Lupus Anticoagulant Test

For research use only

GB

REF 5343005 Lupus Anticoagulant Test

Symbols key			
	Manufacturer		Expiry date
	Storage temperature		Consult instructions for use
AQUA	Distilled water		Determinations
BUF	Reaction buffer	LOT	Lot
CAL	Calibrator	MTP	Microtiter plate
CONJ	Conjugate	REF	Catalogue number
CONT	Control	RTU	Ready to use
DIL	Dilute or dissolve in	STOP	Stop solution
INC	Incubation buffer	SUB	Substrate
RUO	For resarch use only	WASH	Washing solution concentrate

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PRODUCT DESCRIPTION

INTENDEND USE

Reagent Kit for determination of Lupus Inhibitor.

Lupus inhibitors directed against platelet factor III (phospholipids) can be detected by means of a modified activated partial thromboplastin time (aPTT) (3). In the test an activator of SiO_2/Al_2O_3 suspension and two phospholipid concentrations are used. The modified aPTT's are performed with normal plasma (free from platelet contamination), with sample plasma and with a mixture of both plasmas. From the shape of the "aPTT against plasma concentration" curve the probability of the presence of lupus inhibitor can be assessed.

COMPOSITION

Lupus Anticoagulant Test for 6 determinations contains:

mL	Reagent	other indications
1 x 3	Reagent A	Phospholipid
2 x 3	Reagent B	SiO_2/Al_2O_3-Suspension
2 x 2	Reagent C	Platelet poor normal plasma
1 x 1	Lupus Inhibitor plasma Low	Positive control

Lupus Inhibitor Plasma Low is prepared from selected lupus inhibitor plasma positive in most Lupus Inhibitor screening and confirmatory assays. Lupus Inhibitor Plasma contains stabilizers but no bactericide additives.

MATERIAL REQUIRED (not supplied with the kit)

- Pipette
- Distilled water
- Buffer

REF 5279025 $CaCl_2$ 50 mmol/L solution 100 mL

WARNING AND PRECAUTIONS

- For research use only
- All blood and plasma samples and products have to be regarded as potentially infectious and handled with appropriate care and in compliance with the biosafety regulations in force and must be disposed of in the same way as hospital waste.
- Each single donor plasma and each lot of Plasma are tested and found negative for HbSAg, HIV 1/2 Ab and HCV Ab. However, universal precautions (treating all human source materials as if potentially infectious) should be exercised.

STABILITY AND STORAGE

The expiry date printed on the labels applies to storage of the unopened bottles at +2…8°C.

Stability after reconstitution:

RT*	+12°C (Ceveron® alpha)
1 day	1 day

Upon storage, caps should be screwed tightly.

*=room temperature: +18…25°C

TEST PROCEDURE

PREPARATION OF PLASMA SAMPLES

Plasma preparation:
Mix 9 volumes of venous blood and 1 volume of sodium citrate (0.11 mol/L) and centrifuge for 15 minutes at a RCF of 2500 (corresponding to DIN 58905). Transfer the plasma to a clean plastic tube and centrifuge for a further 15 minutes. The plasma should be stored at room temperature and be tested within 4 hours. Shelf life at –20°C: 6 months.

PREPARATION OF REAGENTS

Phospholipid concentration 1:
Reagent A is dissolved with one well shaked vial of *Reagent B* by mixing gently. Allow to stand for 10 minutes at room temperature.
Phospholipid concentration 2:
100 µL of reconstituted and shaked *Reagent A* are further diluted in the second vial of well shaked *Reagent B*. Reagent C is reconstituted in 2 mL of distilled water.

PERFORMANCE OF THE TEST

CEVERON

Technoclone provides Application sheets for Ceveron®. The application sheets contain analyser/assay specific handling and performance information which may differ from that provided in these instruction for use. In this case the information contained in the application sheets supersedes the information in this instruction for use. Please consult the instruction manual of the Ceveron®.

MANUAL

The clotting time for the sample plasma, normal plasma and mixture of both (1+1) is determined using each of the 2 phospholipid concentrations.
The $CaCl_2$-solution 50 mmol/L is preincubated at +37°C.

