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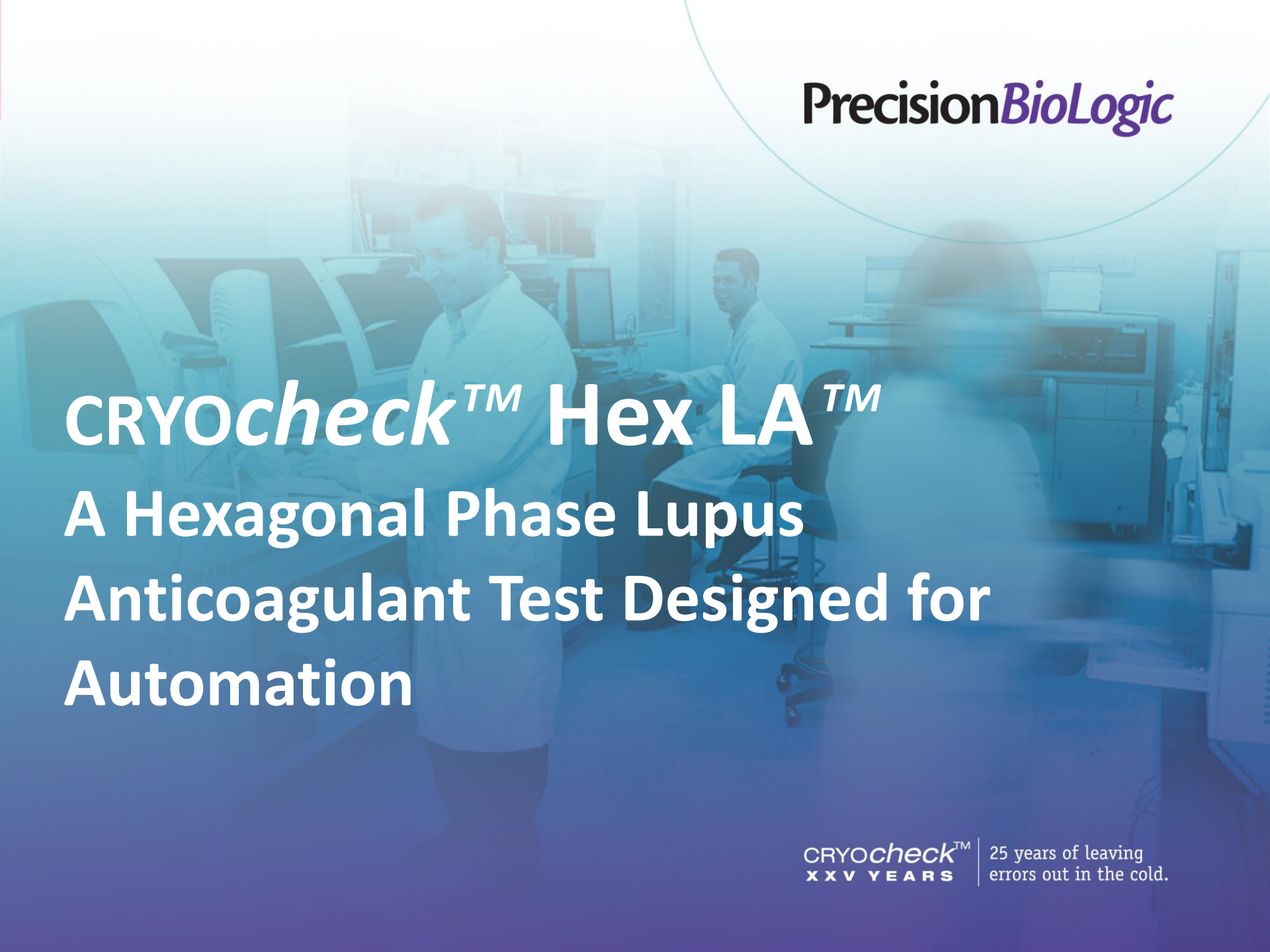
CRYO*check*™ Hex LA™

A Hexagonal Phase Lupus Anticoagulant Test Designed for Automation



CRYO*check*™ XXV YEARS | 25 years of leaving errors out in the cold.

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Overview

Antiphospholipid Syndrome: Overview
Lupus Anticoagulant: History and testing
Introduction to CRYO*check* Hex LA
Competitive comparison
Sales and Marketing strategy

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What is Antiphospholipid Syndrome (APS)?

APS is an acquired autoimmune disorder characterized by the presence of antibodies directed at phospholipids and plasma proteins that bind to phospholipids causing a hypercoagulable state.

- APS is associated with an increased risk for:
  - Venous and arterial thrombosis
  - Recurrent pregnancy failure
  - Stroke or Transient Ischemic Attack (TIA)
  - Rash
- Sometimes associated with specific autoimmune diseases (e.g. SLE) (secondary APS)
- May also be present in isolation (primary APS)

APS often requires treatment with anticoagulant medication to reduce the risk of thrombosis and prevent complications during pregnancy.

