510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K214002

B Applicant

Precision BioLogic Inc.

C Proprietary and Established Names

CRYOcheck 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:

CRYOcheck Chromogenic Factor IX is for clinical laboratory use in the quantitative determination of factor IX activity in 3.2% citrated human plasma. It is intended to be used in identifying factor IX deficiency and as an aid in the management of hemophilia B in individuals aged 2 years and older. For in vitro diagnostic use.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

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

IV Device/System Characteristics:

A Device Description:

CRYO*check* Chromogenic Factor IX is used for determination of FIX activity and contains the following four components, packaged in vials, and provided frozen to preserve the integrity of the components:

Reagent Name	Format (per vial set; each box contains 4 vial sets	Description
Reagent 1	1 x 0.75 mL Frozen	Human FVIII, human FX, bovine FV and a fibrin polymerization inhibitor
Reagent 2	1 x 2.3 mL Frozen	Human FXIa, human FII, calcium chloride and phospholipids
Reagent 3	1 x 1.0 mL Frozen	FXa Substrate containing EDTA and thrombin inhibitor
Diluent Buffer	1x 10.0 mL Frozen	Tris buffer solution containing 1% BSA and a heparin antagonist

B Principle of Operation:

CRYO*check* Chromogenic Factor IX is a chromogenic factor IX assay. In this assay, FIX activity is determined in a chromogenic method, in which human FIX is activated by human FXIa and where formed FIXa activates human FX in the presence of human FVIII, calcium ions and phospholipid. Similar to in vivo conditions, FVIII is activated by thrombin which is generated during the incubation. The amount of FXa formed is related to the FIX activity and is determined from the hydrolysis of a chromogenic FXa substrate. The color produced by the release of p-Nitroanilide (pNA) is measured spectrophotometrically at 405 nm and is proportional to the factor IX in the sample. 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):	K214002	K031829
Device Trade Name	CRYO*check* Chromogenic Factor IX	HemosIL Factor IX Deficient Plasma
General Device Characteristic Similarities		
Intended Use/Indications for Use	CRYO*check* Chromogenic Factor IX is for clinical laboratory use in the quantitative determination of factor IX activity in 3.2% citrated human plasma. It is intended to be used in identifying factor IX deficiency and as an aid in the management of hemophilia B in individuals aged 2 years and older. For in vitro diagnostic use	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 Section	21 CFR 864.7290 Factor Deficiency Test	Same
Type of Test	Quantitative	Same
Measurand	Human Factor IX activity	Same
Expression of Results	Quantitative; Results are expressed as percent activity interpreted relative to a calibration curve.	Same
Instrument	ACL TOP/ACL TOP Family 50 Series	ACL instruments/ACL TOP Family
General Device Characteristic Differences		
Device Description	CRYO*check* Chromogenic Factor IX is used for	The Factor IX deficient plasma kit consists of: Factor IX deficient

	determination of FIX activity and contains the following four components, packaged in vials and provided frozen to preserve the integrity of the components: **Reagent 1:** Human FVIII, human FX, bovine FV and a fibrin polymerization inhibitor. **Reagent 2:** Human FXIa, human FII, calcium chloride and phospholipids. **Reagent 3:** FXa Substrate containing EDTA and a thrombin inhibitor. **Diluent Buffer:** Tris buffer solution containing 1% BSA and a heparin antagonist	plasma (Cat. No. 0020011910): 10 x 1 mL vials of 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
Methodology	FIX activity is determined in a chromogenic method, in which human FIX is activated by human FXIa and where formed FIXa activates human FX in the presence of human FVIII, calcium ions and phospholipid. Similar to in vivo conditions, FVIII is activated by thrombin which is generated during the incubation. The amount of FXa formed is related to the FIX activity and is determined from the hydrolysis of a chromogenic FXa substrate. The color produced by the release of pNA is measured spectrophotometrically	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 is proportional to the concentration (% activity) of that factor in the patient plasma, interpolated from a calibration curve.

