510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K193204

B Applicant

Precision BioLogic

C Proprietary and Established Names

CRYO*check* 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:

Clearance of a new device

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:

CRYO*check* Chromogenic Factor VIII is used for determination of FVIII activity and contains the following four components:

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

B Principle of Operation:

The CRYO*check* 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):
Coatest Sp FVIII

B Predicate 510(k) Number(s):
K042576

C Comparison with Predicate(s):

Device & Predicate Device(s):	K193204	K042576
Device Trade Name	CRYO*check* Chromogenic FVIII	Coatest SP FVIII
General Device Characteristic Similarities		
Intended Use/Indications For Use	CRYO*check* 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.	Coatest SP FVIII is intended for photometric determination of factor VIII activity in citrated plasma, such as when identifying factor VIII deficiency or monitoring patients on replacement therapy, as well as for potency estimation of FVIII concentrates. For in vitro diagnostic use.
Classification	Class II	Class II
Type of Test	Quantitative	Quantitative
Measurand	Human Factor VIII activity	Human Factor VIII activity
Device Description	CRYO*check* Chromogenic Factor VIII is used for determination of FVIII activity and contains the following four components, packaged in glass vials and provided frozen to preserve the integrity of the components: **Reagent 1:** Bovine FX and a fibrin polymerization inhibitor, with activators and stabilizers. **Reagent 2:** Human FIIa, human FIXa, calcium chloride and phospholipids. **Reagent 3:** FXa substrate containing EDTA and a thrombin inhibitor.	Coatest SP FVIII is a modified version of Coatest Factor VIII (K833892) reformulated to European Pharmacopoeia Standards. Coatest SP FVIII is a photometric assay containing a chromogenic substrate, S-2765, with EDTA added as a preservative, lyophilized bovine factors IXa and X with bovine albumin added as a stabilizing agent. The device also contains calcium chloride, Tris buffer stock solution containing sodium chloride, bovine serum albumin with added

	Diluent Buffer: Tris buffer solution containing 1% BSA and a heparin antagonist.	antimicrobial in addition to a mixture of highly purified synthetic phospholipids.
Test Principle	In the presence of calcium and phospholipids, FX is activated to factor Xa by FIXa. This generation is greatly stimulated by FVIII, which may be considered as a cofactor in this reaction. By using optimal amounts of Ca^{2+} and phospholipids and an excess of FIXa and FX, the rate of activation of FX is solely dependent on the amount of FVIII. FXa hydrolyses the chromogenic substrate S- 2765 thus liberating the chromophoric group, pNA. The color is then read photometrically at 405 nm. The generated FXa and thus the intensity of color are proportional to the FVIII activity in the sample. Hydrolysis of S-2765 by thrombin formed is prevented by the addition of the synthetic thrombin inhibitor, I- 2581, together with the substrate.	In the first stage of the chromogenic assay, test plasma (containing an unknown amount of functional FVIII) is added to a reaction mixture comprised of calcium, phospholipids, human purified thrombin and FIXa and bovine FX (Reagent 1 and Reagent 2). This mixture swiftly activates FVIII to FVIIIa, which works in concert with FIXa to activate FX. When the reaction is stopped, FXa production is assumed to be proportional to the amount of functional FVIII present in the sample. The second stage of the assay is to measure FXa through cleavage of an FXa-specific peptide nitroanilide substrate (FXa Substrate). P-nitroaniline is produced, giving a color that can be measured spectrophotometrically by absorbance at 405 nm.
Expression of Results	Quantitative; results are expressed as percent activity interpreted relative to a calibration curve.	Same
General Device Characteristic Differences		
Instrument(s)	IL ACL TOP CTS Series and ACL TOP 50 CTS Series	Manual method, IL ACL 9000
Calibrator and control plasmas	CRYO*check* Reference Control Normal CRYO*check* Abnormal 1 Reference Control CRYO*check* Abnormal 2 Reference Control CRYO*check* Reference Control	HemosIL Normal Control (Assayed) HemosIL High Abnormal Control (Assayed) Calibration plasma calibrated against an International Standard

	Normal, CRYO*check* Abnormal 1 Reference Control, and CRYO*check* Abnormal 2 Reference Control are traceable to the WHO International Standard for Factor VIII/VWF	
Storage	≤-70°C until expiration	2–8°C until expiration
Assay Reportable Range	0–200% FVIII activity	0–150% FVIII activity
Reference Range	43.2–159.3% FVIII activity	Manual Method: 48.6–126% Instrument Application: 55.4–148.9%
Limit of Detection	0.5% FVIII activity	1% FVIII activity

VI Standards/Guidance Documents Referenced:

- CLSI EP07 3rd Edition, Interference Testing in Clinical Chemistry; Approved Guideline, Third Edition, April 2018.
- CLSI EP06, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline, April 2003.
- CLSI EP05-A3, Evaluation of Precision Performance of Quantitative Measurement Procedures; Approved Guideline, Third Edition, October 2014.
- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline, Second Edition, June 2012.
- CLSI EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline, September 2009.
- CLSI EP28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline, Third Edition, October 2010.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

   a. Single-site Study:

   This study was conducted at one internal site over 20 days with two runs per day and two replicates per run, for a total of 80 determinations per sample per lot. The study design included a single representative instrument model, three reagent lots, one normal and two abnormal reference controls, as well as five patient plasma samples representing very low, low, mid, normal and high levels of FVIII activity. Precision estimates were calculated for each of the following variance components: within-run, between-run,

between-day, between-lot and total imprecision. The results for within-run and total imprecision for all lots combined are provided in the summary table below.

