EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR

HemosIL Liquid Anti-Xa

DECISION MEMORANDUM

A. De Novo Number:

DEN190032

B. Purpose for Submission:

De Novo classification request for the evaluation of automatic class III designation of the HemosIL Liquid Anti-Xa

C. Measurands:

Apixaban, Heparin

D. Type of Test:

Anti-Xa chromogenic assay

E. Applicant:

Instrumentation Laboratory Co.

F. Proprietary and Established Names:

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

G. Regulatory Information:

1. Regulation section:

   864.7295

2. Classification:

   Class II (Special Controls)

3. Product codes:

   QLU

4. Panel:

   81 – Hematology

H. Indications For Use:

1. 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, ACL TOP Family 50 Series, and ACL Elite/Elite Pro.

   - **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

   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.

2. Special conditions for use statement(s):

   For prescription use only
   For use in adult population only
   For *in vitro* diagnostic use only

3. Special instrument requirements:

   ACL TOP family: ACL TOP 300 CTS; ACL TOP 500 CTS; ACL TOP 700; ACL TOP

700 CTS; ACL TOP 700 LAS

ACL TOP 50 series family: ACL TOP 350 CTS; ACL TOP 550 CTS; ACL TOP 750; ACL TOP 750 CTS; ACL TOP 750 LAS

I. Device Description:

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

The assay contains:

- Factor Xa reagent – purified bovine Factor Xa, Tris-Buffer, EDTA, dextran sulfate, sodium chloride, and bovine serum albumin
- Chromogenic substrate – liquid chromogenic substrate S-2732 and bulking agent

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

- HemosIL Apixaban Calibrators – two levels ([redacted] and [redacted] ng/mL) of lyophilized calibrators prepared from human citrated plasma containing apixaban, buffers, and stabilizers.
- HemosIL Apixaban Controls – two levels ([redacted] and [redacted] ng/mL) of lyophilized controls prepared from human citrated plasma containing apixaban, buffers, and stabilizers.
- HemosIL Heparin Calibrator – three levels (0, 0.8 and 2.0 IU/mL) of lyophilized calibrators prepared from human citrated plasma containing heparin, buffer and stablizers.
- HemosIL LMW Heparin Controls – two levels (low and high) of lyophilized controls prepared from human citrated plasma containing low molecular weight (LMW) heparin, buffers and stabilizers. Each lot of LMW Heparin Controls is traceable to the 3rd International WHO Standadrd 11/176 for LMW heparin.
- HemosIL UF Heparin Controls – two levels (low and high) of lyophilized controls prepared from human citrated plasma containing unfractionated (UF) heparin, buffers and stabilizers. Each lot of UF Heparin Controls is traceable to the 6th International WHO standard 07/328 for UF heparin.
- Cleaning solution
- Cleaning agent
- Factor diluent

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

- CLSI C62, Liquid Chromatography-Mass Spectrometry Methods; Approved Guideline, First Edition, October 2014.

- CLSI EP05-A3, Evaluation of Precision Performance of Quantitative Measurement Procedures; Approved Guideline, Third Edition, October 2014.

- CLSI EP06, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline, April 2003.
- CLSI EP07-A2, Interference Testing in Clinical Chemistry - Third Edition
- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline, Second Edition, June 2012.
- CLSI EP25, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline, September 2009.
- CLSI EP37, Supplemental Tables for Interference Testing in Clinical Chemistry; First Edition.

K. Test Principle:

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 apixaban, factor Xa is partially neutralized by apixaban. 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 apixaban levels. Thus, maximum absorption occurs with low levels of drug, and minimum absorption occurs with high levels of drug. The same detection principle is employed when low-molecular weight heparin and unfractionated heparin levels are measured. Heparin is analyzed as a complex with antithrombin. Antithrombin present in patient plasma forms an [AT·heparin] complex. The concentration of this complex is dependent on the availability of the patient's endogenous antithrombin. When the synthetic chromogenic substrate is added to the patient sample, the [AT·heparin] complex inhibits FXa and residual FXa reacts with the chromogenic substrate resulting in cleavage of pNA. The released pNA is monitored kinetically at 405 nm. The measured pNA is inversely proportional to the low-molecular weight heparin or unfractionated heparin levels.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

   Analytical performance studies for low-molecular weight heparin and unfractionated heparin were previously reported in K090209.

   a. Precision/Reproducibility:

   Five precision studies were performed following the recommendations in the CLSI EP05-A3 guideline.

