PrecisionBioLogic

CRYO*check*™ IVD

HEX LA™

Intended Use

CRYO*check* 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. CRYO*check* 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.

Summary and Principle

Lupus anticoagulants (LA) are heterogeneous autoantibodies, mainly of the IgG and IgM type, which are directed against phospholipids (PL) or phospholipid-protein complexes involved in coagulation[1]. LA antibodies are detected in patients' plasma by PL-dependent clotting assays. There is a significant association between LA and increased risk of clinical complications such as thrombotic events[2,3] and recurrent fetal loss[4]. Medical diagnosis of LA is based on clinical symptoms and laboratory results. There is no gold standard test for LA. Considering the complexity of mechanism and the heterogeneous nature of LA antibodies, application of different clotting tests that work based on different principles has been recommended[5].

LA prolongs clot formation of PL-dependent coagulation (LA screening) tests in vitro, such as LA-sensitive activated partial thromboplastin time (APTT) or dilute Russell's Viper Venom Time (dRVVT) screen. To confirm the presence of LA in a plasma sample, correction of a prolonged clot time by extra PL (an LA confirmatory test) needs to be performed by laboratories as well as ruling out other abnormalities, such as factor deficiency and heparin presence[5].

CRYO*check* Hex LA is an integrated (screen and confirm) silica-based APTT assay for qualitative LA detection. The presence of LA in a plasma sample is confirmed by the correction of the APTT clot time (CT) of the sample upon the addition of a reaction mixture containing hexagonal phase PL. CRYO*check* Hex LA incorporates a pooled normal plasma (mixing test) and a heparin neutralizer.

Reagents

- **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.

FOR PRESCRIPTION USE ONLY

Storage, Preparation and Handling

When stored at -70 °C or below, CRYO*check* Hex LA is stable to the end of the month indicated on the product packaging.

Thaw one vial each of LA Start, LA Correct and LA APTT at 37 °C (±1 °C) in a waterbath for five minutes using the waterbath "floatie" thawing device (available separately). Thawing times are important and should be strictly adhered to. **The use of a dry bath or heating block for thawing is not recommended.** The use of a timer is recommended.

IMPORTANT: All reagents must be vortexed prior to use. After thawing, immediately vortex all capped reagent vials. Ensure each component is vortexed individually for five seconds, as insufficient vortexing of reagents may reduce the LA sensitivity of the assay. Ensure any large air bubbles produced as a result of vortexing are broken using a transfer pipette.

IMPORTANT: LA APTT must be stirred when in use. Add a micro magnetic stir bar to LA APTT prior to loading in a stirring position on the instrument.

Once thawed, CRYO*check* Hex LA may be used for eight hours on board the analyzer, or for four hours if capped in the original vials and maintained at room temperature (18 to 25 °C). If stored at room temperature, invert capped APTT vial five times prior to loading on board instrument. **Do NOT store CRYO*check* Hex LA reagents in a refrigerator (at 2 to 8 °C).**

NB: CRYO*check Hex LA components are lot-specific and should not be interchanged with other lot numbers.*

Availability

Product	Catalog #	Format
CRYO*check* Hex LA	HEXLA	LA Start: 2 x 1.5 mL (white cap) LA Correct: 2 x 1.5 mL (purple cap) LA APTT: 2 x 3.0 mL (black cap)
	HEXLA-7	LA Start: 2 x 1.5 mL (white cap) LA Correct: 2 x 1.5 mL (purple cap) LA APTT: 3 x 3.0 mL (black cap)
	HEXLA-M	LA Start: 2 x 1.0 mL (white cap) LA Correct: 2 x 1.0 mL (purple cap) LA APTT: 2 x 2.0 mL (black cap)

Instruments

Each lab should prepare the local instrument in accordance with the manufacturer's instructions for use. Protocols for coagulation instruments are available upon request.

