Lupus S, Lupus C

cobas®

Lupus Screen, Lupus Confirm

REF		CONTENT	System-ID	SYSTEM
06504183190	06504183500	Lupus S 60	Lupus S: 07 2005 7	**cobas t** 511 / **cobas t** 711
06504787190	06504787500	Lupus C 60	Lupus C: 07 2005 8	**cobas t** 511 / **cobas t** 711

English

System information

Short name	ACN (application code number)
Lupus S	28390
Lupus C	28391

Intended use

In vitro assays to screen for (Lupus S) and to confirm (Lupus C) the presence of lupus anticoagulants in citrated plasma on the indicated **cobas t** analyzers. The assay is intended as an aid in the diagnosis of anti-phospholipid syndrome.

Summary

These clotting tests use diluted Russell's viper venom reagent for activation of factor X in the patient's plasma according to a standardized method.[1] Lupus anticoagulants (LA) are antibodies against negatively charged phospholipids associated with proteins like β-2-glycoprotein[1] or prothrombin.[2] These antibodies prolong the clotting times of phospholipid-dependent tests like aPTT or PT.

LA are observed in autoimmune disease (systemic lupus erythematosus) or recurrent fetal loss and are considered as a risk factor for thrombotic events.[2,3,4] Presence of LA can be either transient or permanent.[3,4] Presence of LA is an important marker for recurrent thrombosis and may induce anticoagulant therapy.

Test principle

Russell's viper venom directly activates factor X to Xa, which converts prothrombin to thrombin in the presence of phospholipids and factor V. Thrombin transforms fibrinogen into insoluble fibrin. The time between reagent addition to the plasma and clot formation is measured.

Lupus S contains Russell's viper venom and a low phospholipid concentration. The presence of LA in a sample will delay clot formation in this sample. Lupus C contains Russell's viper venom and a high phospholipid concentration which neutralizes the LA in the sample. The clotting time is therefore normalized if LA is present in the sample. If the clotting is still prolonged, mixing studies are recommended to investigate for deficiencies of factors II, V and X or presence of factor Xa inhibitors or thrombin inhibitors.[3,4,5,6]

As Lupus Screen/Confirm reagents directly activate factor X, the test is insensitive to abnormalities of factor VII as well as factors and inhibitors of the intrinsic coagulation path.

Reagents - working solutions

cobas t pack

Lupus S

SR[a)] Lupus Screen: Lyophilized reagent containing Russell's viper venom, low concentration of phospholipids, a heparin-neutralizing substance, calcium chloride, buffers, stabilizers, sodium azide as preservative and a dye.

a) Start reagent

SR is in position A, B and C.

Lupus C

SR[a)] Lupus Confirm: Lyophilized reagent containing Russell's viper venom, high concentration of phospholipids, a heparin-neutralizing substance, calcium chloride, buffers, stabilizers, ciprofloxacin as preservative and a dye.

SR is in position A, B and C.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

The reagents contain sodium azide (< 1 g/L) as a preservative. Reagents containing sodium azide should be discarded with care to prevent the formation of explosive metallic azides. If waste materials are dumped into sinks, use copious quantities of water to flush plumbing thoroughly.

Reagent handling

The reagent in the cassette has been assembled into a ready-for-use unit (**cobas t** pack).

All information required for correct operation is available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Store the **cobas t** pack **upright**.

The unopened **cobas t** pack is stable up to the stated expiration date.

Stability of the opened **cobas t** pack:	
on the **cobas t** analyzer	8 hours after reconstitution

Do not freeze.

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable:
3.2 % citrated human plasma

Use standard sampling tubes made of plastic or siliconized glass. Strictly observe the ratio of blood (9 parts) to sodium citrate solution 0.11 M (1 part).[7,8]

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

The plasma should contain as few plateletes as possible, especially if samples are frozen for later testing. Procoagulant phospholipids from damaged or activated platelets influence the clotting time in the LA assay.[3,4]

Centrifuge 15 minutes at 2500 g or such that the platelet count is < 10000 platelets/µL and assay samples within the given stability period.

Double centrifugation is recommended. If samples are to be frozen, double centrifugation is required.[3,4]

Stability:	
at 15-25 °C	4 hours
at -80 °C (± 5 °C)	12 weeks

Frozen plasma aliquots should be thawed within 5 minutes at 37 °C in a waterbath, homogenized by carefully mixing without foam formation. It is recommended to assay the samples as soon as possible after thawing. Do not refreeze samples.

Materials provided

See "Reagents – working solutions" section.

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Materials required (but not provided)

- REF 07138504190, Lupus Con, 2 x 5 x 1 mL
- General laboratory equipment
- Distilled or deionized water
- **cobas t** coagulation analyzer. See User Assistance of the analyzer concerned for additionally required materials.