   Study 1: This study was conducted over 20 days, with two runs per day and two replicates per run for a total of 240 determinations (i.e. 80 determinations per reagent lot). The study design included three representative instrument models, three reagent

lots (i.e. one lot per instrument), as well as (b)(4) spiked samples and (b)(4) plasma pool. Precision estimates were calculated for each of the following variance components: within-run, between-run, between-day and between-instrument. The results for within-run and total imprecision are provided in the summary table below.

Sample	N	Mean (ng/mL)	Within-Run		Total	
			SD	%CV	SD	%CV
Spiked 1	(b)(4)					
Spiked 2						
Plasma pool						

Study 2: This study was conducted over 20 days, with two runs per day and two replicates per run for a total of (b)(4) determinations (i.e. (b)(4) determinations for the ACL TOP family and (b)(4) determinations for the ACL TOP 50 family). The study design included (b)(4) instrument models from (b)(4) instrument families, multiple reagent lots depending on the instrument family (i.e. (b)(4) reagent lots were assessed on the ACL TOP family whereas (b)(4) reagent lots were assessed on the ACL TOP 50 family), (b)(4) quality controls, (b)(4) spiked samples, and (b)(4) plasma pool. Precision estimates were calculated for each of the following variance components: within-run, between-run, between-day, between-lot and between-instrument. The results for within-run and total imprecision are provided in the summary table below.

Sample	N	Mean (ng/mL)	Within-Run		Total	
			SD	%CV	SD	%CV
Control 1	(b)(4)					
Control 2						
Spiked 1						
Spiked 2						
Spiked 3						
Plasma pool						

Study 3: This study was conducted over 20 days, with two runs per day and two replicates per run for a total of (b)(4) determinations. The study design included (b)(4) instrument models from (b)(4) instrument families, (b)(4) reagent lots, (b)(4) diluted plasma pool and (b)(4) contrived plasma pool. Precision estimates were calculated for each of the following variance components: within-run, between-run, between-day, between-lot and between-instrument. The results for within-run and total imprecision are provided in the summary table below.

Sample	N	Mean (ng/mL)	Within-Run		Total	
			SD	%CV	SD	%CV
Diluted native	(b)(4)					
Contrived pool						

Study 4: This study was conducted at three sites over five days, with two runs per day

and three replicates per run for a total of 270 determinations. The study design included (b)(4) representative instrument model, (b)(4) quality controls, (b)(4) spiked samples and (b)(4) native sample. Precision estimates were calculated for each of the following variance components: within-run, between-run, between-day, between-laboratory and between-lot. The results for within-run, between-laboratory and total imprecision are provided in the summary table below.

Sample	N	Mean (ng/mL)	Within-run		Between-Laboratory		Total	
			SD	%CV	SD	%CV	SD	%CV
Control 1	270	71.00	2.43	3.40	1.22	1.70	3.37	4.70
Control 2	270	290.00	4.73	1.60	3.55	1.20	6.96	2.40
Spiked 1	270	53.90	2.36	4.40	2.32	4.30	4.04	7.50
Spiked 2	270	721.60	23.50	3.30	35.71	4.90	43.7	6.10
Spiked 3	270	954.50	32.10	3.40	33.14	3.50	46.8	4.90
Native	270	199.90	6.07	3.00	9.16	4.60	11.9	5.90

Study 5: This study was conducted at three sites over five days, with one reagent lot per site, two runs per day and three replicates per run for a total of 270 determinations. The study design included one representative instrument model, (b)(4) diluted sample and (b)(4) native samples. Precision estimates were calculated for each of the following variance components: within-run, between-run, between-day, within-laboratory, between-laboratory and between-lot. The results for within-run, between-laboratory and total imprecision are provided in the summary table below.

Sample	N	Mean (ng/mL)	Within-run		Between-laboratory		Total	
			SD	%CV	SD	%CV	SD	%CV
Diluted native	270	59.70	2.52	4.20	1.00	1.70	3.11	5.20
Native 1	270	106.10	3.33	3.10	1.39	1.30	3.88	3.70
Native 2	270	246.90	6.59	2.70	5.34	2.20	8.72	3.50

b. *Linearity/assay reportable range:*

Linearity studies were performed following the CLSI EP06-A guideline using three reagent lots and one representative instrument model from two instrument families. Normal pooled plasma (NPP) spiked with known concentrations of apixaban was tested in four replicates. Two separate linear regressions were performed. For the first, samples with (b)(different concentrations were evaluated to establish the analytical measuring interval; and for the second, samples with (b)(different concentrations were evaluated to establish the extended measuring interval.

