510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K223187

B Applicant

Instrumentation Laboratory Co.

C Proprietary and Established Names

HemosIL Liquid Anti-Xa
HemosIL Rivaroxaban Calibrators
HemosIL Rivaroxaban Controls
HemosIL Apixaban Calibrators
HemosIL Apixaban Controls
HemosIL Heparin Calibrators
HemosIL UF Heparin Controls
HemosIL LMW Heparin Controls

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QLU	Class II	21 CFR 864.7295 - Heparin And Direct Oral Factor Xa Inhibitor Drug Test System	HE - Hematology

II Submission/Device Overview:

A Purpose for Submission:

Clearance of HemosIL Liquid Anti-Xa for Rivaroxaban measurement with HemosIL Rivaroxaban Calibrators.

B Measurand:

Heparin, Apixaban, Rivaroxaban

C Type of Test:

Anti-Factor Xa chromogenic assay

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:

HemosIL Liquid Anti-Xa is an automated chromogenic assay for in vitro diagnostic use by laboratory professionals in clinical laboratories. The assay provides quantitative results on 3.2% citrated human plasma for the following analytes based on the calibrators used:

- **When used with HemosIL Heparin Calibrators:**
  Quantitative determination of unfractionated heparin (UFH) and low molecular weight heparin (LMWH) activity on the ACL TOP Family and ACL TOP Family 50 Series.

- **When used with HemosIL Apixaban Calibrators:**
  Quantitative determination of apixaban on the ACL TOP Family and ACL TOP Family 50 Series through measurement of Factor Xa activity, which is inversely proportional to the apixaban level. With HemosIL Apixaban Calibrators, the assay is intended to measure apixaban concentrations in patients on apixaban therapy in the following situations where measurement of apixaban levels could be useful to have as additional information:

  - Patients at risk for major bleeding

  - Patients experiencing a bleeding episode

- **When used with HemosIL Rivaroxaban Calibrators:**
  Quantitative determination of rivaroxaban on the ACL TOP Family and ACL TOP Family 50 Series through measurement of Factor Xa activity, which is inversely proportional to the rivaroxaban level. With HemosIL Rivaroxaban Calibrators, the assay is intended to measure rivaroxaban concentrations in patients on rivaroxaban therapy in the following situations where measurement of rivaroxaban levels could be useful to have as additional information:

  - Patients at risk for major bleeding

  - Patients experiencing a bleeding episode

The assay is not a stand-alone test and the results should be used in conjunction with other clinical and laboratory findings.

For use in adult population. For prescription use only.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only
For use in adult population only
For in vitro diagnostic use only

D Special Instrument Requirements:

ACL TOP Family (K160276): ACL TOP 300 CTS; ACL TOP 500 CTS; ACL TOP 700; ACL TOP 700 CTS; ACL TOP 700 LAS

ACL TOP Family 50 Series (K150877): ACL TOP 350 CTS; ACL TOP 550 CTS; ACL TOP 750; ACL TOP 750 CTS; ACL TOP 750 LAS

IV Device/System Characteristics:

A Device Description:

HemosIL Liquid Anti-Xa is a one stage chromogenic assay based on a synthetic chromogenic substrate and on Factor Xa inactivation. The assay provides quantitative heparin, apixaban, and rivaroxaban results on 3.2% citrated human plasma when used with HemosIL Heparin Calibrators, HemosIL Apixaban Calibrators and/or Rivaroxaban Calibrators.