	at 405 nm and is proportional to the factor IX in the sample.	
Calibrator and Control Plasma	CRYOcheck Reference Control Normal, CRYOcheck Abnormal 1 and CRYOcheck Abnormal 2 Reference Control	IL Coagulation Normal and Abnormal control
Storage	18 months at ≤-70 ˚C	3 weeks at -20°C
Assay Reportable Range	0.5–200% FIX activity	0.5–150%
Limit of Detection	LoB – 0.4% FIX activity LoD – 0.5% FIX activity LoQ – 0.5% FIX activity	NA

VI Standards/Guidance Documents Referenced:

CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition

CLSI EP06, Evaluation of Linearity of Quantitative Measurement Procedures, 2nd Edition

CLSI EP07, Interference Testing in Clinical Chemistry, 3rd Edition

CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition

CLSI EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline

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

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility

   a. Single Site Precision

   This study was conducted at the internal site over 20 days with two runs per day and two replicates per run, for a total of 80 determinations per sample per reagent lot. Three lots of CRYO*check* Chromogenic Factor IX were tested with six panel members on a single IL ACL TOP 700 CTS by two operators alternating testing days. Test samples included one normal and two abnormal reference controls, as well as three patient plasma samples representing very low, low, and high levels of FIX activity. Precision estimates were calculated for each

of the following variance components: within-run, between-run, between-day, between-lot and within-laboratory precision. The results for within-run and total imprecision for all lots combined are provided in the summary table below.

Sample	N	Mean (%)	Within-Run		Between-Run		Between-Day		Between-lot		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Normal reference control	240	114.9	3.9	3.4	0.2	0.2	1.6	1.4	1.1	0.9	4.2	3.7
Abnormal 1 reference control	240	39.3	1.3	3.4	0.5	1.2	0.4	1.1	1.1	2.8	1.8	4.5
Abnormal 2 reference control	240	10.4	0.7	6.3	0.2	1.6	0.2	2.0	0.3	3.3	0.8	7.3
Plasma sample 1	240	1.2	0.1	9.8	0.0	0.9	0.0	2.4	0.1	10.3	0.2	14.5
Plasma sample 2	240	6.1	0.5	7.9	0.0	0.0	0.2	2.8	0.4	6.3	0.6	10.1
Plasma sample 3	240	174.0	5.4	3.1	3.3	1.9	0.8	0.5	0.4	0.2	6.4	3.7

b. Multi-Site Reproducibility

This study was conducted at three testing sites over five days with two runs per day and three replicates per run for a total of 90 determinations. The study design included three different representative instrument models (one instrument per site). There were two operators at each site alternating test days. The study quantified three reagent lots testing one normal and two abnormal reference controls as well as three patient plasma samples representing very low, low, and high levels of FIX activity. Precision estimates were calculated for each of the following variance components: within-run, between-run, between-day, between-site and reproducibility. The results for overall reproducibility for all the lots combined are provided in the summary table below.

Sample	N	Mean (%)	Within-Run		Between-Run		Between-Day		Between-Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Normal reference control	270	113.1	5.0	4.5	0.9	0.8	1.1	1.0	1.9	1.7	6.3	5.6
Abnormal 1 reference control	270	38.4	1.8	4.8	0.2	0.6	0.1	0.3	1.3	3.4	2.5	6.6
Abnormal 2 reference control	270	10.8	0.8	7.5	0.2	1.7	0.0	0.0	0.2	2.0	0.9	8.6
Plasma sample 1	270	1.2	0.1	6.9	0.0	1.7	0.0	0.0	0.0	0.0	0.2	15.7
Plasma sample 2	270	6.3	0.5	7.9	0.1	1.2	0.1	2.1	0.2	2.4	0.8	13.0
Plasma sample 3	270	168.5	7.6	4.5	0.0	0.0	1.3	0.8	4.4	2.6	10.0	6.0

2. Linearity:

The linearity studies were performed following the CLSI EP06 2nd Ed guideline using three reagent lots at one site on a single IL ACL TOP 700 CTS instrument. Fourteen sample dilutions were prepared using a high FIX plasma (~230%) with congenital FIX deficient plasma (~0%) to create sample dilutions ranging from 0–230% FIX activity. Each dilution was tested in four replicates. The linear range for CRYO*check* Chromogenic Factor FIX assay was determined to be 0–200%.

