510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K222831

B Applicant

Precision BioLogic Inc.

C Proprietary and Established Names

CRYO*check*™ Factor VIII Deficient Plasma with VWF

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
GJT	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

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

CRYO*check* Factor VIII Deficient Plasma with VWF is for clinical laboratory use as a deficient substrate in the quantitative determination of Factor VIII activity in 3.2% citrated human plasma based on the activated partial thromboplastin time (APTT) assay. 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:

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

IV Device/System Characteristics:

A Device Description:

CRYO*check* Factor VIII Deficient Plasma with VWF is normal human citrated plasma which has been immunodepleted of factor VIII and to which an exogenous source of human von Willebrand Factor (vWF) has been added and buffered with HEPES. Factor VIII has been assayed at less than 1% of normal activity levels and vWF antigen and activity are >50%. It is provided frozen to users in small-volume aliquots (25 vials of 1.0 mL, and 25 vials of 1.5 mL).

B Principle of Operation:

CRYO*check* Factor VIII Deficient Plasma with VWF is used in the one-stage APTT clotting assay for the quantification of factor VIII activity. It is used with a modified APTT assay to measure factor VIII activity on the ACL TOP Family and ACL TOP Family 50 Series analyzers. The assay determines the functional activity of Factor VIII by measuring the degree of prolongation of activated partial thromboplastin time in the presence of a contact activator, thromboplastin, phospholipids and calcium ions. Factor VIII activity is correlated with the prolongation of the clotting time of the Factor VIII deficient plasma to which diluted patient sample has been added.

V Substantial Equivalence Information:

A Predicate Device Name(s):

HemosIL Factor VIII Deficient Plasma

B Predicate 510(k) Number(s):

K110237

C Comparison with Predicate(s):

Device & Predicate Device(s):	K222831 Subject Device	K110237 Predicate Device
Device Trade Name	CRYO*check* Factor VIII Deficient plasma with VWF	HemosIL Factor VIII Deficient Plasma
General Device Characteristic Similarities		
Intended Use/Indications For Use	CRYO*check* Factor VIII Deficient Plasma with VWF is for clinical laboratory use as a deficient substrate in the quantitative determination of Factor VIII activity in 3.2% citrated human plasma based on the activated partial thromboplastin time (APTT) assay. 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.	HemosIL Factor VIII deficient plasma is human plasma depleted for Factor VIII and intended for the in vitro diagnostic quantitative determination of Factor VIII activity in citrated plasma, based on the activated partial thromboplastin time (APTT) assay, on the ACL TOP Family analyzers. HemosIL Factor VIII deficient plasma is indicated for use on patients who are suspected of congenital or acquired deficiency based on the activated partial thromboplastin time (APTT) assay results.
Measurand	Human Factor VIII	Same
Product Code	GJT	Same
Assay Type	Quantitative (clot-based measurement of FVIII)	Same
Samples Matrix	3.2% Citrated Plasma	Same
Methodology	Factor VIII 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 plasma deficient in Factor VIII. 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.	Same

Expression of results	Quantitative; results are expressed as percent activity interpreted relative to a calibration curve.	Same
Instruments	ACL TOP/ACL TOP Family 50 Series	Same
General Device Characteristic Differences		
Device Components	CRYO*check* Factor VIII Deficient Plasma with VWF is normal human citrated plasma which has been immunodepleted of factor VIII and to which an exogenous source of human von Willebrand Factor (vWF) has been added and buffered with HEPES. Factor VIII has been assayed at less than 1% of normal activity levels and vWF antigen and activity are >50%. It will be provided to users frozen in small-volume aliquots (25 vials of 1.0 mL, and 25 vials of 1.5 mL). Vials will be packaged into boxes; these will be frozen during the manufacturing process and will be shipped and stored frozen until use to preserve the integrity of the components.	The HemosIL Factor VIII deficient plasma kit contains 10 x 1 mL vials of lyophilized human plasma that has been artificially depleted of factor VIII containing buffer and stabilizers. The residual factor VIII activity is less than or equal to 1% whereas von Willebrand Factor and the remaining intrinsic pathway factor levels are normal.
Expected Values/Reference Range	Factor VIII activity 62%–163%	Factor VIII activity 50%–150%
Linearity	Factor VIII activity 0%–230%	Factor VIII activity 0%–150%
On board Stability (open vial)/In-Use Stability	24 hours at 2 to 8°C	24 hours at 15°C 24 hours at 2 to 8°C 3 weeks at -20°C
Shelf-Life	24 months at -40°C to ≤-70°C	12 months at 2 to 8°C
Calibration	Normal Reference Plasma (K952622)	HemosIL Calibration Plasma (K041905)
Controls	Reference Control Normal (K013708) 96%–130% Factor VIII activity Abnormal Reference Control 1 (K952624) 30%–44% Factor VIII activity	HemosIL Normal Control Plasma (K021023) 99.9%–131.6% Factor VIII activity and HemosIL Special Test Control Level 2 (K040359)

	and Control 2 (K952624) 9%–17% Factor VIII activity	34.4%– 39.2% Factor VIII activity

