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ACTICLOT[®] LA Complete[™]

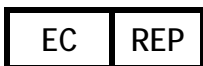
REF 826

**for the Determination of
Lupus Anticoagulants (LA)***



8°C

**(CPT Code No. 85705 x 2)
(CPT Code No. 85613 x 2)**



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INTENDED USE

The ACTICLOT[®] LA Complete[™] is intended for the qualitative determination of Lupus Anticoagulants (LA) in human plasma. The test may be performed using semi-automated and automated coagulation analyzers. The test is for *in vitro* diagnostic use and is not intended for internal use in humans or animals.

EXPLANATION OF THE TEST

Lupus Anticoagulants (LA) are phospholipid-dependent autoantibodies associated with disorders of the autoimmune system such as Antiphospholipid Syndrome (APS).^{1,2} Primary APS is a pathological condition characterized by unexplained thrombosis, recurrent fetal loss, thrombocytopenia, and/or neurological disorders. Secondary APS occurs when LA are present along with other autoimmune diseases such as Systemic Lupus Erythematosus (SLE), as originally described by Conley and Hartmann³.

Lupus Anticoagulants are directed against heterogeneous complexes of anionic phospholipids (e.g. cardiolipin, phosphatidylinositol, phosphatidylethanolamine and phosphatidylserine)⁴ and phospholipid-binding proteins⁵ in plasma. The major protein components of the LA autoantigens include β 2GPI, prothrombin and annexin V. Antiphospholipid antibodies are characterized by their ability to prolong the clot times of coagulation-based *in vitro* tests such as the lupus-sensitive APTT, kaolin clotting time (KCT), dilute Russell's viper venom test (dRVVT) and dilute prothrombin time (dPT) tests.⁶⁻⁸ Due to the heterogeneous nature of the pathological phospholipid-dependent autoantibodies, it is widely acknowledged that no single LA coagulation test identifies all LA antibodies. In 2006, the ISTH Scientific Subcommittee on Antiphospholipid Antibodies enhanced its previous recommendation that each plasma suspected of containing LA should be tested in at least two LA diagnostic assays, representing different assay principles, and a mixing study with pooled normal plasma be performed to determine the presence of an autoantibody or factor deficiency.⁹ In addition, the ISTH SSC recommended that a diagnosis of LA requires a demonstration of the phospholipid-dependent nature of the autoantibodies. This can be accomplished by performing a confirmatory coagulation assay in the presence of high amounts of phospholipids. The presence of LA is "confirmed" by the significant reduction of the plasma's clot time in the presence of a high phospholipid concentration as compared to a lower phospholipid concentration present in an LA "screening" test.

Clinical studies show that a dilute prothrombin time test is an effective LA coagulation assay and can identify LA that are not detected by other tests such as a lupus-sensitive APTT and a dRVVT.¹⁰⁻¹² An LA test panel comprised of a dPT and a dRVVT demonstrated increased sensitivity for detecting LA in patient samples compared to using only a single test.^{8,13}

PRINCIPLE OF THE METHOD

ACTICLOT LA Complete contains a set of reagents that allows for performing a dPT test and a dRVVT test on a patient sample in a single run, thereby increasing the efficiency of LA testing. The reagent set contains DVV Activator[™] for performing a dilute Russell's viper venom time test, and dPT Activator[™] for performing a dilute prothrombin time test. The set also contains LA Phospholipids[™], a uniquely formulated high phospholipid reagent that is used to confirm the phospholipid-dependent nature of the LA detected in the dPT or dRVVT screening tests.