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Risk Factors for APS

Risk factors for antiphospholipid syndrome include:

- **Patient sex**: APS is much more commonly found in women than men
- **Autoimmune disorders**: APS is commonly, although not always, found in patients with another autoimmune disorder such as SLE
- **Infections**: APS is more common in people with certain infections such as syphilis, HIV/AIDS, hepatitis C, or Lyme disease
- **Medications**: Some medications have been linked to APS including hydralazine (high blood pressure), quinidine (heart rhythm-regulating) and phenytoin (anti-seizure), and amoxicillin (antibiotic)
- **Family history**: There is some indication that APS is hereditary

It is possible to have antibodies associated with APS without showing symptoms. When APS antibodies are present, the risk of developing clots increases if a patient becomes pregnant or undergoes surgery.

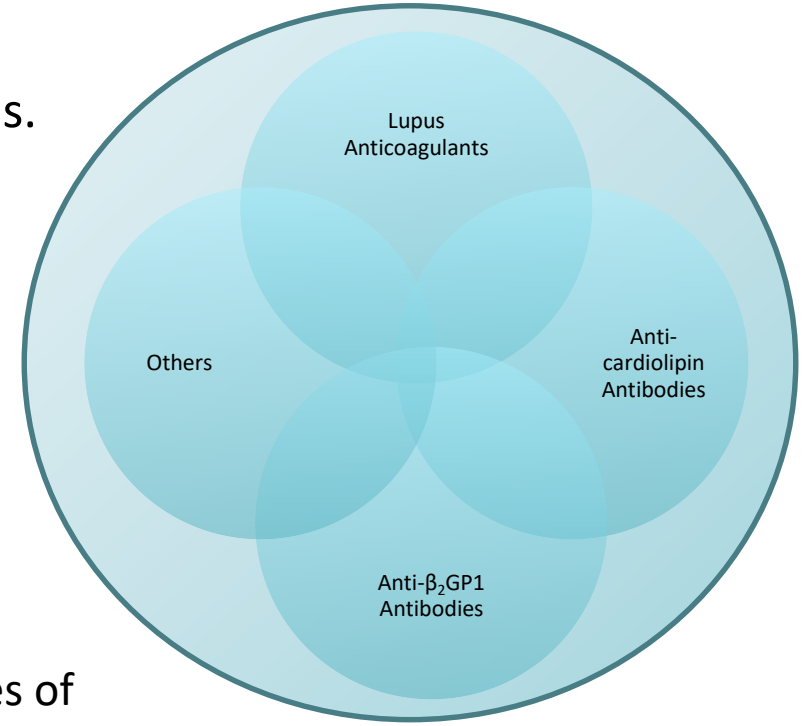
Mayo Clinic: Antiphospholipid syndrome (https://www.mayoclinic.org/diseases-conditions/antiphospholipid-syndrome/symptoms-causes/syc-20355831)

Types of Antiphospholipid Antibodies

- Antiphospholipid antibodies can be classified into several different groups:
  - Anti-β_2GP1 (a subtype of anti-cardiolipin antibodies)
  - Anti-cardiolipin (aCL)
  - Anti-prothrombin (aPT)
  - **Lupus Anticoagulants (LA)**
- Antibodies may be IgA, IgG, or IgM subtypes.

Anti-cardiolipin antibodies can be tested for using solid phase ELISA assays.

Lupus anticoagulant is tested for using a variety of liquid phase coagulation assays.



Categories of Antiphospholipid Antibodies

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What is Lupus Anticoagulant (LA)?

First described more than 50 years ago as an anticoagulant that interfered in whole blood clotting. Lupus anticoagulant is an immunoglobulin that binds to phospholipids and proteins leading to inappropriate blood clotting.

- Sometimes associated with systemic lupus erythematosus (SLE)
- The name lupus anticoagulant is a double misnomer
  - Often found in patients with conditions other than SLE
  - Associated with thrombosis in vivo, not bleeding.
- LA is heterogenous in nature and may also be transient

Clinical Symptoms of Lupus Anticoagulant

- The presence of LA is notably associated with pregnancy-related complications
  - Miscarriage, stillbirth, and pre-term delivery
- Recurrent thrombosis due to LA is often treated with long-term anticoagulant therapy
- Those without a history of recurrent thrombosis should be monitored closely
  - Especially if patient is pregnant because of the increased risk of complications

Due to the heterogeneous and sometimes transient nature of LA, no one test can be used to conclusively test for lupus anticoagulant.

A combination of two screening and confirmatory assays tested 3 months apart are required to form a positive diagnosis for LA.

Guidelines on Lupus Anticoagulant testing

According to the ISTH recommendations for the laboratory detection of LA

- Screening for LA should be performed using two tests based on different principles (i.e.: DRVVT, APTT)
- If one of the two screening tests comes back positive for LA, a mixing study is performed
- If the result of the mixing study is prolonged, a third confirmatory test showing PL dependence should be performed

If an integrated assay is used that includes the screening and confirmatory test in a single procedure, the use of a mixing test is not required. Hexagonal Phase Neutralization Test (HPNT) is an integrated assay that utilizes normal plasma to correct for factor deficiencies in the sample.

