HEMOCLOT™ LA-S

REF CK090K-RUO R 6 x 1 mL

REF CK094K-RUO R 12 x 2 mL

HEMOCLOT™ LA-C

REF CK091K-RUO R 6 x 1 mL

Clotting method for the detection of Lupus Anticoagulant.

FOR RESEARCH USE ONLY.

DO NOT USE IN DIAGNOSTIC PROCEDURES.

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INTENDED USE:

HEMOCLOT™ LA-S and HEMOCLOT™ LA-C kits are diluted Russell's Viper Venom Test (dRVVT) simplified reagents for the specific *in vitro* qualitative detection of lupus anticoagulant (LA), by clotting method on human citrated plasma, using manual or automated 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 must not be used for patient diagnosis or treatment.

SUMMARY AND EXPLANATION:

Technical:

Lupus anticoagulants are antibodies directed against negatively charged phospholipids/protein complexes, therefore yielding prolonged clotting times in phospholipid dependent tests. LA-S (low phospholipids) clotting time (CT) is expected to be prolonged in the presence of LA. LA-C (high phospholipids) is expected to neutralize LA and shorten CT.

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 activated partial thromboplastin time (APTT) for the evaluation of LA.

REAGENTS:

R **HEMOCLOT™ LA-S,** lyophilized with green dyes. Contains RVV, phospholipids, an heparin neutralizing substance, calcium and stabilizers.
REF CK090K-RUO ➔ R 6 vials of 1 mL
REF CK094K-RUO ➔ R 12 vials of 2 mL

R **HEMOCLOT™ LA-C**, lyophilized with pink dyes. Contains RVV, phospholipids, an heparin neutralizing substance, calcium and stabilizers.
REF CK091K-RUO ➔ R 6 vials of 1 mL

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of animal origin. Users of reagents of these types must exercise extreme care in full compliance with safety precautions in the manipulation of these biological materials as if they were infectious.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits.
- Aging studies show that the reagents can be shipped at room temperature without degradation.
- This device of *in vitro* use is intended for professional use in the laboratory.

REAGENT PREPARATION:

Gently remove the freeze-drying stopper, to avoid any product loss when opening the vial.

R Reconstitute the contents of each vial with exactly:
REF CK090K-RUO / CK091K-RUO ➔ 1 mL of distilled water.
REF CK094K-RUO ➔ 2 mL of distilled water.

Shake vigorously until complete dissolution while avoiding formation of foam and load it directly on the analyzer following application guide instruction.
For manual method, allow to stabilize for 30 minutes at room temperature (18-25°C), homogenize before use.

STORAGE AND STABILITY:

Unopened reagents should be stored at 2-8°C in their original packaging. Under these conditions, they can be used until the expiry date printed on the kit.

R Reagent stability after reconstitution, free from any contamination or evaporation, and stored closed, is of:
REF CK090K-RUO / CK091K-RUO / CK094K-RUO:

- **7 days** at 2-8°C.
- **24 hours** at room temperature (18-25°C).
- **2 months** frozen at -20°C or less*
- **Stability on board of the analyzer: see the specific application.**

*Thaw only once, as rapidly as possible at 37°C and use immediately.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:

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

Product Name	Reference
BIOPHEN™ Normal Control Plasma	223201-RUO
LA Control Plasma	SC081K-RUO / SC082K-RUO / SC083K-RUO

Also refer to the specific application guide of the analyzer used.

Materials:

- Electromagnetic water-bath, semi-automatic or automatic analyzer for clotting assays.
- Stopwatch; Calibrated pipettes; silicon glass or plastic test tubes.

SPECIMEN COLLECTION AND PREPARATION:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture. Discard the first tube.
Specimens should be prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5[1] guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references[1,2].

PROCEDURE:

It is recommended to use HEMOCLOT™ LA-S and LA-C together.
The kit can be used in manual or automated method. Perform the test at **37°C±1°C** and the clotting time, triggered by addition of reagent, is measured.

Assay method:

1. Reconstitute the controls using the specific package inserts.
2. The samples should be tested **undiluted**.
3. Principle: detect clotting time by mechanical or optical method.

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

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

If a reaction volume other than that specified above is required for the method used, the ratio of volumes must be strictly observed to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

For an automated method, application guides are available on request. See specific application guide and specific precautions for each analyzer.

QUALITY CONTROL:

The use of quality controls serves to validate method compliance, along with between-test assay homogeneity for a given batch of reagents.
Include the quality controls with each series, as per good laboratory practice, in order to validate the test. 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 must define its acceptance ranges and verify the expected performance in its analytical system.

RESULTS:

Tests and results should be interpreted with regards to recognized recommendations or guidelines (for the United States, see the CLSI H60-A[3]).

- **HEMOCLOT™ LA-S :**

The obtained CT for the sample must be compared with that of the reference normal range for the laboratory (refer to appropriate guideline; 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 (eg **CT > Mean+2SD** compared to reference normal range for the laboratory), confirm the presence of LA with LA-C.

- **HEMOCLOT™ 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 use only and must not be used for patient diagnosis or treatment.

LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully.
- Any reagent presenting an unusual appearance or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
- It is not recommended to perform the LA detection on sample containing heparin. However, 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 under 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 such as anticardiolipin or anti-B_2GPI Elisa.
- For comparative studies it is recommended to test HEMOCLOT™ LA-S and LA-C at the same time.
- Icteric, lipemic, hemolyzed samples or samples with an abnormal aspect (e.g. partial coagulation) may give false results and should be interpreted with caution.

PERFORMANCES:

- 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.
- Performance studies were conducted internally on Sysmex CS-5100. Performance was assessed using laboratory controls over a 6-day period, 2 series per day and 2 repetitions within each series for a control level. The following results were obtained:

	Intra-assay			Inter-assays		
Specimen	**Normal**	**Pathological**		**Normal**	**Pathological**	
Test	**LA-S**	**LA-S**	**LA-C**	**LA-S**	**LA-S**	**LA-C**
n	30	30	30	24	24	24
Mean (sec)	32.1	84.2	37.0	31.6	84.4	37.0
SD (sec)	0.19	1.05	0.26	0.66	1.16	0.33
CV%	0.59	1.24	0.71	2.10	1.38	0.89

- Correlation with reference method (Siemens LA1/LA2 vs HEMOCLOT™ LA-S/LA-C on Sysmex CS-5100) :
  n = 50 y = 1.08x – 0.08 r = 0.930
- Interferences:
  No interference, on the analyzer Sysmex CS-5100 was observed with the molecules and up to following concentrations:

Hemoglobin	Bilirubin (conjugated)	Intralipids	Heparins (UFH/LMWH)
500 mg/dL	25 mg/dL	250 mg/dL	1 IU/mL

REFERENCES:

1. CLSI Document H21-A5: "Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline". 2008.
2. Woodhams B. *et al.* Stability of coagulation proteins in frozen plasma. Blood coagulation and Fibrinolysis. 2001.
3. CLSI Document H60-A: "Laboratory Testing for the Lupus Anticoagulant; Approved Guideline". 2014

SYMBOLS:

Symbols used and signs listed in the ISO 15223-1 standard, see Symbol definitions document.

Changes compared to the previous version.