DVV Activator initiates clotting via direct activation of Factor X to Factor Xa by Russell's viper venom in the presence of low phospholipids and calcium. Factor Xa cleaves prothrombin to thrombin, which converts fibrinogen to fibrin leading to detectable clot formation in plasma. This direct activation of Factor X bypasses the contact and intrinsic pathways in the coagulation cascade, thereby excluding interference from deficiencies of Factors VIII, IX, XI and XII, or their respective inhibitors.

dPT Activator, a unique formulation of relipidated tissue factor in the presence of calcium ions, initiates clotting via activation of the extrinsic coagulation pathway. Tissue Factor binds to Factor VIIa resulting in the activation of Factor IX and Factor X. Factor Xa converts prothrombin to thrombin, which initiates clot formation by cleaving fibrinogen to fibrin. Activation of the tissue factor pathway bypasses the contact (intrinsic) pathway and excludes any interference from deficiencies of Factor XII. The use of recombinant tissue factor in the formulation of the dPT test improves the test's performance.¹²

When running ACTICLOT LA Complete, each patient plasma is first screened for dPT- and dRVVT-sensitive LA. Screening tests are performed using two aliquots of a plasma, each mixed with LA Buffer™. DVV Activator or dPT Activator is added to a respective sample to initiate clotting. The clot time is determined by semi-automated or automated methods. A positive result obtained with either dPT Activator or DVV Activator is indicated by a prolonged clot time relative to an established normal range and is confirmed using the LA Phospholipids reagent. In the Confirmatory Protocols, the plasma is mixed with the LA Phospholipids followed by the addition of either dPT Activator or DVV Activator. A positive result is indicated by a significant reduction of the clot time relative to the clot time in the respective screening protocol.

Plasmas are identified as LA positive when both the Screening and Confirmatory Protocols are positive (see the Decision Algorithm for Diagnosis of LA on page 10). The screening and confirmatory protocols may be performed at the same time, allowing for the most rapid turnaround time for the test results of each plasma.

REAGENTS

- R1** LA Buffer™: 3 vials, 40 tests per vial, contains a unique formulation of buffer, salts and inert ingredients.
- R2** LA Phospholipids™: 3 vials, 40 tests per vial, contains a proprietary mixture of phospholipids, inert preservatives and additives.
- R3** dPT Activator™: 3 vials, 40 tests per vial, contains a proprietary mixture of recombinant human tissue factor, calcium, phospholipids, heparin neutralizer and inert preservatives.
- R4** DVV Activator™: 3 vials, 40 tests per vial, contains a proprietary mixture of Russell's viper venom, calcium, phospholipids, heparin neutralizer and inert preservatives.

WARNING

The reagents contain small amounts of sodium azide that may form explosive compounds upon reaction with copper and lead plumbing. Upon disposal, flush with large amounts of water.



For *in vitro* use only. Not for internal use in humans or animals. Do not use the kit components beyond the printed expiration date. Do not mix reagents from different lots of kits. Avoid microbial contamination of the reagents. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled. Do not pipette reagents by mouth. Wear laboratory coat and disposable gloves throughout the test procedure and wash hands thoroughly afterwards.

REAGENT PREPARATION AND STORAGE

All reagents are supplied lyophilized. Unopened reagents are stable until the expiration date printed on their vial labels when properly stored at 2°-8°C. Reconstitute each reagent according to the following instructions.

- LA Buffer™ (R1):** Reconstitute a vial with 3.0 mL of filtered deionized or distilled water. Mix the reagent well and allow to stand at room temperature for at least 15 minutes to ensure complete dissolution.
- LA Phospholipids™ (R2):** Reconstitute a vial with 2.0 mL of filtered deionized or distilled water. Mix the reagent well and allow to stand at room temperature for at least 15 minutes to ensure complete dissolution.
- dPT Activator™ (R3):** Reconstitute a vial with 2.0 mL of filtered deionized or distilled water. Mix the reagent well and allow to stand at room temperature for at least 15 minutes to ensure complete dissolution. **DO NOT VORTEX! MAINTAIN AT ROOM TEMPERATURE! DO NOT REFRIGERATE! DO NOT FREEZE!**
- DVV Activator™ (R4):** Reconstitute a vial with 2.0 mL of filtered deionized or distilled water. Mix the reagent well and allow to stand at room temperature for at least 15 minutes to ensure complete dissolution.

The stability of the reconstituted reagents are as follows:



	R1	R2	R3	R4
	10 Days	10 Days	24 Hours	24 Hours/7 Days/1 Month
	2°-8°C 18°-25°C	2°-8°C 18°-25°C	18°-25°C	18°-25°C/2°-8°C/-20°C

Reconstituted reagents R1 and R2 may be recovered from the instrument within 10 days. Reconstituted R3 and R4 may be recovered from the instrument within 24 hours.