LA test results may be interpreted by calculating either the percentage correction, or the LA ratio between screen and confirm tests.

Guidelines on Lupus Anticoagulant testing

CLSI places less importance on the Mixing Test and recommends that this is performed only if the confirmatory test for an abnormal screen fails to show PL dependence. In this instance, a mixing test would be used to run both the screen and confirm assays to determine if the clotting abnormality is due to LA, another inhibitor, or a factor deficiency.

ISTH™
International Society on Thrombosis and Haemostasis

- Screening assay
- Mixing Test
- Confirmatory Assay

CLSI

- Screening assay
- Confirmatory Assay
- Mixing Test

Classification of Lupus Anticoagulant Tests

There are four classifications of lupus anticoagulation tests that satisfy different testing criteria:

- **Independent Screening**: Assay with low level of PL showing a prolonged clot time.
- **Independent Confirmatory**: Assay with increased level of PL showing a shortening of the clot time.
- **Paired**: Screening assay with low levels of PL is recapitulated in a confirmatory assay with increased levels of PL.
- **Integrated**: Screening, confirmatory, and mixing tests are performed in parallel as part of the same test system.

Let's look at some examples of each of the three classifications might work together in a lupus anticoagulant testing panel...

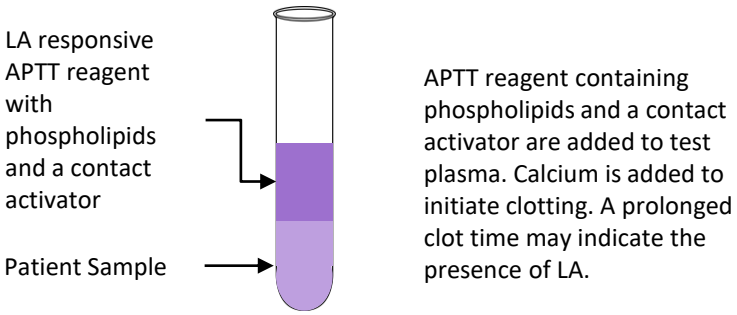
Independent Screening Test

APTT- Activated Partial Thromboplastin Time

About the Test

- Based on activation of the intrinsic pathway
- APTT reagent used must demonstrate responsiveness to LA
- Recommended as a first-line test by CLSI and ISTH (along with dRVVT)
- Can be paired with PNP as a confirmatory assay
- Can also be paired with a Hexagonal Phase Neutralization Test as a confirmatory assay
- Affected by presence of UFH

Screening Assay

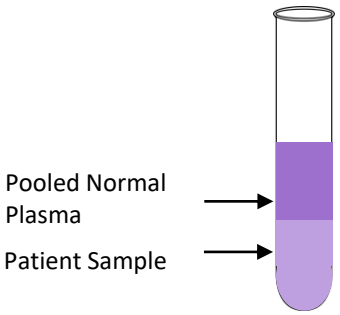


Mixing Test

About the Test

- Mixing test can help identify if factor deficiency is present in sample
- ISTH recommends running a mixing study immediately after the screening step
- CLSI has placed lower priority on the mixing test and recommends performing only if the confirm assay failed to show PL dependence (run on screen and confirm assays)
- Mixing test can be performed on both screen and confirm assays to help guide diagnosis

Mixing Test



A 1:1 mix of patient plasma and normal pooled plasma is prepared and then used to perform the desired screening or confirmatory test.

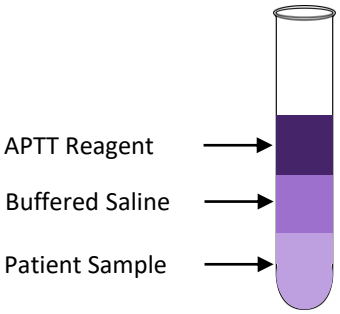
Independent Confirmatory Test

PNP- Platelet Neutralization Procedure

About the Test

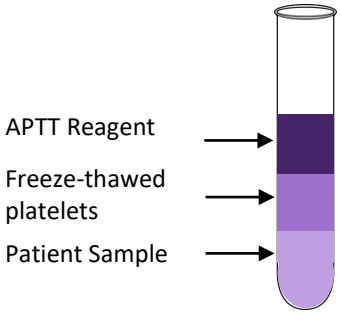
- Based on activation of the intrinsic pathway
- APTT-based LA confirmatory test
- Uses negatively charged platelet PL to dampen or neutralize effect of LA
- Results are expressed as a delta between tube 1 (patient plasma and buffer) and tube 2 (patient plasma and platelet lysate)
- Affected by presence of UFH

Tube 1



In tube one, an APTT reagent is used to initiate clotting in a mixture of patient sample and buffered saline solution.

Tube 2



In tube two, an APTT reagent is used to initiate clotting in a mixture of patient sample and freeze-thawed platelets. The platelet PL present in the mix will neutralize any LA in the sample and shorten the clotting time.