The HemosIL Liquid Anti-Xa assay consists:

4mL Kit Vial Size

- Factor Xa reagent 5 x 2.5 mL vial of a liquid preparation containing purified bovine Factor Xa, Tris-Buffer, EDTA, dextran sulfate, sodium chloride and bovine serum albumin
- Chromogenic substrate 5 x 3 mL vial of liquid chromogenic substrate S-2732 and bulking agent

10mL Kit Vial Size

- Factor Xa reagent 5 x 5 mL vial of a liquid preparation containing purified bovine Factor Xa, Tris-Buffer, EDTA, dextran sulfate, sodium chloride and bovine serum albumin
- Chromogenic substrate 5 x 6 mL vial of liquid chromogenic substrate S-2732 and bulking agent

The assay requires the following components which are not included in the assay kit:

- HemosIL Rivaroxaban Calibrators- two levels (0 and 500 ng/mL) of lyophilized calibrators prepared from human citrated plasma containing rivaroxaban, buffers, and stabilizers.
- HemosIL Rivaroxaban Controls- two levels (80 and 300 ng/mL) of lyophilized controls prepared from human citrated plasma containing rivaroxaban, buffers, and stabilizers.
- HemosIL Apixaban Calibrators and Controls refer to DEN190032.
- HemosIL Heparin Calibrator and Controls (LMWH and UFH) refer to K090209.
- Cleaning solution
- Cleaning agent
- Factor diluent

B Principle of Operation:

The HemosIL Liquid Anti-Xa is an anti-factor Xa one-stage chromogenic assay. When an excess amount of factor Xa is added to a sample containing rivaroxaban, factor Xa is partially

neutralized by rivaroxaban. Residual factor Xa is quantified when the synthetic chromogenic substrate is added to the patient sample. The released paranitroaniline (pNA) is released and monitored kinetically at 405 nm. The measured pNA is inversely proportional to rivaroxaban levels. Thus, maximum absorption occurs with low levels of drug, and minimum absorption occurs with high levels of drug.

V Substantial Equivalence Information:

A Predicate Device Name(s):
HemosIL Liquid Anti-Xa

B Predicate 510(k) Number(s):
DEN190032, K213464

C Comparison with Predicate(s):

Device & Predicate Device(s):	K223187	K213464
General Device Characteristic Similarities		
Device Trade Name	HemosIL Liquid Anti-Xa	HemosIL Liquid Anti-Xa
Intended Use/Indications For Use	HemosIL Liquid Anti-Xa is an automated chromogenic assay for *in vitro* diagnostic use by laboratory professionals in clinical laboratories. The assay provides quantitative results on 3.2% citrated human plasma for the following analytes based on the calibrators used: **• When used with HemosIL Heparin Calibrators:** Quantitative determination of unfractionated heparin (UFH) and low molecular weight heparin (LMWH) activity on the ACL TOP Family and ACL TOP Family 50 Series. **• When used with HemosIL Apixaban Calibrators:** Quantitative determination of apixaban on the ACL TOP Family and ACL TOP Family 50 Series through measurement of Factor Xa activity, which is inversely proportional to the apixaban level. With HemosIL Apixaban Calibrators, the assay is intended to measure apixaban concentrations in patients on apixaban therapy in the following situations where measurement of apixaban levels could be useful to have as additional information: - Patients at risk for major bleeding	HemosIL Liquid Anti-Xa is an automated chromogenic assay for *in vitro* diagnostic use by laboratory professionals in clinical laboratories. The assay provides quantitative results on 3.2% citrated human plasma for the following analytes based on the calibrators used: **• When used with HemosIL Heparin Calibrators:** Quantitative determination of unfractionated heparin (UFH) and low molecular weight heparin (LMWH) activity on the ACL TOP Family, ACL TOP Family 50 Series. **• When used with HemosIL Apixaban Calibrators:** Quantitative determination of apixaban on the ACL TOP Family and ACL TOP Family 50 Series through measurement of Factor Xa activity, which is inversely proportional to the apixaban level. With HemosIL Apixaban Calibrators, the assay is intended to measure apixaban concentrations in patients on apixaban therapy in the following situations where measurement of apixaban levels could be useful to have as additional information:

	- Patients experiencing a bleeding episode • **When used with HemosIL Rivaroxaban Calibrators:** Quantitative determination of rivaroxaban on the ACL TOP Family and ACL TOP Family 50 Series through measurement of Factor Xa activity, which is inversely proportional to the rivaroxaban level. With HemosIL Rivaroxaban Calibrators, the assay is intended to measure rivaroxaban concentrations in patients on rivaroxaban therapy in the following situations where measurement of rivaroxaban levels could be useful to have as additional information: - Patients at risk for major bleeding - Patients experiencing a bleeding episode The assay is not a stand-alone test and the results should be used in conjunction with other clinical and laboratory findings. For use in adult population. For prescription use only. **Note: Same as predicate except for addition of Rivaroxaban.*	- Patients at risk for major bleeding - Patients experiencing a bleeding episode The assay is not a stand-alone test and the results should be used in conjunction with other clinical and laboratory findings. For use in adult population. For prescription use only.
Technical Method/ Test Methodology	One stage chromogenic assay based on a synthetic chromogenic substrate and on factor Xa inactivation. The test detects residual factor Xa using a chromogenic substrate. The signal or optical density is compared to a drug-specific calibration curve and results are reported as nanograms per milliliter (ng/mL).	Same
Measurement	Quantitative	Same
Sample Matrix	3.2% citrated plasma	Same
Composition	The HemosIL Anti-Xa includes the following components: • Factor Xa reagent: Liquid preparation containing purified bovine Factor Xa (approximately 5.5 nkat/mL), Tris-Buffer, EDTA, dextran sulfate, sodium chloride and bovine serum albumin.	Same

	• Chromogenic substrate: liquid chromogenic substrate S-2732 (approximately 1.2 mg/mL) and bulking agent.	
Instrumentation	ACL TOP Family (K160276) ACL TOP 50 Series (K150877)	Same
Quality Control	Automated QC	Same
Open Reagent Stability	1 month	Same
On-board Stability	4 Days	Same
General Device Characteristic Differences		
Measurand	Heparin Apixaban Rivaroxaban	Heparin Apixaban
Assay Volume	4 mL Vial Size Factor Xa reagent 5 x 2.5 mL Chromogenic substrate 5 x 3 mL 10 mL Kit Vial Size Factor Xa reagent 5 x 5 mL Chromogenic substrate 5 x 6 mL	4 mL Vial Size Factor Xa reagent 5 x 2.5 mL Chromogenic substrate 5 x 3 mL
Limit of Detection	8 ng/mL for Rivaroxaban	N/A
Linearity	20 ng/mL to 1000 ng/mL for Rivaroxaban	N/A
Calibrators (Sold Separately)	HemosIL Heparin Calibrators and HemosIL Apixaban Calibrators, HemosIL Rivaroxaban Calibrators; Target Levels: 0 and 500 ng/mL	HemosIL Heparin Calibrators; Targe levels: 0, 0.8 and 2.0 IU/mL HemosIL Apixaban Calibrators; Target Levels: 0 and 500 ng/mL
Controls (Sold Separately)	HemosIL Heparin Controls and HemosIL Apixaban Controls, HemosIL Rivaroxaban Controls; Target Levels: 80 and 300 ng/mL	HemosIL Heparin (LMW and UF) Controls; Target Levels: low and high HemosIL Apixaban Controls; Target Levels: 75 and 300 ng/mL

VI Standards/Guidance Documents Referenced:

EP05-A3. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline−Third Edition, October 2014 (R2019).

EP06-A2. Evaluation of Linearity of Quantitative Measurement Procedure-Second Edition, April 2003.

EP07-Ed. 3. Interference Testing in Clinical Chemistry-Third Edition, November 2005 (R2022).

EP17-A2. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline−Second Edition, October 2004 (R2017).

EP34-Ed. 1. Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking-First Edition, August 2018.

