Editorial

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Trials and tribulations in lupus anticoagulant testing

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This issue of *Clinical Chemistry and Laboratory Medicine* includes a report by Pradella and colleagues regarding a cooperative multicentre study to define the upper limits of normal for several tests used in the diagnostic assessment of lupus anticoagulant (LA) [1]. In total, 200 normal samples were assessed, comprising 40 from each of five centres, for a total of six functional LA assays. Each centre used the same tests, dilute Russell viper venom time (dRVVT) (screen and confirm), and silica clotting time (SCT), the same reagents and the same type of instrument from a single manufacturer. This is a notable report for several reasons, apart an interesting observational exercise around the concepts of standardisation (use of common products, procedures, processes, and practices) and harmonisation (the process whereby different analytic systems are determined to provide clinically similar results) of LA testing.

The study explores an interesting approach to a common laboratory problem – undertaking studies with sufficient numbers of normal individuals to establish a critical cut-off value that will help define patients 'with' or 'without' LA. This is an important component of the laboratory evaluation of the antiphospholipid syndrome (APS) [2, 3], in turn having significant implications for patient management [2–4]. In brief, patients identified clinically to have APS, as diagnostically aided using laboratory assessment by LA, can be subjected to long-term anticoagulant therapy, which has significant implications for both lifestyle changes as well as bleeding and thrombosis risk. In essence, an incorrect diagnosis will have significant clinical and social implications; a false-positive diagnosis of APS may subject a patient to unnecessarily long-term anticoagulant therapy with risk of bleeding, whereas a false-negative diagnosis may mean the patient is not appropriately anti-coagulated and therefore at future risk of thrombosis.

Current LA guidelines [5] suggest that laboratories use 'at least 40 adult healthy donors' and take 'the cut-off as the value above the 99th percentile of the distribution'. This has caused some contention in the field, given that a statistically valid 99th percentile evaluation of a non-Gaussian distributed normal population would require a minimum of nearly 400 samples [6]. Pradella and colleagues [1] comparatively evaluated the data obtained in each assay, as well as assay ratios, in each center, as well as the composite of all study data. Notably, when assay data was expressed in seconds, some test results showed a normal Gaussian distribution, whereas others did not, and there were also some statistically significant differences in results obtained between centers. In contrast, when results were expressed as normalised ratios, the test results were normally distributed and there were no longer any statistically significant differences in data between centres. Thus, better agreement between centres, and in essence standardisation and harmonisation, was obtained when data was interpreted in terms of normalised ratios.

In LA testing, there is actually very limited consensus in terms of inter-laboratory processes. Thus, different laboratories use different test procedures [e.g., based on dRVVT, SCT, activated partial thromboplastin time (APTT), kaolin clotting time (KCT) and/or other tests], different test panels (e.g., APTT ± dRVVT ± KCT ± SCT, etc.), different test approaches [testing of neat plasma vs. testing of mixed plasma (i.e., patient plus normal), sometimes using different test plasma:normal plasma mixtures], different ways of interpreting test results (e.g., test ratios [division], test differences [subtraction], percent corrections, Rosner Index), and different cut-off values [6–9].

Of interest, Pradella and colleagues [1] determined a cut-off value of 1.22 for the dRVVT normalised ratio. This value is actually very close to that value 'nominally' indicated on many dRVVT product information sheets, as well as generally used by laboratories. For example, Figure 1A shows data from the Australasian College of Pathologists of Australasia (RCPA) Haematology Quality Assurance Program (QAP) [10]. Most laboratories report a negative LA