3. Analytical Specificity/Interference:

Interference study was conducted in accordance with the CLSI EP07-A3 guideline. A single lot of CRYO*check* Chromogenic Factor IX was used on an IL ACL TOP 700 CTS instrument.

Potentially interfering endogenous and exogenous substances were spiked into the control samples (one normal and one abnormal reference control plasma). Ten replicates were evaluated for each spiked plasma or matrix-blank plasma. The maximum concentration of each interferent was determined per CLSI EP37 guideline. None of the substances in the following table (endogenous or exogenous) were found to lead to clinically significant interference (up to the concentrations indicated in the table below).

Interferent	**Concentration**
Unconjugated Bilirubin	40 mg/dL
Conjugated Bilirubin	23 mg/dL
Hemoglobin	1000 mg/dL
Intralipid	2000 mg/dL
Dabigatran	0.04 mg/L
Fondaparinux	0.26 mg/L
Low Molecular Weight Heparin (LMWH)	1.5 IU/mL
Unfractionated Heparin	1.2 IU/mL
Lupus Anticoagulant	dRVVT ratio = 1.8

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

Traceability

Three levels of CRYO*check* Reference controls are prepared from human citrated plasma with three different concentrations of hemostatic parameters. CRYO*check* Reference Control Normal (K013708) was normal human citrated plasma while CRYO*check* Abnormal 1 Reference Control (K952624) and CRYO*check* Abnormal 2 Reference Control (K952624) were the citrated human plasma with hemostatic parameter values in the borderline pathological range 30% to 44% and 9% to 17%, respectively. Calibrator value assignments are traceable to the WHO International Standard for Factor IX (4th IS Factors II, VII, IX, X, Plasma, 09/172).

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, room temperature (18–22°C) and below -70°C. The study was performed using two reagent lots of CRYOcheck Chromogenic Factor IX on IL ACL TOP 300 and IL ACL TOP 700 analyzers with five replicate measurements at each time point for each sample. The study data demonstrate that citrated plasma samples are stable for four hours when stored at room temperature (18–22°C), three months when stored at <-70°C, including up to two freeze thaw cycles.

Shelf-life Stability

The shelf-life stability study was performed to determine the shelf-life stability of CRYOcheck Chromogenic Factor IX when stored at ≤-70 °C. The study was conducted with five plasma samples (one normal, two abnormal reference controls and two patient plasmas)

using three lots of CRYOcheck Chromogenic Factor IX on an IL ACL TOP 700 CTS analyzer. Samples were thawed from ≤-70 °C storage and tested in five replicates for FIX activity at the following timepoints: 0, 6, 7, 12, 13, 18, 19, 24, 25, 36 and 37 months. The study is ongoing and current data supports the shelf-life stability claim of 18 months when the product is stored at ≤-70 °C.

In Use Stability

The in-use stability studies were performed to characterize in-use stability of CRYOcheck Chromogenic Factor IX assay when maintained at three different in-use conditions: on-board ACL TOP analyzer (12–18°C), in a refrigerator (2–8°C) and refrozen at ≤-70°C after maintaining on board for 3 hours. Five plasma samples (one normal, two abnormal reference controls and two patient plasmas) were tested by one operator on one IL ACL TOP 700 CTS analyzer. Samples were tested in five replicates by using three lots of CRYOcheck Chromogenic Factor IX at each storage condition at each timepoint. The study data supports the in-use stability claim of 24-hours when the product is stored on-board (12–18°C), 48 hours at 2–8°C and 1 month stored at ≤-70°C if the product is stored on board and refrozen within 4 hours of the initial thaw. The refrozen product must be used within 8 hours of next thawing while kept on-board.

