510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K230852

B Applicant

Instrumentation Laboratory Company

C Proprietary and Established Names

HemosIL Chromogenic Factor IX

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
GGP	Class II	21 CFR 864.7290 - Factor Deficiency Test	HE - Hematology

II Submission/Device Overview:

A Purpose for Submission:
Clearance of a new device

B Measurand:
Factor IX activity

C Type of Test:
Quantitative Chromogenic Assay

III Intended Use/Indications for Use:

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

B Indication(s) for Use:

HemosIL Chromogenic Factor IX is an automated assay for the photometric, quantitative determination of factor IX activity in 3.2% citrated plasma on the ACL TOP Family and ACL TOP Family 50 Series in the laboratory setting by a healthcare professional. HemosIL Chromogenic Factor IX is indicated for use on patients when identifying factor IX deficiency or measuring factor IX activity from patients on replacement therapy.

For adult population only. For prescription use only.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

Instrumentation Laboratory (IL) ACL TOP Family (K160276) and ACL TOP Family 50 Series (K150877)

IV Device/System Characteristics:

A Device Description:

The HemosIL Chromogenic Factor IX is used for determination of Factor IX activity measured from 3.2% citrated plasma samples and the assay kit contains the following components in the table below:

Reagent Name	Packaging Unit(s) and Volume	Description
Reagent A:	2 vials x 1.4 mL	Lyophilized preparation containing human FVIII, human FX, bovine FV, bovine serum albumin and a fibrin polymerization inhibitor.
Reagent B:	2 vials x 8 mL	Lyophilized preparation containing human FXIa, human FII, bovine serum albumin, calcium chloride and phospholipids.
Substrate:	1 vial x 6 mL	Solution containing 2.5 mmol/L chromogenic FXa substrate (Z-D-Arg-Gly-Arg-pNA), a thrombin inhibitor and preservative.
Buffer:	1 vial x 20 mL	Stock solution of buffer containing a heparin antagonist, bovine serum albumin and preservative.
Barcode Labeled Vials: **Diluted Buffer**	2 x 30 mL empty vials	Empty vials with linear barcode labels for Diluted Buffer (to be prepared).

B Principle of Operation:

Factor IX activity in a patient's plasma is determined using a chromogenic method, in which human factor IX is activated by human factor XIa, and when formed, factor IXa activates human factor X in the presence of human factor VIII, calcium and phospholipid. The amount of

factor Xa generated is proportionate to the factor IX activity and is determined from the hydrolysis of a chromogenic factor Xa substrate. Results are determined by comparing a chromogenic signal to a calibration curve. FIX results are reported in percent activity where 100% FIX activity is equivalent to 1.0 IU/mL.

V Substantial Equivalence Information:

A Predicate Device Name(s):
HemosIL Factor IX Deficient Plasma

B Predicate 510(k) Number(s):
K031829

C Comparison with Predicate(s):

Device & Predicate Device(s):	K230852	K031829
Device Trade Name	HemosIL Chromogenic Factor IX	HemosIL Factor IX Deficient Plasma
General Device Characteristic Similarities		
Intended Use/Indications For Use	HemosIL Chromogenic Factor IX is an automated assay for the photometric, quantitative determination of factor IX activity in 3.2% citrated plasma on the ACL TOP Family and ACL TOP Family 50 Series in the laboratory setting by a healthcare professional. HemosIL Chromogenic Factor IX is indicated for use on patients when identifying factor IX deficiency or measuring factor IX activity from patients on replacement therapy. For adult population only. For prescription use only.	HemosIL Factor IX deficient plasma is human plasma immunodepleted of factor IX for the quantitative determination of factor IX activity in citrated plasma, based on activated partial thromboplastin time (APTT) assay, on IL Coagulation Systems.
Classification	Class II	Same
Regulation	21 CFR 864.7290	Same
Measurand	Factor IX activity	Same
Measurement Type	Quantitative	Same
Sample Matrix	Citrated Plasma	Same
Reporting Units	% Activity and/or IU/mL	% Activity, U/mL and/or sec
Instruments	ACL TOP Family (K160276);	ACL Elite/Elite Pro (K060162);