EP37-Ed. 1. Supplement Tables for Interference Testing in Clinical Chemistry-First Edition, April 2018.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

Analytical performance studies for apixaban, low-molecular weight heparin (LMWH) and unfractionated heparin (UFH) were previously reported in DEN190032 (apixaban) and K090209 (LMWH, UFH), respectively.

1. Precision/Reproducibility:

Four precision studies were performed following the recommendations of the CLSI EP05-A3 guideline.

Study 1: This study was conducted over 20 days, two runs per day and two replicates per run for a total of 480 determinations (i.e., 80 determinations per reagent lot). The study design included three representative ACL TOP Family instrument models, two reagent lots (each lot was tested on all instrument models), as well as three spiked plasma samples and one patient pool. Precision estimates were calculated for each of the following variance component: within-run, between-run, between-day, between-lot and within laboratory. The results are provided in the summary table below.

Sample	N	Rivaroxaban Mean ng/mL	Repeatability (Within-Run)		Between-Run		Between-Day		Between-Lot		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Spiked 1	480	50.2	1.57	3.1	1.12	2.2	0.59	1.2	1.15	2.3	2.32	4.6
Spiked 2	480	787.2	11.25	1.4	12.00	1.5	6.18	0.8	6.70	0.9	23.71	3.0
Spiked 3	480	928.2	11.91	1.3	17.18	1.9	17.55	1.9	0.00	0.0	33.77	3.6
Plasma Pool	480	127.2	1.77	1.4	0.98	0.8	1.00	0.8	2.04	1.6	3.04	2.4

Study 2: This study was conducted over 20 days, two runs per day and two replicates per run for a total of 720 determinations (i.e., 80 determinations per reagent lot). The study design included three representative ACL TOP Family 50 Series instrument models, three reagent lots (each lot was tested on all instrument models), as well as three spiked plasma samples and one patient pool. Precision estimates were calculated for each of the following variance component: within-run, between-run, between-day, between-lot and within laboratory. The results are provided in the summary table below.

Sample	N	Rivaroxaban Mean ng/mL	Repeatability (Within-Run)		Between-Run		Between-Day		Between-Lot		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Spiked 1	720	50.7	2.10	4.1	0.71	1.4	0.93	1.8	0.75	1.5	2.78	5.5
Spiked 2	720	777.2	19.90	2.6	4.48	0.6	6.28	0.8	11.03	1.4	38.80	5.0
Spiked 3	720	923.4	17.40	1.9	4.44	0.5	10.32	1.1	4.52	0.5	40.36	4.4
Plasma Pool	720	127.9	2.44	1.9	1.39	1.1	1.19	0.9	1.92	1.5	5.19	4.1

Study 3: This study was conducted over 20 days, two runs per day and two replicates per run for a total of 480 determinations (i.e., 80 determinations per reagent lot). The study design included two representative ACL TOP Family and ACL TOP Family 50 Series instrument models, three reagent lots (each lot was tested on all instrument models), native MDL trough and peak rivaroxaban plasma samples. Precision estimates were calculated for each of the following variance component: within-run, between-run, between-day, between-lot and within laboratory. The results are provided in the summary table below.

Sample	N	Rivaroxaban Mean ng/mL	Repeatability (Within-Run)		Between-Run		Between-Day		Between-Lot		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Native 5 (Trough)	480	32.4	1.41	4.4	0.39	1.2	0.66	2.0	0.00	0.0	1.70	5.2
Native 6 (Peak)	480	428.0	6.32	1.5	0.00	0.0	1.64	0.4	5.54	1.3	11.06	2.6

Study 4: This study was conducted at three sites by three operators (1 operator per site), over 5 days, two runs per day and three replicates per run for a total of 270 determinations (i.e., 30 determinations per reagent level). The study design included three different ACL TOP Family instruments, three reagent lots (i.e., one per instrument and the same reagent lots were used across all sites), as well as two quality controls, three spiked plasma samples and two patient pools. Precision estimates were calculated for each of the following variance component: within-run, between-run, between-day, between-site, between-lot, and reproducibility. The results are provided in the summary table below.