5. Detection Limit:

The limit of blank (LoB), limit of detection (LoD), and limit of quantification (LoQ) for test system were determined following the CLSI EP17-A2 guideline. Each study design included three reagent lots and one representative instrument model (IL ACL TOP 700 CTS).

The LoB was determined using four blank plasma samples obtained from individuals with severe congenital hemophilia B. Each sample was tested in triplicate using three lots of CRYO*check* Chromogenic Factor IX over five days on an IL ACL TOP instrument for N=15 determinations per reagent lot. The LoB was determined to be 0.4% FIX activity.

The LoD was determined using four plasma samples with low FIX activity obtained from individuals with congenital hemophilia B donors. Each sample was tested in triplicate using three lots of CRYO*check* Chromogenic Factor IX over five days on an IL ACL TOP instrument for N=15 determinations per reagent lot. The LoD was determined to be 0.5% FIX activity.

The LoQ was determined using four plasma samples with low FIX activity obtained from congenital hemophilia B donors. Samples were measured in triplicate using three lots of CRYO*check* Chromogenic Factor IX over five days on an IL ACL TOP 700 instrument. The samples were also measured in triplicate using a validated laboratory-developed chromogenic factor IX assay (ROX Factor IX) over five days to determine the assigned values. The LoQ was determined to be 0.5% FIX activity.

6. Assay Cut-Off:
Not Applicable

7. Recovery of FIX Replacement Therapies

The recovery of replacement therapy study was conducted to evaluate the recovery of FIX activity of seven FIX replacement therapies with one lot of CRYO*check* Chromogenic Factor IX. A congenital FIX deficiency plasma was used to prepare seven concentration levels for each FIX replacement therapy (1.0, 0.8, 0.6, 0.4, 0.2, 0.1, 0.05 IU/mL). Ten replicates of each level of each product were tested on an IL ACL TOP 700 CTS analyzer. The FIX percent recovery was determined from the measured versus expected FIX activity (%) of each product at each level. CRYO*check* Chromogenic Factor IX recovered FIX activity levels in plasma containing AlphaNine, Alprolix, BeneFIX, Ixinity, Rebinyn and Rixubiz at concentrations ranging from 0.05 to 1.0 IU/mL. There was an over estimation of Idelvion across all concentration relative to labeled potency.

Product	Mean Percent Recovery (%)
AlphaNine SD	96
Alprolix	116
BeneFIX	93
Ixinity	82
Rebinyn	117
Rixubis	102
Idelvion	153

B Comparison Studies:

1. Method Comparison with Predicate Device:

The method comparison study was conducted using the CRYO*check* Chromogenic FIX on the Instrumentation Laboratory (IL) ACL TOP 700 and IL ACL TOP 750 CTS by testing n=386 clinical samples (citrated plasma) collected from the intended use population. Results from the CRYO*check* Chromogenic FIX were compared to results from the ROX Factor IX validated laboratory developed test. 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
368	0.7–190.7	1.10 (1.08, 1.12)	0.64 (0.20, 1.34)	0.992

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 reference interval study was conducted at one laboratory site. The FIX activity of plasma samples collected from 128 normal, ostensibly healthy individuals (≥ 18 years) was tested by two operators using three lots of CRYO*check* Chromogenic FIX on two Instrumentation Laboratory ACL TOP instruments. The reference interval was established by calculating the non-parametric 95% confidence interval (2.5^{th} to 97.5^{th} percentiles). The calculated normal reference range for CRYOcheck Chromogenic FIX is 78.8–154.9%.

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.