

# ACTICLOT® dPT™

REF 824

## ***Dilute Prothrombin Time Test for the Determination of Lupus Anticoagulants (LA)***



### INTENDED USE

The ACTICLOT® dPT™ 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).<sup>1,2</sup> 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 Hartmann<sup>3</sup>.

Lupus Anticoagulants are directed against heterogeneous complexes of anionic phospholipids (e.g. cardiolipin, phosphatidylinositol, phosphatidylethanolamine and phosphatidylserine)<sup>4</sup> and phospholipid-binding proteins<sup>5</sup> in plasma. The major protein components of the LA autoantigens include  $\beta$ 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.<sup>6-8</sup> 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 1995, the ISTH Scientific Subcommittee on Antiphospholipid Antibodies recommended that each plasma suspected of containing LA should be tested in at least two LA diagnostic assays and a mixing study with pooled normal plasma be performed to determine the presence of an autoantibody or factor deficiency.<sup>9</sup> 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.<sup>10-12</sup> Adding a dilute prothrombin time test to an LA testing panel increased the sensitivity of detecting LA in patient samples.<sup>8,13</sup> ACTICLOT dPT is a fully integrated dilute prothrombin time test for screening and confirming the presence of phospholipid-dependent LA autoantibodies. The screening protocol utilizes an activator reagent that contains a unique formulation of relipidated recombinant tissue factor and calcium. The use of recombinant tissue factor in the formulation of the dPT test improves the test's performance.<sup>12</sup> In the confirmatory protocol, a uniquely formulated phospholipid reagent is used to demonstrate the phospholipid-dependent nature of the LA detected in samples that tested positive in the screening protocol.

### PRINCIPLE OF THE METHOD

The ACTICLOT dPT is a coagulation assay that identifies the presence of LA in plasma. Clotting is initiated by activating the tissue factor (extrinsic) coagulation pathway with tissue factor in the presence of calcium ions. 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.

In the Screening Protocol, the patient plasma is mixed with the ACTICLOT LA Buffer™ and ACTICLOT dPT Activator™. The clot time is determined by semi-automated or automated methods. A positive result is indicated by a prolonged clot time relative to an established normal range. In the Confirmatory Protocol, the patient plasma is mixed with the ACTICLOT LA Phospholipids™ and ACTICLOT dPT Activator. A positive result is indicated by a significant reduction of the clot time relative to the clot time in the screening protocol.

Plasmas are identified as possessing LA when both the Screening and Confirmatory Protocols are performed and both tests 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. Alternatively, plasmas may first be screened for the presence of LA and then retested for confirmation of the phospholipid-dependence of the autoantibodies detected.


## 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™:** 6 vials, 40 tests per vial, contains a proprietary mixture of recombinant human tissue factor, calcium, phospholipids, inert preservatives and additives.

## WARNINGS and PRECAUTIONS

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.

LA Buffer	-	-	H412, P273
LA Phospholipids	-	-	H412, P273
dPT Activator	Danger		<div>CONT Cadmium chloride</div> H315, H319, H335, H340, H350, H360, H373, P202, P260, P264, P280, P305+P351+P338, P332+P313, P308+P313



- Hazard Statements:**
- H315 Causes skin irritation.
  - H319 Causes serious eye irritation.
  - H335 May cause respiratory irritation.
  - H340 May cause genetic defects.
  - H350 May cause cancer.
  - H360 May damage fertility or the unborn child.
  - H373 May cause damage to organs through prolonged or repeated exposure.
  - H412 Harmful to aquatic life with long lasting effects.
- Precautionary statements:**
- P202 Do not handle until all safety precautions have been read and understood.
  - P260 Do not breathe mist or vapor.
  - P264 Wash thoroughly after handling.
  - P273 Avoid release to the environment.
  - P280 Wear protective gloves/protective clothing/eye protection/face protection.
  - P305+P351+P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
  - P332+P313 If skin irritation occurs: Get medical advice/attention.
  - P308+P313 If exposed or concerned: Get medical advice/attention.