Pooled 3 Site Data														
Level	Mean (ng/mL)	N	Repeatability (Within-Run)		Between-Run		Between-Day		Between-Site		Between-Lot		Reproducibility (Total)	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Control	72.6	270	2.28	3.1%	1.03	1.4%	0.00	0.0%	1.44	2.0%	0.80	1.1%	3.00	4.1%
High Control	281	270	4.58	1.6%	4.10	1.5%	0.00	0.0%	2.15	0.8%	0.17	0.1%	6.51	2.3%
Spiked 1	45.0	270	2.55	5.7%	0.88	2.0%	0.56	1.3%	3.38	7.5%	0.66	1.5%	4.41	9.8%
Spiked 2	750	270	19.68	2.6%	1.44	0.2%	6.30	0.8%	22.12	3.0%	5.26	0.7%	30.76	4.1%
Spiked 3	939	270	25.49	2.7%	3.43	0.4%	7.41	0.8%	22.98	2.4%	8.83	0.9%	36.36	3.9%
Plasma Pool 1	51.8	270	2.46	4.7%	1.30	2.5%	0.84	1.6%	1.55	3.0%	0.83	1.6%	3.39	6.5%
Plasma Pool 2	118	270	3.71	3.1%	4.29	3.6%	2.76	2.3%	5.71	4.8%	1.64	1.4%	8.67	7.3%

2. Linearity:

Linearity studies were performed following the CLSI EP06-A2 guideline using three different reagent lots tested on a representative ACL TOP Family and an ACL TOP Family 50 series model. Normal Pooled Plasma spiked with known concentrations of rivaroxaban was tested in four replicates. Two separate linear regressions were performed. For the first, samples with thirteen different concentrations were evaluated to establish the analytical measuring interval; and for the second, samples with nine different concentrations were evaluated to establish the extended measuring interval.

Based on the results of the Linearity studies and Detection Limit studies, the claimed assay reportable range is 20–1000 ng/mL.

3. Analytical Specificity/Interference:

Interference studies were conducted based on the CLSI EP07-A2 guideline and CLSI EP37 guideline. The study design included one representative instrument model and one regent lot. Potentially interfering endogenous and exogenous substances were spiked into the test samples (rivaroxaban at 50 ng/mL, 180 ng/mL or 400 ng/mL native donor samples collected at peak and trough), and each sample level were run in five replicates on an ACL TOP family instrument.

Rivaroxaban results on ACL TOP Family and ACL TOP Family 50 Series are not affected by the interferents listed up to the concentrations detailed in the table below.

Interfering Substances	Concentration without Interference
Hemoglobin	600 mg/dL
Bilirubin (unconjugated)	40 mg/dL
Bilirubin (conjugated)	40 mg/dL
Triglycerides	921 mg/dL
Lupus anticoagulant	dRVVT Screen/Confirm Ratio 2.47 (dRVVT: Diluted Russell Viper Venom Time)
Acetylsalicylic acid	3.00 mg/dL
Atorvastatin	0.075 mg/dL
Isosorbide dinitrate	0.600 mg/dL
Ticagrelor	0.188 mg/dL
Warfarin	7.50 mg/dL

Rivaroxaban results may be falsely elevated in samples tested post-andexanet alfa administration.

The assay is not intended for the monitoring and dosage adjustment of rivaroxaban.

Administer a reversal agent based on current clinical guidance, and take into consideration other clinical and laboratory findings including the Factor Xa inhibitor dose and time since last dose. For additional information, refer to current clinical guidance and reversal agent prescribing information.

