510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K193556

B Applicant

Precision BioLogic

C Proprietary and Established Names

Cryocheck Hex LA

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
GFO	Class II	21 CFR 864.7925 - Partial Thromboplastin Time Tests	HE - Hematology

II Submission/Device Overview:

A Purpose for Submission:
New device

B Measurand:
Lupus anticoagulant

C Type of Test:
Qualitative integrated test based on hexagonal-phase phospholipid neutralization (HPNT)

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:

CRYOcheck Hex LA is for clinical laboratory use as a qualitative test kit intended to aid in the detection of lupus anticoagulants (LA) in 3.2% citrated human plasma by the application of hexagonal phase phospholipids. CRYOcheck Hex LA should be used as an integrated test for lupus anticoagulant detection. For in vitro diagnostic use. The performance of this device has not been established in neonate and pediatric patient populations.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

D Special Instrument Requirements:
Diagnostica Stago STA-R Evolution

IV Device/System Characteristics:

A Device Description:
CRYOcheck Hex LA is used to perform a qualitative test for the detection of lupus anticoagulants and consists of the following three components:

- LA Start: Pooled normal plasma with buffer and a heparin neutralizer
- LA Correct: Pooled normal plasma with buffer, a heparin neutralizer, and inverted hexagonal phase phospholipid
- LA APTT: Silica-based LA sensitive APTT reagent with stabilizer(s)

B Principle of Operation:

CRYOcheck Hex LA is a hexagonal-phase phospholipid neutralization test (HPNT), which is an integrated test that combines screening, confirmatory and mixing test procedures into a single assay. The HPNT works on the principle that lupus anticoagulants (LA) are neutralized by hexagonal phase phospholipids (PL). The presence of LA in plasma samples is confirmed by the correction of prolonged APTT clot times in the presence of a reaction mixture containing hexagonal phase phospholipids.

In the CRYOcheck Hex LA assay, the test plasma suspected to contain LA is incubated in two reaction cuvettes, both of which involve dilution with pooled normal plasma. In the first cuvette, the screening test component is performed by mixing the test plasma with the LA Start reagent (pooled normal plasma with a heparin neutralizer). In the second cuvette, the confirmatory test component is performed by mixing the test plasma with the LA Correct reagent (pooled normal plasma with a heparin neutralizer and hexagonal phase phospholipid). The LA APTT reagent is then added to each cuvette, followed by 0.025 M CaCl2 to activate clotting via the intrinsic pathway. Clot times are recorded for the reaction mixture containing LA Start (screen) and the reaction mixture containing LA Correct (confirm). The result is reported as the difference in clot time in seconds (“delta correction”) between LA Start and LA Correct cuvettes.

Delta Correction = (CT LA Start) – (CT LA Correct)

The result is then compared to an established cutoff. A result greater than or equal to the established cutoff is considered LA positive, while a result less than the established cutoff is considered LA negative.

V Substantial Equivalence Information:

A Predicate Device Name(s):
STACLOT LA TEST KIT

B Predicate 510(k) Number(s):
K923731

C Comparison with Predicate(s):

Device & Predicate Device(s):	K193556	K923731
Device Trade Name	CRYO*check* Hex LA	STACLOT-LA
General Device Characteristic Similarities		
Intended Use/Indications For Use	CRYOcheck Hex LA is for clinical laboratory use as a qualitative test kit intended to aid in the detection of lupus anticoagulants (LA) in 3.2% citrated human plasma by the application of hexagonal phase phospholipids. CRYOcheck Hex LA should be used as an integrated test for lupus anticoagulant detection. For in vitro diagnostic use. The performance of this device has not been established in neonate and pediatric patient populations.	The STACLOT-LA test kit is a reagent system designed for the qualitative detection of lupus anticoagulants (LA) in plasma by the use of hexagonal HII phase phospholipid molecules.
Type of Test	Qualitative; hexagonal phase neutralization test	Qualitative; hexagonal phase neutralization test
Measurand	clot time delta (seconds)	clot time delta (seconds)
Results	Qualitative; results are reported as clot time delta (seconds) and are interpreted as positive or negative relative to an established cut-off value.	Qualitative; results are reported as clot time delta (seconds) and are interpreted as positive or negative relative to an established cut-off value.
General Device Characteristic Differences		
Device Description	CRYO*check* Hex LA is comprised	Staclot LA is comprised of three

