510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K183440

B. Purpose for Submission:

New Device

C. Measurand:

Factor VIII inhibitory antibodies (BU/mL)

D. Type of Test:

Quantitative

E. Applicant:

Precision BioLogic

F. Proprietary and Established Names:

CRYOcheck™ Factor VIII Inhibitor Kit

G. Regulatory Information:

1. Regulation section:

   21 CFR 864.7290, Factor deficiency test

2. Classification:

   Class II

3. Product code:

   GGP, Test, qualitative and quantitative factor deficiency

4. Panel:

   Hematology (81)

H. Intended Use:

1. Intended use(s):

   The CRYOcheck FVIII Inhibitor Kit is for clinical laboratory use in conjunction with a Factor VIII activity assay to enable performance of a modified Nijmegen-Bethesda assay using 3.2% citrated human plasma. It enables the determination of a functional FVIII inhibitor titer to aid in the clinical management of congenital hemophilia A in individuals aged 2 years or older. For in vitro diagnostic use.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Siemens Healthcare Diagnostics BCS XP Analyzer

I. Device Description:

The FVIII Inhibitor Kit is used in conjunction with a FVIII activity assay to perform the Centers for Disease Control (CDC) modification of the Nijmegen-Bethesda assay for determination of FVIII inhibitor activity and consists of the following four components:

- Imidazole Buffered Pooled Normal Plasma (IB-PNP): Pooled normal plasma from a minimum of twenty donors with a factor VIII activity value of 95–113% and buffered with imidazole to a pH of 7.3–7.5.

- Imidazole Buffered Bovine Serum Albumin (IB-BSA): A 4% BSA solution buffered with imidazole to a pH of 7.3–7.5.

- Negative Factor VIII Inhibitor Control: Pooled normal plasma from a minimum of five donors buffered with HEPES to a pH of 6.2–8.2.

- Positive Factor VIII Inhibitor Control: HEPES buffered (pH 6.2–8.2) immunodepleted FVIII deficient plasma to which anti-human FVIII antibodies have been added.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Factor VIII Antibody Screen

2. Predicate 510(k) number(s):

   K082205

3. Comparison with predicate:

Similarities		
Item	Device CRYOcheck FVIII Inhibitor Kit	Predicate Factor VIII Antibody Screen
Intended Use	The CRYO*check* FVIII Inhibitor Kit is for clinical laboratory use in conjunction with a Factor VIII activity assay to enable performance of a modified Nijmegen-Bethesda assay using 3.2% citrated human plasma. It enables the determination of a functional FVIII inhibitor titer to aid in the clinical management of congenital hemophilia A in individuals aged 2 years or older. For in vitro diagnostic use.	The Factor VIII Antibody Screen is a qualitative solid phase enzyme linked immunosorbent assay (ELISA) designed to detect IgG antibodies reactive with recombinant human VIII (FVIII) in human serum and plasma.
Measurand	Factor VIII Inhibitor	Antibodies to human Factor VIII

Differences		
Item	Device CRYOcheck FVIII Inhibitor Kit	Predicate Factor VIII Antibody Screen
Assay Type	Quantitative (BU/mL)	ELISA (Qualitative)
Device Description	The FVIII Inhibitor Kit is used in the CDC modification of the Nijmegen-Bethesda assay and contains the following components: • Imidazole Buffered Pooled Normal Plasma (IB-PNP): Pooled normal plasma from a minimum of twenty donors with a factor VIII activity value of 95–113% and buffered with imidazole to a pH of 7.3–7.5.	The Factor VIII Antibody Screen assay is an ELISA with a colorimetric endpoint. The assay kit is made of a 96 welled microwell plate (in 1 x 8 strips), alkaline phosphatase conjugated goat antibody to human immunoglobulin G, p-nitrophenyl phosphate substrate, positive and negative control, Stop Solution, diluents, buffers, wash solutions, and plate sealers.

