PrecisionBioLogic

CRYO*check*™ IVD

dRVTT CONFIRMATORY REAGENT

LA SURE™

Intended Use

CRYO*check* LA Sure is a dilute Russell's Viper Venom Time (dRVVT) reagent intended to confirm the presence of lupus anticoagulants (LA) in citrated human plasma.

Summary and Principle

LA are autoantibodies of the IgG and IgM types that are specifically directed against negatively charged phospholipids, such as phosphatidylinositols and phosphatidylserines, or complexes of phospholipids with either β2-glycoprotein-1 or clotting factors such as prothrombin. They occur in various clinical conditions, especially autoimmune diseases[1]. LA have traditionally been detected using phospholipid sensitive *in vitro* clotting tests, such as the activated partial thromboplastin time (APTT), kaolin clotting time (KVT), and dRVVT[2]. The dRVVT was introduced in 1986 and showed improved sensitivity to LA over the APTT partially due to a reduced phospholipid concentration[3].

LA are usually indicated by a prolonged clotting time result that is not corrected by mixing patient plasma with normal plasma. The correction of a prolonged result by the addition of phospholipids to the plasma is a more specific characteristic of LA[3].

LA prolong phospholipid-sensitive clotting tests; however, they are paradoxically associated with thrombotic problems[4]. LA are a common cause of unexplained prolonged APTTs and need to be carefully distinguished from idiopathic antibodies against factor VIII associated with bleeding.

LA are now considered to be a significant risk factor in patients with otherwise unexplained thrombosis and are often present in women who have recurrent fetal loss[4, 5]. They are also associated with a variety of hemostatic problems such as thrombocytopenia and neurological disorders[6].

Russell's viper venom directly activates factor X, bypassing factor VII of the extrinsic pathway and the contact and antihemophilic factors of the intrinsic pathway. Therefore, dRVVT tests are more specific for LA than APTTs as they are not affected by contact factor abnormalities or by factor VIII deficiencies or antibodies[3]. Excess phospholipid is present in CRYO*check* LA Sure to neutralize LA.

Reagents

CRYO*check* LA Sure contains Russell's viper venom, phospholipids, antiheparin agents, calcium, buffers, stabilizers, sodium azide, and red dye (<0.001%).

Sodium azide may react with lead and copper plumbing to form highly explosive metal compounds. Ensure proper disposal of reagent according to federal, state, and local regulations.

Storage and Handling

When stored at -40 to -80 °C, CRYO*check* LA Sure is stable to the end of the month indicated on the product packaging.

Thaw each vial at 37 °C (± 1 °C) in a waterbath. **The use of a dry bath or heating block for thawing is not recommended**. Thaw times are important and should be strictly adhered to. The use of a timer is recommended. Refer to the Thawing Table for recommended thawing times based on aliquot size. Allow thawed plasma to acclimate to room temperature (18 to 25 °C) and invert gently prior to use.

Thawing Table	
Aliquot Size	**37 °C (± 1 °C) Waterbath**
1.0 mL	4 minutes

CRYO*check* LA Sure may be used for up to 48 hours after thawing, if capped in the original vial and maintained at 2 to 8 °C. Allow refrigerated reagent to acclimate to room temperature (18 to 25 °C) and invert gently prior to use. **Thawed material may be refrozen once and stored at -20 °C for up to one month.**

Availability

Product	Catalog #	Format
CRYO*check* LA Sure	SUR-10	25 vials x 1.0 mL

Instruments

Each lab should prepare the local instrument in accordance with the manufacturer's instructions for use.

Procedure

After thawing and preparing CRYO*check* LA Sure, use in accordance with established laboratory procedures.

Materials Provided

- CRYO*check* LA Sure

Materials Required but not Provided

- Waterbath capable of maintaining 37 °C (± 1 °C)
- Coagulation instrument or assay system
- 12 mm x 75 mm glass test tubes
- CRYO*check* LA Check
- Quality control material (e.g. CRYO*check* Lupus Positive Control, CRYO*check* Weak Lupus Positive Control)
- Plastic disposable pipettes

- Volumetric pipette
- Timer
- Stopwatch

Specimen collection and Preparation

Patient samples should be collected into 105 - 109 mmol/L sodium citrate dihydrate anticoagulant (3.2%) in a ratio of 9 parts blood to 1 part anticoagulant. Patient plasma is derived by 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 2 to 4 °C in accordance with CLSI guidelines[7]. If samples are to be frozen before testing, plasmas should be centrifuged a second time, and stored at -20 °c or below.

