HEMOCLOT™ LA-S
REF CK090K (6 x 1 mL)
HEMOCLOT™ LA-C
REF CK091K (6 x 1 mL)

Detection of Lupus Anticoagulant by clotting assay

HYPHEN BioMed
Sales and Support:
CoaChrom Diagnostica GmbH
www.coachrom.com | info@coachrom.com
Tel: +43-1-236 222 1 | Fax: +43-1-236 222 111
Toll-free contact for Germany:
Tel: 0800-24 66 33-0 | Fax: 0800-24 66 33-3

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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.

SUMMARY AND EXPLANATION:
Lupus anticoagulants are antibodies directed against negatively charged phospholipids/protein complexes, therefore yielding prolonged clotting times in phospholipid dependent tests. They are associated with numerous clinical states including eg lupus, autoimmune diseases, thrombosis, fœtal loss and must usually be confirmed from multiple assays.

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:
R1: HEMOCLOT™ LA-S (lyophilized with green dyes; contains RVV, phospholipids, an heparin neutralizing substance, calcium, stabilizers, and preservative).
6 vials of 1 mL.

R1: 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:
- Biological products must be handled with all necessary precautions and considered as being potentially infectious.
- Waste should be disposed of in accordance with applicable 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.
- Handle the reagents with care to avoid contamination during use. If possible, avoid reagent evaporation during use by limiting the liquid-air exchange surface. Evaporation reduces the reagent's stability in the analyzer.
- To preserve reagent stability, seal the vials after use with their respective caps.
- Aging studies, conducted over a 3-week period at 30°C, show that the reagents can be shipped at room temperature over a short period of time, without degradation.
- 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* diagnostic use.

PREPARATION AND STABILITY OF REAGENTS:
The reagents are lyophilized under vacuum in their vials. To avoid any product loss when opening the vial of lyophilized reagents, gently remove the freeze-drying stopper.

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.
Reagent stability after reconstitution, excluding any contamination or evaporation, and stored in the original vial, is of:
- **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 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.

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	SC081K SC082K SC083K

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 (In the USA, refer to CLSI Document H21-A5 for further instructions on specimen collection, handling and storage).
- Specimens:

Human plasma obtained from trisodium citrate anticoagulated blood.
- Collection:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M) by clean venipuncture. Discard the first tube.
- 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 plasma:
  - 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 for various analyzers are available on request. **See the specific application and specific precautions for each analyzer.**

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:

The use of quality controls serves to validate method compliance, along with between-test assay homogeneity for a given batch 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:

Tests and results should be interpreted with regards to recognized recommendations or guidelines, such as CLSI guideline H60, and according to the patient's clinical and biological states.

- **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.

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.
- It is not recommended to perform the LA detection on sample from patient receiving 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 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.
- Test result should always be interpreted according to the patient's clinical and biological states and other findings.
- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully. The laboratory is responsible for validating any changes made to these instructions for use.
- Any reagent presenting an unusual appearance or showing signs of contamination must be rejected.
- Any plasma displaying a coagulum or showing signs of contamination must be rejected.

EXPECTED VALUE:

LA are absent from normal human plasmas.
As an indication, for normal plasma, CT value is usually expected **< 45 sec**, and normalized ratio **< 1.20.**
Each laboratory should determine its own normal range for each combination of lot, instrument and protocol used.

PERFORMANCES:

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

	Intra assay			**Inter assay**		
Control	**Normal**	**Pathological**		**Normal**	**Pathological**	
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.

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SYMBOLS:

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

Changes compared to the previous version.