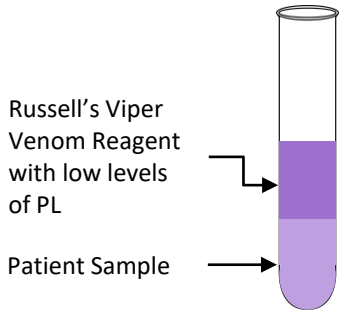
Paired Screening and Confirmatory Test

dRVVT- Dilute Russell's Viper Venom Time

About the Test

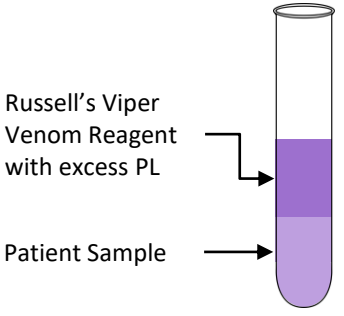
- Based on activation of the common pathway
- Robust and widely used
- Recommended as a first-line test by CLSI and ISTH (along with APTT)
- More specific for LA than APTT, KCT, or dPT
- Results calculated and reported as a ratio between tube 1 and tube 2
- If result is below the cut-off, confirmatory testing is not obligatory

Screening Assay



Russel's Viper Venom directly activates factor X, bypassing factor VII of the extrinsic pathway making the test more specific for LA.

Confirmatory Assay



Excess PL in the confirmatory assay will neutralize LA if present and shorten the clotting time. Results are expressed by calculating the ratio between the screen and confirm tests and comparing to the established cut-off.

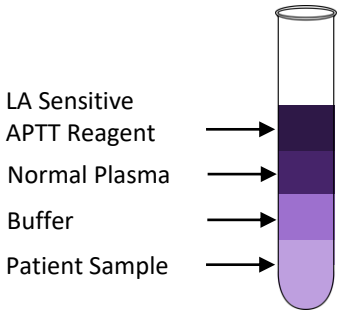
Integrated Screen/Confirm

HPNT- Hexagonal Phase PL Neutralization Test

About the Test

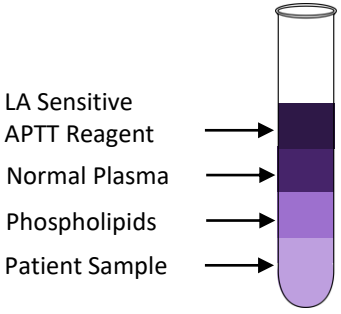
- Based on activation of the intrinsic pathway
- Incorporates a screening, confirmatory, and mixing step into one assay system (no need for a discreet mixing test)
- Results expressed as a delta between tube 1 and tube 2
- Can be used as a secondary screen and confirm test as part of LA testing panel

Tube 1



In tube one, clotting is initiated by an LA sensitive APTT reagent with normal plasma and buffer.

Tube 2



In tube two, phospholipids are added in place of buffer. If the clot time corrects, this may indicate the presence of LA.

