, twice a day for 20 days. A total of 240 measurements per sample level/reagent lot were performed and are summarized below:

| Sample | N | Mean | Within-Run | | Between-Run | | Between-Day | | Between-Lot | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| LMWH CO 1 | 240 | 0.443 | 0.010 | 2.25 | 0.004 | 1.00 | 0.007 | 1.60 | 0.005 | 1.06 | 0.014 | 3.12 |
| LMW CO 2 | 239 | 1.024 | 0.013 | 1.23 | 0.007 | 0.66 | 0.012 | 1.15 | 0.004 | 0.36 | 0.019 | 1.85 |
| PP LMWH 1 | 240 | 0.151 | 0.009 | 5.71 | 0.006 | 4.05 | 0.008 | 4.97 | 0.005 | 3.60 | 0.014 | 9.30 |
| PP LMWH 2 | 240 | 0.703 | 0.010 | 1.37 | 0.011 | 1.54 | 0.011 | 1.60 | 0.008 | 1.11 | 0.020 | 2.83 |
| PP LMWH 3 | 240 | 1.409 | 0.17 | 1.19 | 0.021 | 1.50 | 0.023 | 1.64 | 0.008 | 0.54 | 0.036 | 2.58 |
| UF CO 1 | 240 | 0.315 | 0.007 | 2.34 | 0.002 | 0.58 | 0.008 | 2.48 | 0.004 | 1.34 | 0.012 | 3.71 |
| UF CO 2 | 240 | 0.699 | 0.009 | 1.35 | 0.009 | 1.29 | 0.010 | 1.41 | 0.019 | 2.70 | 0.025 | 3.58 |
| PP UFH 1 | 240 | 0.152 | 0.006 | 4.18 | 0.004 | 2.84 | 0.008 | 5.09 | 0.004 | 2.45 | 0.012 | 7.58 |
| PP UFH 2 | 240 | 0.677 | 0.009 | 1.35 | 0.007 | 1.01 | 0.013 | 1.86 | 0.014 | 1.99 | 0.022 | 3.21 |
| PP UFH 3 | 240 | 1.327 | 0.016 | 1.18 | 0.022 | 1.64 | 0.012 | 0.92 | 0.032 | 2.44 | 0.044 | 3.30 |

Reproducibility

Reproducibility studies were conducted at three external clinical sites for both UFH and LMWH with different operators on three different BCS XP analyzers (one instrument per site). One lot of INNOVANCE Heparin reagent was tested with two LMWH controls, two UFH controls, three LMWH plasma pool samples and three UFH plasma pool samples according to the CLSI EP05-A2 guideline (two runs per day, two single determinations per run for 20 days). The study design and testing data for combined sites are presented below.

| Sample | N | Mean | Within-Run | | Between-Run | | Between-Day | | Between-Lot | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| LMWH CO 1 | 240 | 0.448 | 0.010 | 2.17 | 0.005 | 1.07 | 0.006 | 1.34 | 0.015 | 3.42 | 0.020 | 4.40 |
| LMWH CO 2 | 240 | 1.045 | 0.013 | 1.24 | 0.010 | 01.00 | 0.00 | 0.89 | 0.024 | 2.34 | 0.031 | 2.97 |
| PP LMWH 1 | 240 | 0.160 | 0.009 | 5.56 | 0.010 | 6.33 | 0.000 | 0.00 | 0.031 | 19.40 | 0.034 | 21.15 |
| PP LMWH 2 | 240 | 0.717 | 0.010 | 1.35 | 0.010 | 1.33 | 0.008 | 1.14 | 0.019 | 2.67 | 0.025 | 3.47 |
| PP LMWH 3 | 240 | 1.425 | 0.17 | 0.97 | 0.014 | 1.02 | 0.018 | 1.26 | 0.042 | 2.94 | 0.050 | 3.49 |
| UFH CO 1 | 240 | 0.330 | 0.007 | 2.16 | 0.003 | 0.85 | 0.006 | 1.96 | 0.022 | 6.64 | 0.024 | 7.30 |
| UFH CO 2 | 240 | 0.731 | 0.009 | 1.13 | 0.007 | 0.93 | 0.009 | 1.30 | 0.019 | 2.64 | 0.024 | 3.29 |
| PP UFH 1 | 240 | 0.161 | 0.006 | 5.31 | 0.002 | 1.06 | 0.006 | 3.50 | 0.031 | 19.57 | 0.033 | 20.6 |
| PP UFH 2 | 240 | 0.719 | 0.009 | 1.30 | 0.008 | 1.13 | 0.009 | 1.30 | 0.027 | 3.72 | 0.031 | 4.30 |
| PP UFH 3 | 240 | 1.403 | 0.016 | 0.86 | 0.018 | 1.26 | 0.013 | 0.92 | 0.062 | 0.062 | 0.067 | 4.79 |