Procedure

Material Provided

- CRYO*check* Hex LA (LA Start, LA Correct, LA APTT)

Materials Required but not Provided

- 0.025 M $CaCl_2$

- Waterbath capable of maintaining 37 °C (±1 °C)
- Floatie for thawing vials in waterbath
- Timer
- Vortex
- Coagulometer (IL ACL TOP series, Stago STA-R Evolution, Stago STA-R Max, Siemens BCS XP, and Sysmex CS Series)
- Micro magnetic stir bar
- Quality control materials (e.g. CRYO*check* Lupus Positive Control, CRYO*check* Weak Lupus Positive Control, CRYO*check* Lupus Negative Control)

Specimen Collection and Preparation

Collect and process blood in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines[6] and the guidelines for lupus anticoagulant detection[7]. Patient samples should be collected into 105 to 109 mmol/L trisodium citrate dihydrate anticoagulant (3.2 %) in a ratio of 9 parts blood to 1 part anticoagulant. Patient plasma is derived by double centrifugation at 1500 x g for 15 minutes in order to achieve platelet poor plasma (<10,000 platelets/µL) and should be tested within four hours of collection when maintained at room temperature. If samples are not to be tested within four hours, then plasma should be removed from the cells and frozen at ≤-70 °C for up to two months. Samples should not undergo more than one freeze-thaw cycle prior to testing.

Assay Procedure

1. Prepare CRYO*check* Hex LA reagents according to Storage, Preparation and Handling instructions above. **IMPORTANT: after thawing, ensure each component is vortexed individually for five seconds.** Ensure any large air bubbles produced as a result of vortexing are broken using a transfer pipette.
2. Prepare instrument according to the manufacturer's instructions for use. Protocols for coagulation instruments are available upon request.
3. **IMPORTANT: LA APTT must be stirred**. Uncap LA APTT vial, add a micro magnetic stir bar to LA APTT reagent and load in a stirring position on the instrument.
4. Uncap LA Start and LA Correct vials and load on the instrument.
5. Allow uncapped reagents to acclimate to the instrument temperature for at least 10 minutes before testing.
6. Load samples on the instrument.
7. Measure LA Start and LA Correct clot times using the appropriate instrument protocol.

Results and Interpretation

Calculate the difference in clot time (CT) in seconds ("delta correction") obtained with the LA Start and LA Correct reagents:

Delta Correction = CT LA Start - CT LA Correct

The delta correction result is then compared to an established assay cut-off[7,8]. A result greater than or equal to the established cut-off is considered LA positive, while a result less than the established cut-off is considered LA negative.

On normal samples, the CT of LA Correct may be longer than the CT of LA Start, resulting in a negative delta correction value. This is of no consequence, and the sample in question should be considered LA negative.

Refer to Pengo et al, 2009 and CLSI H60 for guidelines on lupus anticoagulant detection[5,7].

Quality Control

For all coagulation tests, the laboratory must include at least two levels of control for every eight hours of operation and any time a change in reagents occurs[9].

Assay controls are available for purchase separately. These include CRYO*check* Lupus Negative Control, CRYO*check* Weak Lupus Positive Control and CRYO*check* Lupus Positive Control. Refer to the Quality Control Certificate for results with CRYO*check* Hex LA specific to each lot of control.

Expected Values

A normal range study was performed in-house on two analyzers using normal samples (Analyzer A, n=137; Analyzer B, n=126) according to CLSI EP28: A3c[8]. Each sample was tested using three lots of CRYO*check* Hex LA. A pooled mean ±2 SD range was determined for delta correction results and is shown in the table below.

Normal Range	
Lower Range (s)	**Upper Range (s)**
-5.9	2.0

Cut-Off

The cut-off for the assay delta correction was determined using pooled data from the normal range study and calculating the mean + 4 SD, with the following results:

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

The results were obtained using specific lots of reagent. The cut-off is calculated as the mean of the delta correction + 4 SD, consistent with accepted methods for hexagonal phase neutralization tests. This method of establishing cut-off is different than that indicated for confirmatory tests in Pengo et al., 2009[5]. Each laboratory should verify its own cut-off, by testing the plasma of at least 20 normal individuals.

Performance Characteristics

All studies were performed using CRYO*check* Hex LA on Stago STA-R Evolution analyzer(s).

Method Comparison

A method comparison study was conducted to assess the efficacy of CRYO*check* Hex LA in the qualitative detection of LA relative to a comparator assay, Staclot LA. A total of 446 samples were included in the study: 124 known (previously characterized) LA positive samples, 75 normal (presumed LA negative) samples, 27 samples from individuals with other medical conditions including autoimmune disorders, and 220 LA target screening population samples. The study was conducted at one internal and three external sites. Each site performed the investigational device assay on their assigned portion of the samples using a single lot of CRYO*check* Hex LA. One external site, acting as the central laboratory, performed the comparator device testing on all 446 samples using the Staclot LA assay on a STA-R

Evolution. The data demonstrated positive percent agreement of 95.6 % (95 % CI, 91 to 98 %), negative percent agreement of 95.2 % (95 % CI, 92 to 97 %), and overall agreement of 95.3 % (95 % CI, 93 to 97 %) as summarized below.