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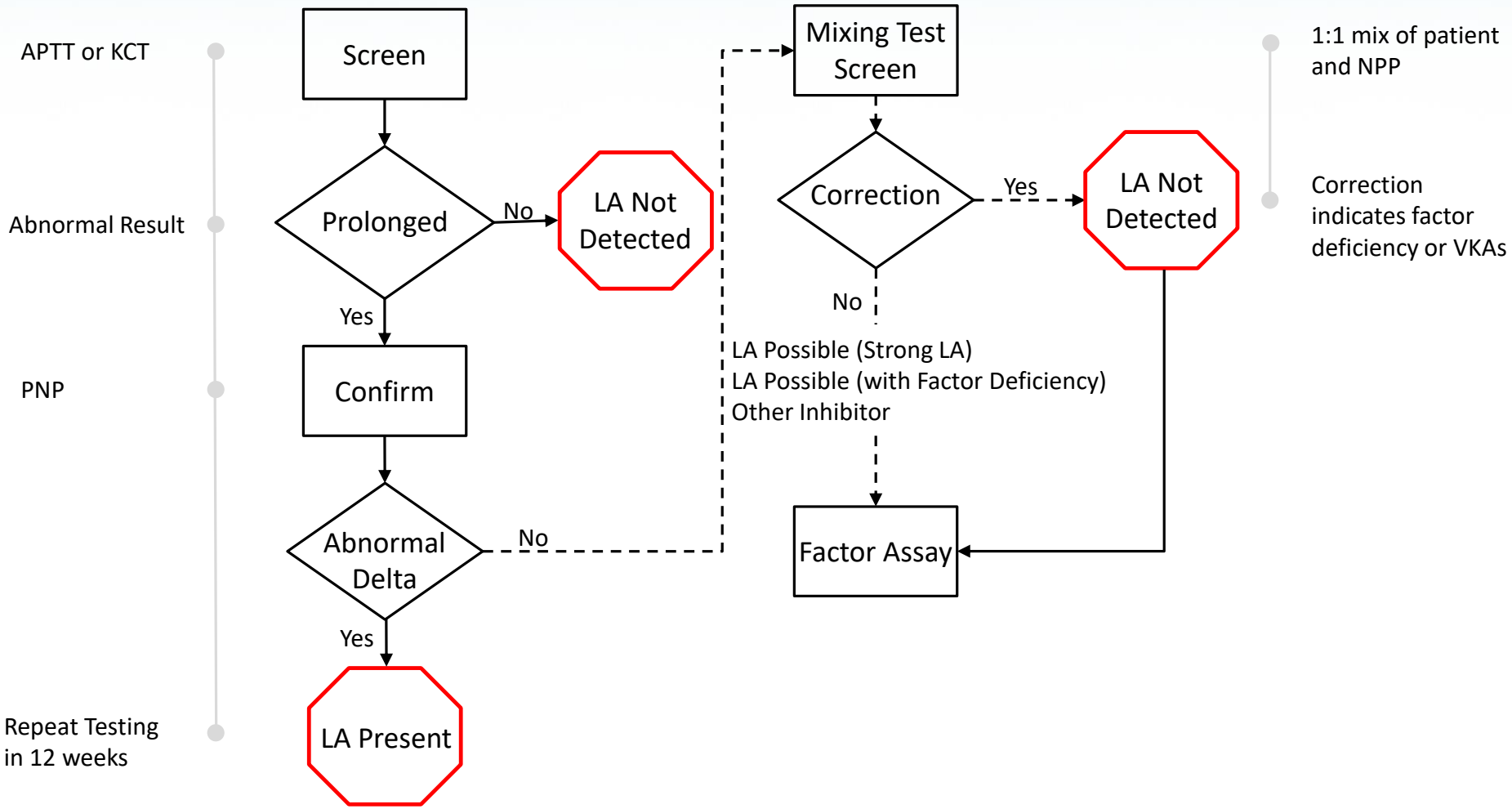
LA Testing Algorithms – Independent System



CLSI Guideline for Lupus Anticoagulant testing

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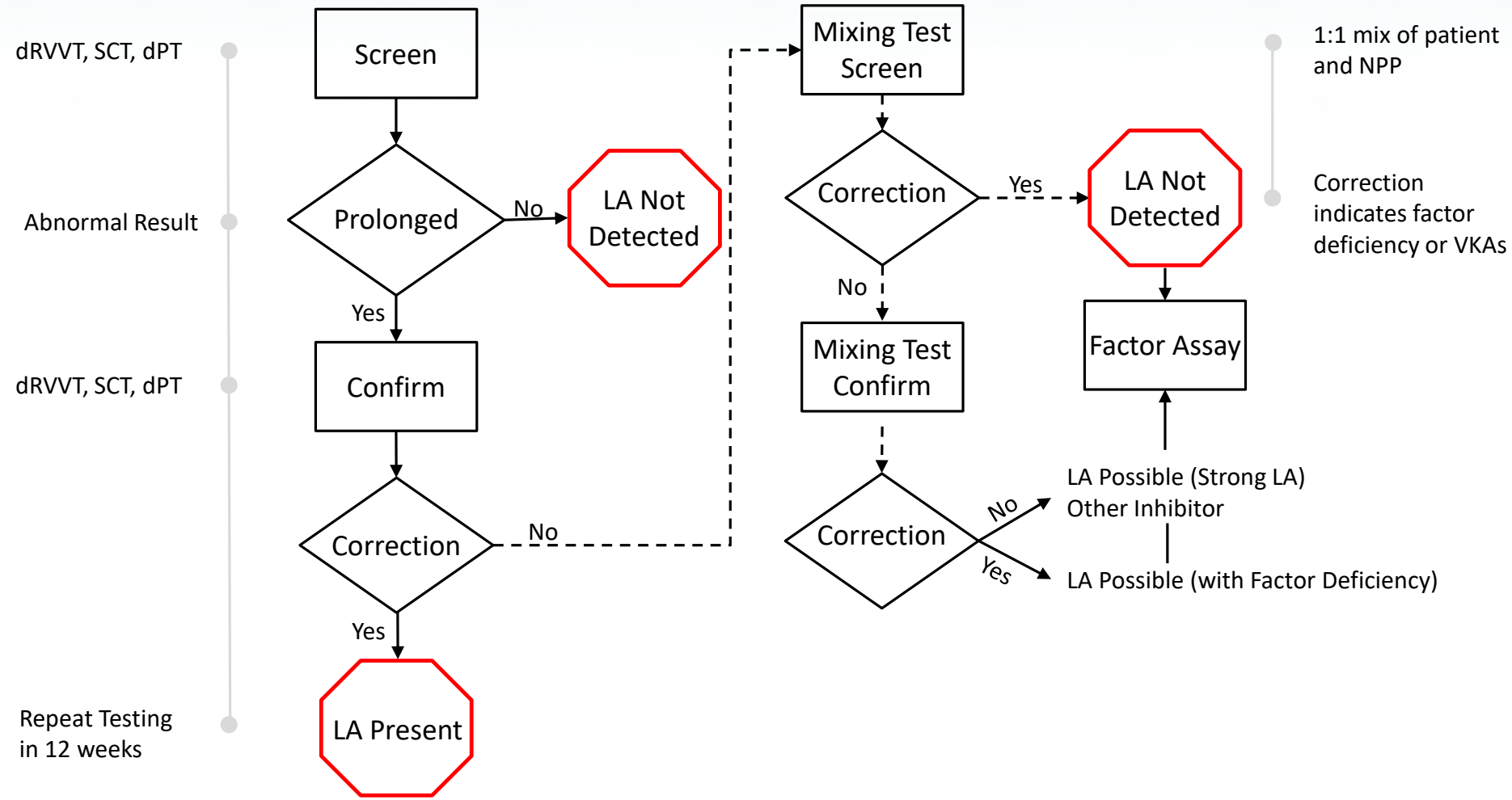
LA Testing Algorithms – Paired System