Based on the results of the linearity studies, the claimed assay reportable range is 20–1000 ng/mL.

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

Traceability

Two levels of lyophilized HemosIL Apixaban Calibrators are prepared from human citrated plasma by means of a dedicated process at two different concentrations of apixaban. Calibrator 1 contains no apixaban. A house standard lot of calibrators is used to value assign each new lot of Apixaban Calibrator 2. Calibrator value assignments are traceable to apixaban supplied by the manufacturer and quantitated in plasma assayed by Liquid Chroatography – tandem Mass Spectrometry (LC-MS/MS).

Expected values:
Calibrator (b)(4)
Calibrator 2: (b)(4)

Two levels of lyophilized HemosIL Apixaban controls are prepared from human citrated plasma by means of a dedicated process at two different concentrations of apixaban. A house standard lot of Apixaban Controls is used to value assign each new lot of Apixaban Controls. Calibrator value assignments are traceable to apixaban supplied by the manufacturer and quantitated in plasma assayed by LC-MS/MS.

Expected values:
Low Control: (b)(4)
High Control: (b)(4)

Freeze-thaw cycle
Three lots of Liquid Anti-Xa reagents were tested before frozen (baseline) and after each of the three freeze-thaw cycles. The study was performed with three reagent lots and two vials per reagent lot for each sample at each time point. For each freeze-thaw cycle, the reagents were frozen at (b)(4) for (b)(4) hours and thawed at 2–8°C for (b)(4) hours. The Liquid Anti-Xa demonstrated stability of up to two freeze-thaw cycles, with no more than 24 hours between each cycle at (b)(4).

Sample stability

Sample stability studies were performed to support the recommended storage and handling instructions found in the 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 at least four replicate measurements at each time point for each sample. The study data demonstrate that citrated plasma samples are stable for 24 hours when stored at 15–25°C and 7 days when stored at 2–8°C.

d. *Detection limit:*

The limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) for the test system was determined following the CLSI EP17-A2 guideline. Each study

design included (b)(4) reagent lots and (b)(4) representative instrument model from each instrument family.

The limit of blank (LoB) was determined using the four citrated plasma pools containing no apixaban. Three replicate measurements were tested for each of the four plasma pools on five different days on two different instrument models for n=60 determinations per reagent lot per instrument model. The sponsor determined the LoB to be (b)(4).

The limit of detection (LoD) was determined using four citrated plasma pools containing low levels of apixaban around 10 ng/mL. Each of the four plasma pools were tested in triplicate on five different days on two instrument models for n=60 determinations per reagent lot per instrument model. The sponsor determined the LoD to be (b)(4).

The limit of quantitation (LoQ) was determined using four citrated plasma pools spiked apixaban at four targeted concentrations: (b)(4), (b)(4), (b)(4) and (b)(4). Each of the four plasma pools were tested in eight replicates per day on five different days, for n=40 determinations per reagent lot per instrument model. The sponsor determined the LoQ to be (b)(4).

e. *Analytical specificity:*

Interference studies were conducted based on the CLSI EP07-A2 guideline. The study design included one representative instrument model and three different reagent lots. Potentially interfering endogenous substances were spiked into the test samples (spiked with apixaban at (b)(4), (b)(4) or (b)(4)) and the control samples. Potentially interfering exogenous substances were spiked into the test samples (i.e. native pooled plasma with apixaban at (b)(4) and (b)(4); pooled plasma spiked with apixaban at (b)(4)) and the control samples. Each sample was tested in a minimum of four replicates.

None of the substances in the following table (endogenous or exogenous substances) were found to lead to clinicially significant interference.

