

White Paper

New CLSI Guideline on Testing for Lupus Anticoagulant (LA): What is Different from the SSC Guideline?

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The new Approved Guideline H60-A, Laboratory Testing for the Lupus Anticoagulant, was released by the Clinical and Laboratory Standards Institute (CLSI) in April 2014. Committee members included U.S. and international key opinion leaders in lupus testing.

The major differences in the CLSI 2014 guideline¹ versus the SSC 2009 guideline² are outlined in Table 1 below.

Key Provisions of the CLSI Guideline Establishment of reference intervals and cutoff values

In LA testing, the upper limit of normal determines the test cutoff, while the mean of the reference interval (RI) is used for normalizing LA assays. The RI is defined as the mean \pm 2SD range.

For local determination of the RI, at least 40 samples from normal, healthy donors are required.

Alternatively, an RI established by the assay's manufacturer can be transferred for a defined instrument/reagent combination. A prerequisite is that manufacturers provide a well-established RI based on a minimum of 120 normal samples, which is the case for all Siemens methods. For verification of the manufacturer-recommended RI, a minimum of 20 reference individuals should be tested according to CLSI document EP28.

Use of normalized ratios is recommended for expressing results of all LA tests; the mean of the RI must be used as the denominator.

Patient selection

For routine clinical use (general coagulation screening), APTT reagents developed with high LA sensitivity should be avoided. APTT reagents with high LA sensitivity are indicated only in patients for whom LA testing is requested.

Table 1. Major differences in the CLSI Guideline vs. the SSC 2009 Guideline

| Category | CLSI 2014 | SSC 2009 |
|-------------------------------------|--|---|
| Screening tests | Recommends dRVVT and APTT as first choice, but does not exclude other assays, such as dPT, KCT, or other snake venom assays. | First choice dRVVT, followed by APTT. No other tests allowed. |
| APTT reagents | Does not restrict the type of APTT activator to be used. Allows combination of high and low LA-sensitive APTT for screening and confirmation. | Limits APTT reagents to those using silica as activator; recommends not using ellagic acid-based reagents. Requires integrated assays for screening and confirmation (same type of assay, differing only in phospholipid content). |
| Test sequence | <ul style="list-style-type: none"> • Screening • Confirmation • Optional: Mixing if required/indicated | <ul style="list-style-type: none"> • Screening • Mixing • Confirmation All three steps are mandatory in this sequence. |
| Cutoff values | Defines cutoff as mean plus 2SD. Recommends applying cutoff provided by manufacturer. | Defines cutoff as 99th percentile. Requires cutoff determination by each individual lab. |
| Result expression | Normalized ratio to the mean of the reference interval. | Normalized ratio to mean of NPP (normal pool plasma). |
| Result screen to confirm expression | Normalized ratio screen/confirm or percent correction. | Percent correction: (screen-confirm)/screen x 100 |
| Interpretation | Interpretation should be provided with individual test results; permits use of "indeterminate" for results not clearly indicating presence of absence of LA. | Interpretation should be provided with individual test results; discourages use of borderline results. |

Ideally, patients should not be under anticoagulant therapy. Vitamin K antagonists and the new direct thrombin and FXa inhibitors prolong dRVVT, APTT, and PT, which makes interpretation challenging because of the risk of false-positive results.

Sample preparation

Specimens should be processed within 4 hours of collection; storage should be at room temperature. Platelet-poor plasma (PPP) with $<10 \times 10^9/L$ platelets is critical for testing accuracy, as residual platelets may neutralize LA. Residual platelets are more problematic in frozen samples that have been thawed, as platelets may burst and release excess phospholipids (PL) into the plasma. This may cause false-negative results by shortening clotting times. To ensure minimal residual platelets in plasma, double centrifugation is strongly recommended (regardless of whether or not samples will be frozen), while the use of filters to obtain platelet-poor plasma is discouraged.

Furthermore, it is critical that the normal pool plasma (NPP) used for mixing tests does not exceed the recommended residual platelet count.

If samples cannot be tested within 4 hours, they should be frozen (preferably at -70°C , but -20°C is acceptable). Frozen samples should be thawed in a 37°C water bath for five minutes to prepare for testing.

Recommended assays

APTT (using an LA-sensitive reagent) and dRVVT are the preferred screening tests. However, second-line tests such as KCT or dPT may detect LA that does not manifest in APTT or dRVVT testing.