SPECIMEN COLLECTION AND PREPARATION

Citrate collected platelet poor plasma must be used for this assay. See "Collection, Transport and Processing of Blood Specimens for Testing Plasma-based Coagulation Assays; Approved Guidelines-Fourth Edition", NCCLS Document H21-A4, Vol. 23, No. 35, December 2003.¹⁴ Plasma collection should be performed as follows:

- Using a syringe or evacuated siliconized tubes, collect 9 parts of blood into 1 part of 3.2% (0.109 M) trisodium citrate (dihydrate form) anticoagulant solution.
- Centrifuge the blood sample at a minimum of 5,000 x g for 10 minutes to yield platelet poor plasma. The plasma should have fewer than 10⁴ platelets/μL. Platelets may also be removed by passing the plasma through a 0.22 micron filter.
- Plasma should be stored at 2°-8°C and assayed within 4 hours. Alternatively, plasma may be stored at -70°C for up to 6 months.

	4 hours	6 months
	2°-8°C	-70°C

- Frozen plasma should be thawed rapidly at 37°C. Thawed plasmas should be stored at 2°- 8°C and assayed within 4 hours.

Notes:

- If the hematocrit of the original blood sample is greater than 55%, the results of the ACTICLOT LA Complete may be inaccurate and an adjustment of the blood-to-anticoagulant ratio may be indicated.
- Do not test samples with substantial icterus or lipemia using photo-optical instruments as they may yield false clot times. An alternative manual or semi-automated testing method is advisable.
- Do not perform the ACTICLOT LA Complete on hemolyzed samples.

PROCEDURE

Materials Provided – See Reagents

Materials Required But Not Provided

LArol™ Normal Control Plasma (American Diagnostica Inc. REF 816N)
 LArol™ Abnormal Control plasma (American Diagnostica Inc. REF 816A)
 Pooled Normal Plasma (PNP)
 0-200 μL, 200-1000 μL, 1000-5000 μL single pipettes
 0.22 μm filtered deionized or distilled water
 Automated or semi-automated coagulation analyzer

ACTICLOT LA Complete Screening Protocol

The ACTICLOT LA Complete Screening Protocol can be performed on most automated coagulation analyzers. Applications for selected automated analyzers are available upon request.

1. Transfer LA Buffer, DVV Activator and dPT Activator to the reagent wells of the automated coagulation analyzer.
2. Pipette 100 µL of test plasma (e.g. patient plasma, PNP, LA positive control or LA negative control) into each of two coagulation cuvettes.
3. Add 50 µL of LA Buffer to each cuvette containing the test plasma. Incubate for 2 minutes at 37°C.
4. Add 50 µL of DVV Activator or dPT Activator prewarmed to 37°C to the respective cuvettes containing the test plasma. Record the clot time.

ACTICLOT LA Complete Confirmatory Protocol

The ACTICLOT LA Complete Confirmatory Protocol can be performed on most automated coagulation analyzers. Applications for selected automated analyzers are available upon request.

1. Transfer LA Phospholipids and DVV Activator or dPT Activator to the reagent wells of the automated coagulation analyzer.
2. Pipette 100 µL of test plasma (e.g. patient plasma, PNP, LA positive control or LA negative control) into each of two coagulation cuvettes.
3. Add 50 µL of LA Phospholipids to each cuvette containing the test plasma. Incubate for 2 minutes at 37°C.
4. Add 50 µL of DVV Activator or dPT Activator prewarmed to 37°C to the respective cuvettes containing the test plasma. Record the clot time.

NORMAL REFERENCE RANGES

The proper performance of ACTICLOT LA Complete requires that each laboratory establish its own Normal Reference Ranges for the screening and confirmatory protocols. A minimum of 20 healthy blood donors, including both men and women and spanning the adult age range, should be used to establish the normal reference range (see EXPECTED VALUES). When establishing the normal reference ranges, collection and preparation of the normal plasma samples must be in the same manner as the plasma samples to be tested. If frozen samples are tested exclusively, then the normal reference ranges should be established using frozen normal samples. It is not recommended to test mixed plasma populations of fresh and frozen samples for establishing the normal reference ranges or for routine testing. The normal reference range must be re-established with each change in reagent lot, coagulation analyzer, or at least once a year. The normal reference range data should be obtained over a period of several days to account for day-to-day variations.