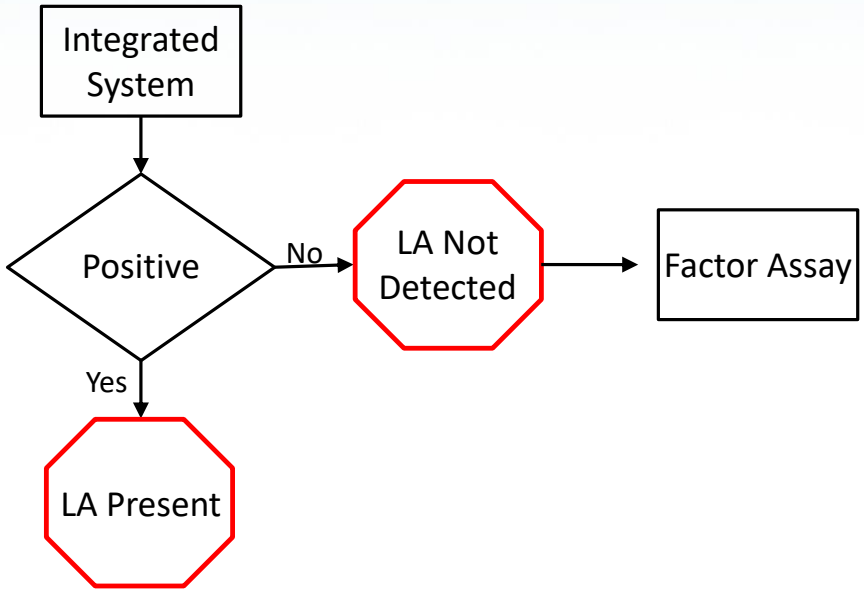
CLSI Guideline for Lupus Anticoagulant testing

LA Testing Algorithms – Integrated System

HPNT

Qualitative Result

Repeat Testing in 12 weeks



- A much simplified testing algorithm with an integrated test system.

CLSI Guideline for Lupus Anticoagulant testing

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About Hexagonal Phase Lupus Anticoagulant Testing

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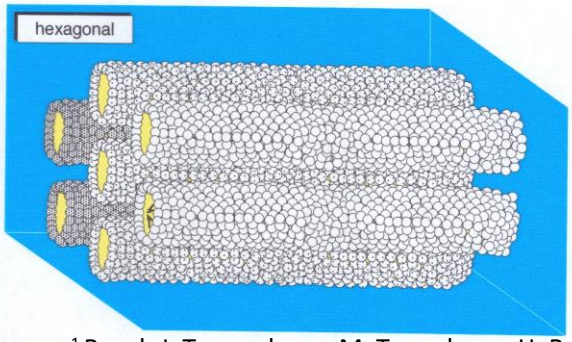
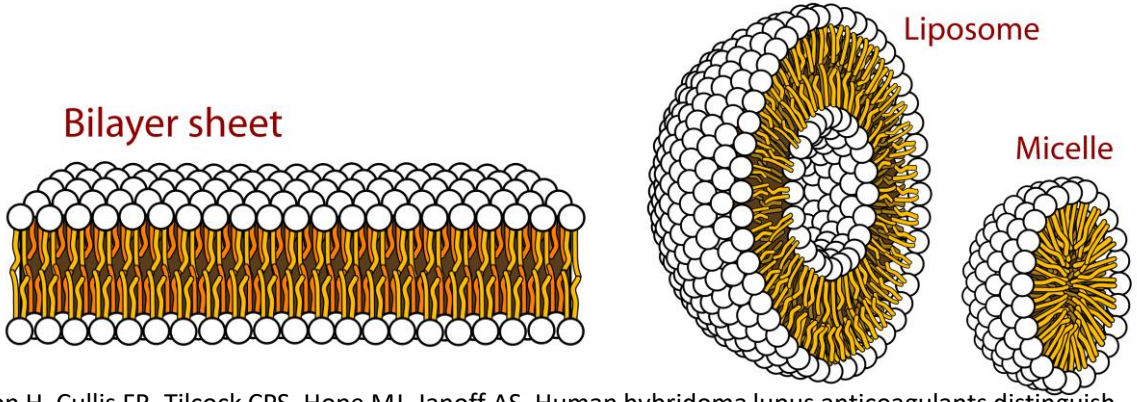
About Hexagonal Phase Phospholipids

Phospholipids may aggregate in a variety of ways, with common structures being bilayer, and hexagonal phase.

