510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K251440

B Applicant

Precision BioLogic Inc.

C Proprietary and Established Names

CRYOcheck Chromogenic Factor VIII

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:

Change in the formulation of the Reagent 2 component of CRYOcheck Chromogenic Factor VIII to replace the human FIXa ingredient with bovine FIXa.

B Measurand:

Factor VIII 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 VIII is for clinical laboratory use in the quantitative determination of factor VIII activity in 3.2% citrated human plasma. It is intended to be used in identifying factor VIII deficiency and as an aid in the management of hemophilia A 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:

Instrumentation Laboratory (IL) ACL TOP CTS Series and ACL TOP 50 CTS Series

IV Device/System Characteristics:

A Device Description:

CRYOcheck Chromogenic Factor VIII is used for determination of FVIII activity. The assay contains the following four components, packaged in glass vials and provided frozen to preserve the integrity of the components:

Reagent Name	Format (per vial set; each box contains 4 vials sets)	Description
Reagent 1	1 x 1.25 mL	Bovine FX and a fibrin polymerization inhibitor, with activators and stabilizer.
Reagent 2	1 x 1.25 mL	Human FIIa, bovine FIXa, calcium chloride and phospholipids.
Reagent 3	1 x 1.25 mL	FXa substrate containing EDTA and a thrombin inhibitor.
Diluent Buffer	1 x 7.0 mL	Tris buffer solution containing 1% BSA and a heparin antagonist.

B Principle of Operation:

The CRYOcheck Chromogenic FVIII is a two-stage factor VIII (FVIII) assay. In the first stage, patient plasma (containing an unknown amount of functional FVIII) is diluted with Diluent Buffer and combined with Reagents 1 and 2, containing Factors IXa and X, thrombin, calcium, and phospholipids. The FVIII in the patient's plasma is activated and works in concert with FIXa to cause the activation of FX to FXa. Following an incubation period to allow activation of FX to FXa, the second stage of the assay occurs through the addition of the chromogenic FXa Substrate to this mixture. The FXa, present from the previous step, hydrolyzes the substrate into a peptide and p-Nitroaniline (pNA). The color produced by the release of pNA is measured spectrophotometrically at 405 nm and is proportional to the Factor VIII in the sample. FVIII results are reported in percent activity where 100% FVIII activity is equivalent to 1.0 IU/mL.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Cryocheck Chromogenic Factor VIII

B Predicate 510(k) Number(s):
K193204

C Comparison with Predicate(s):

Device & Predicate Device(s):	K251440	K193204
Device Trade Name	CRYOcheck Chromogenic Factor VIII	CRYOcheck Chromogenic Factor VIII
General Device Characteristic Similarities		
Intended Use/Indications For Use	CRYOcheck Chromogenic Factor VIII is for clinical laboratory use in the quantitative determination of factor VIII activity in 3.2% citrated human plasma. It is intended to be used in identifying factor VIII deficiency and as an aid in the management of hemophilia A in individuals aged 2 years and older. For in vitro diagnostic use.	Same
Assay Type	Quantitative	Same
Measurand	Human Factor VIII activity	Same
Expression of results	Quantitative; results are expressed as percent activity interpreted relative to a calibration curve.	Same
Instrument(s)	IL ACL TOP CTS Series and IL ACL TOP 50 CTS Series	Same
Calibrator and control plasmas	CRYOcheck Reference Control Normal CRYOcheck Abnormal 1 Reference Control CRYOcheck Abnormal 2 Reference Control CRYOcheck Reference	Same

	Control Normal, CRYOcheck Abnormal 1 Reference Control, and CRYOcheck Abnormal 2 Reference Control are traceable to the WHO International Standard for Factor VIII/VWF	
Storage	≤-70°C until expiration	Same
Assay Reportable Range	0–200% FVIII activity	Same
Reagents	Reagent 1: Bovine FX and a fibrin polymerization inhibitor, with activators and stabilizer. Reagent 3: FXa substrate containing EDTA and a thrombin inhibitor. Diluent Buffer: Tris buffer solution containing 1% BSA and a heparin antagonist.	Same
General Device Characteristic Differences		
Reagents	Reagent 2: Human FIIa, bovine FIXa, calcium chloride and phospholipids	Reagent 2: Human FIIa, human FIXa, calcium chloride and phospholipids
Interferences	• Hemoglobin: ≤1000 mg/dL • Intralipid: ≤830 mg/dL • Bilirubin (unconjugated): ≤40 mg/dL • Bilirubin (conjugated): ≤11 mg/dL • von Willebrand factor: ≤20 µg/mL • Unfractionated heparin: ≤3.3 IU/mL • Low molecular weight heparin: ≤5 IU/mL • Fondaparinux: ≤0.2	• Hemoglobin: ≤500 mg/dL • Intralipid: ≤500 mg/dL • Bilirubin (unconjugated): ≤29 mg/dL • Bilirubin (conjugated): ≤2 mg/dL • von Willebrand factor: ≤20 µg/mL • Unfractionated heparin: ≤2 IU/mL • Low molecular weight heparin: ≤2 IU/mL • Fondaparinux: ≤1.25