Assay

For optimum performance of the assays follow the directions given in this document. Refer to the appropriate User Assistance for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Procedure

Reference interval (RI)

Establish a reference interval for each lot of Lupus S and Lupus C reagent, respectively. Use at least 40 individual normal plasmas from healthy donors and calculate the mean and the 2 SD range.[3] The mean clotting time of the RI (MRI) should be used for normalization. A new reference interval has to be created upon change of the reagent lot or the analyzer. To account for intra-assay variability, data for the reference interval should be collected over several days. It is recommended to establish a new reference interval for both reagents, Lupus S and Lupus C, even if there is a lot change for only 1 of the 2 reagents, in order to account for variability between the sample populations used.

Calculation

The clotting times [s] of both the screening and confirmatory test are to be normalized against the MRI and are reported as ratios:

Screen Ratio = clotting time Lupus S of the patient plasma / MRI Lupus S.

Confirm Ratio = clotting time Lupus C of the patient plasma / MRI Lupus C.

Final results are expressed as Normalized Ratio = Screen Ratio / Confirm Ratio.

A Normalized Ratio of > 1.2 is considered a positive result and implies presence of LA.

The Screen Ratio, Confirm Ratio and Normalized Ratio are calculated tests and will not be automatically configured on the system after installation of the Lupus S and Lupus C application e-barcodes. For configuration of the calculated tests, follow the instruction in **cobas t** User Assistance under “Creating a calculated test”.

Interpretation of the results

Testing for LA is started with the Lupus S assay.

If the Screen Ratio is < 1.2, presence of LA is not detected.

If the Screen Ratio is > 1.2, presence of LA is suspected. In this case further investigations using the Lupus C assay and, optionally, by mixing studies are required. If the Confirm Ratio is < 1.2 and the Normalized Ratio is > 1.2, presence of LA is detected.

The recommendations of the CLSI on Laboratory testing for the Lupus Anticoagulant and of the Scientific and Standardization Committee of Lupus Anticoagulant have to be considered.[3,4]

Mixing studies

If the Screen Ratio and/or Normalized Ratio are borderline, or the Confirm Ratio is > 1.2, or the Normalized Ratio is < 1.2, or clotting times in both screen and confirm tests are prolonged, mixing tests may be helpful for further differentiation. Mixing studies allow differentiation between factor deficiencies and the presence of an inhibition[3,4] but may be compromised by anticoagulant antibody dilution.[9] Mixing studies are performed on a 1:1 mixture of patient plasma with the reference pool.

Quality control

Controls are required for checking the accuracy and reproducibility of the results.

For quality control, use control kits as listed in the "Materials required (but not provided)" section.

The control intervals and limits should be adapted to each laboratory’s individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. No impact on results was observed up to the listed concentrations.

Lupus S and Lupus C:

Endogenous substances

Compound	Concentration
Conjugated bilirubin	5 mg/dL
Unconjugated bilirubin	5 mg/dL
Hemoglobin	200 mg/dL
Intralipid	120 mg/dL

Criterion: Recovery within ± 10 % of initial value.

The L-index flagging is deactivated in instrument software versions lower than 2.3. In these instances the L-index flagging is deactivated in the e-barcode for Lupus S and Lupus C assays. Therefore, Lupus S and Lupus C assay results will only be flagged for H-and I-indices.

The impact of lipemia, hemoglobin and bilirubin was tested according to Glick.[10]

No significant interference has been observed in a plasma pool spiked with heparin up to a concentration of 0.8 IU/mL for unfractionated heparin (UFH) and 1.0 IU/mL for low molecular weight heparin (LMWH).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[11,12]

The presence of direct thrombin inhibitors, such as dabigatran, argatroban and bivalirudin, or activated factor X (FXa) inhibitors, such as apixaban, edoxaban and rivaroxaban, in the sample influences the assay results which can be of clinical importance.

The fibrinolytic action of streptokinase (fibrin clot and fibrinogen destruction) prolongs the clotting times and thus alters the normalized ratio (NR).

The presence of oritavancin in the sample influences the assay results of Lupus Screen and Lupus Confirm.

In patients under oral anticoagulant therapy the activity of FXa and thrombin is reduced and therefore LA results from these patients can be misleading.[4] Persistence of LA antibodies should be determined 12 weeks after the initial finding to exclude the presence of transient antibodies that are not clinically significant.[13]

Commercially available normal control plasmas with unspecified levels of citrate and platelets are not recommended as quality control in mixing studies.

At least 2 screening assays based on different test setups should be performed before exclusion of LA.[3,4]

For diagnostic purposes, the results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

Extra wash cycle: The use of special wash steps is mandatory when certain test combinations are run together on **cobas t** analyzers. Refer to the latest version of the carry over evasion list found with the CLEAN and Deproteinizer Method Sheets and the User Assistance for further instructions. Where required, special wash/carry over evasion cycles must be implemented prior to reporting results with this test.

