The CLSI & BCSH algorithms for Lupus anticoagulant testing

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The Clinical and Laboratory Standards Institute (CLSI) is a global, not-for-profit, standards-developing organisation that promotes the development and use of voluntary consensus standards and guidelines within the health care community. Its first guideline for lupus anticoagulant (LA) testing is due for publication in early 2013. As it is not yet published, content may be subject to change. The recently published guidelines on antiphospholipid syndrome (APS) from the British Committee for Standards in Haematology (BCSH) in 2012 update and replace the previous guideline published in 2000 based on relevant publications since then. The main recommendations are summarised below:

Pre-analytical issues

Both documents recommend double centrifugation of blood collected into 3.2% tri-sodium citrate to ensure generation of platelet poor plasma with a platelet count of $<10 \times 10^9$/L. Filtration is not recommended due to loss of some clotting factors and potential to generate microparticles. Standard routine coagulation screening tests are valuable to exclude undiagnosed coagulopathies and anticoagulant therapy. CLSI further recommends use of a LA-unresponsive APTT reagent to reduce serendipitous finding of LAs in asymptomatic patients and permit interpretation of LA assays unencumbered by the possibility of a co-existing abnormality.

Lupus anticoagulant assays

CLSI recommends that both dRVVT and LA-responsive APTT are performed as first-line screening tests whilst use of other assays is not excluded providing they each employ different pathways in their design. BCSH specifically recommends dRVVT and suggests a suitable APTT would normally be the second assay but others are not excluded. The BCSH guideline intentionally describes laboratory criteria as 'classical findings': (i) prolongation of a phospholipid-dependent clotting assay (ii) demonstration of the presence of an inhibitor in mixing studies (iii) demonstration of phospholipid-dependence. This is because recognition is given to the potential to dilute LAs in mixing studies, thus reducing sensitivity, and that screen & confirm results from undiluted plasma alone can demonstrate the presence of a LA when no other causes of elevated clotting times are present. This parallels the ISTH (2009) suggestion that performing screen and confirm on every patient does not, in principle, require mixing tests. The CLSI guideline goes one step further and re-prioritises the testing sequence to screen, confirm and then the mix only if required. Using the mix result as a decision point to complete the LA test medley risks reporting false negative interpretations when a fundamental limitation of mixing test design is masking a genuine antibody. However, mixing tests increase specificity and diagnostic accuracy when there are co-existing abnormalities, the confirm test on undiluted plasma does not return to the reference range and the co-factor effect is present and continue to have a place in the analytical armoury.

Cut-offs

Cut-offs must be locally derived based on specific reagent/analyser pairings. Obtaining sufficient normal donors to generate an accurate 99^{th} centile cut-off for screen and mixing

tests is beyond the reach of most diagnostic departments and CLSI maintains that 97.5th centile can be adopted and points readers to its own reference range guideline (CLSI C28-A3) for further detail and discussion. BCSH considers inaccuracy in relation to sample numbers and suggests that previously established cut-offs can be validated with smaller numbers. It should be recognised that increasing cut-offs to the 99th centile improves specificity but reduces sensitivity. Furthermore, whilst use of 99th centile will reduce frequency of false positive screening tests it will also increase that of false negatives. Any elevated screening test will receive a confirm test that will be similarly elevated if not due to a LA, so ultimately, a false positive interpretation will not ensue. Both documents describe calculations for assessing phospholipid dependence.

LA testing during anticoagulant therapy

BCSH specifically states that the majority of patients on VKA therapy can receive LA testing upon cessation of the treatment to avoid analytical complications. Despite this, it is not uncommon for laboratories to receive requests to test on such patients and guidance is given in both documents. The utility of LA assays performed on undiluted plasma is disputed. Undertaking screen and confirm assays on 1:1 mixtures of test and control plasma can reveal a LA if the antibody is sufficiently potent to overcome the dilution and the confirm step will reveal phospholipid dependence. No restrictions are suggested based on INR. Negative testing does not exclude a LA due to the dilution effect. Both guidelines accept that TSVT screening, with either Ecarin time or platelet neutralisation procedure as confirmatory tests, can be a useful adjunct. BCSH discourages testing on patients receiving unfractionated heparin whilst CLSI gives examples where heparin neutralisers in dRVVT reagents are successful in revealing a LA and when they are not.