- In 1986, Dr. J. Rauch demonstrated the ability of hexagonal (II) phase phospholipids to neutralize the anticoagulant activity of 11 LA antibodies from patients with systemic lupus erythematosus (SLE)[1].
  - In contrast, lamellar phase and bilayer phospholipids had no effect on the anticoagulant activity of these antibodies.
- Dr. Rauch concluded that antibodies are able to distinguish between different structures, and hexagonal phase showed a superior immunogenic response.





[1] Rauch J, Tannenbaum M, Tannebaum H, Ramelson H, Cullis FR, Tilcock CPS, Hope MJ, Janoff AS. Human hybridoma lupus anticoagulants distinguish between lamellar and hexagonal phase lipid systems. **J Biol Chem**.1986; *261*:9672–9677.

About Hexagonal Phase LA Testing

- The Hexagonal Phase Lupus Anticoagulant test is an integrated (screen and confirm) APTT assay for the qualitative detection of LA.
- Hexagonal phase phospholipids have a neutralizing effect on Lupus Anticoagulant in-vitro.
- By testing a patient sample both in the presence and absence of Hexagonal Phase Phospholipids, the presence of LA can be confirmed by comparing the clot time correction in seconds and to the cut-off.
- Hexagonal Phase LA tests also include a source of normal plasma, correcting for any factor deficiencies that may be present in the sample.
  - This effectively replaces any mixing test that may be required as part of the LA testing algorithm.

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Advantages of an Integrated Hexagonal Phase Assay

- **Guidelines**: Includes an integrated mixing step, satisfying all three testing criteria from CLSI and ISTH for LA diagnosis (Screening, Confirmatory, and Mixing tests).
- **Sensitivity**[1]: With an integrated assay "weak" LA that prolongs clotting times above a patients baseline, but not above the RI, are more likely to be detected. In this situation, non agreement between screen and confirm components of the test may reveal LA where a APTT based screening test alone would not.
- **Immunogenic Response**[2]: Hex phase phospholipids have been shown to have a superior immunogenic response to LA when compared to bilayer or lamellar phospholipids arrangements.
- **Simplicity**: A testing algorithm with easy interpretation of positive results and less ambiguity around negative results.

[1] CLSI Guideline for Lupus Anticoagulant testing

[2] Rauch J, Tannenbaum M, Tannebaum H, Ramelson H, Cullis FR, Tilcock CPS, Hope MJ, Janoff AS. Human hybridoma lupus anticoagulants distinguish between lamellar and hexagonal phase lipid systems. **J Biol Chem**.1986; *261*:9672–9677.

Current Hexagonal Phase Test Availability

- Staclot LA From Stago is the only commercially available Hexagonal Phase Lupus Anticoagulant test available.
- Available in two formats as a lyophilized reagent set:
  - **Staclot LA 10**
  - **Staclot LA 20**
- As labs have moved to running all tests on automated coagulation analyzers, Staclot LA has been adapted to work on most platforms.
  - Most of these applications are laboratory developed

Staclot LA protocols have changed considerably from the original manual method described in the direction insert.

- Incubation time and reagent volumes often differ on automated analyzers

Performance - Precision

Precision studies were carried out over 20 days using three lots of CRYO*check* Hex LA

- Each sample was measured with each product lot in duplicate twice a day

Sample	Within-Laboratory Precision			Within-Laboratory Precision		
	Mean Clot Time (s)	SD	%CV	Mean Clot Time (s)	SD	%CV
CRYO*check* Lupus Negative Control	53.0	1.6	3.0	52.8	2.8	5.3
CRYO*check* Weak Lupus Positive Control	87.3	3.2	3.7	65.4	2.8	4.2
CRYO*check* Lupus Positive Control	125.4	5.2	4.2	79.8	4.5	5.7
LA Negative Plasma Sample	55.9	1.7	3.1	55.1	2.5	4.5
LA Near Cut-Off Plasma Sample	67.6	2.5	3.8	58.4	2.6	4.5
LA Weak Positive Plasma Sample	89.8	3.3	3.7	66.4	3.0	4.6
LA Moderate Positive Plasma Sample	146.5	6.0	4.1	85.9	5.8	6.7
LA Strong Positive Plasma Sample	270.7	9.6	3.6	118.0	9.0	7.6

Performance claims subject to change pending FDA review

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Performance – Reproducibility LA Start

Reproducibility studies were carried out at three sites (one internal and two external) over five days using three lots of CRYO*check* Hex LA

