The importance of using an acceptable linear calibration curve for factor assays

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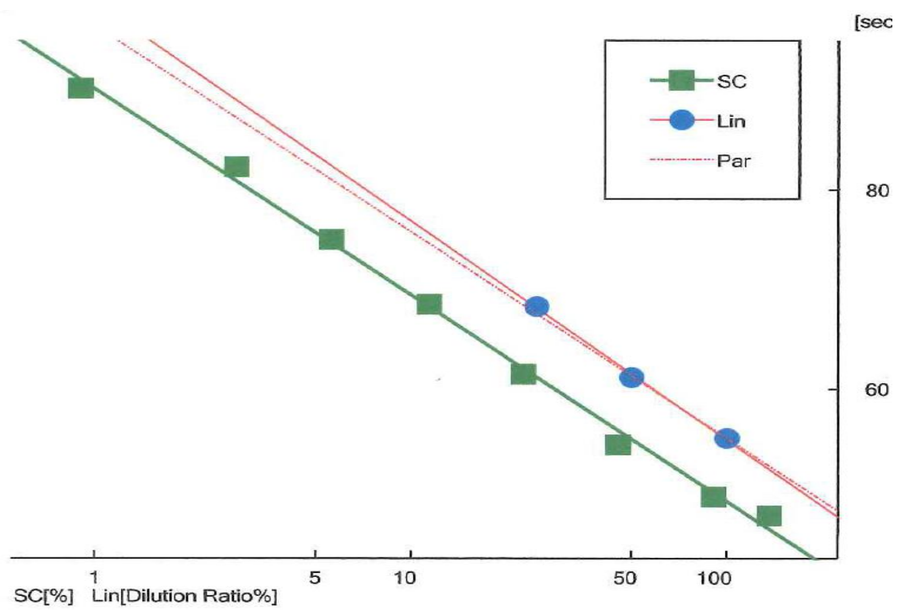
Introduction

It has been widely reported that in order to determine accurate factor assay results there are several recommendations to achieve this [1]. At least three dilutions of test plasma are needed to determine linearity and parallelism. The coagulation data must be checked by looking at the slope value R2 and the shape of the curve. By using the parallel line method to determine relative factor potency this may show evidence of inhibition (most commonly the presence of antibodies to coagulation factors or lupus anticoagulants). A point to point curve or a single point curve can give a misleading result and inhibitors can be missed.



Method

All evaluations were performed on the Dynex DS2 (programmable) automated ELISA analyser (Werfen Group). The assay consisted of use of the reagents: Inova Diagnostics Quanta Lite® IgG and IgM ELISA reagents (Werfen Group, California, USA). The presence or absence of aCL was determined by comparison of the sample optical density with that of a 6 point calibration curve. An absorbance (OD) of 450nm, and reference wavelength of 620nm, was used. The clinical performance of the assay was investigated by determining the following: intra-precision assay (n=12) and the coefficients of variation (CV) for inter-precision assay (n=6), using ACA IgG and IgM controls (containing preservative and human serum antibodies to cardiolipin); and lower limit of quantification. The cut-off for the assays was determined by analysis of 66 normal donor sera samples (which was kept frozen at -80°C). A comparison study was also performed by analysis of 30 patient serum samples with different reagents (Aeskulisa, Wendelsheim, Germany) and analyser (Grifols UK Triturus, Cambridge, United Kingdom).

Results

The standard curve for the IgG assay was linear throughout the standard range (*Figure 1a*) whilst the IgM assay exhibited plateauing at the higher standard (*Figure 1b*), common to many ELISA assays. The intra precision assay on control samples of IgG and IgM were 3.2% and 3.5% respectively. The CV for the inter precision IgG and IgM aCL were 8.2% and 6.6% respectively. *Table 1* shows the summary of the precision run data and reproducibility of the assays. The lower limit of quantification was determined to be 2.7GPL (IgG) and 2.9MPL (IgM). Cut-offs were derived (n=66) using 95th-99th percentile for low positives in relation to obstetric APS, moderate positives as between 99% - 80G/MPL, and strong positive, >80G/MPL. The 95th-99th percentile for IgG and IgM were 10-15G/MPL. *Table 2* summarises the in-house therapeutic ranges generated. The comparison study with different reagent and analyser had shown good agreement, with values ranging from normal to strong positive. In view of improved linearity of standard curves on the Dynex DS2 at higher levels where Triturus standard curves begin to plateau, direct comparison of patient data with higher values was not possible. However, in terms of results being 'normal, slightly, moderately or markedly elevated' results were diagnostically equivalent.

