

HEMOCLOT™ LA-S

Ref CK090K-RUO (6 x 1 mL)

HEMOCLOT™ LA-C

Ref CK091K-RUO (6 x 1 mL)

Detection of Lupus Anticoagulant by clotting assay

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

English, last revision: 07-2016

INTENDED USE:

Diluted Russell's Viper Venom Test (dRVVT) are simplified reagents for the specific detection of lupus anticoagulant (LA), using a manual, semi automated or automated clotting method.

- **HEMOCLOT™ LA-S:** Simplified dRVV reagent to screen for the presence of Lupus Anticoagulants.
- **HEMOCLOT™ LA-C:** dRVV reagent with high Phospholipid content to confirm the presence of Lupus Anticoagulants.

This kit is for research use only and should not be used for patient diagnosis or treatment.

ASSAY PRINCIPLE:

In the presence of calcium, Factor X present in the tested sample is directly activated into FXa by RVV. In the presence of Factor V, calcium and phospholipids, FXa activates prothrombin to thrombin which rapidly clots fibrinogen. Consequently, contact factor abnormalities, FVII, FVIII and FIX deficiencies or inhibitors are not expected to affect the results.

HEMOCLOT™ LA-S is performed with low concentration of phospholipids, thus LA-S clotting time is expected to be prolonged in the presence of LA.

HEMOCLOT™ LA-C contains a higher phospholipid concentration, expected to neutralize LA present in the test plasma, and thus shorten clotting time.

An heparin neutralizing substance is also included (no significant Heparin interference up to 1 IU/mL in the tested sample). Therefore, HEMOCLOT™ LA-S and HEMOCLOT™ LA-C are more specific tests than APTT for the evaluation of LA.

REAGENTS:

CK090K: HEMOCLOT™ LA-S (lyophilized with green dyes; contains RVV, phospholipids, an heparin neutralizing substance, calcium, stabilizers, and preservative).
6 vials of 1 mL.

CK091K: HEMOCLOT™ LA-C (lyophilized with pink dyes; contains RVV, phospholipids, an heparin neutralizing substance, calcium, stabilizers, and preservative).
6 vials of 1 mL.

CAUTIONS AND WARNINGS:

- Any product of biological origin must then be handled with all the required cautions, as being potentially infectious.
- The disposal of waste materials must be carried out according to current local regulations
- Use only reagents from kits with the same lot number. Do not mix reagents from kits with different lots when running the assay; they are optimized for each lot of kits.
- Reagents must be handled with care, in order to avoid any contamination during use. Take care to limit as much as possible any evaporation of the reagents during use, by limiting the liquid-air surface exchange. Evaporation reduces reagent stability on instrument board.
- In order to improve stability, reagents must be closed with their original screw cap following each use.
- Stability studies for 3 weeks at 30°C show that the reagents can be shipped at room temperature for a short period without damage.
- Incubating the reconstituted vials at room temperature allows stabilizing the reagents, and obtaining a homogeneous reactivity.
- It is recommended to homogenize each vial before use, in order to have a good reproducibility, all the time.
- For in vitro use.

PREPARATION AND STABILITY OF REAGENTS:

Vials are closed under vacuum. Remove carefully the stopper, in order to avoid any loss of powder when opening the vials.

Reconstitute each vial with exactly 1 mL of distilled water, shake thoroughly for complete homogenization, let the reagent stabilize for 30 min at room temperature (18-25°C); while shaking the vial from time to time.

Homogenize before each use.

Stability of reagent, provided that any contamination or evaporation is avoided, kept in its original vial or in a closed plastic microcentrifuge tube:

- 48 hours at 2-8°C.
- 24 hours at room temperature (18-25 °C).
- 1 month frozen at -20°C or below*

*Thaw once as rapidly as possible at 37°C, adapt duration to the volume of reagent. The stability of the thawed reagent should be verified in the working conditions of the user laboratory.

STORAGE CONDITIONS:

Unopened reagents must be stored at 2-8°C, in their original packaging box. They are then usable until the expiration date printed on the label.

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water, preferentially sterile
- Suitable quality controls normal and abnormal for LA, e.g.:

| Controls | BIOPHEN® Normal Control Plasma | LA Control Plasma |
|-----------|--------------------------------|-------------------|
| Reference | 223201-RUO | SC081K-RUO |

Materials:

- Electromagnetic Water-bath, semi-automatic or automatic instrument for clotting assays
- Stopwatch; Calibrated pipettes; test tubes.

SPECIMEN COLLECTION:

Preparation and storage of specimens must be performed according to the current local regulations.

Specimens:

Human plasma obtained from trisodium citrate anticoagulated blood.

Collection:

Blood (9 vol.) must be collected on trisodium citrate anticoagulant (1 vol.) (0.109M) in order to avoid any activation, through a net venipuncture. The first tube must be discarded.

Centrifugation:

Within 2 hours, use a validated method in the laboratory to obtain a platelet-poor plasma, e.g., a minimum of 15 minutes at 2500 g at room temperature (18-25°C) and plasma must be decanted into a plastic tube.

Storage of plasma¹:

- 4 hours at room temperature (18-25°C)
- 1 month at -20°C.
- 18 months at -70°C.

Frozen plasma specimens should be rapidly thawed at 37°C, then gently mixed and tested immediately. Resuspend any precipitation by thorough mixing immediately after thawing and before testing.

TEST PROCEDURE:

It is recommended to use HEMOCLOT™ LA-S and LA-C together, and to perform all testing in duplicate.