Determination of the Mean Screening Time and Normal Reference Range for the Screening Protocol

Test the 20 normal plasmas using the Screening Protocol and determine the mean screening time (in seconds) for the 20 plasmas. The mean screening time + 2 SD is the upper limit of the normal reference range and is used as the cut-off to determine if a patient sample is positive for LA in the screening protocol.

Determination of the Mean Confirmatory Time for the Confirmatory Protocol

Test the 20 normal plasmas using the Confirmatory Protocol and determine the mean confirmatory time. Determine the mean confirmatory time (in seconds) for the same 20 normal plasmas tested using the Screening Protocol.

NOTE: Determination of LA positivity requires testing each plasma using the Screening and Confirmatory Protocols. The mean screening and confirmatory times of the normal plasmas are used in calculations to diagnose the presence of LA in the patient samples. Two methods of calculations for patient samples are described below: Screening Time/Confirmatory Time (S/C) and the Normalized Ratio. Alternative methods for calculating results of LA tests have been described.¹⁵

Determination of Normal Reference Range for the Confirmatory Protocol Using the S/C Ratio Method

The S/C Ratio is calculated for each of the twenty normal plasmas by dividing the screening time by the confirmatory time:

$$\text{S/C Ratio} = \text{Screening Time (sec)} \div \text{Confirmatory Time (sec)}$$

Determine the mean S/C Ratio (± 2 S.D) of the twenty normal plasmas. The mean normal S/C Ratio + 2 SD is used to determine the upper limit of the normal reference range for diagnosing the presence of LA.

Determination of the Normal Reference Range for the Confirmatory Protocol Using the Normalized Ratio Method

Divide the screening time of each normal plasma by the normal reference range mean screening time (see above). Next, divide the confirmatory time of each normal plasmas by the normal reference range mean confirmatory time (see above). The Normalized Ratio is determined by dividing the normalized screening ratio by the normalized confirmatory ratio:

$$\text{Normalized Ratio} = \frac{\text{Normal Plasma Screening Time (sec)} \div \text{Mean Normal Screening Time (sec)}}{\text{Normal Plasma Confirmatory Time (sec)} \div \text{Mean Normal Confirmatory Time (sec)}}$$

Determine the mean normalized ratio (± 2 SD) of the twenty normal samples. The mean Normalized Ratio + 2 SD is used to determine the upper limit of the normal reference range for diagnosing the presence of LA.

INTERPRETATION OF RESULTS

A Decision Algorithm for testing patient plasmas is provided at the end of this product insert for clarity in interpreting results.

- A. Patient plasmas are tested using the both the DVV and dPT Screening Protocols. Determine if the clot time of the plasma sample falls above or below the upper limit of the established normal reference ranges.
 1. If the plasma has a screening clot time (sec) that is below the upper limit of the established normal reference range (mean + 2 SD) for both DVV and dPT Activators, the test result is negative for LA.
 2. If the plasma has a screening clot time (sec) that is above the upper limit of the established normal reference range (mean + 2 SD) for the DVV and/or dPT Screening Protocol, then the sample is suspected of being positive for LA.

A plasma with a positive DVV and/or dPT Screening Protocol result must be tested using the Confirmatory Protocol for DVV and/or dPT in order to identify the presence of LA in the plasma sample. A positive finding of LA using the ACTICLOT LA Complete Screening Protocol can only be confirmed using the ACTICLOT LA Complete Confirmatory Protocol.

- B. Each plasma that tested positive in the Screening Protocol is tested in the Confirmatory Protocol. Determine the S/C Ratio or the Normalized Ratio of the test plasma.
 1. If the S/C Ratio or the Normalized Ratio of the test plasma is greater than the upper limit established for the normal reference range (mean + 2 SD), the plasma is confirmed positive for LA.
 2. If the S/C Ratio or the Normalized Ratio of the test plasma is within normal reference range (mean \pm 2 SD), then the plasma is not confirmed for LA. Mixing Studies should be performed on the sample.