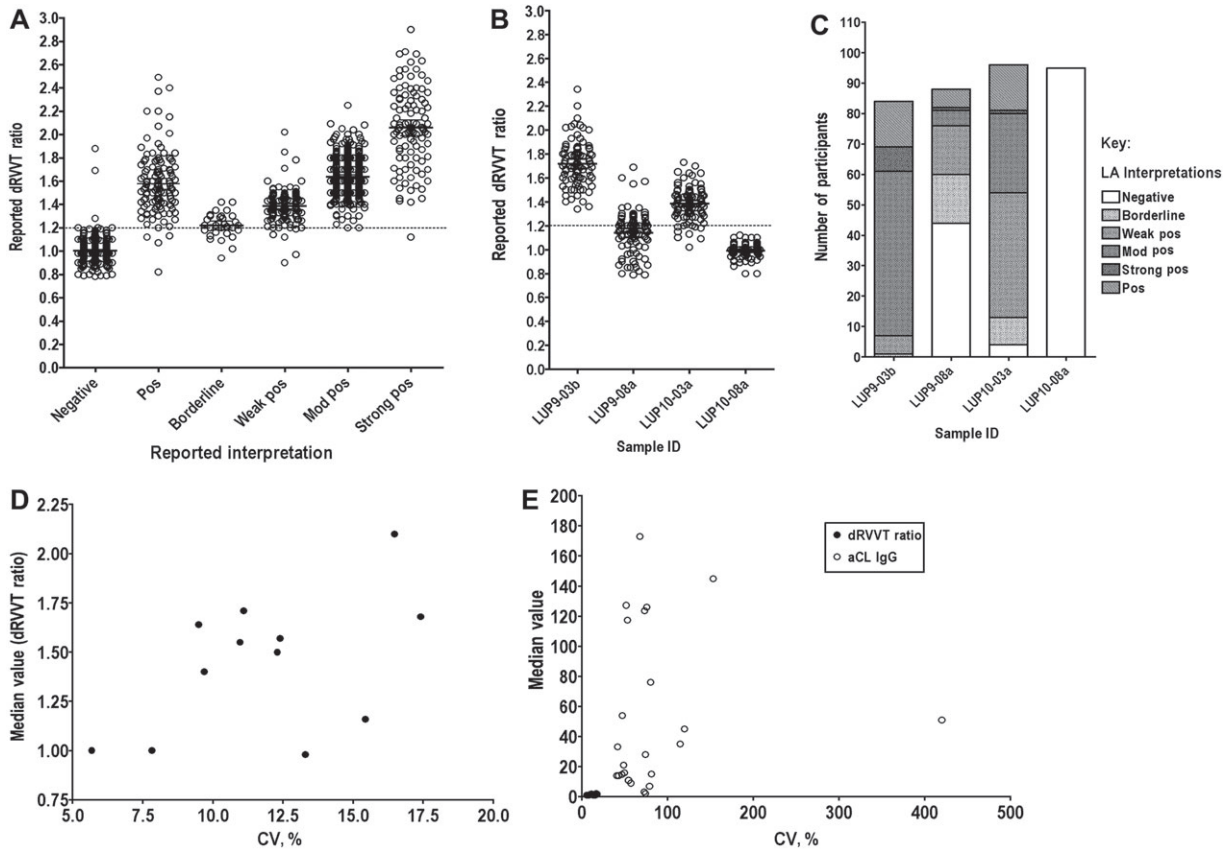

Figure 1 Representative data from the RCPA QAP to illustrate important points of discussion; data capture years =2009–2011 inclusive, for all or select representative cross-laboratory tested samples.
(A) Comparison of participant reported normalised dRVVT ratio vs. interpretation (all samples). (B) Participant reported normalised dRVVT ratios for select samples representing clearly LA positive (LUP9-03b), clearly LA negative (LUP10-08a), or weak LA positive samples providing equivocal findings from participants (LUP9-08a and LUP10-03a). (C) Participant reported interpretations for samples identified in Figure B. (D) Inter-laboratory CVs vs. median of reported normalised dRVVT ratios for LA testing (all samples). (E) Inter-laboratory CVs vs. median of reported values for aCL IgG testing contrasted to normalised dRVVT ratio for LA testing (all samples). For additional detail regarding findings from the RCPA QAP in this area of testing see elsewhere [10, 11].

interpretation when they identify a normalised LA ratio below 1.2, and most report an LA positive finding for LA ratios above 1.2. There is some inter-laboratory variation, however, in interpretations of LA positivity vs. negativity when ratio values are very close to the cut-off value of 1.2; thus, some laboratories will call these negative, and some will call these positive or equivocal ('borderline' or 'indeterminant').

Indeed, the cut-off value – what value is assigned, how it is determined and subsequently the interpretation of test results close to the cut-off value – often causes the greatest problems in LA testing. This can be illustrated by Figures 1B and 1C, again using data from the RCPA QAP [10]. Thus, samples with a normalised dRVVT ratio above 1.3 are invariably considered LA positive by participants of this program, although the grade of positivity designated may differ between laboratories (see data for sample LUP9-03b as representative example); similarly, samples with a normalised dRVVT ratio below 1.1 are invariably considered LA negative by participants of this program (see data for sample LUP10-08a as representative example). However, when a sample with a normalised dRVVT ratio close to 1.2 is tested by participants, a mixture of interpretations (LA-negative and LA-positive) is always returned; this is dependant on inter-laboratory assay variation, the normalised dRVVT ratio actually obtained by the laboratory, and whether this value is above or below their own designated cut-off value (albeit recognising that this value will be close to 1.2 for most laboratories; see data for samples LUP9-08a and LUP10-03a as representative examples).

This brings this discussion to two other critical considerations in LA testing, and namely inter-assay variability and standardisation/harmonisation. As noted at

the start of this commentary, the report from Pradella and colleagues [1] identified a statistically significant inter-laboratory difference in some test times when these were expressed in seconds, but not when expressed as ratios. In this study, all laboratories used the same tests, the same reagents and the same type of instrument. Notably, when laboratories use different tests or different reagents or instruments, one would expect a larger variation in test results, reflecting a relative lack of standardisation or harmonisation. When results are expressed as a ratio, such as a normalised dRVVT ratio, the resultant inter-laboratory coefficients of variation (CVs) range from approximately 5%–20% (see Figure 1D for examples from the RCPA QAP). Interestingly, comparative data for solid phase antiphospholipid antibody (aPL) testing, which represents another facet of testing in APS [2, 3, 7], shows substantially worse inter-laboratory CVs ranging from approximately 30% to over 200% (see Figure 1E for examples from the RCPA QAP; [11]).

All of the discussed elements above suggest that although LA testing is considered not well standardised or harmonised [12], it is on comparably better standardised than solid phase aPL testing. This may also help to explain why LA is more strongly associated with adverse clinical events, such as thrombosis than other aPL, such as anticardiolipin or anti-beta-2-glycoprotein I antibodies [13, 14]. In essence, this means that we can be better assured about the test outcome and clinical 'quality' of LA testing by dRVVT than that of aPL testing by solid phase assays. Nevertheless, much remains to be done. For example, we need to train clinicians better in terms of test requests; to avoid inappropriate testing that is both costly and which may lead to false-positive diagnosis of APS and adverse patient treatments [15, 16]. We also need to develop better assays for APS diagnosis [12]. Additionally, but certainly not finally, we need to continue to improve harmonisation and standardisation in laboratory testing for all APS associated tests including LA [17].

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