VI Standards/Guidance Documents Referenced:

- CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition, October 2014 (R2019).
- CLSI EP06, Evaluation of Linearity of Quantitative Measurement Procedures; Second Edition, November 2020.
- CLSI EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline, September 2009.
- CLSI EP07, Interference Testing in Clinical Chemistry; 3rd Edition, November 2005 (R2022).
- 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:

All studies were performed using CRYO*check* Factor VIII Deficient Plasma with VWF in a modified APTT assay with Instrumentation Laboratories' APTT SynthASil reagent to measure FVIII activity on Instrumentation Laboratories' ACL TOP Series or TOP 50 Series Instruments; the specific instrument(s) used for each study are indicated in the summary reports below.

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 ACL TOP 700 CTS, three reagent lots, one normal (RCN) and two abnormal reference controls (ARP1 and ARP2), as well as three patient plasma samples (PM) representing very low (<1%), low (1%), and high levels (>100%) of FVIII 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.

Single-Site Study Summary Table

Sample	Mean FVIII (%)	N	Repeatability (Within-Run)		Between-Run		Between-Day		Between-Lot		Within-Lab	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
RCN	100.4	240	3.5	3.4	3.2	3.2	3.2	3.2	0.0	0.0	5.7	5.7
ARP1	37.7	240	1.7	4.6	1.1	3.0	0.9	2.5	0.5	1.4	2.3	6.2
ARP2	11.3	240	0.4	3.7	0.3	2.8	0.2	1.5	0.2	1.9	0.6	5.2
High PM	164.0	240	6.1	3.7	4.7	2.9	5.8	3.5	1.3	0.8	9.8	6.0
Low PM	2.0	240	0.2	9.3	0.1	4.2	0.1	3.3	0.1	2.8	0.2	11.1
Very Low PM	0.1	240	0.0	18.2	0.0	2.2	0.0	15.3	0.0	2.9	0.0	24.1

b) Multi-site 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 three different representative instrument models ACL TOP 500, ACL TOP 700 CTS, and ACL TOP 750 CTS (one instrument per site), three reagent lots, two operators at each site, one normal (RCN) and two abnormal reference controls (ARP1 and ARP2), as well as three patient plasma samples (PM) representing very low (<1%) , low (1%), and high levels (>100%) of FVIII activity. Precision estimates were calculated for each of the following variance components: within-run, between-run, between-day, between-lot, within-site, between-site, and total imprecision. The results for total imprecision of all lots combined are provided in the summary table below.

Multi-Site Study Summary Table

Sample	N	Mean FVIII (%)	Repeatability (Within-Run)		Between-Run		Between-Day		Between-Lot		Between-Site		Reproducibility	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
RCN	270	98.97	3.00	3.03	2.07	2.09	1.78	1.79	1.92	1.94	3.95	3.99	5.98	6.04
ARP1	270	37.40	1.25	3.33	0.63	1.68	0.61	1.63	0.80	2.14	0.44	1.16	1.77	4.75
ARP2	270	11.87	0.41	3.48	0.18	1.55	0.28	2.43	0.33	2.81	0.66	5.54	0.91	7.68
High PM	269	166.3	6.54	3.93	2.08	1.25	1.45	0.87	4.34	2.61	1.78	1.07	8.44	5.07
Low PM	270	1.93	0.11	5.84	0.06	3.08	0.03	1.81	0.08	4.01	0.09	5.01	0.18	9.39
Very Low PM	270	0.11	0.03	30.24	0.01	5.90	0.01	10.16	0.01	4.52	0.02	20.34	0.04	38.56

2. Linearity:

A linearity study was conducted in accordance with CLSI EP06-Ed2 using three lots of CRYO*check* Factor VIII Deficient Plasma with VWF in a modified APTT assay to quantify FVIII activity of fifteen samples created by combining plasma with a high FVIII concentration (~260%) with congenital hemophilia A patient plasma (0% FVIII). The results support a linear range of 0 to 230%.

3. Analytical Specificity/Interference:

Interference studies were conducted based on the CLSI EP07, 3rd Ed guideline. The study design included a single reagent lot and one representative instrument model ACL TOP 700 CTS. Potentially interfering endogenous and exogenous substances were spiked into the test samples (one normal and one abnormal reference control plasma sample) and tested with the control samples. Screening and dose-response testing were performed and none of the substances in the following table were found to lead to clinically significant interference.

Rivaroxaban, fondaparinux, dabigatran, unfractionated heparin, low molecular weight heparin, and emicizumab were found to interfere with the quantification of FVIII activity using CRYO*check* Factor VIII Deficient Plasma with VWF in a modified APTT assay. Limitations regarding the interference of these substances are warranted for the product instructions for use.