Sample	N	Mean (%)	Within-Run		Within-Laboratory	
			SD	%CV	SD	%CV
Normal reference control	240	80.8	3.3	4.1	4.0	5.0
Abnormal 1 reference control	240	26.1	1.5	5.6	1.9	7.1
Abnormal 2 reference control	240	7.8	0.7	8.4	0.8	9.9
Plasma sample 1	240	1.0	0.1	8.2	0.1	N/A
Plasma sample 2	240	5.4	0.4	6.6	0.4	7.4
Plasma sample 3	240	26.0	1.4	5.4	1.7	6.7
Plasma sample 4	240	85.3	3.5	4.1	4.3	5.1
Plasma sample 5	240	152.1	4.9	3.2	5.7	3.8

b. Multisite Study

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

Sample	N	Mean (%)	Within-Run		Between-Site		Total	
			SD	%CV	SD	%CV	SD	%CV
Normal reference control	270	80.3	3.6	4.5	0.0	0.0	4.1	5.1
Abnormal 1 reference control	270	25.4	1.3	5.2	1.5	5.7	2.1	8.2
Abnormal 2 reference control	270	7.7	0.5	7.0	0.4	4.9	0.7	9.6
Very low plasma sample	270	1.1	0.1	N/A	0.0	N/A	0.2	N/A
Normal plasma sample	270	85.4	4.3	5.1	0.0	0.0	5.0	5.8
High plasma sample	270	156.9	7.8	5.0	6.1	3.9	11.4	7.3

2. Linearity/assay reportable range:

Linearity studies were performed following the CLSI EP06-A guideline using three reagent lots on a representative instrument model. Fifteen sample dilutions were prepared using a high FVIII (260%) and a congenital FVIII deficient plasma sample (0%). Each dilution was tested in four replicates. Based on the results of the linearity study, the claimed linear range was determined to be 0–200%.

3. Analytical Specificity/Interference:

Interference studies were conducted based on the CLSI EP07-A2 guideline. The study design included a single reagent lot and one representative instrument model. Potentially interfering endogenous and exogenous substances were spiked into the test samples (one normal and one

abnormal reference control plasma sample) and the control samples. None of the substances in the following table (endogenous or exogenous substances) were found to lead to clinically significant interference.

Interfering Substance	Concentration Tested
Hemoglobin	≤ 500 mg/dL
Intralipid	≤ 500 mg/dL
Unconjugated bilirubin	≤ 29 mg/dL
vWF	≤ 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

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

Traceability

Three levels of CRYOcheck Reference controls are prepared from human citrated plasma with three different concentrations of hemostatic parameters. CRYOcheck Reference Control Normal (K013708) was normal human citrated plasma while CRYOcheck Abnormal 1 Reference Control (K952624) and CRYOcheck Abnormal 2 Reference Control (K952624) were the citrated human plasma with hemostatic parameters values in the borderline pathological range 30% to 40% and 5% to 10%, respectively. Calibrator value assignments are traceable to the WHO International Standard for Factor VIII/VWF (6th IS Factor VIII/VWF Plasma, 07/316).

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 (15–25°C) and below -70°C. The study was performed using one reagent lot of CRYOcheck Chromogenic Factor VIII on IL ACL TOP 300 and IL ACL TOP 700 (K160276) analyzers with four replicate measurements at each time point for each sample. The study data demonstrate that citrated plasma samples are stable for two hours when stored at room temperature (15–25°C), three months when stored at <-70°C, including up to two freeze thaw cycles.

5. 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 three reagent lots and one representative instrument model.

The limit of blank (LoB) was determined using four plasma samples obtained from individuals with severe congenital Hemophilia A. Each sample was tested in three replicates on five different days on one representative instrument model for n=15 determinations per reagent lot. The sponsor determined the LoB to be 0.4% FVIII activity.

The limit of detection (LoD) was determined using four plasma samples obtained from individuals with congenital Hemophilia A. Each sample was tested in three replicates on five different days on one representative instrument model for n=15 determinations per reagent lot. The sponsor determined the LoD to be 0.5% FVIII activity.

The limit of quantitation (LoQ) was determined using four plasma samples with low FVIII activity at the following target concentrations: 0.4% FVIII, 0.4% FVIII, 0.5% FVIII and 0.5% FVIII. Each of the four plasma samples were tested in three replicates per day on five different days, for n=15 determinations per reagent lot. The sponsor determined the LoQ to be 0.5% FVIII activity.

6. Assay Cut-Off:

Not applicable

B Comparison Studies:

1. Method Comparison with Predicate Device:

The method comparison study was conducted using the CRYO*check* Chromogenic Factor VIII on the Instrumentation Laboratory ACL TOP CTS and ACL TOP 750 CTS by testing n=318 clinical samples (citrated plasma) collected from the intended use population. Results from the CRYO*check* Chromogenic Factor VIII were compared to results from the Instrumentation Laboratory Coatest SP FVIII on the Instrumentation Laboratory ACL TOP 700. The following tables summarize the line equation from the Passing-Bablok regression analysis performed for the combined dataset and the absolute predicted bias.

N	Pearson Correlation Coefficient	Slope (95% CI)	Intercept (95% CI)
318	0.994	1.041 (1.027, 1.058)	0.720 (0.252, 1.205)

%FVIII activity	Absolute Predicted Bias (95% CI)
1	-1.11 (-1.87, -0.35)
5	-0.81 (-1.53, -0.08)
45	2.20 (1.71, 2.69)
50	2.57 (2.09, 3.06)

100	6.33 (5.51, 7.15)
150	10.09 (8.71, 11.47)

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 FVIII activity of plasma samples collected from 120 normal, ostensibly healthy individuals (≥ 18 years) was tested by two operators using three lots of CRYOcheck Chromogenic Factor VIII on an Instrumentation Laboratory ACL TOP CTS. 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 Factor VIII is 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.