Mixing Studies

The presence of biological abnormalities such as a blood factor deficiency (e.g. factor II, factor V or factor X deficiency), a factor inhibitor (e.g. fVIII inhibitor), or that the patient is on oral anticoagulant (OAC) medication (e.g. Coumadin®) may be suspected when the plasma has a prolonged ACTICLOT LA Complete screening time but the S/C Ratio or Normalized Ratio falls within the normal reference range. In this event, it is recommended to perform a mixing study. A mixing study is performed by adding equal proportions of test plasma and pooled normal plasma. The screening and confirmatory protocols should be performed on the 1:1 mixed plasma.

1. If the screening time of the 1:1 mixture is greater than the laboratory's normal reference range (mean + 2 SD), then the plasma is suspected positive for LA. If the screening time of the 1:1 mixture is within the normal range (mean ± 2 SD), then the presence of a blood factor deficiency is suspected. Appropriate factor assays may be carried out if desired (see Decision Algorithm).
2. If the S/C Ratio or Normalized Ratio of the 1:1 mixture is greater than the laboratory's normal reference range (mean + 2 SD), then the plasma is confirmed as positive for LA. If the S/C Ratio or Normalized Ratio of the mixture is within the laboratory's normal reference range (mean ratio ± 2 SD), then other abnormalities (e.g. Coumadin, factor inhibitor) are suspected.

EXPECTED VALUES

Typical normal reference ranges for ACTICLOT LA Complete Screening and Confirmatory Protocols, clot times and the S/C Ratio, using commercial coagulation analyzers are provided in Tables 1A and 1B. These results are to be used only as a guide. Frozen plasma samples from individual donors were either purchased from commercial sources or collected and prepared by the laboratory performing the test in accordance with NCCLS Document H21-A4.¹⁴ Donors included both men and women, spanning the adult age range and having normal PT and APTT values. Further demographic data was unavailable.

TABLE 1A. Normal Reference Ranges for DVV Screening and Confirmatory Protocols Using Commercial Coagulation Analyzers^a

Coagulation Analyzer	ACL® 300+ (n=25)	STA Compact® (n=25)
Screening Time, sec (mean ± 2 SD)	30.0 ± 5.6	34.2 ± 4.8
Confirmatory Time, sec (mean ± 2 SD)	23.0 ± 3.9	30.2 ± 4.4
S/C Ratio (mean ± 2 SD)	1.3 ± 0.13	1.13 ± 0.12

TABLE 1B. Normal Reference Ranges for dPT Screening and Confirmatory Protocols Using Commercial Coagulation Analyzers^a

Coagulation Analyzer	ACL® 300+ (n=25)	STA Compact® (n=25)
Screening Time, sec (mean ± 2 SD)	34.4 ± 4.5	36.6 ± 5.9
Confirmatory Time, sec (mean ± 2 SD)	29.2 ± 4.8	35.7 ± 3.1
S/C Ratio (mean ± 2 SD)	1.18 ± 0.12	1.03 ± 0.09

^a data from American Diagnostica Inc.¹⁶

QUALITY CONTROL

A normal LA control plasma and abnormal LA control plasma should be tested with each group of tests performed, with a change in personnel or work shift, or according to the testing laboratory's guidelines. The controls must be platelet poor, with fewer than 10⁴ platelets/ μ L. Normal and abnormal LA control plasmas are available from American Diagnostica Inc. (REF 816N and REF 816A respectively). The values for both the normal and abnormal LA control plasmas should fall within the laboratory's established control ranges. If the values for the controls do not meet the laboratory's previously established control limits and it has been determined that the equipment is performing properly, the results should be discarded and samples should be retested with fresh reagents. Correct normal and abnormal control values should be obtained before patient samples are tested.