	mg/L • Lupus Anticoagulant: ≤1.8 dRVVT ratio • Dabigatran and Rivaroxaban interfere with the quantification of FVIII activity. • Emicizumab: ≤150 µg/mL • Mim8: ≤8 µg/mL • Warfarin: INR ≤7	mg/L • Lupus Anticoagulant: ≤1.8 dRVVT ratio • Dabigatran and Rivaroxaban interfere with the quantification of FVIII activity.
Recovery of FVIII Replacement Therapies	CRYOcheck Chromogenic Factor VIII evaluated the recovery of FVIII levels in replacement products including ADVATE, ADYNOVATE, AFSTYLA, ALTUVIIO, ESPEROCT, HUMATE-P, JIVI, KOVALTRY, Novoeight, Nuwiq, and wilate at concentrations ranging from 0.05 to 1.0 IU/mL. It evaluated recovery of Factor VIII levels in replacement products ELOCTATE, and XYNTHA from 0.05 to 0.6 IU/mL, with an over recovery observed at 0.8 and 1.0 IU/mL. There was an underestimation of OBIZUR.	The performance of this device has not been established in evaluating the potency of FVIII concentrates.

VI Standards/Guidance Documents Referenced:

- CLSI EP07, Interference Testing in Clinical Chemistry; Approved Guideline, Third Edition
- CLSI EP06, Evaluation of the Linearity of Quantitative Measurement Procedures, Approved Guideline, Second Edition
- CLSI EP05-A3, Evaluation of Precision Performance of Quantitative Measurement Procedures; Approved Guideline, Third 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* Medical Laboratory Test Reagents; Approved Guideline, Second Edition
- CLSI EP28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline, Third Edition
- CLSI EP-09, Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline, Third Edition
- CLSI EP37, Supplemental Tables for Interference Testing in Clinical Chemistry; Approved Guideline, First Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision

a) Repeatability: Study was conducted at one internal site over 20 days with two runs per day and two replicates per run with two operators. Panel of seven samples with one IL ACL TOP 700 CTS instrument and three lots of CRYOcheck Chromogenic Factor VIII were used. Precision estimates were calculated for each of the following variance components: within-run, between-run, between-day, between-lot and total imprecision. Study met pre-defined acceptance criteria.

Sample	N	Mean FVIII (%)	Within-Run		Between- Run		Between- Day		Between- Lot		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma sample 1	240	1.3	0.1	10.6	0.1	4.0	0.0	0.0	0.0	0.0	0.1	11.4
Plasma sample 2	240	4.1	0.3	6.8	0.1	1.4	0.1	2.5	0.2	5.6	0.4	9.3
Plasma sample 3	240	145.8	4.7	3.2	3.3	2.3	2.2	1.5	5.3	3.6	8.1	5.6
Plasma sample 4	240	24.1	1.3	5.2	0.7	2.9	0.4	1.8	0.9	3.7	1.7	7.2
Normal reference control	240	80.4	3.3	4.1	1.9	2.3	1.0	1.3	2.1	2.6	4.4	5.5
Abnormal 1 reference control	240	25.7	1.2	4.6	0.5	2.0	0.7	2.6	0.7	2.7	1.6	6.3
Abnormal 2 reference control	240	7.0	0.5	6.8	0.0	0.0	0.3	3.6	0.1	0.9	0.5	7.7

b) Reproducibility: Study was conducted at one site using a panel of six samples with two different instruments and three lots of CRYOcheck Chromogenic Factor VIII. The study was performed over 5 days with two runs and three replicates per day by two operators. Precision estimates were calculated for each of the following variance components: within-run, between-run, between-day, between-site and total imprecision. Study met pre-defined acceptance criteria.

Sample	N	Mean FVIII (%)	Within-Run		Between-Run		Between-Day		Between-instrument		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma sample 1	180	1.3	0.2	13.4	0.1	4.8	0.0	2.8	0.0	2.9	0.2	15.4
Plasma sample 2	180	4.2	0.3	6.0	0.1	3.1	0.0	0.0	0.0	0.0	0.4	9.6
Plasma sample 3	180	145.3	5.5	3.8	2.2	1.5	0.0	0.0	0.0	0.0	9.3	6.4
Normal reference control	180	79.4	3.0	3.7	1.2	1.6	0.0	0.0	2.2	2.8	4.9	6.2
Abnormal 1 reference control	180	27.4	1.5	5.3	0.5	1.8	0.1	0.3	1.1	4.1	2.0	7.3
Abnormal 2 reference control	180	7.7	0.6	7.4	0.1	1.7	0.0	0.5	0.5	6.3	0.8	9.9

2. Linearity: The linearity study was performed following the CLSI EP06 (second edition) guideline using one reagent lot and instrument. Nine sample dilutions were prepared by combining high FVIII plasma (~260%) with congenital FVIII deficient plasma (~0%). Each dilution was tested in four replicates. Based on the results of the linearity study, the linear range for the assay is 0–200%.

3. Analytical Specificity/Interference: The interference study was conducted in accordance with the CLSI EP07-A3 and CLSI EP37 guidelines. Study was performed with two plasma samples (normal, abnormal) spiked with potential interfering substances using a single lot of CRYOcheck Chromogenic Factor VIII. Ten replicates were evaluated for each sample.