## 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.

- 1. LA Buffer™ (R1):** Reconstitute a vial with 3.0 mL of filtered deionized or distilled water. Mix the reagents well and allow to stand at room temperature for at least 15 minutes to ensure complete dissolution.
- 2. LA Phospholipids™ (R2):** Reconstitute a vial with 2.0 mL of filtered deionized or distilled water. Mix the reagents well and allow to stand at room temperature for at least 15 minutes to ensure complete dissolution.
- 3. dPT™ Activator™ (R3):** Reconstitute a vial with 2.0 mL of filtered deionized or distilled water. Mix the reagents 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!**

The stability of the reconstituted reagents are as follows:



	R1	R2	R3
	10 Days	10 Days	8 Hours
	2°-8°C 18°-25°C	2°-8°C 18°-25°C	18°-25°C

Reconstituted reagents R1 and R2 may be recovered from the instrument within 10 days. Reconstituted R3 may be recovered from the instrument within 8 hours. (See the above table for each temperature requirement.)

## 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 and Molecular Hemostasis Assays; Approved Guidelines-Fifth Edition", CLSI Document H21-A5, Vol. 28, No. 5.<sup>14</sup> 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 10<sup>4</sup> 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 dPT 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 dPT on hemolyzed samples.

## PROCEDURE

### Materials Provided – See Reagents

#### Materials Required But Not Provided

LArol™ Normal Control Plasma (BioMedica Diagnostics REF 816N)  
 LArol™ Abnormal Control plasma (BioMedica Diagnostics 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 dPT Screening Protocol

##### Semi-Automated Procedure for ST4

1. Transfer sufficient ACTICLOT dPT Activator and ACTICLOT LA Buffer to the 37°C reagent wells of an ST4.
2. Pipette 100 µL of test plasma (e.g. patient plasma, PNP, LA positive control or LA negative control) into a coagulation cuvette. Incubate for 2 minutes at 37°C.
3. Add 50 µL of prewarmed ACTICLOT LA Buffer to the cuvette containing the test plasma.
4. Add 50 µL of prewarmed ACTICLOT dPT Activator to the cuvette containing the test plasma. Immediately start the timer and record the clot time.

#### Automated Procedure

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

1. Transfer ACTICLOT LA Buffer and ACTICLOT 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 a coagulation cuvette.
3. Add 50 µL of ACTICLOT LA Buffer to the cuvette containing the test plasma. Incubate for 2 minutes at 37°C.
4. Add 50 µL of ACTICLOT dPT Activator prewarmed to 37°C to the cuvette containing the test plasma. Record the clot time.

#### ACTICLOT dPT Confirmatory Protocol

##### Semi-Automated Procedure for ST4

1. Transfer sufficient ACTICLOT dPT Activator and ACTICLOT LA Phospholipids to the 37°C reagent wells of an ST4.
2. Pipette 100 µL of test plasma (e.g. patient plasma, PNP, LA positive control or LA negative control) into a coagulation cuvette. Incubate for 2 minutes at 37°C.
3. Add 50 µL of prewarmed ACTICLOT LA Phospholipids to the cuvette containing the test plasma.
4. Add 50 µL of prewarmed ACTICLOT dPT Activator to the cuvette containing the test plasma. Immediately start the timer and record the clot time.

#### Automated Procedure

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

1. Transfer ACTICLOT LA Phospholipids and ACTICLOT 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 a coagulation cuvette.
3. Add 50 µL of ACTICLOT LA Phospholipids to the cuvette containing the test plasma. Incubate for 2 minutes at 37°C.
4. Add 50 µL of ACTICLOT dPT Activator prewarmed to 37°C to the cuvette containing the test plasma. Record the clot time.