Expected values

Lupus S: 20.5-33.6 s

Lupus C: 24.0-31.6 s

These values correspond to the 2.5th and 97.5th percentiles of results obtained from a total of 200 human plasma samples. Normalized Ratio cutoff: 1.2. The cutoff was determined according to CLSI H60A using a total of 200 samples. The cutoff was calculated using the mean + 2 SD.

Normal values may vary due to local conditions and patient populations. Factors such as the sampling procedure, sample storage conditions and the method of measurement can also affect the results.

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Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Repeatability and intermediate precision were determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05 requirements (2 aliquots per run, 2 runs per day, 21 days). The following results were obtained:

Lupus Screen	Repeatability			Intermediate precision	
Sample	**Mean (s)**	**SD (s)**	**CV (%)**	**SD (s)**	**CV (%)**
LA Con Low	35.0	0.304	0.9	0.533	1.6
LA Con High	89.7	1.08	1.2	1.73	1.9
Plasma 1	32.3	0.217	0.7	0.470	1.5
Plasma 2	45.4	0.501	1.1	0.963	2.1
Plasma 3	54.2	0.381	0.7	0.989	1.8
Plasma 4	68.5	0.560	0.8	1.14	1.7
Plasma 5	95.5	0.622	0.7	1.38	1.4

Lupus Confirm	Repeatability			Intermediate precision	
Sample	**Mean (s)**	**SD (s)**	**CV (%)**	**SD (s)**	**CV (%)**
LA Con Low	31.4	0.113	0.4	0.162	0.5
LA Con High	36.1	0.0982	0.3	0.189	0.5
Plasma 1	30.7	0.145	0.5	0.176	0.6
Plasma 2	33.9	0.169	0.5	0.219	0.6
Plasma 3	34.2	0.109	0.3	0.202	0.6
Plasma 4	33.0	0.210	0.6	0.275	0.8
Plasma 5	40.5	0.210	0.5	0.360	0.9

Normalized Ratio	Repeatability			Intermediate precision	
Sample	**Mean (NR)**	**SD (NR)**	**CV (%)**	**SD (NR)**	**CV (%)**
LA Con Low	1.10	0.00988	0.9	0.0199	1.8
LA Con High	2.45	0.0295	1.2	0.0485	2.0
Plasma 1	1.04	0.00816	0.8	0.0184	1.8
Plasma 2	1.32	0.0145	1.1	0.0300	2.3
Plasma 3	1.56	0.0117	0.7	0.0338	2.2
Plasma 4	2.05	0.0159	0.8	0.0365	1.8
Plasma 5	2.33	0.0187	0.8	0.0416	1.8

Method comparison

A comparison of the Lupus S and Lupus C assays, with paired test calculation and the normalized ratio as final result, on a **cobas t** 711 analyzer (y) with an automated coagulation assay (x) gave the following correlation (%):

Number of samples measured: 286

Total agreement (%): 90.6

The normalized ratios with the Lupus S and Lupus C assays were between 0.770 and 3.08.

References

1 Exner T, Papadopoulos G, Koutts J. Use of a simplified dilute Russell's viper venom time (DRVVT) confirms heterogeneity among "lupus anticoagulants". Bl. Coag. Fibrinol. 1990; 1: 259-266.

2 Devreese KMJ, Ortel TL, Pengo V et al. Laboratory criteria for antiphospholipid syndrome: communication from the SSC of the ISTH, J. Thromb Haemostasis 2018; 16: 809-

3 CLSI document H60-A. Laboratory testing for the Lupus Anticoagulant; Approved guideline (April 2014).

4 Pengo V, Tripodi A, Reber G, et al. Official communication of the scientific and standardization committee on lupus anticoagulant/phospholipid-dependent antibodies: update of the guidelines for lupus anticoagulant detection. J Thromb Haemostasis 2009;7:1737-1740.

5 Genzen JR, Miller JL. Presence of direct thrombin inhibitors can affect the results and interpretation of lupus anticoagulant testing. Am J Clin Pathol. 2005;124(4):586-593.

6 Moore GW, Recent Guidelines and Recommendations for Laboratory Detection of Lupus Anticoagulants, Semin Thromb Hemost 2014;40:163–171

7 CLSI Document H21-A5, Vol.28, No.5, 2008. Collection, transport, and processing of blood specimens for testing plasma-based coagulation assays and molecular hemostasis assays; approved guideline, 5th edition.

8 CLSI Document H3-A6. Procedures for the collection of diagnostic blood specimens by venipuncture; approved standard - Sixth Edition, vol. 27, No. 26, 2007.

9 Moore GW, Savidge GF. The dilution effect of equal volume mixing studies compromises confirmation of inhibition by lupus anticoagulant even when mixture specific reference ranges are applied. Thrombosis Research 2006;118:523-528.

10 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

11 Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.

12 Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.

13 Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemostasis 2006;4: 295-306.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

For further information, please refer to the appropriate User Assistance for the relevant analyzer and Method Sheets of all necessary components.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number

Additions, deletions or changes are indicated by a change bar in the margin.

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CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606