ii. INNOVANCE Heparin Calibrator

Repeatability

An internal single-site precision study was conducted on three INNOVANCE Heparin Calibrator levels (Calibrators 2, 3 and 4) using a single instrument (BCS XP) with three operators. Testing was performed with two runs per day and two replicates per run for 20 days following the CLSI EP05-A2 guideline for the study and data analysis. The data for within-run, between-run, between-lot, between-day, and total precision of each level of calibrator were calculated for all three operators combined and the results met the pre-defined acceptance criteria. The repeatability of Calibrator 1 and 5 were not performed as their values are outside of the assay measuring range (0.10 – 1.50 IU/mL).

Reproducibility

The reproducibility study was conducted at three separate internal sites on the BCS XP analyzers with three INNOVANCE Heparin Calibrator levels (Calibrators 2, 3 and 4) for five days with two runs per day and four replicates per run. The data for within-run, between-run, between-lot, between-day, and total precision of each calibrator level were calculated following the CLSI EP05-A2 guideline for all three sites combined and the results met the pre-defined acceptance criteria. The reproducibility of Calibrator 1 and 5 were not performed as their values are outside of the assay measuring range (0.10 – 1.50 IU/mL).

iii. INNOVANCE Heparin UF and LMW Controls

Repeatability

A single-site precision study was conducted with three lots of INNOVANCE Heparin Control (LMWH and UFH) on a single BCS XP analyzer at Siemens with three operators. Testing was performed with two runs per day and two replicates per run for 20 days for each level of each control (LMWH level 1 and 2; UFH level 1 and 2) following the CLSI EP05-A2 guideline for the study and data analysis. The data of within-run, between-run, between-lot, between-day, and total precision of each level of calibrator were calculated for all three operators combined and and the results met the pre-defined acceptance criteria.

Reproducibility

The reproducibility study was conducted at three internal sites on the BCS XP analyzer with four INNOVANCE Heparin Control (LMWH level 1 and 2; UFH level 1 and 2) for 20 days with two runs per day and two replicates per run. The data of within-run, between-run, between-lot, between-day, and total precision of each calibrator were calculated following the CLSI EP05-A2 guideline for all three sites

combined and the results met the pre-defined acceptance criteria.

*b. Linearity/assay reportable range:*

A linearity study was conducted as described in CLSI EP06-A "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach." Three lots of the assay reagent were tested on BCS XP analyzers. Human plasma samples spiked with an increased concentration (0.00–1.90 IU/mL) of the international UFH and LMWH standard were used to assess linearity of the assay. The tests were performed in four replicates at each dilution level of both UFH and LMWH using the INNOVANCE Heparin assay on two BCS XP coagulation analyzers. The measuring range was established as 0.10–1.5 IU/mL.

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

i. <u>INNOVANCE Heparin Calibrator</u>

Traceability:

The INNOVANCE Heparin Calibrator consists of five calibrator levels and the heparin activity was defined with LMWH, which was calibrated against the WHO International Standard for UFH (NIBSC code 07/328)and LMWH (NIBSC code 05/112). INNOVANCE Heparin Calibrator 1 is plasma without heparin. INNOVANCE Heparin Calibrators 2–5 are plasmas with a heparin concentration of 0.3–1.8 IU/mL.