#### NORMAL REFERENCE RANGES

The proper performance of ACTICLOT dPT 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 dPT 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.<sup>15</sup>

#### Determination of Normal Reference Range 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:

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

Determine the mean S/C Ratio ( $\pm 2$  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 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:

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

Determine the mean normalized ratio ( $\pm 2$  SD) of the twenty normal samples. The mean Normalized Ratio  $\pm 2$  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 Screening Protocol. Determine if the clot time of the plasma sample falls above or below the upper limit of the established normal reference range.

1. If the plasma has a screening clot time (sec) that is within the established normal reference range (mean  $\pm 2$  SD), 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  $\pm 2$  SD), then the sample is suspected of being positive for LA.

A plasma with a positive ACTICLOT dPT Screening Protocol result must be tested using the ACTICLOT dPT Confirmatory Protocol in order to identify the presence of LA in the plasma sample. A positive finding of LA using ACTICLOT dPT Screening Protocol can only be confirmed using the ACTICLOT dPT 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  $\pm 2$  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, V or 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 dPT 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 ACTICLOT dPT screening and confirmatory protocols should be performed on the 1:1 mixed plasma.

1. If the dPT screening time of the 1:1 mixture is greater than the laboratory's normal reference range (mean  $\pm 2$  SD), then the plasma is suspected positive for LA. If the dPT screening time of the 1:1 mixture is within the normal range (mean  $\pm 2$  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  $\pm 2$  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  $\pm 2$  SD), then other abnormalities (e.g. Coumadin, factor inhibitor) are suspected.

### EXPECTED VALUES

Typical normal reference ranges for ACTICLOT dPT Screening and Confirmatory Protocols, clot times and the S/C Ratio, using commercial coagulation analyzers are provided in Table 1. 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.<sup>14</sup> Donors included both men and women, spanning the adult age range and having normal PT and APTT values. Further demographic data was unavailable.

**TABLE 1. Normal Reference Ranges for ACTICLOT dPT Using Commercial Coagulation Analyzers<sup>a</sup>**

Coagulation Analyzer	ACL® 300+ (n=25)	BCT® (n=32)	CA7000 (n=20)	MLA® 900C (n=93)	ST4 (n=25)	STA Compact® (n=25)
Screening Time, sec (mean $\pm 2$ SD)	34.4 $\pm$ 4.5	51.2 $\pm$ 7.8	38.8 $\pm$ 8.8	26.7 $\pm$ 4.8	38.4 $\pm$ 4.2	36.6 $\pm$ 5.9
Confirmatory Time, sec (mean $\pm 2$ SD)	29.2 $\pm$ 4.8	50.5 $\pm$ 11.0	35.8 $\pm$ 4.0	26.2 $\pm$ 5.2	34.9 $\pm$ 8.5	35.7 $\pm$ 3.1
S/C Ratio (mean $\pm 2$ SD)	1.18 $\pm$ 0.12	1.02 $\pm$ 0.12	1.08 $\pm$ 0.26	1.02 $\pm$ 0.16	1.11 $\pm$ 0.19	1.03 $\pm$ 0.09

<sup>a</sup> data from BioMedica Diagnostics<sup>16</sup>

### QUALITY CONTROL

A normal LA control plasma and abnormal LA control plasma should be tested with each group of tests run, 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 10<sup>4</sup> platelets/ $\mu$ L. Normal and abnormal LA control plasmas are available from BioMedica Diagnostics (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 rerun 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 BioMedica Diagnostics.<sup>16</sup>

### LIMITATIONS OF THE PROCEDURE

The ACTICLOT dPT screening time 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 ACTICLOT dPT Activator contains 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 ACTICLOT dPT 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.<sup>9</sup>

## PERFORMANCE CHARACTERISTICS

### Precision

ACTICLOT dPT precision studies were performed by BioMedica Diagnostics and two independent laboratories using various coagulation analyzers: the Instrumentation Laboratory ACL™ 300R, the Dade Behring BCT®, the Sysmex® CA-7000, the Instrumentation Laboratory MLA® 900C coagulation analyzer, the Diagnostica Stago STart® 4 and STA Compact®. The studies included multiple tests performed over several days using commercial control plasmas. The results are summarized in Table 2.