Device & Predicate Device(s):	K193556	K923731
Device Trade Name	CRYO*check* Hex LA	STACLOT-LA
	of three reagents supplied in a ready to use frozen format as follows: LA Start: Pooled normal plasma with buffer and a heparin neutralizer. LA Correct: Pooled normal plasma with buffer, a heparin neutralizer, and inverted hexagonal phase phospholipid. LA APTT: Silica-based LA sensitive APTT reagent with stabilizer.	lyophilized reagents and two reconstitution liquids as follows: Reagent 1: ready-for-use buffer Reagent 2: lyophilized hexagonal phase phosphatidylethanolamine Reagent 3: lyophilized normal human plasma containing a heparin inhibitor Reagent 4: lyophilized PTT-LS reagent consisting of cephalin prepared from rabbit cerebral tissues and a particulate siliceous activator Reagent 5: solvent for reconstitution of Reagent 4.
Normal Range and Cutoff	Delta correction: 6 seconds	Delta correction: 8 seconds

VI Standards/Guidance Documents Referenced:

- CLSI EP07 3rd Edition, Interference Testing in Clinical Chemistry; Approved Guideline, Third Edition, April 2018.
- CLSI EP05-A3, Evaluation of Precision Performance of Quantitative Measurement Procedures; Approved Guideline, Third Edition, October 2014.
- 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 Precision

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 STA-R Evolution instrument, three reagent lots, one normal and two abnormal reference controls, as well as five patient plasma samples representing negative, near cut-off, weak positive, moderate positive and strong positive levels of lupus anticoagulant. 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	Within Laboratory Precision LA Start			Within Laboratory Precision LA Correct		
	Mean Clot Time (s)	SD	%CV	Mean Clot Time (s)	SD	%CV
CRYO*check* Lupus Negative Control	53.0	1.6	3.0	52.8	2.8	5.3
CRYO*check* Weak Lupus Positive Control	87.3	3.2	3.7	65.4	2.8	4.2
CRYO*check* Lupus Positive Control	125.4	5.2	4.2	79.8	4.5	5.7
LA Negative Plasma Sample	55.9	1.7	3.1	55.1	2.5	4.5
LA Near Cut-Off Plasma Sample	67.6	2.5	3.8	58.4	2.6	4.5
LA Weak Positive Plasma Sample	89.8	3.3	3.7	66.4	3.0	4.6
LA Moderate Positive Plasma Sample	146.5	6.0	4.1	85.9	5.8	6.7
LA Strong Positive Plasma Sample	270.7	9.6	3.6	118.0	9.0	7.6

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 a single STA-R Evolution instrument (one instrument per site), three reagent lots, three operators, one normal and two abnormal reference controls, as well as four pooled patient plasmas (single donor LA positive plasma diluted with single donor normal plasma) representing negative, near cut-off, weak positive, and strong positive levels of lupus anticoagulant. 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.

Reproducibility: LA Start											
Sample	Mean Clot Time (s)	Within-Run		Between-Run		Between-Day		Between-Site		Reproducibility	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
CRYO*check* Lupus Negative Control	52.8	1.4	2.7	0.3	0.6	0	0	0.4	0.7	1.6	3.0
CRYO*check* Weak Lupus Positive Control	85.6	3.3	3.9	0.5	0.6	0.5	0.6	1.9	2.2	3.9	4.6
CRYO*check* Lupus Positive Control	123.6	5.0	4.0	0	0	1.5	1.3	2.1	1.7	5.7	4.6
LA Negative Plasma Sample	55.8	1.5	2.6	0.1	0.2	0.3	0.5	0.7	1.3	1.8	3.2
LA Near Cut-Off Plasma Sample	66.9	2.3	3.5	0.4	0.6	0.8	1.2	0.8	1.2	2.7	4.0
LA Weak Positive Plasma Sample	88.3	3.7	4.1	0	0	1.1	1.3	1.8	2.0	4.3	4.8
LA Strong Positive Plasma Sample	264.9	6.9	2.6	0.3	0.1	2.3	0.9	4.5	1.7	10.3	3.9

Reproducibility: LA Correct											
Sample	Mean Clot Time (s)	Within-Run		Between-Run		Between-Day		Between-Site		Reproducibility	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
CRYO*check* Lupus Negative Control	53.7	1.7	3.1	0.8	1.6	0	0	0	0	3.1	5.8
CRYO*check* Weak Lupus Positive Control	65.7	2.4	3.6	1.0	1.5	0.7	1.0	0.7	1.1	3.1	4.8
CRYO*check* Lupus Positive Control	80.2	3.2	4.0	1.5	1.8	1.4	1.7	1.8	2.2	4.8	5.9
LA Negative Plasma Sample	56.0	1.7	3.0	0.6	1.1	0.2	0.4	0	0	2.9	5.2
LA Near Cut-Off Plasma Sample	59.2	2.2	3.7	1.2	2.0	0.7	1.1	0.3	0.6	3.0	5.0
LA Weak Positive Plasma Sample	66.9	2.7	4.0	1.3	2.0	1.2	1.8	2.2	3.3	4.0	6.0
LA Strong Positive Plasma Sample	117.4	4.5	3.9	3.1	2.6	2.0	1.7	2.6	2.2	9.4	8.0