Differences		
Item	Device CRYOcheck FVIII Inhibitor Kit	Predicate Factor VIII Antibody Screen
	• Imidazole Buffered Bovine Serum Albumin (IB-BSA): A 4% BSA solution buffered with imidazole to a pH of 7.3–7.5. • Negative Factor VIII Inhibitor Control: Pooled normal plasma from a minimum of five donors buffered with HEPES to a pH of 6.2–8.2. • Positive Factor VIII Inhibitor Control: HEPES buffered (pH 6.2–8.2) immunodepleted FVIII deficient plasma to which antihuman FVIII antibodies have been added.	
Methodology	Diluted heat inactivated patient plasma is mixed with an exogenous source of FVIII (IB-PNP) and prepared alongside a control mix, consisting of a diluent (IB-BSA) and IB-PNP. Following a 2-hour incubation, the FVIII activity of the test plasma relative to the control mix is determined and used to calculate FVIII inhibitor activity expressed as Bethesda units per milliliter.	Diluted patient sample is added to microwells coated with recombinant FVIII, and antibody, if present will bind. Unbound material is washed away, and an alkaline phosphatase labeled anti-human immunoglobulin reagent (anti IgG) is added to the wells and incubated. Unbound anti-IgG is washed away and then PNPP (p-nitrophenyl phosphate) substrate is added. After a 30 minute incubation period, the reaction is stopped, and the optical density of the color that develops is measured at 405nm.
Test results	Quantitative; results are expressed in Bethesda units per milliliter (BU/mL)	Qualitative; results are reported as positive or negative.

K. Standard/Guidance Document Referenced (if applicable):

CLSI EP05-A3, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition.

CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.

CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline-Second 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.

L. Test Principle:

The FVIII Inhibitor Kit components are used to perform a sample pretreatment process, including negative and positive assay controls that also undergo the pretreatment process. Diluted heat inactivated patient plasma is mixed with an exogenous source of FVIII (IB-PNP) and prepared alongside a control mix, consisting of a diluent (IB-BSA) and IB-PNP. Following a 2-hour incubation, the FVIII activity of the test plasma relative to the control mix is determined and used to calculate FVIII inhibitor activity expressed as Bethesda units per milliliter.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Repeatability:
   An internal precision study was performed using three different lots of CRYOcheck FVIII Inhibitor Kit by one operator on a Siemens BCS XP analyzer. The study included the kit's Positive and Negative Control as test samples as well as four plasma samples collected from congenital hemophilia A patients representing negative, low, mid and high levels of FVIII inhibitor. Each sample was measured with each product lot in duplicate, twice a day for 20 days for a total of 80 replicates per sample per lot. All results met the predefined acceptance criteria and were determined to be acceptable.

Overall Precision (Lots 1, 2 and 3)			
Sample	Mean (BU/mL)	Within - Laboratory	
		SD	%CV
Negative Plasma Sample	0.3	0.1	29.0
Low Plasma Sample	1.2	0.1	8.2
Mid Plasma Sample	5.3	0.4	8.3
High Plasma Sample	8.6	0.7	8.4

Reproducibility

Reproducibility studies were conducted at three sites by three different operators on three different Siemens BCS XP analyzers using a single lot of CRYOcheck FVIII Inhibitor Kit. The study included the kit's Positive and Negative Control as test samples as well as four plasma samples collected from congenital hemophilia A patients representing negative, low, mid and high levels of FVIII inhibitor. Each sample was measured in triplicate, twice a day for five days for a total of 30 replicates per sample per site. The data across three sites is summarized below. All results met the predefined acceptance criteria and were determined to be acceptable.

All Sites Combined											
Sample	Mean (BU/mL)	Within-Run		Between- Run		Between-Day		Between-Site		Overall	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Kit Negative Control	0.0	0.1	399.4	0.0	138.3	0.0	0.0	0.0	0.0	0.1	422.7
Kit Positive Control	1.8	0.2	8.9	0.1	3.3	0.1	3.4	0.2	12.4	0.3	16.0
Negative Plasma Sample	0.3	0.1	26.9	0.0	0.0	0.0	9.0	0.0	2.2	0.1	28.4
Low Plasma Sample	1.4	0.1	10.2	0.0	0.0	0.0	2.9	0.0	0.0	0.1	10.6
Mid Plasma Sample	5.5	0.4	7.8	0.1	1.9	0.1	2.2	0.0	0.0	0.5	8.3
High Plasma Sample	9.6	0.7	7.8	0.4	4.0	0.3	3.4	0.9	9.2	1.3	13.2

b. Linearity/assay reportable range:

Not applicable[1]

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Shelf Life Stability

A shelf-life stability study was conducted using three lots of CRYOcheck FVIII

[1] Calculations are based on FVIII linearity. Linearity has been demonstrated for the factor VIII assay on the BCS-XP analyzer (0–100%).

