NORDIC BIOMARKER

How to Test the Linearity of an Assay

The calibration curves in many methods are not perfectly linear; sometimes clearly hooked or even sigmoidal! Despite this, the method should deliver linear assay results to the user.

The definition of linearity in CLSI EP06 is simple and straight forward: "Linearity is the ability of a quantitative analytical method to provide results that are directly proportional to the concentration or activity of an analyte in test samples, within a given measuring interval. A measurement procedure is linear when there is a mathematically verified straight-line relationship between the measured values and the true values."

In other words, if you analyze a sample, then dilute it 2-fold and measure again, the analysis result should be half of the original value. After another 2-fold dilution the analysis result should be one-fourth of the original value, and so on.

The calibration curve itself, however, does not need to be linear to get linear results. This may be obvious to most people who have worked with this before. But we find that many persons within the coagulation field not are used to the concept. The misunderstanding could be because early coagulation instruments lacked computational power and sometimes only had linear options for the calibration curve, which may have given the notion that the calibration curve must be linear. In fact, with the instrument of today, it can even sometimes be an advantage to intentionally design a method with a non-linear calibration curve, e.g. to get a better slope/signal in the lower end of the measuring range.

Linear or not, the whole point of a calibration curve is to deliver linear results. You test this by carefully (preferably using an analytical balance) making serial dilutions of samples. If the system is robust, the dose-response curve you get should definitely be linear.