2. Linearity:
   Not applicable in Section.

3. Analytical Specificity/Interference:

   Interference studies were conducted based on the CLSI EP07 guideline. The study design included a single reagent lot and one STA-R Evolution instrument. Potentially interfering endogenous and exogenous substances were spiked into the control samples (one normal and one abnormal reference control plasma sample). 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
Bilirubin (unconjugated)	≤ 20 mg/dL
Bilirubin (conjugated)	≤ 2 mg/dL
Intralipid	≤ 500 mg/dL
Unfractionated heparin	≤ 2 IU/mL
Low molecular weight heparin	≤ 2 IU/mL
Elevated factor VIII activity	≤ 180%
Factor VIII inhibitor antibodies	≤ 15 BU/mL
Abnormally low factor II	≥ 50%
elevated INR	≤ 4.5

Interfering Substance	Concentration Tested
C-reactive protein	≤ 15 μg/mL
platelet counts	<10,000/ μL

4. Assay Reportable Range:
Not Applied in Section.

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

a. Shelf-Life Stability:

Shelf-life stability studies were performed to support the recommended storage and handling instructions found in the device labeling. Three control samples were tested by using three lots of CRYOcheck Hex LA after storage in the following temperature ranges up to 37 months, -70°C (-66 to -72°C) and -80°C (-76 to -82°C). The study was performed using a single STA-R Evolution instrument with ten replicate measurements at each time point for each sample. The study data demonstrate that a shelf-life stability claim of 12 months when stored at -70°C or colder.

b. In-Use Stability:

In-use stability studies were performed to support the recommended storage and handling instructions found in the device labeling. Three control plasmas and four test plasmas with varying levels of LA (Negative, Near Cut-off, Weak Positive and Strong Positive) were tested by using three lots of CRYOcheck Hex LA after storage in the following conditions, room temperature (18–25°C) or on-board the instrument. The study was performed using three reagent lots on a STA-R Evolution instrument with five replicate measurements at each time point for each sample. The in-use stability claim was established at 4 hours when reagents are maintained at room temperature or 8 hours when stored on-board the instrument.

c. Sample Stability

Sample stability studies were performed to support the recommended storage and handling instructions found in the device labeling. Sixty-four 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 Hex LA on STA-R Evolution instrument with four 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 (15–25°C), two months when stored at <-70°C, including one freeze thaw cycle.

d. Expected Values – Controls

CRYO*check* Lupus Negative Control (CCLN): a HEPES-buffered pooled normal citrated human plasma, which is negative for Lupus Anticoagulant.

CRYO*check* Weak Lupus Positive Control (CCWLP, K032804): a HEPES-buffered pooled normal citrated human plasma, mixed with LA positive plasma to give a weakly positive LA test result which is reported on its assay certificate.

CRYO*check* Lupus Positive Control (CCLP, K952623): a HEPES buffered pooled normal citrated human plasma, to give a moderate-strong LA test result which is reported on its assay certificate.

6. Detection Limit:
Not applicable

7. Normal Range and Assay Cut-Off:

The reference interval study was conducted at one laboratory site. The LA level of plasma samples collected from 137 normal, ostensibly healthy individuals (≥ 18 years) were tested by two operators using three lots of CRYOcheck Hex LA on a STA-R Evolution instrument. The reference interval for delta correction was established by calculating the pooled mean ±2 SD. The calculated normal reference range for delta correction results of CRYOcheck Hex LA is shown in the table below:

Normal Range	
Lower Range	Upper Range
-5.9	2.0

The cut-off results were determined by using pooled data from the normal range study. The cut-off is calculated as the mean of the delta correction + 4 SD.

Delta Correction	Interpretation
< 6.0 seconds	LA Negative
≥ 6.0 seconds	LA Positive

This method of establishing cut-off is different than that indicated for confirmatory tests. Each laboratory should verify its own cut-off, for example, by testing the plasma of at least 20 normal individuals.

B Comparison Studies:

1. Method Comparison with Predicate Device:

The method comparison study was conducted using the CRYOcheck Hex LA on the STA-R Evolution by testing n=446 clinical samples (citrated plasma) collected from the intended use population. Results from the CRYOcheck Hex LA were compared to results from the STACLOT LA assay on a STA-R Evolution. The following tables summarize the 95% confidence intervals for PPA, NPA, and overall agreement were calculated using the Clopper-Pearson (exact) method.

		CRYOcheck Hex LA		
		Negative	Positive	Total
STACLOT-LA	Negative	295	15	310
	Positive	6	130	136
	Total	301	145	446

Agreement	Point Estimate (95% Confidence Interval)
Positive Percent Agreement	95.6% (91% - 98%)
Negative Percent Agreement	95.2% (92% - 97%)
Overall Agreement	95.3% (93% - 97%)

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:
Not applicable

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.