- Each sample was measured with each product lot in triplicate twice a day

Sample	Mean CT (s)	Within-Run		Between-Run		Between-Day		Between-Site		Across-Site	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
CRYO*check* Lupus Negative Control	52.8	1.3	2.5	0.7	1.4	0.0	0.0	0.4	0.7	1.6	3.0
CRYO*check* Weak Lupus Positive Control	85.7	3.3	3.9	0.0	0.0	1.0	1.2	1.9	2.2	4.0	4.6
CRYO*check* Lupus Positive Control	123.6	4.5	3.6	2.0	1.6	1.7	1.4	2.1	1.7	5.6	4.5
LA Negative Plasma Sample	55.8	1.3	2.3	0.8	1.5	0.1	0.2	0.7	1.2	1.8	3.2
LA Near Cut-Off Plasma Sample	66.9	2.1	3.1	1.0	1.5	1.0	1.6	0.8	1.1	2.7	4.0
LA Weak Positive Plasma Sample	88.3	3.6	4.1	0.0	0.0	1.4	1.5	1.7	2.0	4.3	4.8
LA Strong Positive Plasma Sample	264.9	6.0	2.3	3.1	1.2	2.8	1.0	4.4	1.7	10.1	3.8

Performance claims subject to change pending FDA review

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Performance – Reproducibility LA Correct

Reproducibility studies were carried out at three sites (one internal and two external) over five days using three lots of CRYO*check* Hex LA

- Each sample was measured with each product lot in triplicate twice a day

Sample	Mean CT (s)	Within-Run		Between-Run		Between-Day		Between-Site		Across-Site	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
CRYO*check* Lupus Negative Control	53.7	1.5	2.8	0.8	1.6	0.0	0.0	0.0	0.0	3.1	5.8
CRYO*check* Weak Lupus Positive Control	65.8	2.1	3.3	1.0	1.5	0.7	1.0	0.7	1.1	3.1	4.8
CRYO*check* Lupus Positive Control	80.2	2.8	3.5	1.5	1.8	1.4	1.7	1.8	2.2	4.8	5.9
LA Negative Plasma Sample	55.9	1.6	2.9	0.6	1.1	0.2	0.4	0.0	0.0	2.9	5.2
LA Near Cut-Off Plasma Sample	59.2	1.9	3.2	1.2	2.0	0.7	1.1	0.3	0.6	3.0	5.0
LA Weak Positive Plasma Sample	66.9	2.2	3.2	1.3	2.0	1.2	1.8	2.2	3.3	4.0	6.0
LA Strong Positive Plasma Sample	117.6	3.0	2.6	3.1	2.6	2.0	1.7	2.6	2.2	9.4	8.0

Performance claims subject to change pending FDA review

Performance – Normal Range and Assay Cutoff

Normal Range: A normal range study was performed in-house using 137 normal samples according to CLSI EP28: A3c. Each sample was tested using three lots of CRYO*check* Hex LA. A pooled mean ±2 SD range was determined for delta correction results and is shown in the table below:

Lower Range (s)	Upper Range (s)
-5.0	2.2

Assay Cut-Off: The cut-off for the assay delta correction was determined using 137 normal samples and calculating the mean + 4 SD, with the following results:

Delta Correction	Interpretation
<7 seconds	LA Negative
>7 seconds	LA Positive

Performance claims subject to change pending FDA review

Performance - Interferences

Interference studies were conducted according to CLSI EP07, 3rd ed. using a single lot of CRYO*check* Hex LA. Patient plasma samples were spiked with possible interferents and 20 replicates were tested alongside 20 replicates of the corresponding blank matrix control. The following substances showed no interference up to the concentrations indicated:

Substance Tested	Test Concentration
Hemoglobin	≤ 500 mg/dL
Intralipid	≤ 500 mg/dL
Bilirubin	≤ 20 mg/dL
Unfractionated heparin	≤ 2 IU/mL
Low molecular weight heparin	≤ 2 IU/mL

Performance claims subject to change pending FDA review

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Performance - Method Comparison

A method comparison study was conducted to assess the efficacy of CRYO*check* Hex LA in the qualitative detection of LA relative to a comparator assay, Staclot® LA. A total of 226 samples were included in the study: 124 known (previously characterized) LA positive samples, 75 normal (presumed LA negative) samples and 27 samples from individuals with other medical conditions including autoimmune disorders. The data demonstrated positive percent agreement of 94% (95% CI, 88-97%), negative percent agreement of 99% (95% CI, 94%-100%), and overall agreement of 96% (95% CI, 93%-98%) as summarized below.

		CRYO*check* Hex LA results		
		Negative	Positive	Total
Staclot LA	Negative	95	1	96
	Positive	8	122	130
	Total	103	123	226

Agreement	Point Estimate (95% Confidence Interval)
Positive Percent Agreement	94% (88% - 97%)
Negative Percent Agreement	99% (94% - 100%)
Overall Agreement	96% (93% - 98%)

Performance claims subject to change pending FDA review