	ACL TOP Family 50 Series (K150877)	ACL TOP Family (K160276); ACL TOP Family 50 Series (K150877)
Controls	HemosIL Normal Control Assayed (K021023) ≥ 70% FIX Activity HemosIL Special Test Control Level 2 (K040359) 22%–42% FIX Activity	Same
Calibrator	HemosIL Calibration Plasma (K041905)	Same
Assay Reportable Range	1.0–150% FIX Activity	Same
General Device Characteristic Differences		
Device Description/ Test Principle	Factor IX activity in a patient's plasma is determined using a chromogenic method, in which human factor IX is activated by human factor XIa, and, when formed, factor IXa activates human factor X in the presence of human factor VIII, calcium and phospholipid. The amount of factor Xa generated is proportionate to the factor IX activity and is determined from the hydrolysis of a chromogenic factor Xa substrate. Results are determined by comparing a chromogenic signal to a calibration curve.	Factor IX activity in a patient's plasma is determined by performing a modified activated partial thromboplastin time test (APTT). Patient plasma is diluted and added to a plasma deficient in factor IX. Correction of the clotting time of the deficient plasma is proportional to the concentration (% activity) of that factor in the patient plasma, interpolated from a calibration curve.
Kit Components	Reagent A: Lyophilized preparation containing human factor VIII, human factor X, bovine factor V, bovine serum albumin and a fibrin polymerization inhibitor. Reagent B: Lyophilized preparation containing human factor XIa, human factor II, bovine serum albumin, calcium chloride and phospholipids. Substrate: Solution containing 2.5 mmol/L chromogenic factor Xa substrate (Z-D-Arg-Gly-Arg-	Factor IX deficient plasma: Lyophilized human plasma that has been artificially depleted of factor IX containing buffer and stabilizers. The residual factor IX activity is less than or equal to 1% whereas all other coagulation factors have normal levels.

	pNA), a thrombin inhibitor and preservative. Buffer: Stock solution of buffer containing a heparin antagonist, bovine serum albumin and preservative. Diluted Buffer Barcode Labeled Vials: Empty vials with linear barcode labels for Diluted Buffer (to be prepared).	
Limit of Detection	LoB 0.1% Activity LoD 0.3 % Activity LoQ 0.6% Activity	Not Applicable
Storage/Stability	Unopened reagents are stable until the expiration date shown on the vial when stored at 2–8°C. <u>Stability after reconstitution</u>: 4 months at ≤ -65°C for Reagent A and B 12 months at ≤ -65°C for Substrate and Buffer	Unopened reagents are stable until the expiration date shown on the vial when stored at 2–8°C (K031829 FIX Deficient Plasma, and K953981 HemosIL SynthASil). <u>Stability after reconstitution</u>: K031829: 24 hours 2–8 °C K953981: APTT Reagent 30 days at 2–8 °C, Calcium Chloride 30 days at 2–30°C

VI Standards/Guidance Documents Referenced:

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

CLSI EP06-A2, Evaluation of the Linearity of Quantitative Measurement Procedures, Second Edition, November 2020.

CLSI EP07-Ed.3, Interference Testing in Clinical Chemistry, Third Edition November 2005.

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

CLSI EP25-A, Evaluation of Stability of In Vitro Diagnostic Approved Guideline, September 2009.

CLSI EP28-A3c Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline, Third Edition, October 2010.

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

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

a. Single Site Precision:

A precision study was performed at one site testing the controls and samples with each reagent/instrument combination for 20 days, with two runs a day and two replicates per run (n=80 per material for each reagent lot). Three different reagent lots of HemosIL Chromogenic Factor IX were tested on three ACL TOP Family models, and one reagent lot of HemosIL Chromogenic Factor IX tested on three ACL TOP Family 50 Series models. Each reagent lot was tested with the same control lots (normal and abnormal) and the same three factor IX plasma sample pools, with approximate target concentrations of 4%, 65%, and 100% activity. The precision estimates were calculated and the results for repeatability are provided in the summary table below.

Sample	N	Mean % Activity	Within-Run		Between-Run		Between-Day		Between-Instrument		Between-series		Between-Lot		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Normal Control	960	112.5	4.0	3.5	1.4	1.2	4.5	4.0	2.3	2.0	0.8	0.7	0.5	0.5	6.6	5.9
Special Test Control Level 2	960	31.8	1.0	3.2	0.5	1.6	0.9	2.8	1.3	4.0	1.4	4.3	0.0	0.0	2.4	7.4
Sample 1 Pool	960	4.1	0.2	4.6	0.0	0.0	0.1	2.9	0.2	4.5	0.0	0.0	0.1	1.9	0.3	7.3
Sample 2 Pool	960	64.9	2.5	3.9	0.9	1.4	1.8	2.7	2.3	3.6	0.0	0.0	0.0	0.0	4.0	6.1
Sample 3 Pool	960	103.8	3.6	3.5	2.2	2.1	3.3	3.2	2.9	2.8	0.0	0.0	0.2	0.2	6.1	5.9

b. Reproducibility:

The reproducibility performance of HemosIL Chromogenic Factor IX was conducted at one internal and two external sites all located in the US. The testing was performed by one operator with one representative instrument and one reagent lot per site. At each site there were two control levels (normal and abnormal), five sample pools covering the claimed analytical range, with approximate target concentrations of <1%, 3%, 30%, 65%, and 100% activity and three sample pools spiked with a factor IX concentrate, with approximate target concentrations of 3%, 30%, and 100% activity. Each material was tested in triplicate, twice a day for 5 days, for a total of 30 replicates per level. The precision estimates were calculated and results for reproducibility are provided in the summary table below.

Level	Mean (% Activity)	N	Repeatability		Between-Run		Between-Day		Between Site/lot/operator		Reproducibility	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Normal Control Assayed	108.9	90	5.5	5.0	4.4	4.0	3.7	3.4	4.3	3.9	9.0	8.3
Special Test Control Level 2	30.7	90	1.2	3.9	0.5	1.6	1.0	3.3	0.5	1.5	1.7	5.6
Sample Pool 1	1.3	90	0.2	15.2	0.0	0.0	0.2	12.7	0.1	7.1	0.3	21.1
Sample Pool 2	4.0	90	0.2	5.3	0.0	0.0	0.1	3.3	0.1	3.3	0.3	7.1
Sample Pool 3	33.6	90	1.2	3.6	0.9	2.7	0.7	2.1	0.4	1.2	1.7	5.1
Sample Pool 4	73.9	90	3.0	4.1	1.4	1.9	3.1	4.2	0.0	0.0	4.5	6.1
Sample Pool 5	109.7	90	4.9	4.5	1.7	1.5	5.4	4.9	0.0	0.0	7.5	6.8
Concentrate Sample 1 Pool	3.7	90	0.2	6.2	0.0	0.0	0.1	1.7	0.1	3.4	0.3	7.3
Concentrate Sample 2 Pool	30.1	90	1.1	3.6	0.1	0.3	0.9	3.1	0.4	1.3	1.5	4.9
Concentrate Sample 3 Pool	98.8	90	3.8	3.8	1.5	1.5	4.1	4.1	0.3	0.3	5.8	5.8

2. Linearity:

A linearity study was performed with three different lots of HemosIL Chromogenic Factor IX tested on a representative ACL TOP Family model (ACL TOP 700), and one lot of HemosIL Chromogenic Factor IX tested on a representative ACL TOP Family 50 Series model (ACL TOP 750). For each panel, a high plasma pool was created using a high factor IX activity plasma pool (~250% activity). If the high plasma pool had to be diluted, a low pool of immunodepleted FIX deficient plasma (< 1% activity) was used. The panels, which consisted of at least nine levels, were made by mixing portions of the high factor plasma pool with the low plasma pool. Four replicates of each level were run from lowest factor concentration to highest factor concentration. Samples were run on the same day as preparation.

The linear range for Chromogenic FIX assay was determined to be 1.0%–150% FIX activity.

3. Analytical Specificity/Interference:

An interference study was performed internally to establish limits for the HemosIL Chromogenic Factor IX using three reagent lots on a representative ACL TOP Family model (ACL TOP 700) and ACL TOP Family 50 Series model (ACL TOP 750 CTS). The study included the following interfering substances: hemoglobin, bilirubin, triglycerides,

unfractionated (UF) heparin and low molecular weight (LMW) heparin. An interference stock solution was created for each interfering substance. Subsequently, three-level concentration panels were created to test each interferent in normal, abnormal, and a 65% activity MDL pool ±20% plasma pool samples. All levels for all interferents with all reagent lots and instruments were tested in five replicates. Samples tested: 100% Normal Plasma Pool Normal Pooled Plasma, 30% Abnormal Plasma Pool Normal Pooled Plasma diluted in immunodepleted Factor IX deficient plasma from Hematologic Technologies, Inc, Plasma Pool (MDL ±20%) Normal Pooled Plasma diluted into Factor IX Deficient Plasma.

The study result is presented in the table below.