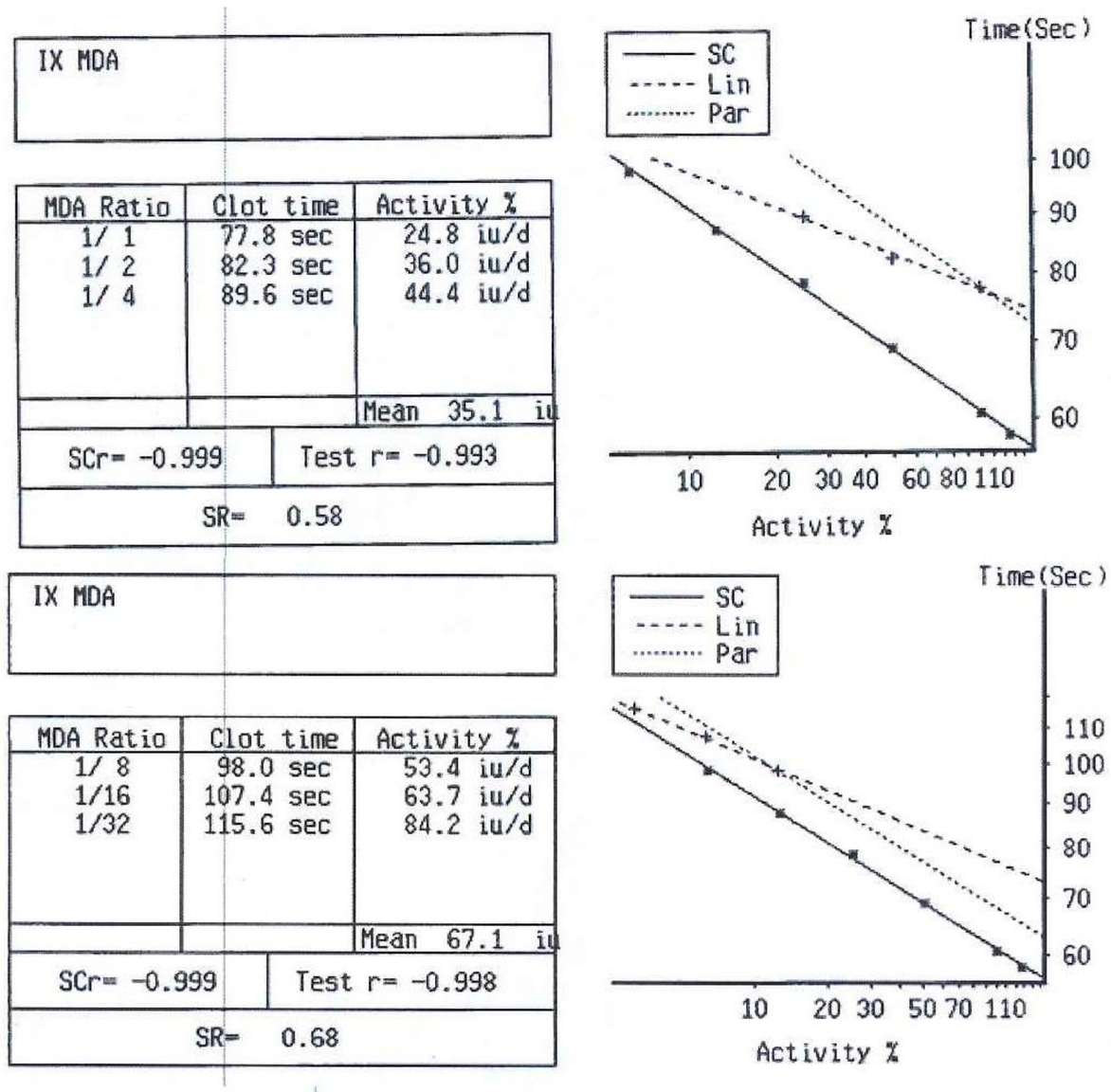


Figure 1b – Standard curve for aCL IgM assay

	aCL IgG	aCL IgM
Intra Assay (n = 12)	Range for positive GPL is 35 - 75GPL CV = 3.2% Mean = 47.54 SD = 1.51	Range for positive MPL is 35 – 75MPL CV = 3.5% Mean = 48.85 SD = 1.70
Inter Assay (n = 6)	Range for positive GPL is 35 - 75GPL CV = 8.2% Mean = 45.39 SD = 3.73	Range for positive MPL is 35 – 75MPL CV = 6.6% Mean = 47.46 SD = 3.13

Table 1 – Summary of precision studies for aCL IgG and IgM assay

IgG & IgM aCL	Range
Cut-Off	10G/MPL
Low Positive	10 – 15G/MPL
Moderate Positive	>15 - <80G/MPL
High Positive	>80G/MPL

Table 2 – Description of reference range

Conclusion

From our data, we confirmed the pairing of Inova aCL reagents on Dynex DS2 analyser generated robust assay suitable for detecting and quantifying aCL according to current guidelines. It is a simple and reliable assay which has vastly improved turn around times for testing. It provides ideal patient monitoring for suspected cases of APS, SLE or other thrombotic disorders, within the diagnostic laboratory.

Reference

[1] http://www.nhs.uk/Conditions/hughes-syndrome/Pages/Introduction.aspx (2013), last accessed on 16th August 2015.