**TABLE 2. Precision Data for ACTICLOT dPT 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 (%)
ACL 300R	Normal	32.8	2.5	5.1	30.2	3.8	5.3
	Abnormal	63.8	1.9	7.1	36.9	3.2	3.8
BCT	Normal	47.5	0.5	3.2	51.6	1.7	4.5
	Abnormal	89.2	0.6	5.2	61.9	1.2	3.7
CA-7000	Normal	44.3	0.9	ND	37.9	2.0	ND
	Abnormal	84.1	5.4	ND	48.6	3.3	ND
MLA 900C	Normal	27.9	2.5	3.7	27.2	2.8	4.1
	Abnormal	51.6	2.4	8.6	30.5	1.5	3.7
STart 4	Normal	38.1	0.7	ND	37.3	3.8	ND
	Abnormal	60.6	6.5	ND	44.0	6.7	ND
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

\* BioMedica Diagnostics REF 816N, 816A ND = Not Determined

### Specificity and Sensitivity

In Clinical Study I, the specificity and sensitivity of ACTICLOT dPT were determined using twenty normal plasmas and 23 known weak LA plasmas. The results are shown in Table 3. The results of ACTICLOT dPT combined with the commercially available LA tests *DVVtest/DVVconfirm* (from BioMedica Diagnostics) are also shown.

**TABLE 3. Clinical Study I – Specificity and Sensitivity<sup>a</sup>**

	ACTICLOT dPT Screening Protocol	ACTICLOT dPT Confirmatory Protocol	ACTICLOT dPT (S/C) + DVV (T/C)
<u>No. of Normal Plasmas Tested Negative</u> No. of Normal Plasmas Tested	20/20	20/20	NR <sup>b</sup>
Specificity	100%	100%	NR <sup>b</sup>
<u>No. of Known LA Plasmas Tested Positive</u> No. of Known LA Plasmas Tested	21/23	18/23	22/23
Sensitivity	91%	78%	95.6%

<sup>a</sup> performed using CA-7000 coagulation analyzer

<sup>b</sup> Not Reported

In Clinical Study II, twenty-nine known LA positive plasmas were tested with ACTICLOT dPT, *DVVtest/DVVconfirm* and a commercially available LA-sensitive aPTT reagent. The sensitivity of ACTICLOT dPT when used in a panel with these tests is shown in TABLE 4. The sensitivity increased when results from each test were combined.

**TABLE 4. Clinical Study II: Sensitivity of an LA Test Panel of ACTICLOT dPT, *DVVtest/DVVconfirm* and aPTT.**

	ACTICLOT dPT <sup>a</sup>	ACTICLOT dPT <sup>a</sup> + DVV <sup>b</sup>	ACTICLOT dPT <sup>a</sup> + DVV <sup>b</sup> + aPTT <sup>b</sup>
<u>No. of Known LA Plasmas Tested Positive</u> No. of Known LA Plasmas Tested	23/29	25/29	28/29
Sensitivity	78%	86%	97%

<sup>a</sup> performed on the ACL® 300R coagulation analyzer

<sup>b</sup> performed on the Sysmex CA-1500 coagulation analyzer

### Method Comparison

Two method comparison studies were performed with ACTICLOT dPT and *DVVtest/DVVconfirm*. Both studies utilized patient samples that had previously tested positive for LA or found to be within the normal range. In the first study, conducted on an STA Compact, the results of 49 out of 54 patient samples tested (90.7%) were in concordance. In the second study, conducted on a BCT, the results of 82 out of 93 patient samples tested (88.2%) were in concordance.<sup>16</sup>