Interferent	**Maximum Concentration with No Interference**
Hemoglobin	1000 mg/dL
Bilirubin (unconjugated)	40 mg/dL
Bilirubin (conjugated)	40 mg/dL
Triglycerides	1500 mg/dL
Unfractionated Heparin	2.0 IU/mL
Low Molecular Weight Heparin	2.0 IU/mL
Dabigatran	5.0 mg/L
Rivaroxaban	0.05 mg/L
Fondaparinux	1.02 mg/L
Lupus anticoagulant	*dRVVT Screen/Confirm Ratio 1.8

***dRVVT** Dilute Russell Viper Venom Time

Warfarin inhibits vitamin K dependent coagulation factors and interferes with the quantification of factor IX activity.

4. Assay Reportable Range:

1% to 150% Factor IX Activity

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

Traceability

The HemosIL Calibration Plasma (sold separately) is traceable to NIBSC WHO 4th International Standard 09/172. The HemosIL Normal Control Assayed and HemosIL Special Test Control Level 2 (sold separately) are traceable to NIBSC WHO 4th International Standard 09/172.

Expected Values:

Control Material	Target Value Range	Value Assignment Range
HemosIL Normal Control Assayed	≥ 70%	± 20% (Absolute)
HemosIL Special Test Control 2	22–42%	± 10% (Absolute)

Sample Stability

Studies were performed to support the recommended storage and handling instructions detailed in the device labeling. Internal sample stability studies were performed at 2–8°C and 15–25°C, with one reagent lot of HemosIL Chromogenic Factor IX tested on one representative ACL TOP Family or ACL TOP Family 50 Series. For the study, four sample plasma pools (~<1%, ~15%, ~65%, ~100% factor IX activity) were prepared. Specimens are stable up to 8 hours at 15–25°C and up to 8 hours at 2–8°C.

Shelf-life Stability

Three lots of HemosIL Chromogenic Factor IX (whole kits) were stored at 2–8°C for the duration of the study. At time zero (t_0) and periodic time intervals, a factor IX activity plasma sample pool at ~65% activity and two freshly prepared control levels (normal and abnormal) were tested on a representative ACL TOP Family/ACL TOP Family 50 Series model. At t_0 for each kit lot, the plasma sample pool and control levels were tested in 10 replicates to establish the baseline means, then in triplicate at each successive time point, and these mean results compared to the t_0 baseline means. The study supports a shelf-life stability claim of 28 months for HemosIL Chromogenic Factor IX at 2–8°C.

Open Vial Stability

The open vial or reconstituted claims were established by testing three lots of HemosIL Chromogenic Factor IX kit components reconstituted (Reagent A and Reagent B) or opened (Substrate) and stored at ≤ -65°C for the duration of the study and reconstituted (Reagent A and Reagent B) or opened (Substrate and Buffer) and then stored at 2–8°C for the duration of the study. At time zero (t_0) and each successive time point, a factor IX activity plasma sample pool at ~65% activity and two freshly prepared control levels (normal and abnormal) were tested with the stressed individual kit components on a representative ACL TOP Family/ACL TOP Family 50 Series model. The plasma sample pool and control levels were tested in 10 replicates to establish baseline means, then in triplicate (triplicate or six replicates for 2–8°C), at each successive time point and these mean results compared to the t_0 baseline means. Between t_0 and the successive time points, the applicable kit component was capped and stored in its original vial at ≤ -65°C and 2–8°C, then removed from storage, thawed, uncapped, and placed on the instrument for testing.

Reagent A and Reagent B: Stability after reconstitution: 72 hours at 2–8°C in the closed original vial or 4 months at ≤ -65°C. Frozen reagent may be thawed once and gently mixed before use. Do not refreeze.

Substrate: Opened reagent is stable 1 month at 2–8°C in the closed original vial or 12 months at ≤ -65°C. Frozen reagent may be thawed once and gently mixed before use. Do not refreeze.

Buffer: Opened reagent is stable for 12 months at 2–8°C.

On-Board Stability

The on-board stability claims were established for the individual HemosIL Chromogenic Factor IX kit components: for Reagent A and B, Substrate and Diluted Buffer. Three lots of HemosIL Chromogenic Factor IX kit components were reconstituted (Reagent A and Reagent B) and opened/prepared (Substrate and Diluted Buffer) and then placed on-board the instrument for the duration of the study. At time zero (t_0) and periodic time intervals, a factor IX activity plasma sample pool at ~65% activity and two freshly prepared control levels (normal and abnormal) were tested with the stressed individual kit components on a representative ACL TOP Family/ACL TOP Family 50 Series model. At t0 for each kit lot using one of the reconstituted or opened/prepared components, the plasma sample pool and control levels were tested in 10 replicates to establish baseline means, then in triplicate at each successive time point, and these mean results compared to the t_0 baseline means. Between t_0 and successive time points, the applicable kit component remained on-board the instrument uncapped.