Manual Method – Tilt Tube

1. In a 37 °C (± 1 °C) waterbath, prewarm a slight excess of CRYO*check* LA Sure allowing 200 µL per test.
2. Dispense 200 µL of test plasma into a test tube and warm for one minute at 37 °C (± 1 °C).
3. Add 200 µL of prewarmed CRYO*check* LA Sure to the plasma and simultaneously initiate the clot timer. Record clotting times in seconds.
4. Repeat for duplicate test values and report the average of these as the result.

Automated Methods

Reagent preparation instructions and instrument settings for variety of analyzers are available upon request from Precision BioLogic.

Results

If the CRYO*check* LA Sure test is indicated in the event that the CRYO*check* LA Check™ test result is prolonged (i.e. greater than three standard deviations (SD) above the laboratory-established normal reference mean). Perform the CRYO*check* LA Sure test and calculate the ratio result by dividing the CRYO*check* LA Check clotting time by the CRYO*check* LA Sure clotting time as follows:

$$\text{LA Check/LA Sure ratio} = \frac{\text{LA Check clotting time (sec.)}}{\text{LA Sure clotting time (sec.)}}$$

If the LA Check/LA Sure ratio is greater than the upper limit of the established 3 SD normal reference range, the test is positive for LA. If the LA Check/LA Sure ratio is less than or equal to the upper limit of the 3 SD normal reference range, then LA is absent.

In accordance with the SSC Subcommittee for the Standardization of LA guidelines[8], testing should be performed and results interpreted in the context of a multi-test algorithm performed on the same sample using tests based on different principles, since no single assay can guarantee, with certainty, that LA is present or absent.

Quality Control

Each laboratory should establish its own quality control (QC) ranges using acceptable statistical methods. These QC ranges may then be used to monitor and validate the integrity of the test system[9].

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[10].

Commercial lyophilized quality control plasmas containing unspecified levels of citrate and platelets are not recommended as they may give erroneous results[11, 12].

Limitations of the Procedures

Patients with deficiencies of factors II, V, or X or patients on anti- vitamin K therapy may exhibit prolonged CRYO*check* LA Sure times. Typically, the final ratio (LA check/LA Sure) should be normal unless LA is present. However, false-positive results have been known to occur with patients on anti-vitamin K therapy. This may be overcome by mixing the patient's plasma sample with pooled normal plasma and repeating the CRYO*check* LA Check screening and CRYO*check* LA Sure confirmatory tests[13]. CRYO*check* LA Check results on plasmas subjected to mixing studies should be interpreted with care as published data has demonstrated that further dilution of weak inhibitors can produce false-negative results[14].

CRYO*check* LA Sure is unaffected by heparin levels up to 1.0 unit/mL. Plasmas containing heparin levels greater than 1.0 unit/mL may give false-positive results and should not be tested with this reagent.

Plasma samples with visible hemolysis should not be used due to possible clotting factor activation and endpoint measurement interference[7]. Icteric or lipemic samples may also interfere with endpoint determination on some optical instruments[7].

Expected Values

In a study of 20 healthy males and females using a Diagnostica Stago ST4® analyzer, an LA Check/LA Sure ratio normal reference range (3 SD confidence interval) of 0.72 - 1.26 was established. These values should be used as a guide only. Each laboratory should establish its own normal reference range.

Performance Characteristics

In precision studies over 48 hours at 2 to 8 °C with CRYO*check* Lupus Positive Control plasma on a Diagnostica Stago ST4® analyzer, CRYO*check* LA Sure exhibited an overall coefficient of variation (CV) of 4.03%.

An R^2=0.991 was derived in a correlation study using Gradipore LA confirm™ dRVVT confirmatory test involving OAT patient plasmas (n=15), known LA positive samples (n=12), and plasmas with depleted levels of factors II (n=3), V (n=3), and X (n=3). An R^2=0.956 was derived for the corresponding ratios.

Bibliography

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Symbols Used

IVD	In vitro diagnostic medical device		Biological risks
LOT	Batch code		Manufacturer
REF	Catalogue number	EC REP	Authorized representative in the European Community / European Union
	Use by Date	**Rx ONLY**	For prescription use only
	Temperature limit		Consult electronic instructions for use

EC REP European Authorized Representative (Regulatory affairs only)
Emergo Europe— Westervoortsedijk 60, 6827 AT Arnhem, The Netherlands

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