### BIBLIOGRAPHY

- Harris, E. N. The Antiphospholipid Syndrome. Diagnosis, Management, and Pathogenesis. *Clin Rev Allergy Immunol* 1995, **13**(1): 39-48.
- Levine, J. S., Branch, D. W. and Rauch, J. The Antiphospholipid Syndrome. *NEJM* 2002, **346**: 752-763.
- Conley, C. L. and Hartmann, R. C. A Hemorrhagic Disorder Caused by a Circulating Anticoagulant in Patients with Disseminated Lupus Erythematosus. *J Clin Invest* 1952, **31**: 621-622.
- Thiagarajan, P., Shapiro, S. S. and DeMarco, L. Monoclonal Immunoglobulin M lambda Coagulation Inhibitor with Phospholipid Specificity: Mechanism of a Lupus Anticoagulant. *J Clin Invest* 1980, **66**: 397-405.
- Triplett, D. A. Antiphospholipid Antibodies. *Arch Pathol Lab Med* 2002, **126**(11): 1424-1429.
- Thiagarajan, P., Pengo, V. and Shapiro, S. S. The use of the Dilute Russell Viper Venom Time for the Diagnosis of Lupus Anticoagulants. *Blood* 1986, **68**: 869-874.
- Exner, T., Papadopoulos, G. and Koutts, J. Use of a Simplified Dilute Russell Viper Venom Time (dRVVT) Confirms Heterogeneity among 'Lupus Anticoagulants.' *Blood Coag Fibrinol* 1990, **1**: 259-266.
- Johns, A. S., Chamley, L., Ockelford, P. A., Pattison, N. S., McKay, E. J., Corkill, M. and Hart, H. Comparison of tests for the lupus anticoagulant and antiphospholipid antibodies in systemic lupus erythematosus. *Clinical and Experimental Rheumatology* 1994, **12**: 523-526.
- Brandt, J. T., Triplett, D. A., Alving, B. and Scharer, I. Criteria for the Diagnosis of Lupus Anticoagulants: An Update. *Thromb Haemost* 1995, **74**(4): 1185-1190.
- Liestøl, S., Jacobsen, E. M. and Wisloff, F. Dilute Prothrombin Time-based Lupus Ratio Test. Integrated LA Testing with Recombinant Tissue Thromboplastin. *Thromb Res* 2002, **105**: 177-182.
- Triplett, D. A., Brandt, J. T., Kaczor, D., and Schaeffer, J. Laboratory Diagnosis of Lupus Inhibitors: a Comparison of the Tissue Thromboplastin Inhibition Procedure with a New Platelet Neutralization Procedure. *Am J Clin Path* 1983, **79**: 678-682.



12. Arnout, J., Vanrusselt, M., Huybrechts, E. and Vermeylen, J. Optimization of the Dilute Prothrombin Time for the Detection of the Lupus Anticoagulant by Use of a Recombinant Tissue Thromboplastin. *Br J Haematol* 1994, **87**: 94-9.
13. Mackie, I. J., Lawrie, A. S., Greenfield, R. S., Guinto, E. R. and Machin, S. J. A New Lupus Anticoagulant Test Based on Dilute Prothrombin Time. *Thromb Res* 2004, **114**: 673-674.
14. CLSI. Collection, Transport and Processing of Blood Specimens for Testing Plasma-based Coagulation Assays and Molecular Hemostasis Assays; Approved Guidelines-Fifth Edition", CLSI document H21-A5, Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
15. Lawrie, A. A., Mackie, I. J., Purdy, G. and Machin, S. J. The Sensitivity and Specificity of Commercial Reagents for the Detection of Lupus Anticoagulants Show Marked Differences in Performance between Photo-optical and Mechanical Coagulometers. *Thromb Haemost* 1999, **81**: 758-762.
16. Data on file, BioMedica Diagnostics, Stamford, Connecticut 06902

ACL is a trademark of Instrumentation Laboratory, SpA












BCT is a registered trademark of Dade Behring Inc.