Substance Tested	Concentration with No Interference
Hemoglobin	≤1000 mg/dL
Intralipid	≤2000 mg/dL
Bilirubin (conjugated)	≤4.0 mg/dL
Bilirubin (unconjugated)	40 mg/dL
Lupus Anticoagulant	≤1.8 *dRVVT ratio (*Diluted Russell Viper Venom Time)
Warfarin	≤ INR ratio 2.72

4. Assay Reportable Range:

0 to 230% FVIII activity

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

Sample stability
A sample integrity study was conducted at two external sites to assess the stability of fresh plasma samples at room temperature, when stored frozen at ≤-70°C and after up to two freeze-thaw cycles. The FVIII activity of eighty plasma samples was measured using one lot of CRYO*check* Factor VIII Deficient Plasma with VWF on IL ACL TOP 300 and IL ACL TOP 700 (K160276) analyzers. Results were compared using weighted Deming regression analysis and support a fresh sample stability claim of 2 hours at room temperature and a frozen storage claim of 1.5 months at ≤-70°C, including up to two freeze-thaw cycles.

Shelf-Life Stability
A shelf-life stability study was conducted in accordance with CLSI EP25-A using an IL ACL TOP 700 CTS instrument (K160276). Three lots of CRYO*check* Factor VIII Deficient Plasma with VWF were stored at -40°C and ≤-70°C and tested at t=0 and regular intervals defined by the lot-specific pull schedule up to 37 months (real time study is ongoing). At each timepoint, five replicates of one normal and two abnormal reference controls and two patient plasma samples representing very low and high levels of FVIII activity levels were quantified in a modified APTT assay. The study has been completed up to 25 months and supports a shelf-life stability claim of 24 months when the product is stored at -40°C and 80°C.

In-Use Stability
An in-use stability study was conducted in accordance with CLSI EP25-A using an IL ACL TOP 700 CTS instrument (K160276). Three lots of CRYO*check* Factor VIII Deficient Plasma with VWF were maintained on board the analyzer (14.5–15.5°C) for up to 25 hours and in a refrigerator (2–8°C) for up to 25 hours. Each lot was used in a modified APTT assay to quantify five replicates of one normal and two abnormal reference controls and two patient plasma samples representing low and high levels of FVIII activity from each storage condition at defined timepoints. The data support a stability claim of 24 hours on board the instrument at 2–8°C.

6. Detection Limit:
Not applicable

7. Recovery of FVIII Replacement Therapies:

A recovery study was conducted using a single lot of CRYO*check* Factor VIII Deficient Plasma with VWF in a modified APTT assay on an IL ACL TOP 700 CTS instrument (K160276). Congenital FVIII deficient plasma was spiked with six FVIII replacement therapies at seven concentrations and percent recovery was determined. CRYO*check* Factor VIII Deficient Plasma with VWF met the predefined acceptance criteria to evaluate the potency of FVIII concentrates including Advate, Afstyla, Elocate, Jivi, Novoeight, and Wilate at concentrations ranging from 0.05 to 1.0 IU/mL. There was an underestimation of Afstyla*, and a 2x correction factor were applied to the results based on the manufacturer's recommendations.

Product	Mean Percent Recovery (%)
Advate	92.84
Afstyla*	97.38
Eloctate	94.90
Jivi	104.49
Novoeight	113.33
Wilate	94.92

**Per the manufacturer's recommendation, a chromogenic assay is recommended for measurement of Afstyla. Mean percent recovery value includes 2x correction based on manufacturer's recommendation that one stage clotting assay shows an under recovery of 50%.*

B Comparison Studies:

1. Method Comparison with Predicate Device:

   A method comparison study was conducted at four sites (one internal and three external) to compare the accuracy of factor VIII activity measurement when using CRYO*check* Factor VIII Deficient Plasma with VWF in a modified APTT assay to a comparator device. Three hundred and sixty-six human plasma samples from normal ostensibly healthy individuals, patients with congenital or acquired hemophilia A, patients with hemophilia B, patients with von Willebrand disease, patients on recombinant FVIII replacement therapies, and patients with other factor deficiencies were distributed across four sites and tested to quantify FVIII activity using a single lot of CRYO*check* Factor VIII Deficient Plasma with VWF on IL ACL TOP 500, IL ACL TOP 700 CTS (K160276), and IL ACL TOP 750 CTS (K150877) analyzers. A second aliquot of each sample was tested using a modified APTT assay with HemosIL Factor VIII Deficient Plasma on an IL ACL TOP 750 instrument (K150877). Results were compared by Passing-Bablok regression analysis. The method comparison results met the pre-defined acceptance criteria.

N	Slope		Intercept		Pearson Correlation Coefficient (r)
	Value	95% CI	Value	95% CI	
366	1.16	1.15, 1.18	-0.08	-0.25, 0.13	0.96

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 interval study was conducted by two operators on two IL ACL TOP 700 CTS (K160276) analyzers in accordance with CLSI EP28-A3c using three lots of CRYO*check* Factor VIII Deficient Plasma with VWF and citrated plasma samples from 136 normal, ostensibly

healthy individuals. The reference interval was determined to be 62–163% FVIII activity by calculating the non-parametric 95% confidence interval (2.5th to 97.5th percentiles).

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.