Reagent A and Reagent B: 8 hours at 15°C on the ACL TOP Family and ACL TOP Family 50 Series.

Substrate: 24 hours at 15°C on the ACL TOP Family and ACL TOP Family 50 Series.

Diluted Buffer: Stability after dilution: 24 hours on the ACL TOP Family and ACL TOP Family 50 Series.

6. Detection Limit:

The detection limit was performed to test HemosIL Chromogenic Factor IX on the ACL TOP Family and ACL TOP 50 Series for Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ). The study was performed using three different lots of HemosIL Chromogenic Factor IX tested on a representative ACL TOP Family model (ACL TOP 700) and ACL TOP Family 50 Series model (ACL TOP 750 CTS). Four blank samples were prepared from FIX immunodepleted plasma for the LoB study, and six low level samples, each targeting a different % activity level were prepared using Factor IX Deficient Plasma <1% Activity (Immunodepleted), and WHO 5th International Standard for Blood Coagulation Factor IX Concentrate for the LoD and LoQ studies. Aliquots maintained at ≤ -65°C for the duration of the study.

LoB determination: Four different blank samples were run in six replicates each across three days for a total of n=72 replicates for each of the three reagent lots and two instruments.

LoD determination: Six low level samples, each targeting a different FIX % activity level, were run in four replicates each across three days for a total of n=24 replicates for each of the three reagent lots and two instruments.

LoQ determination: Five low level samples, each targeting a different FIX % activity level, were run in five replicates each across five days for a total of n=25 replicates per respective lot and instrument, and n=50 overall across all testing days and instrument systems per reagent lot.

HemosIL Chromogenic Factor IX Determination (% Activity)		
LoB	**LoD**	**LoQ**
0.1	0.3	0.6

7. Assay Cut-Off:

Not Applicable

8. Recovery of Factor FIX Replacement Concentrates:

The recovery of replacement therapy concentrates study was performed to compare dilutions of different factor IX concentrates (AlphaNine SD, BeneFIX, IDELVION and Rebinyn), tested with HemosIL Chromogenic Factor IX on one ACL TOP Family model. There were panels tested consisting of at least seven to fourteen levels made by mixing high factor IX concentrate plasma pool with the low factor IX plasma pool. For each panel, the low factor plasma pool was factor IX immunodepleted plasma. Four replicates of each level were run. The FIX percent recovery was determined from the measured versus expected FIX activity (%) of each product at each level. HemosIL Chromogenic Factor IX recovered FIX activity levels in plasma containing AlphaNine SD, BeneFIX, IDELVION and Rebinyn at concentrations ranging from 0.5% to 200% of FIX activity. There was an over-estimation of Idelvion across all concentrations relative to labeled potency.

Product	Mean Percent Recovery (%)
AlphaNine SD	90
BeneFIX	93
Rebinyn	112
Idelvion*	159

* Per the manufacturer's recommendations, a one stage clotting assay is recommended for measurement of Idelvion and results may vary based on the APTT reagent in use.

B Comparison Studies:

1. Method Comparison and Predicted Device:

The Method Comparison study was performed at four clinical, research and/or satellite laboratories. There was a total of 344 citrated plasma samples assayed by HemosIL Chromogenic Factor IX (subject device) and HemosIL Factor IX deficient plasma (predicate device). The study used two reagent lots of HemosIL Chromogenic Factor IX tested on representative members of the ACL TOP Family and ACL TOP Family 50 series, using retrospective banked frozen samples from patients with von Willebrand disease, patients with hemophilia A and B and patients on factor IX replacement therapies. Each sample was analyzed in singlicate on both the subject and predicate devices at each site. The following table summarizes the line equation from the Passing-Bablok regression analysis performed for the combined dataset.

N	FIX Activity Range (%)	Slope (95% CI)	Intercept (95% CI)	Pearson Correlation Coefficient
344	1.0–148.6	1.015 (0.994, 1.037)	-0.920 (-2.064, -0.185)	0.972

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:

A reference range interval study was performed with one lot of HemosIL Chromogenic Factor IX tested on a representative ACL TOP Family model (ACL TOP 700). A total of 120 normal individual plasma samples were used in the final calculations. All samples were run in singlicate. The established reference range for HemosIL Chromogenic Factor IX is 71.1% to 134.1%.

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.