TRACEABILITY OF CONTROL MATERIALS

Information regarding traceability of control materials is available upon request from American Diagnostica Inc.¹⁶

LIMITATIONS OF THE PROCEDURE

The ACTICLOT LA Complete screening times may be prolonged in patients with congenital or acquired factor deficiencies. Congenital factor deficiencies can be determined by performing the mixing studies as previously described. Plasmas that contain factor inhibitors (acquired deficiencies) may or may not be identified in mixing studies because the inhibitor may not be fully neutralized by the PNP used in the mixing studies.

The DVV Activator and the dPT Activator contain agents that neutralize unfractionated heparin up to and including 1.0 U/mL. Plasmas that contain unfractionated heparin levels greater than 1.0 U/mL may give incorrect results and should not be evaluated with the test.

Plasmas from patients treated with Coumadin and other oral anticoagulants may have prolonged screening and confirmatory clot times. Mixing studies may shorten these clot times to within the normal range provided that LA is not present.

No single LA test identifies all LA positive samples. The ISTH SSC recommends that any sample suspected of having LA be tested using two or more LA screening tests and at least one high phospholipid containing confirmatory test.⁹

PERFORMANCE CHARACTERISTICS

Precision

ACTICLOT LA Complete precision studies were performed by American Diagnostica Inc. and two independent laboratories using the Diagnostica Stago STA Compact®. The studies included multiple tests performed over several days using commercial control plasmas. The results are summarized in Tables 2A and 2B.

TABLE 2A. Precision Data for DVV Activator using Normal and Abnormal (Lupus Positive) Control Plasmas.

Coagulation Analyzer	Control*	Mean DVV Screening Time (sec)	Intra-Assay CV (%)	Inter-Assay CV (%)	Mean DVV Confirmatory Time (sec)	Intra-Assay CV (%)	Inter-Assay CV (%)
STA Compact	Normal	35.7	1.1	2.6	33.0	1.6	3.8
	Abnormal	77.4	1.2	3.7	39.3	1.3	2.8

* American Diagnostica Inc. REF 816N, 816A

TABLE 2B. Precision Data for dPT Activator using Normal and Abnormal (Lupus Positive) Control Plasmas.

Coagulation Analyzer	Control*	Mean dPT Screening Time (sec)	Intra-Assay CV (%)	Inter-Assay CV (%)	Mean dPT Confirmatory Time (sec)	Intra-Assay CV (%)	Inter-Assay CV (%)
STA Compact	Normal	40.2	0.8	3.4	39.7	0.9	4.3
	Abnormal	77.9	1.1	7.2	46.0	1.0	4.8

* American Diagnostica Inc. REF 816N, 816A

Specificity and Sensitivity

In Clinical Study I, the specificity and sensitivity of the DVV Screening and Confirmatory Protocols were determined using 25 normal plasmas and 67 known weak LA plasmas. The results are shown in Table 3A.

TABLE 3A. Clinical Study I – Specificity and Sensitivity^a

	DVV Activator Screening Protocol	DVV Activator Confirmatory Protocol
<u>No. of Normal Plasmas Tested Negative</u> No. of Normal Plasmas Tested	25/25	25/25
Specificity	100%	100%
<u>No. of Known LA Plasmas Tested Positive</u> No. of Known LA Plasmas Tested	67/67	49/62
Sensitivity	100%	79%

^a performed using STA Compact

In Clinical Study II, the specificity and sensitivity of the dPT Screening and Confirmatory Protocols were determined using twenty normal plasmas and 23 known weak LA plasmas. The results are shown in Table 3B.

TABLE 3B. Clinical Study II – Specificity and Sensitivity^a

	dPT Activator Screening Protocol	dPT Activator Confirmatory Protocol
<u>No. of Normal Plasmas Tested Negative</u> No. of Normal Plasmas Tested	20/20	20/20
Specificity	100%	100%
<u>No. of Known LA Plasmas Tested Positive</u> No. of Known LA Plasmas Tested	21/23	18/23
Sensitivity	91%	78%

^a performed using a Sysmex® CA-7000 coagulation analyzer

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- Data on file, American Diagnostica Inc., Stamford, Connecticut 06902