4. Assay Reportable Range:
HemosIL Liquid Anti-Xa Assay Reportable Range: 20–1000 ng/mL rivaroxaban.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Traceability
Calibrator value assignments are traceable to Rivaroxaban supplied by the manufacturer and quantitated in plasmas assayed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Two levels of lyophilized calibrators prepared from human citrated plasma by means of a dedicated process at two different concentrations containing rivaroxaban, buffers, and stabilizers. A house standard lot of HemosIL Rivaroxaban Calibrators is used to value assign each new lot of Rivaroxaban Calibrator 2.

Expected values:
Calibrator 1: 0 ng/mL
Calibrator 2: 500 ng/mL

HemosIL Liquid Anti-Xa assay uses Rivaroxaban Controls when calibrated with HemosIL Rivaroxaban Calibrators. Two levels of lyophilized HemosIL Rivaroxaban controls are prepared from human citrated plasma by means of a dedicated process at two different concentrations containing rivaroxaban, buffers, and stabilizers.

Expected values:
Low Control : 80 ng/mL
High Control : 300 ng/mL

Sample Stability
Sample stability studies were performed to support the recommended storage and handling instructions detailed in device labeling. Citrated plasma samples were tested after storage in the following temperature ranges 15–25°C and 2–8°C. The study was performed using one reagent lot with three contrived samples and two native (trough and peak) samples. The contrived samples were tested in quadruplicate and native samples were tested in 8 replicates for baseline then in quadruplicate for various time intervals. The study demonstrated specimens are stable up to 24 hours at 15–25°C and up to 7 days at 2–8°C.

Reagent Stability
The results support the following claim: Open Vial 2–8°C for 1 month, On-Board Instrument 15–25°C for 4 days, Shelf-life 2–8°C for 30 Months.

6. Detection Limit:
The limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) for the test system was determined following CLSI EP17-A2 guideline. Each study design included three reagent lots, an ACL TOP Family and ACL TOP Family 50 Series instruments.

The LoB was determined using four citrated plasma pools containing no rivaroxaban. Three replicate measurements were tested for each of the four plasma pools on five different days on two different instrument models for 60 determinations per reagent lot per instrument model. The LoB was determined to be 2.4 ng/mL.

The LoD was determined using four citrated plasma pools containing low levels of rivaroxaban around 10 ng/mL. Three replicate measurements were tested for each of the four

plasma pools on five different days on two different instrument models for 60 determinations per reagent lot per instrument model. The LoD was determined to be 8 ng/mL.

The LoQ was determined using four citrated plasma pools spiked with four target concentration 15, 20, 30, 50 ng/mL of rivaroxaban. Eight replicate measurements were tested for each of the four plasma pools on five different days on two different instrument models for 40 determinations per reagent lot per instrument model. The LoQ was determined to be 20 ng/mL.

7. Assay Cut-Off:

   Not Applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:
   The method comparison study was conducted using the HemosIL Liquid Anti-Xa assay on three ACL TOP Family instrument models by testing 337 clinical samples collected from patients receiving rivaroxaban at three different US clinical sites. Results from the HemosIL Liquid Anti-Xa were compared to results from a validated rivaroxaban liquid chromatography tandem mass spectrometry (LC-MS/MS) method. Weighted Deming regression analysis was performed for the dataset collected and the result is shown in the following table.

Sample Number	Rivaroxaban Range (ng/mL)	r	Slope (95% CI)	Intercept (95% CI)
337	20.3–940.4	0.995	0.971 (0.953, 0.990)	-0.697 (-3.087, 1.692)

2. Matrix Comparison:
   Not Applicable.

C Clinical Studies:

1. Clinical Sensitivity:
   Not Applicable.

2. Clinical Specificity:
   Not Applicable.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):
   Not Applicable.

D Clinical Cut-Off:

Not Applicable.

E Expected Values/Reference Range:

The HemosIL Liquid Anti-Xa assay is not intended to monitor patients on rivaroxaban therapy. Rivaroxaban does not require therapeutic monitoring, thus, there is no standard therapeutic range as the on-therapy range may be different for each individual patient.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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