The new CLSI guideline for Lupus Anticoagulant testing

G. Moore
Haemostasis & Thrombosis, Viapath at Guy's & St. Thomas' Hospitals, London, UK

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. They generate standards and guidelines for all the main disciplines of pathology. Although based in the USA, the guidelines are written by international panels of recognised experts, and once published, are commonly adopted by the US FDA and recommended in other countries. The first CLSI guideline for LA testing, H60-A, was published in April 2014. The main recommendations are summarised below:

Pre-analytical issues
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 is recommended, although it is acknowledged that a properly conducted and verified single centrifugation may achieve the requisite plasma quality. Filtration is not recommended due to loss of some clotting factors. Standard routine coagulation screening tests are valuable to exclude undiagnosed coagulopathies and undisclosed anticoagulant therapy. H60-A 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 screening assays
H60-A recommends that both dRVVT and LA-responsive APTT are performed as first-line screening tests. In contrast to the ISTH guideline update of 2009, use of other assays is not excluded providing they each employ different pathways in their design. There is some debate about the possibility of generating an increase in false-positives if more than two assays are employed at the screening stage. This is countered in H60-A by indication that although false-positive screening tests may ensue when employing a wider repertoire, it is the interpretation of the screening test, mixing test and confirmatory test composite that secures the laboratory diagnosis. Some LA will not manifest in the tried and tested dRVVT + APTT pairing, or at least in a given reagent pairing, so additional tests may be valuable in certain patients.

Mixing and confirmatory tests
A common response to an elevated LA screening test is to perform a mixing test to evidence inhibition. H60-A recommends use of 1:1 volumes of test and normal pooled plasma with interpretation via the Index of Circulating Anticoagulant formula or application of mixing test-specific cut-offs. Confirmatory tests usually employ concentrated phospholipid to overwhelm any LA, assessment of phospholipid-dependence being obtained from the normalised screen/confirm ratio or percent correction formulae. Whilst many would cease testing upon finding a negative mixing test, H60-A acknowledges potential for false-negative results due to antibody dilution. Consequently, H60-A recommends that test order is re-prioritised to screen, confirm, and then mix only if testing on undiluted plasma is not clear cut, such as an elevated confirmatory test or known co-existing abnormality.

Cut-offs and ratios
Cut-offs must be locally derived based on specific reagent/analyser pairings. Obtaining sufficient normal donors to generate an accurate 99^{th} percentile cut-off for screen and mixing tests is beyond the reach of most diagnostic departments and H60-A maintains that 97.5^{th} percentile can be adopted and points readers to its own reference range guideline (CLSI C28-A3) for further detail and discussion. It should be recognised that increasing cut-offs to the 99^{th} percentile improves specificity but reduces sensitivity, thereby risking false-negative screening. Again, false-positives occurring due to application of the 97^{th} percentile will be exposed upon interpretation of the composite. H60-A recommends application of reference interval mean clotting times as denominator to generate normalised ratios in preference to batch-specific normal pooled plasma clotting times.

LA testing during anticoagulant therapy
Whilst the vast majority of patients on VKA therapy can wait to receive LA testing upon treatment cessation, it is not uncommon for laboratories to receive requests to test on such patients and guidance is required. The utility of LA assays performed on undiluted plasma is disputed and thus not currently recommended. 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 effect, but negative testing does not exclude a weaker antibody. TSVT screening, with either Ecarin time or platelet neutralisation procedure as confirmatory tests can be a useful adjunct. It can be possible to detect LA in patients receiving unfractionated heparin with many dRVVT reagents provided that heparin neutralisers are not quenched. Data are still emerging for LA testing in patients receiving direct oral anticoagulants. Direct FXa inhibitors will particularly interfere with dRVVT, and variably with other reagents, although they have no effect on TSVT and ecarin time. Direct thrombin inhibitors may, perhaps inevitably, interfere with all assays to some degree.