   Rivaroxaban and dabigatran demonstrated interference with the assay.

   Following endogenous or exogenous substances were found not to lead to clinically significant interference up to the concentrations indicated in the table below.

Substance Tested	Concentration tested
Hemoglobin	≤1000 mg/dL
Intralipid	≤830 mg/dL
Bilirubin (unconjugated)	≤40 mg/dL
Bilirubin (conjugated)	≤11 mg/dL
von Willebrand factor	≤20 µg/mL
Unfractionated heparin	≤3.3 IU/mL
Low molecular weight heparin	≤5 IU/mL
Fondaparinux	≤0.2 mg/L
Lupus Anticoagulant	≤1.8 dRVVT ratio
Emicizumab	≤150 µg/mL
Mim8	≤8 µg/mL
Warfarin	INR ≤7

4. Recovery of FVIII Replacement Therapies: A recovery study was conducted using a single lot of CRYOcheck Chromogenic Factor VIII on an IL ACL TOP 550 CTS instrument. 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). Five replicates of each level of each product were tested. Congenital FVIII deficient plasma was spiked with 14 FVIII replacement therapies. The FVIII percent recovery was determined from the mean measured versus expected FVIII activity of each product at each level. CRYOcheck Chromogenic Factor VIII recovered Factor VIII activity levels in plasma containing Advate, ADYNOVATE, AFSTYLA, ALTUVIIO, Esperoct, HUMATE-P, JIVI, KOVALTRY, Novoeight, Nuwiq, and wilate at concentrations ranging from 0.05 to 1.0 IU/mL. CRYOcheck Chromogenic Factor VIII recovered Factor VIII activity levels in plasma containing ELOCTATE, and XYNTHA from 0.05 to 0.6 IU/mL, with an over recovery observed at 0.8 and 1.0 IU/mL. There was an underestimation of OBIZUR.

Factor replacement product	Mean Percent Recovery (%)
Advate	90.3
ADYNOVATE	97.7
AFSTYLA	89.8
ALTUVIIIO	96.2*
ELOCTATE	116.3
Esperoct	93.2
HUMATE-P	95.0
JIVI	98.5
KOVALTRY	92.0
Novoeight	115.6
Nuwiq	90.2
OBIZUR	49.5
Wilate	95.2
XYNTHA	114.7

*Per the manufacturer's recommendations, the mean percent recovery value includes dividing by a correction factor of 2.5 for chromogenic measurement.

5. Assay Reportable Range:

   Not applicable

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

   a) Sample stability:
   Please refer to K193204.

   b) Shelf-life stability: The shelf-life of the CRYOcheck Chromogenic Factor VIII was conducted on six plasma samples with five replicates per sample using one instrument with three lots of. Product lots were stored at ≤-70°C and testing will continue for up to 37 months from the date of manufacture. The study is ongoing, and the current data supports a shelf-life stability of 6 months when the product is stored at ≤-70 °C.

   c) In-use stability: Study was performed with six panel samples using one IL ACL TOP 700 CTS instrument with one lot of CRYOcheck Chromogenic Factor VIII. The in-use

stability studies were performed to characterize in-use stability when maintained at three different conditions (on-board (12–18°C), refrigerated at 2-8°C, and refrozen at ≤-70°C). The study data supports on-board stability for 8 hours when the product is stored on-board (12–18°C), 120 hours at refrigerated conditions (2–8°C), and 1 month (4 hours from the initial thaw) for frozen conditions (≤-70°C).

7. Detection Limit: The limit of blank (LoB), limit of detection (LoD), and limit of quantification (LoQ) for test system was verified following the CLSI EP17-A2 guideline. Study was conducted with one instrument using three low positive samples (~0.5% FVIII) and three blank (~0% FVIII) plasma samples in triplicates using a single lot of CRYOcheck Chromogenic Factor VIII for three days. The study verified the limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) determined in K193204. The LoB is 0.4% FVIII activity, LoD/LoQ is 0.5% FVIII activity.

8. Assay Cut-Off:

   Not applicable

B Comparison Studies:

1. Method Comparison with Predicate Device: Study was conducted with 238 clinical plasma specimens collected from the intended use population at two testing sites using CRYOcheck Chromogenic Factor VIII (after reagent change) and CRYOcheck Chromogenic Factor VIII (current in-market product) as the comparator device. Each site used a different IL ACL TOP analyzer (700 CTS and 700 series). Assays were performed by one or more operators per site. The following tables summarize Passing-Bablok regression analysis performed for the combined dataset and predicted bias.

	N	Slope (95% CI)	Intercept (95% CI)	Pearson Correlation Coefficient
Combined sites	238	0.99 (0.97, 1.01)	0.32 (0.21, 0.52)	0.996

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:

1. Reference Interval: Reference verification study was conducted with 20 plasma samples collected from healthy normal adults (≥18 years) using one instrument. The study was performed using three lots of CRYOcheck Chromogenic Factor VIII. All samples were within the originally established reference interval of 43.2-159.3%.

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.