		CRYO*check* Hex LA results		
		Negative	**Positive**	**Total**
Comparator device results	**Negative**	295	15	310
	Positive	6	130	136
	Total	301	145	446

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

Precision

An internal precision study was performed using three different lots of CRYO*check* Hex LA on a STA-R Evolution analyzer in accordance with CLSI EP05-A3[10]. Three lot numbers of CRYO*check* Hex LA were used to test three control plasmas and five plasmas with varying LA positivity, in duplicate, twice a day for 20 days. The results demonstrated a pooled precision of <5 % CV for LA Start and <8 % CV for LA Correct.

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

Reproducibility

Reproducibility studies were conducted at three sites (one internal and two external) using three lots of CRYO*check* Hex LA in accordance with CLSI EP05-A3[10]. The study tested three control plasmas as well as five plasmas with varying LA positivity. Each sample was tested in triplicate, twice a day for five days for each of the three lots of CRYO*check* Hex LA. The data across three sites demonstrated a pooled reproducibility of <5 % CV for LA Start and ≤8% CV for LA Correct as summarized in the reproducibility tables below.

Reproducibility: LA Start											
Sample	Mean Clot Time (s)	Within-Run (Repeatability)		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 (Repeatability)		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

Interferences

Interference studies were conducted according to CLSI EP07, 3rd ed. using a single lot of CRYO*check* Hex LA[11]. Patient plasma samples were spiked with possible interferents and 20 replicates were tested alongside 20 replicates of the corresponding blank matrix control. The following substances showed no interference up to the concentrations indicated:

Substance Tested	Test Concentration
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

- Dabigatran, rivaroxaban, and fondaparinux do not interfere with the interpretation of CRYO*check* Hex LA results but may increase the delta correction of LA positive samples.
- Elevated factor VIII activity (up to 180 %) does not interfere with CRYO*check* Hex LA.
- Elevated fibrinogen concentrations do not interfere with the interpretation of CRYO*check* Hex LA results but may increase the delta correction of LA positive samples.
- C-reactive protein does not interfere with the interpretation of CRYO*check* Hex LA results but at concentrations above 15 µg/mL may increase the delta correction of LA positive samples.
- Factor VIII inhibitor antibodies do not interfere with the interpretation of CRYO*check* Hex LA results, but at titers above 15 BU/mL may increase the delta correction of LA positive samples.

- Plasma samples with elevated INR (up to 4.5) do not interfere with the interpretation of CRYO*check* Hex LA results.
- High platelet counts (>10 000 platelets/µL) showed interference with CRYO*check* Hex LA results when compared with platelet poor (<10 000 platelets/µL, single centrifuged) or platelet free (double centrifuged) plasma samples from the same donors.
- Abnormally low factor II activities (below 50%) may interfere with the interpretation of CRYO*check* Hex LA, potentially resulting in false negative results for weak LA positive plasmas.
- Factor VII and factor IX deficiencies do not interfere with CRYO*check* Hex LA.
- Abnormally low factor X activities (below 50%) do not interfere with the interpretation of CRYO*check* Hex LA results but may increase the delta correction for LA positive samples.

Precautions/Warnings

Do not use the product if it is thawed upon receipt or if the vials appear cracked, or if upon thawing the product appears to have clotted. Transferring the material into another container other than siliconized glass could have a performance impact and is not recommended.

Any serious incident that has occurred in relation to the use of this device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or patient is established.

A Summary of the Safety and Performance of this device can be found on the EUDAMED database.

All blood products should be treated as potentially infectious. Source material from which this product was derived was found to be negative when tested in accordance with current required tests for transfusion-transmitted diseases. No known test method can offer assurance that products derived from human blood will not transmit infectious agents. Accordingly, these human blood-based products should be handled and discarded as recommended for any potentially infectious human specimen[12].

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Symbols Used

IVD	In vitro diagnostic medical device		Manufacturer
LOT	Batch code	EC REP	Authorized representative in the European Community / European Union
REF	Catalogue number	CH REP	Authorized representative in Switzerland
	Use by date	Rx Only	For prescription use only
	Upper temperature limit		Consult electronic instructions for use
	Biological risks		

CE 0123

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