Value Assignment:

Heparin activities of the calibration plasmas spiked with the International Standard for UFH and the International Standard for LMWH were determined using the INNOVANCE Heparin Assay on the BCS XP instrument and the reference calibration curves were generated. For each heparin type, the activities of three lots of calibrator were evaluated directly against the LMWH and UFH international reference curves and the results indicated that the activity of the INNOVANCE Heparin Calibrator 2–5 met the pre-defined heparin specification ranges:

INNOVANCE Heparin Calibrator 2: 0.30–0.50 IU/mL
INNOVANCE Heparin Calibrator 3: 0.65–0.95 IU/mL
INNOVANCE Heparin Calibrator 4: 1.05–1.35 IU/mL
INNOVANCE Heparin Calibrator 5: 1.50–1.80 IU/mL

On-Board Stability:

On-board stability studies for the INNOVANCE Heparin Calibrators were conducted at internally according to CLSI EP25-A. Three lots of INNOVANCE Heparin Calibrators were evaluated for on-board stability on the BCS XP analyzer. The calibrator lots were tested at intervals spanning the shelf-life. The calibrators were placed in the cooled section of the instrument and 10 citrated human plasma samples spiked with UFH (4 samples) and LMWH (6 samples) were tested in

triplicate after each calibration at the indicated timepoints in hours (0, 2, 4, 6, 8, 9). The results met the acceptance criterion and support 8 hours on-board stability.

Reconstituted Stability at 2–8°C:

Stability studies for reconstituted INNOVANCE Heparin Calibrators was conducted internally according to CLSI EP25-A. Three lots of INNOVANCE Heparin Calibrators were evaluated for on-board stability on the BCS XP analyzer. The calibrator lots at various points of shelf-life (0, 11 and 18 months) were used in the reconstituted stability studies for a good representation of the overall product stability claim. Seven citrated human plasma samples spiked with UFH (4 samples) and LMWH (6 samples) were tested in triplicate after each calibration at the indicated timepoints in hours (0, 2, 4, 5, 8, 24, 32, 48 and 49). The results met the acceptance criterion and support 48 hours reconstituted stability at 2–8°C.

Shelf-life:

Shelf-life stability studies for the INNOVANCE Heparin Calibrators were conducted internally according to CLSI EP25-A. Two calibrator groups were designated for shelf-life stability testing. One group of calibrators was stored at 2–8°C, the other was stored at -70°C or below. For comparison, the INNOVANCE Heparin Reagent were also stored at -70°C or below. Three lots of INNOVANCE Heparin Calibrators were evaluated for shelf-life stability on the BCS XP analyzer. Seven citrated human plasma samples spiked with UFH (4 samples) and LMWH (6 samples) were tested in triplicate after each calibration at the indicated timepoints in months (0, 3, 8, 11, 12, 18, 19, 24 and 25). The results of the study met the acceptance criterion and support the 24 months shelf-life stability at 2–8°C.

ii. <u>INNOVANCE Heparin Controls</u>

Value Assignment:

The heparin value of INNOVANCE Heparin (UFH or LMWH) Controls have been determined using three lots of INNOVANCE Heparin Calibrator on three BCS XP System. The INNOVANCE Heparin Calibrator is traceable to both LMWH and UFH International Standards; therefore, the LMWH and UFH controls are also traceable to the International Standard for LMWH and UFH. Recovery of the controls within their assigned ranges indicates proper functionality of the assay system. The pre-defined heparin specification ranges are:

INNOVANCE Heparin LMW Control 1: 0.30–0.50 IU/mL
INNOVANCE Heparin LMW Control 2: 0.85–1.15 IU/mL
INNOVANCE Heparin UF Control 1: 0.20–0.40 IU/mL
INNOVANCE Heparin UF Control 2: 0.60–0.80 IU/mL

On-board Stability:

On-board stability studies for INNOVANCE Heparin Control were conducted internally according to CLSI EP25-A. Three lots of INNOVANCE Heparin

Controls were evaluated for on-board stability on the BCS XP analyzer. The control lots at the various points of the shelf-life, such as the beginning, mid (11-months) and end (24-months), were used in the on-board stability studies for a good representation of the overall product stability claim and the stability tests were performed at the indicated hours (0, 2, 4, 6, 8, 9). The results met the acceptance criterion for UFH and LMWH and support 8 hours on-board stability.