Pipetting scheme:

200 µL of sample (test plasma/mixture/Reagent C) 100 µL phospholipid (concentration 1/2) ---------- 3 minutes at +37°C ---------- 100 µL $CaCl_2$-solution (50 mmol/L, +37°C)
The coagulation end point is determined in the usual way

When using ball coagulometers (e.g. Ceveron® manual instruments) reagent B or phospholipid (concentration 1+2) have to be stirred for 15 min.

Before each use the phospholipid concentration 1 and 2 must be shaked-up.

ANALYSES RESULTS

CALCULATION OF THE RESULTS

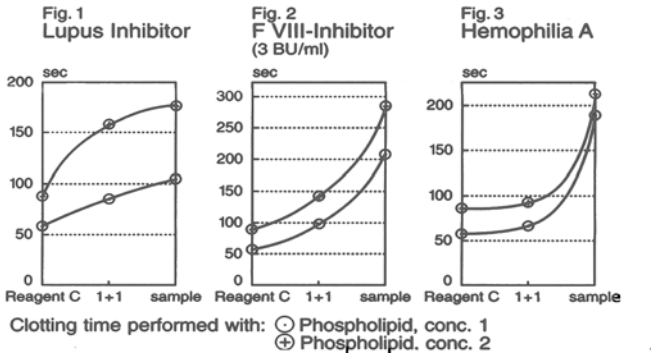
The coagulation times are evaluated both on the basis of the graph shape (1) and a numerical index (LCA-index) (2).
To produce the graph the various coagulation times of the samples determined are plotted on graph paper.
y-axis: coagulation time in sec.;
x-axis: Reagent C (0%), mixture (50%), test plasma (100%)
In a typical sample with lupus anticoagulant the resulting curve is shown in figure 1. The presence of F VIII-inhibitor is shown in figure 2, and of F VIII deficiency in figure 3.

In addition the lupus anticoagulant can be determined numerically using the LCA index which is calculated by the formula:

$$\text{LCA index} = \frac{(b-c)}{a} \times 100$$

a = clotting time of sample plasma
b = clotting time of sample plasma mixed with Reagent C
c = clotting time of Reagent C

The presence of lupus inhibitor is characterised by the LCA index value of above 15. A convex curve means an additional confirmation of the test results. In most cases the clotting time produced with phospholipid concentration 2 are used but phospholipid concentration 1 can be used if the clotting time of the 1+1-mixture is greater than 100 seconds.



QUALITY CONTROL

To control the assay the Lupus Inhibitor Plasma Low is tested in the same way as sample plasma. The LCA Index found should be compared to the value indicated on the vial label of the Lupus Inhibitor Plasma Low.
If the results obtained are <15, avoid measuring samples until the problem is solved.

STANDARDISATION

No international calibrator is available for the standardization of Lupus Anticoagulant tests. The Lupus Anticoagulant Test documentation is based on studies testing plasma samples from normal subjects and plasma samples with lupus inhibitor. A house reference batch is established in order to avoid batch-to-batch variation.

PRECISION

Reproducibility was determined and following results were obtained :

	Lupus Inhibitor Plasma		Lupus Inhibitor Plasma Low		Platelet Poor Plasma	
	Intra-assay (CV%)	Inter-assay (CV%)	Intra-assay (CV%)	Inter-assay (CV%)	Intra-assay (CV%)	Inter-assay (CV%)
Lupus Test PL 2	0.9	2.5	1.4	1.9	1.6	1.2
Lupus Test PL 1	2.1	1.0	1.5	1.6	0.7	0.4

LIMITATIONS OF THE TEST

Insufficiently centrifuged plasmas contain thrombocytes that may release phospholipids during thrombocytolysis.

LITERATURE

(1) T. EXNER, K. A. RICKARD, H. KRONENBERG: A Sensitive Test Demonstrating Lupus Anticoagulant and its Behavioural Patterns. Brit. J. Haem. 40 (1978); 143
(2) E. ROSNER, R. PAUZNER, A. LUSKY, M. MODAN, A. MANY: Detection and Quantitative Evaluation of Lupus Circulating Anticoagulant Activity. Thrombos. Haemostas. 57 (1987); 144
(3) D. A. TRIPLETT: Screening for the Lupus Anticoagulant. Research in Clin. and Lab. 19 (1989); 379