The HEMOCLOT™ LA-S and LA-C kit is a clotting method, manual or automated. The assay is performed at **37±1°C**, and the clotting time, triggered by addition of reagent, is measured

Automated methods:

Applications to the various analyzers are available upon request. **Refer to each specific applications and specific cautions for each instrument**

Assay method:

1. Reconstitute the controls using the specific package inserts.
2. The samples should be tested **undiluted**.

Manual method:

Principle: detect clotting time by mechanic or optic method. The test is performed at 37±1°C.

Prewarm to 37°C appropriate volume of reagent (0.2mL per test).
Into a small test tube, introduce:

| | Test tube |
|--|-----------|
| Plasma to test | 200 µL |
| Incubate at 37°C, for 1-2 minutes, then introduce (starting the stop-watch): | |
| Reagent preincubated at 37°C | 200 µL |
| Record the exact clotting time (CT, sec) | |

QUALITY CONTROL:

Using quality controls, allows validating the homogeneous reactivity from run to run, when using a same lot of reagents.

Quality control must be included in each series, as per good laboratory practice, in order to validate generated results. A new verification of the normal range must be carried out at least for each new lot of reagents or, after each important analyzer's maintenance, or when quality controls values are measured outside the acceptance range determined for the method. The clotting time obtained with the same reagent lot can vary slightly according to the instrument used and the clot detection sensitivity.

Each laboratory should establish and verify its own normal range, target values, acceptance ranges and expected performances, according to the lots, the instruments and protocols used.

RESULTS:

- **HEMOCLLOT™ LA-S :**

The obtained CT for the sample must be compared with that of the reference normal range for the laboratory (normal range ideally established from individual normal plasmas; alternatively, reference pool of normal human plasma for which the result must be in this range and tested in each series).

Results can be reported as a ratio:

LA-S ratio = Sample LA-S (CT, sec) / Mean of normal range for LA-S (CT, sec).

If LA-S result is abnormally prolonged (e.g. **CT > Mean+2SD** compared to reference normal range for the laboratory), confirm the presence of LA with LA-C.

- **HEMOCLLOT™ LA-C:**

Results can be reported as a ratio:

LA-C ratio = Sample LA-C (CT, sec) / Mean of normal range for LA-C (CT, sec).

- **Normalized LA ratio**

Establish Normalized LA ratio = LA-S ratio / LA-C ratio.

- **Mixing studies :**

To confirm presence of LA, mixing studies may be used, as 50:50 mixture of test plasma and normal plasma.

Interpretation:

Plasmas which contain lupus anticoagulant usually give a prolonged result with LA-S and a shorter result with LA-C reagent.

As an indication:

- **Normalized ratio ≥ 1.20** indicates LA presence (and increasing presence with increased ratio).
- **Normalized ratio < 1.20** (or borderline) and LA-S and LA-C Clotting times prolonged: results should be confirmed by additional investigation as mixing studies.
- Mixing normal plasma with the test plasma (50:50 mixture) replaces the factors potentially lacking in the test plasma. If the mixing test is still prolonged, an anticoagulant or other inhibitor is present in the test plasma.

The results obtained should be for research purposes only and not used for patient diagnosis or treatment.

LIMITATIONS:

- Icteric, lipemic, hemolyzed samples or samples with an abnormal aspect (e.g. partial coagulation) may give false results and should be interpreted with caution. In spiking study on CS-5100, there was no significant effect up to 25mg/dL Bilirubin C, 250 mg/dL Intralipid and 500 mg/dL Hemoglobin.
- Both HEMOCLOT™ LA-S and LA-C reagents contain an heparin neutralizing substance which neutralizes up to 1 IU/ml Heparin.
- Other new antithrombotics agents may have unexpected effects on test and ratio.
- In an external study, results were less influenced by low coagulation activity of warfarin and rivaroxaban than other commercial dRVV Test Screen devices.
- Commercially available normal quality control plasmas with unspecified citrate and platelet levels are not recommended for use in mixing studies.
- An additional investigation should be conducted to determine the origin of each unexpected or abnormal result. At least 2 screening assays with different properties and sensitivity should be performed before the possibility of LA is excluded. Borderline results should be considered in line with other markers for APS such as anticardiolipin or anti-B2GPI Elisas.
- For comparative studies it is recommended to test HEMOCLOT™ LA-S and LA-C at the same time.
- In order to get the optimal performances of the assay, the procedural instructions must be strictly respected.
- Any reagent presenting an unusual aspect or a contamination sign must be rejected.
- Any plasma containing a coagulum or contamination must be rejected.

PERFORMANCES:

- Example of intra- and inter-assay reproducibility data obtained on normal and pathological controls using Stago STA-R instrument:

| Control | Intra assay | | | Inter assay | | |
|-------------------|-------------|--------------|-------------|-------------|--------------|-------------|
| | Normal | Pathological | LA-C | Normal | Pathological | LA-C |
| Test | LA-S | LA-S | LA-C | LA-S | LA-S | LA-C |
| n | 30 | 30 | 30 | 14 | 14 | 14 |
| Mean (sec) | 37.6 | 89.4 | 42.2 | 36.6 | 89.2 | 41.5 |
| SD (sec) | 0.4 | 2.0 | 0.5 | 0.7 | 2.4 | 0.4 |
| CV% | 1.1 | 2.2 | 1.1 | 1.8 | 2.7 | 1.0 |

- In an external study, the results showed similar distribution on 59 dRVVT positive samples and 62 normal samples compared to commercial dRVV Screen and Confirm devices.

REFERENCES:

1. Woodhams B, Girardot O, Blanco M-J, Colesse G, Gourmelin Y. Stability of coagulation proteins in frozen plasma. Blood coagulation and Fibrinolysis. 2001. Vol 12, No 4. 229-236.

SYMBOLS:

Used symbols and signs listed in the ISO standard 15223-1.