Endogenous Substances

Interfering Substance	Concentration Tested
Hemoglobin	300 mg/dL
Bilirubin	25 mg/dL
Triglycerides	1150 mg/dL
Lupus anticoagulant	2.11 (dRVVT screen/confirm ratio)

Exogenous Substances

Interfering Substance	Concentration Tested
Acetylsalicylic acid	3 mg/dL
Atorvastatin	0.075 mg/dL
Isosorbide dinitrate	0.6 mg/dL
Ticagrelor	0.188 mg/dL
Warfarin	7.5 mg/dL

f. Assay cut-off:

Not applicable

2. Clinical performance:

Method comparison performance studies using clinical samples for low-molecular weight heparin and unfractionated heparin were previously reported in K090209.

a. Accuracy

The method comparison study was conducted using the HemosIL Liquid Anti-Xa assay on the Instrumentation Laboratory ACL TOP 300 CTS, ACL TOP 500 CTS, and ACL TOP 700 CTS by testing n=367 clinical samples collected from patients receiving apixaban at three different U.S. clinical sites. Results from the HemosIL Liquid Anti-Xa were compared to results from a validated apixaban LC-MS/MS method. Linear regression analyses were performed for the dataset collected for each site. The following table summarizes the line equation from the linear regression analysis performed for the combined dataset.

N	r	Slope (95% CI)	Intercept (95% CI)
367	0.995	1.101 ((b)(4) , (b)(4))	-2.458 ((b)(4) , (b)(4))

A summary of device performance at different medically important concentrations througout the reportable range for the combined dataset is shown below.

Relative Predicted Bias (95% CI)			
30 ng/mL	50 ng/mL	100 ng/mL	350 ng/mL
(b)(4)			

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:

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

N. Proposed Labeling

The labeling supports the decision to grant the De Novo request for this device.

O. Identified Risks to Health and Mitigation Measures

Identified Risk	Mitigation Measures
False positive/false negative/failed to provide a result for diagnostics	Certain analytical studies and clinical studies in design verification and validation. Certain labeling information.

P. Benefit/Risk Determination

Patient perspectives

This submission did not include specific information on patient perspectives for this device.

Summary of the Assessment of Benefit

The HemosIL Liquid Anti-Xa assay is intended for the measurement of apixaban levels in patients at risk of major bleeding or experiencing a bleeding episode. The performance characteristics of the test are evaluated in analytical and clinical validation studies and the data demonstrate acceptable analytical and clinical performance for this intended use. The

benefit of the test is to provide clinicians with an additional assessment tool that is used in conjunction with clinical and other laboratory findings in the management of patients receiving apixaban. Drug levels that are within expected ranges/on-therapy ranges may provide clinicians with reassurance and resulting in continuation of treatment. Detection of drug levels below or above expected ranges/on-therapy range may lead to a change in the anticoagulant regimen. In the preoperative settings, a drug concentration of (b)(4) is currently accepted as the safe-for-treatment threshold for invasive procedures and surgeries. In patients with drug concentrations (b)(4), the procedures/sugeries may be postponed until the drug concentrations are below this threshold. Several factors contribute to the uncertainty for the benefits. The on-therapy range shows (b)(4) variability between patients. There is limited information on the relationship between drug concentrations and clinical outcomes. The (b)(4) threshold has not been fully evaluated for its impact on patient outcomes. In addition, apixaban does not require routine monitoring and the drug levels are not used as the basis of dose-adjustment and reversal. Therefore, the extent of uncertainty for the benefits is medium.

Summary of the Assessment of Risk

The risk of the device is associated with inaccurate test results. For apixaban levels around the "on-therapy range", falsely high and falsely low results may lead to inappropriate switching of anticoagulant regimen or continuation of apixaban. At drug levels close to the cutoff for surgeries and invasive procedures ((b)(4)), falsely high results may cause delay of life saving surgeries or invasive procedures, whereas falsely low results may increased the risk for perioperative bleeding.

Summary of the Assessment of Benefit-Risk

Considerations of risk mitigation include special controls for performance characteristics addressing analytical and clinical validation, and labeling describing device performance and limitations (e.g. not intended for the monitoring and dosage adjustment of apixaban). Additionally, the device is labeled "prescription only", "not a stand-alone test" and "should be used in conjunction with other clinical and laboratory findings." Given the combination of the device's indications for use, labeling, and the required general controls and special controls established for this device, the probable benefits would outweigh the probable risks.

Q. Conclusion

The De Novo request is granted and the device is classified under the following and subject to the special controls identified in the letter granting the De Novo request:

Product Code: QLU
Device Type: Heparin and direct oral factor Xa inhibitor drug test system
Class: II
Regulation: 21 CFR 864.7295