Reconstituted Stability at 2–8ºC:

The stability studies for reconstituted INNOVANCE Heparin Controls stored at 2–8ºC, were conducted internally according to CLSI EP25-A. Three lots of the INNOVANCE Heparin (UFH and LMWH) Controls were evaluated on the BCS XP analyzer. Control lots were selected at various points across the shelf-life and used in the study. Each control was tested in triplicate at the indicated timepoints in hours (0, 2, 4, 5, 8, 24, 32, 48 and 49). The results met the acceptance criterion for UFH and LMWH and support 48 hours reconstituted stability at 2–8ºC.

Reconstituted Stability at 15–25ºC:

The stability studies for reconstituted INNOVANCE Heparin Controls stored at 15–25ºC, were conducted internally according to CLSI EP25-A. Three lots of INNOVANCE Heparin (UFH and LMWH) Controls were evaluated on the BCS XP analyzer. Control lots were selected at various points across the shelf-life and used in the study. Each control was tested in triplicate at the indicated timepoints in hours (0, 2, 3, 8, 24 and 25). The results met the acceptance criterion for UFH and LMWH and support 24 hours reconstituted stability at 15–25ºC.

Shelf-Life:

Shelf-life stability studies for the INNOVANCE Heparin Controls were conducted internally according to CLSI EP25-A. Two control groups were designated and stored under different conditions. One group of controls was stored at 2–8°C, the other was stored at -70°C or below. Three lots of INNOVANCE Heparin Controls were evaluated for shelf-life stability on the BCS XP analyzer. Seven citrated human plasma samples spiked with UFH (4 samples) and LMWH (6 samples) were tested in triplicate after each calibration at the indicated timepoints in months (0, 3, 8, 11, 12, 18, 19, 24 and 25). The results met the acceptance criterion for UFH and LMWH and support 24 months shelf-life stability at 2–8ºC.

iii. INNOVANCE Heparin Reagents

On-board Stability:

On-board stability studies for INNOVANCE Heparin reagents were conducted at the Siemens Marburg, Germany site according to CLSI guideline EP25-A. Three lots of INNOVANCE Heparin reagents were evaluated for on-board stability on the BCS XP analyzer. The reagent lots at the various points of the shelf-life, such

as the beginning, mid (11-months) and end (24-months), were used in on-board stability studies for a good representation of the overall product stability claim. The reagents were placed in the cooled section of the instrument and 10 citrated human plasma samples spiked with UHF (4 samples) and LMWH (6 samples) were tested in triplicate after each calibration on the indicated hours (0, 2, 4, 6, 18, 21, 24, 25). The results met the acceptance criterion and support 24 hours on-board stability.

Open-vial Stability at 2–8ºC:

Open-vial stability studies for INNOVANCE Heparin reagent were conducted at the Siemens Marburg, Germany site according to CLSI guideline EP25-A. Three lots of INNOVANCE Heparin reagent were evaluated on the BCS XP analyzer. The reagent and substrate were opened for 30 minutes at 15–25ºC and then closed to store at 2–8ºC for final stability testing. The reagent lots at the various points of the shelf-life, such as 0-month, 11-months and 18-months, were used in the open-vial stability studies for a good representation of the overall product stability claim. Seven citrated human plasma samples spiked with UHF (3 samples) and LMWH (4 samples) were tested triplicate after each calibration on the indicated weeks (0, 2, 4, 6, 8, 9). The results met the acceptance criterion and support 8 weeks open-vial stability at 2–8ºC.