Inhibitor Kit stored at < −70°C (monitored condition -76 to -82°C). At each timepoint (0, 6, 7, 12, 13 months), five replicates of the kit's Positive and Negative Control as well as four plasma samples collected from congenital hemophilia A patients representing negative, low, mid and high levels of FVIII inhibitor were quantified. The study was performed up to 13 months and the data were found to be acceptable to support a 12-month shelf-life claim.

In-Use Stability

An in-use stability study was conducted using three lots of CRYOcheck FVIII Inhibitor Kit maintained at room temperature (18–25 °C) or in a refrigerator (2–8 °C) post-thaw. At each time point (0, 2, 4, 6 and 7 hours), six replicates of the kit's Positive and Negative Control as well as three plasma samples collected from congenital hemophilia A patients representing negative, low and high levels of FVIII inhibitor were evaluated. The data support a 4-hour in-use stability of the product when maintained at room temperature or at 2–8 °C post thaw.

d. Detection limit:

Not applicable

e. Analytical specificity:

Interference studies were conducted using a single lot of CRYOcheck FVIII Inhibitor Kit. Patient plasma samples (concentrations shown in the table below) were spiked with potential interferents (hemoglobin, intralipid, bilirubin, human von Willebrand factor, rheumatoid factor (RF) and Lupus Anticoagulant (LA)) and ten replicates were tested alongside ten replicates of the corresponding blank matrix control. To screen pathogenic autoantibodies as interferents, RF and LA spiked inhibitor-positive and negative samples were prepared by adding a high titer FVIII inhibitor-positive plasma (~100 BU/mL) from a single donor to a pool of RF+ patient plasma or a pool of heat inactivated (30 min at 56°C) LA+ patient plasma to target inhibitor levels similar to the described panel members in the table below. The corresponding matrix blank was prepared by adding the same quantity of a high titer FVIII inhibitor-positive plasma to normal plasma (RF blank) or heat inactivated normal plasma (LA blank).

Table: Patient inhibitor titer sample concentrations

Sample	Approximate Titer
Negative Inhibitor	0.3 BU/mL
Borderline	0.6 BU/mL
Low Positive Inhibitor	1.0 BU/mL
Mid Positive	5.0 BU/mL
High Positive Inhibitor	9.0 BU/mL

The interference study results demonstrate that the following interferents do not interfere with test results up to the following concentrations:

Interferent	Concentration
Hemoglobin	≤ 500 mg/dL
Bilirubin	≤ 500 mg/dL
Intralipid	≤ 29 mg/dL
vWF	≤ 20 µg/mL

The presence of lupus anticoagulant autoantibodies may interfere with the quantification of low titer FVIII inhibitors.

Rheumatoid factor demonstrated interference at ≥ 82 IU/mL with quantification of FVIII inhibitors.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Method comparison studies were performed at three sites to compare the accuracy of the CRYO*check* FVIII Inhibitor Kit relative to a comparator device (a validated CDC modified FVIII Chromogenic Nijmegen Bethesda assay). Aliquots of 3.2% sodium citrate frozen plasma samples from individuals with congenital hemophilia A (N=210) were distributed across three sites and tested using a single lot of CRYO*check* FVIII Inhibitor Kit. A second aliquot of each sample was tested at a central reference laboratory using a validated chromogenic CDC Modified Nijmegen-Bethesda Assay on a Siemens BCS XP instrument. Prior to testing, samples tested on the comparator and candidate devices underwent the same number of freeze-thaw cycles.

Determination of titer inhibition:

CRYOcheck FVIII Inhibitor Kit

The Chromogenic FVIII activity of each sample was measured using the Siemens Factor VIII Chromogenic Assay on a BCS-XP analyzer. To determine the inhibitor titer of each test sample, residual FVIII activity (%RA) was calculated as the relative percentage of FVIII activity of the test mixture compared to the control mix. Using the sample dilution with the %RA closest to 50%, the Bethesda Units (BU/mL) was calculated using the following formula: BU/mL= [(2-Log %RA)/0.30103] x dilution factor.

Passing-Bablok regression analysis was performed for each site and all sites combined (results summarized below). The results met the predefined acceptance criteria and were determined to be acceptable.

	N	Slope		Intercept		Pearson Correlation Coefficient (r)
		Point Estimate	95% CI	Point Estimate	95% CI	
Site 1	68	1.543	1.404, 1.660	-0.224	-0.434, -0.127	0.977
Site 2	72	1.065	0.998, 1.190	-0.113	-0.138, -0.043	0.985
Site 3	70	1.373	1.225, 1.455	-0.075	-0.159, -0.023	0.980
Overall	210	1.341	1.265, 1.406	-0.145	-0.180, -0.070	0.970

The predicted bias at the predefined medical decision points was calculated for all sites. The observed predicted bias at the medical decision points for all sites combined met the predefined acceptance criteria and were determined to be acceptable.