ACL is a trademark of Instrumentation Laboratory, SpA

STA Compact is a registered trademark of Diagnostica Stago SA

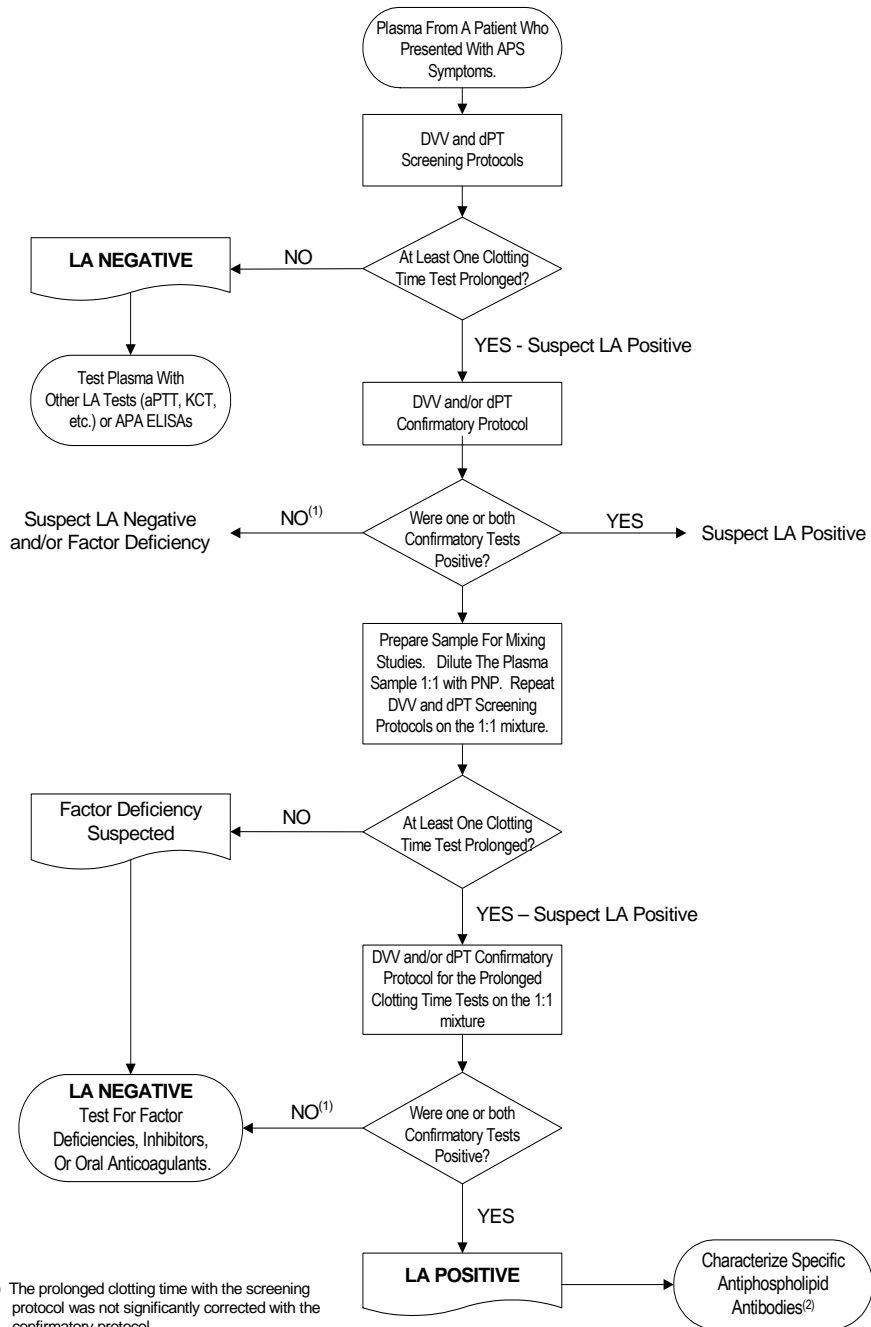
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ACTICLOT® LA Complete™ Decision Algorithm for Diagnosis of LA



(1) The prolonged clotting time with the screening protocol was not significantly corrected with the confirmatory protocol.

(2) ELISA assays for anti-β2GP1, anti-prothrombin, anti-cardiolipin, anti-annexin V, etc.