Shelf-life at 2–8ºC:

Shelf-life stability studies for INNOVANCE Heparin reagents were conducted at the Siemens Marburg, Germany site according to CLSI guideline EP25-A. Two groups were designed for shelf-life stability testing. One group of reagent was stored at 2–8°C, the other was stored at -70°C or below. The reagents of the two groups were compared to each other at each time point of testing. Three lots of INNOVANCE Heparin reagents were evaluated for shelf-life stability on the BCS XP analyzer at 0, 3, 8, 11, 12, 18, 19 and 25 months. Seven citrated human plasma samples spiked with UHF (3 samples) and LMWH (4 samples) were tested in triplicate after each calibration. The INNOVANCE Heparin Controls including both UFH and LMWH were also used in the shelf-life stability assay. The results met the acceptance criterion and support 24 months shelf-life stability at 2–8ºC.

iv. Sample Stability

Sample stability studies were performed at one internal site and two external sites according to CLSI EP25-A. Thirty (UFH, 15 samples and LMWH, 15 samples) therapeutic patient plasma samples covering the analytical measuring range (AMR) and medical decision points (MDPs) were included in the study. Sample stability testing at 15–25ºC was performed at the indicated hours (0, 4 and 5). For the frozen storage conditions, testing was performed at the following timepoints: 0, 3 and 4 months at –20ºC and 0, 3, 4, 6 and 7 months at –74ºC. At each time point, four single determinations were performed. The results met the predetermined acceptable criteria UFH and LMWHand support 4-hour sample stability at 15–

25ºC, 3 months stability at –20ºC and 6 months stability at –70ºC.

*d. Detection limit:*

The Limit of Blank (LoB) and Limit of Detection (LoD) of the INNOVANCE Heparin Assay were determined using one BCS XP analyzer with two lots of reagents and substrates according to CLSI EP17-A2. Two lots of INNOVANCE Heparin Calibrator were used for assay calibration. Five independent blank samples (normal donor plasmas containing no heparin) were used as true blank and tested for 3 days with four replicates per day for LoB determination. To determine LoD, five independent low level samples for each heparin type (normal donor plasmas spiked with 0.05 IU/mL of the International Standards for UFH and LMWH) were used and tested with two lots of reagents for three days with four replicates per day for LoD determination.

The Limit of Quantitation (LoQ) of the INNOVANCE Heparin Assay was determined by following CLSI EP17-A2. Five independent low level samples for each heparin type (normal donor plasmas spiked with 0.10 IU/mL of the International Standards for UFH and LMWH) were tested with three lots of the INNOVANCE Heparin Assay (containing INNOVANCE Heparin reagent and INNOVANCE Heparin substrate) on two BCS XP analyzers and the samples were tested over 3 days with two replicates per BCS XP analyzer, per day and per lot of the INNOVANCE Heparin Assay. The final results of LoB, LoD and LoQ are summarized below.

| **LoB, LoD and LoQ** | | |
|---|---|---|
| LoB | LoD | LoQ |
| < 0.10 IU/mL | ≤ 0.10 IU/mL | ≤ 0.10 IU/mL |

*e. Analytical specificity:*

The study was conducted internally according to CLSI EP07-A2. A total of 12 plasma samples spiked with various levels of UFH and LMWH to cover the AMR (0.10–1.50 IU/mL) and MDPs for UFH (0.30 IU/mL and 0.70 IU/mL for UFH) and LMWH (0.40 IU/mL, 0.60 IU/mL and 1.00 IU/mL). Five test samples with increased interference substance concentration were prepared for each heparin concentration level. Each sample was tested in four replicates on the BCS XP analyzer using one lot of INNOVANCE Heparin reagents and one lot of INNOVANCE Heparin Calibrator. A total of 240 measurements (12 samples x 5 interference substances x 4 replicates) were performed for each interferent. The analytical specificity for each interferent was determined to be the highest concentration of interferent in which the UFH and LMWH results were not affected.

The common endogenous and exogenous interfering substances and their interference results are listed below and showed no significant interference up to the indicated concentration for all samples tested. Additional interference studies for anticoagulation medications were also performed.

| Endogenous Substances | |
|---|---|
| Interferent | Concentration |
| Ascorbic Acid | 176 mg/dL |
| Unconjuated Bilirubin | 60 mg/dL |
| Conjugated Bilirubin | 40 mg/dL |
| Hemoglobin | 347 mg/dL |
| Rheumatoid Factor | 220 IU/mL |
| Triglycerides | 807 mg/dL |
| Platelet Factor 4 | 0.7ug/mL |

| Exogenous Substances | |
|---|---|
| Interferent | Concentration |
| Danaparoid Sodium | 0.03 IU/mL |
| Rivaroxaban | 7.1ng/mL |
| Apixaban | 4.1 ng/mL |
| Fondaparinux | 35.5 ng/mL |