MLA is a registered trademark of Instrumentation Laboratory, SpA

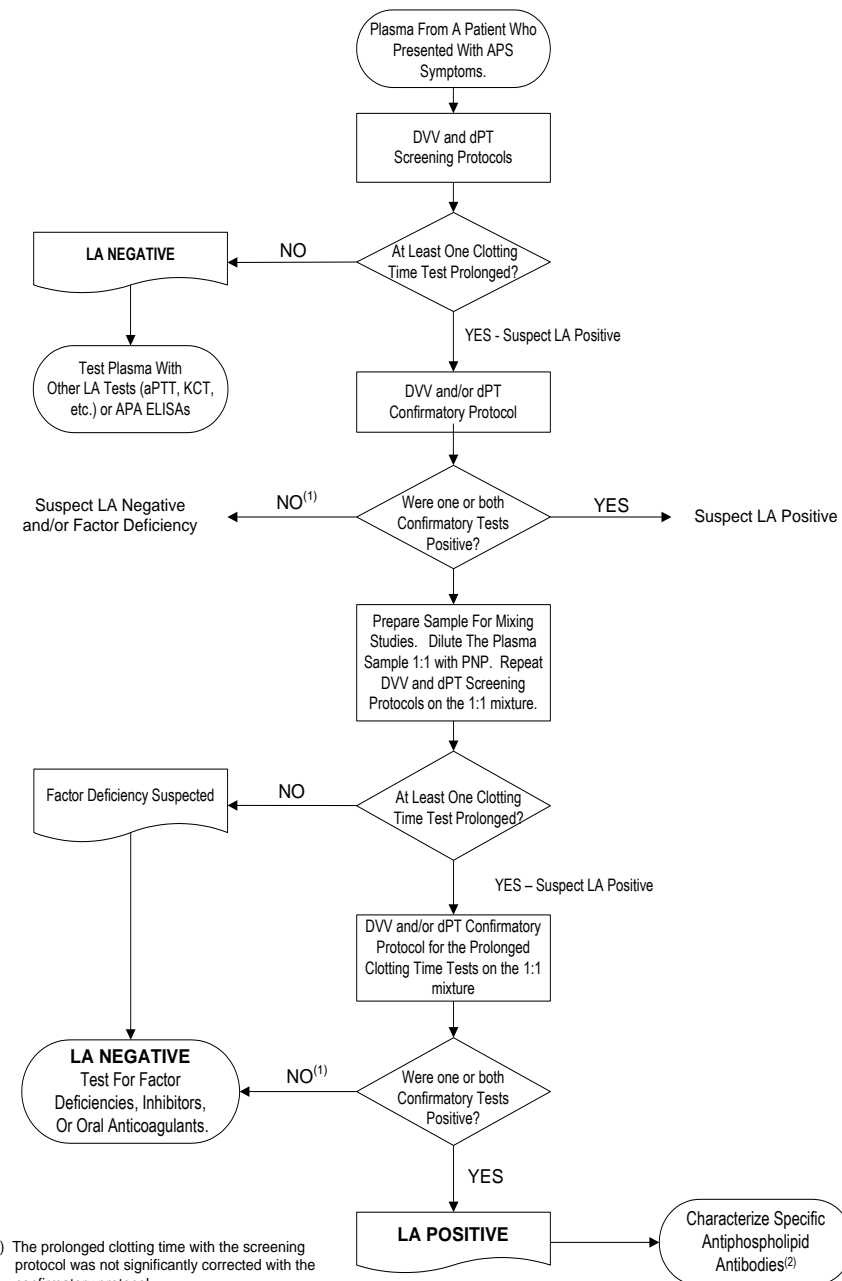
STACLOT and STA Compact are registered trademarks of Diagnostica Stago SA

Sysmex is a registered trademark of the Sysmex Corporation

### KEY TO SYMBOLS

	Consult instructions for use		Lot number
	Refer to SDS		Expiration Date
	In vitro diagnostic medical device		Catalog number
	Manufactured by		Contains sufficient for <n> tests
	Temperature limitation Store at 2°C to 8°C		Contains...
	European Authorized Representative		

### ACTICLOT dPT 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.