Agreement between the candidate device (CRYOcheck FVIII Inhibitor Kit) and comparator method results are summarized below based on a cut-off of <0.6 BU/mL[2] for a negative assay result. As shown in the table below, 100% (N=133) of the samples that tested positive by the comparator method also tested positive by the candidate device. Of the 77 samples that tested negative by the comparator method, 76 also tested negative by the candidate device. The single sample that tested positive by the candidate method did so because of rounding the calculated BU/mL to one decimal place. The candidate device result was 0.55 BU/mL (rounding to 0.6 BU/mL) versus a result of 0.41 BU/mL (rounding to 0.4 BU/mL) by the comparator method.

		CRYOcheck FVIII Inhibitor Kit		
		Positive	Negative	Total
Comparator Method	Positive	133	0	133
	Negative	1	76	77
	Total	134	76	210

Test vs Reference Method	Proportion	95% CI	
Positive Percent	100%	97%	100%
Negative Percent	99%	93%	100%
Total Percent Agreement	99%	97%	100%

b. Matrix comparison:

Fresh versus Frozen and freeze-thaw cycles studies

The purpose of this study was to observe the effects of freeze-thaw cycles and storage at different freezing temperatures on the stability of samples tested with the CRYOcheck FVIII Inhibitor Kit. Two stability studies were performed, one study was

[2] Miller CH, Payne AB, Driggers J, Ellingsen D, Boylan B, Bean CJ. Reagent substitutions in the Centers for Disease Control and Prevention Nijmegen-Bethesda assay for factor VIII inhibitors.*Haemophilia*. 2018;24(3):e116-e119.

performed using samples contrived from immunodepleted factor FVIII-deficient plasma and monoclonal FVIII inhibitor antibodies and a second study was performed using samples from congenital hemophilia A patients.

In the first stability study, three test plasma samples to represent negative (0.0 BU/mL), low (2.0 BU/mL) and high titer (11.0 BU/mL) were created using immunodepleted Factor VIII deficient plasma mixed with a high-titer sample (0.31 µg/mL of anti-FVIII monoclonal antibody). The titer of each freshly prepared plasma sample was quantified at baseline (t=0) and then subjected to five different storage conditions that tested fresh versus frozen and multiple rounds of freeze thaw cycles as outlined in the table below. Conditions 1 and 2 were evaluated at 6, 7, 24 and 25 hours. Condition 3 was evaluated at 7, 8, 14, 15 days and conditions 4 and 5 was evaluated for three freeze-thaw cycles.

Condition	Storage Conditions
1	Fresh samples stored at 2−8°C for up to 25 hours
2	Frozen at −80°C, thawed and stored at 2−8°C for up to 25 hours
3	Frozen at −80°C, transferred to -20°C and stored for up to 15 days
4	Frozen at −80°C, three freeze thaw cycles at -20°C
5	Frozen at −80°C, three freeze thaw cycles at -80°C

Linear regression analysis was performed for each measured titer under the specified storage conditions in the table above. The study results demonstrated comparability between fresh and frozen samples and met the predefined acceptance criteria. These findings support that samples for use with the FVIII Inhibitor Kit can be stored at 2−8°C for up to 24 hours, at −20°C for up to 14 days. Patient samples can also be subjected to up to three freeze-thaw cycles prior to use with the CRYOcheck FVIII Inhibitor Kit.

In the second study, a retrospective analysis was performed examining FVIII inhibitor titer results for four plasma samples from congenital hemophilia A patients representing negative, low, mid and high levels of FVIII inhibitor quantified using three lots of CRYOcheck FVIII Inhibitor Kit. Four plasma samples derived from congenital hemophilia A patients including a non-zero negative panel member (0.3 BU/mL) and three FVIII inhibitor-positive samples (low (1.0 BU/mL), mid (5.0 BU/mL) and high panel (9.0 BU/mL) members were prepared. These samples were tested at baseline (t=0) and stored at <−70 °C for up to 13 months. At various time points, plasma samples were taken from storage, thawed (37 ± 1°C) and tested. The results demonstrated sample stability of 12 months when stored frozen at < −70°C.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

Not applicable

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.