*f. Carryover*

i. Reagent Carryover:

Reagent carryover effect was assessed internally. The pipetting probe was washed between steps. All possible carryover events, e.g., from INNOVANCE Heparin Assay to other assays (PT, APTT, FVIII, FX, FXII and Antithrombin) and from other assays (same as above) to the INNOVANCE Heparin Assay were evaluated. The results indicated that reagent carryover was within the acceptable criteria.

ii. Sample Carryover:

Sample carryover effect was assessed internally. The pipetting probe was washed between steps. All possible carryover events, e.g., carryover between high and low heparin plasma samples with the same INNOVANCE Heparin Assay reagents; carryover between heparin containing plasma samples from the INNOVANCE Heparin Assay to other assays (PT and FX, FXII) and the carryover between heparin containing plasma samples from other assays (same as above) to INNOVANCE Heparin Assay were analyzed. The results indicated that sample carryover was within the acceptable criteria.

*g. Assay cut-off:*

Not applicable

2. Comparison studies:

*a. Method comparison with predicate device:*

Method comparison studies were conducted at three sites including one site in the United States and two internal sites (two physically separated labs, two different instruments and two different operators). Testing at each site compared the INNOVANCE Heparin Assay on the BCS XP to the Comatic Heparin assay on the ACL TOP (comparator device) using both fresh and frozen UFH and LMWH patient samples representing the intended use population. One lot of reagents and calibrators were used across the three testing sites. All samples were tested in singlet with both methods. The intended use population, patient demographics with disease states were provided. All samples were collected in 3.2% sodium citrate and samples excluded according to the specified exclusion criteria were documented and reported.

A total of 165 plasma samples from UFH patients and 155 plasma samples from LMWH patients were analyzed in the study. Data analysis was performed using the Passing-Bablok regression method analysis for each site and all sites combined. The predicted bias (deviation) at each site and combined sites at the defined medical decision points met the predefined acceptance criteria.

Passing-Bablok Regression Analysis and and Deviation for LMWH

| Study Site | N | Slope | Intercept IU/mL | r | Relative Bias at 0.40 IU/mL | Relative Bias at 0.60 IU/mL | Relative Bias at 1.00 IU/mL |
|---|---|---|---|---|---|---|---|
| Site 1 | 55 | 1.07 | -0.02 | 0.98 | 0.01 | 3.66% | 4.97% |
| Site 2 | 50 | 1.06 | -0.03 | 1.00 | 0.00 | 1.80% | 3.62% |
| Site 3 | 50 | 1.08 | 0.00 | 0.99 | 0.03 | 7.34% | 7.46% |
| Combined | 155 | 1.06 | -0.01 | 0.99 | 0.01 | 4.62% | 5.35% |

Passing-Bablok Regression Analysis and Deviation for UFH

| Study Site | N | Slope | Intercept IU/mL | r | Deviation at 0.30 IU/mL | Deviation at 0.40 IU/mL | Deviation at 0.7 IU/mL |
|---|---|---|---|---|---|---|---|
| Site 1 | 55 | 0.97 | 0.05 | 0.98 | 0.04 | 0.04 | 4.29% |
| Site 2 | 58 | 0.98 | -0.05 | 0.99 | -0.06 | -0.06 | -8.99% |
| Site 3 | 52 | 0.89 | 0.03 | 0.99 | 0.00 | 0.01 | -6.59% |
| Combined | 165 | 0.93 | 0.03 | 0.98 | 0.00 | 0.00 | -3.81% |

*b. Matrix comparison:*

A comparison study between fresh versus frozen plasma samples was performed with a total of 69 samples (33 LMWH and 36 UFH). The frozen samples were collected fresh and stored at -74ºC for one week before testing. The results were analyzed by Passing-Bablok regression analysis and Bland Altman plots. The results of the Passing-Bablok regression and Bland-Altman plots met the predetermined acceptance criteria.

3. Clinical studies:

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.