The composition of the APTT reagent plays a significant role in detecting LA, which depends primarily on the phospholipid (PL) content. The CLSI guideline does not restrict the choice of activator, permitting silica, ellagic acid, and other contact activators.

Differences in the LA responsiveness of APTT reagents can be exploited for diagnostic testing purposes; **a combination of two matching APTT tests, one responsive and another not responsive to LA, provides a simple system for detecting LA.**

APTT is an independent screening test; however, a paired test can be built using LA-responsive and nonresponsive APTT reagents. (Note: Any APTT reagent can be responsive to LA if the antibody is sufficiently strong.)

Alternatively an APTT-based hexagonal phase PL neutralization test can be performed.

DRVVT tests of low and high PL content are considered to be paired tests, as screening and confirmation tests differ only in PL content. DRVVT seems to detect mostly B2GPI-dependent LA and is more specific than APTT, KCT, or dPT. However, direct thrombin and FXa inhibitors prolong dRVVT as well.

For diluted prothrombin time (dPT), the use of recombinant tissue factor reagents is recommended; however, an optimal dilution for PT reagents is not provided.

A mixing test is required only if the confirmation assay cannot demonstrate PL dependence. In that case, a mixing test is performed to assess the inhibitory action of LA in patient plasma against NPP. The mixing test should use equal amounts of patient PPP and NPP (1+1). Incubated mixing tests are not recommended. Mixing tests may be used for confirmation tests as well to increase specificity. In all mixing tests, quality of the NPP is critically important; lyophilized, platelet-poor normal plasma prepared specifically for use as NPP can be used.

Test interpretation

All LA test results should be accompanied by an interpretation. For LA-negative samples, the preferred term is "LA not detected"; "LA present" is preferred for samples in which LA activity is detected.

| Method | BCS [®] XP System, BCS System | Sysmex [®] CA-1500 System | Sysmex CS-2000i [†] System |
|--|--|------------------------------------|-------------------------------------|
| LA1, screen (sec) | 35.0 27.7–42.2 | 33.7 27.8–39.6 | 35.9 28.6–43.1 |
| LA2, confirm (sec) | 28.6 25.6–31.7 | 35.5 32.4–38.4 | 30.6 27.7–33.5 |
| LA1/2 ratio | 1.22 1.04–1.40 | 0.95 0.83–1.07 | 1.20 1.00–1.40 |
| Dade [®] Actin [®] FSL Reagent, screen (sec) | 28.8 23.8–33.7 | 27.6 23.8–31.4 | 27.5 24.3–30.6 |
| Pathromtin [®] SL Reagent, screen (sec) | 30.4 24.7–36.2 | 31.2 24.8–37.5 | 30.6 24.6–36.5 |
| Dade Actin FS Reagent, confirm (sec) | 27.2 22.4–32.0 | 25.2 21.7–28.8 | 24.7 20.9–28.4 |

Conclusion

The new CLSI guideline allows the lab much more flexibility. Furthermore, the CLSI guideline is much less stringent regarding local reference-range determination and more straightforward in diagnosing presence of LA using the combination of screening and confirmation assays, requiring mixing tests only in certain cases. Application of the new CLSI guideline for LA testing will substantially benefit the laboratory, and Siemens therefore recommends implementation of this guideline.

Abbreviations

| | |
|-------|--|
| CLSI | Clinical and Laboratory Standards Institute |
| dPT | Dilute prothrombin time |
| dRRVT | Dilute Russell viper venom time |
| LA | Lupus anticoagulant |
| KCT | Kaolin clotting time |
| NPP | Normal pooled plasma |
| PL | Phospholipids |
| PPP | Platelet-poor plasma |
| RI | Reference interval |
| SSC | Scientific Subcommittee of the ISTH (International Society of Thrombosis and Hemostasis) |

Disclaimers

*Not available for sale in the U.S.

** These reference intervals were obtained in a study with apparently healthy individuals. Reference intervals vary from laboratory to laboratory depending on the population served and the technique, method, equipment, and reagent lot used. Therefore, each laboratory must establish its own reference intervals or verify them whenever one or more of the aforementioned variables are changed.

This white paper provides additional information.

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References and Recommended Readings

1. Ledford-Kraemer MR, Moore GW, Bottenus R, Daniele C, de Groot PG, Exner T, Favalaro EJ, Moffat KA, Nichols WL. Laboratory Testing for the Lupus Anticoagulant. Approved guideline – first edition. CLSI document H60. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
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Open access: <http://onlinelibrary.wiley.com/doi/10.1111/ijlh.12227/pdf>

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