

Recalibration of immunoassays

Things can go wrong when you run our immunoassays, or assays from other manufacturers. Potential problems can originate from the reagents as well as from malfunctions within the instruments used. Either way, as an end-user, you should have a quality control program to pick up abnormalities in the assays. Each laboratory should establish a control range to determine the allowable variation in the day to day performance of the test. Recalibration is suggested whenever control materials are not within the acceptable range.

If you have tight control ranges, the natural aging of the reagents might be one reason why the controls fall out of their range. If you have wider ranges, the aging process will probably not be seen as a problem, if other errors of the assay/instrument are not added on top of it. The document you have in front of you is written specifically to explain the background to how the aging process affects the assay results, and how you can avoid the problem.

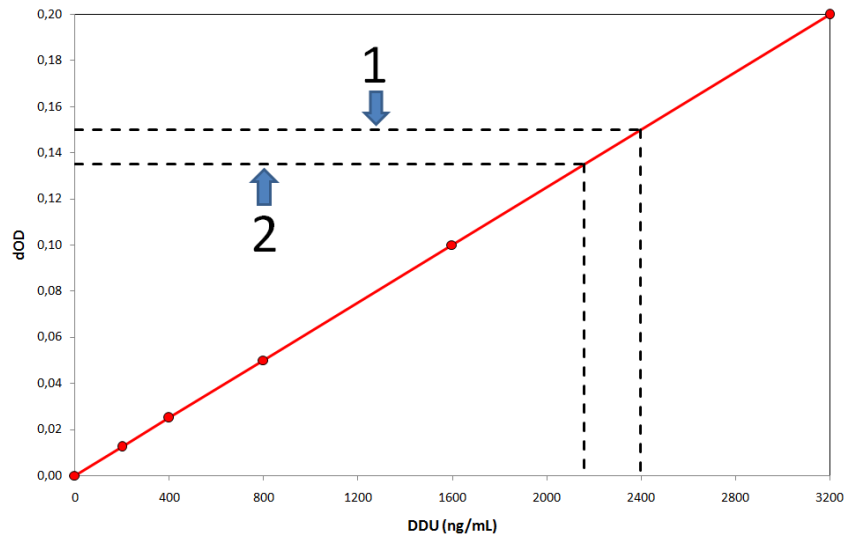
All immunologically based assays have a limited shelf-life. Particle-enhanced immunoassays, so called latex assays, are no exception. This is why you typically see shelf-lives of 12 – 24 months for the latex kits. Does this mean that the performance of a specific lot of a latex kit is identical over its life-span, before the date of expiry is reached? No, it doesn't. What it ought to mean, however, is that the performance, although it deteriorates over time, is still good enough, at the date of expiry, to meet the requirements and expectations of the product.

So, if the Latex Reagent is not completely stable over time, does this have any practical significance? Yes, while a re-calibration must always be done whenever a new lot of reagents is being used, a re-calibration may also be needed after some time, usually several months, despite that the same lot is being used. The good news, on the other hand, is that calibrators in the kits from Nordic Biomarker is very stable. In fact, the calibrators show no significant change in activity at all, over the life-span of the kit. Therefore, this allows for a re-calibration of the kits to be accurately done even close to their dates of expiry. The re-calibration will be accurate despite the fact that the Latex Reagent has lost some of its activity at that point.

Here is an example, constructed for the argument but still a realistic example. A brand new D-dimer kit was calibrated (Figure 1, red line). The same day, a control material was assayed with the newly calibrated kit and reported to hold 2400 ng/mL D-dimer Units (Figure 1, arrow 1).



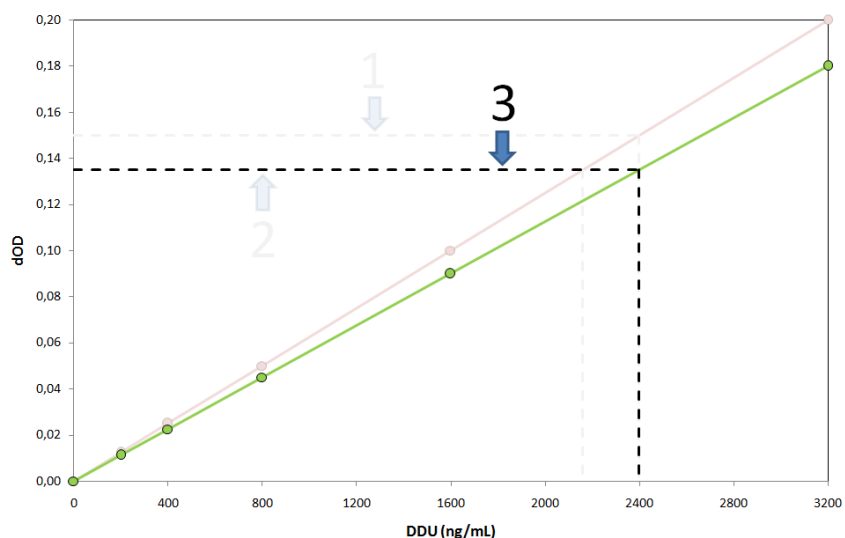
Figure 1: Calibration curve of a fresh lot



A year later, the instrument had not yet been re-calibrated, and the same lot of the D-dimer kit was being used. Again, the same control material as before was assayed (Figure 1, arrow 2). Now, the control material was reported to hold 10 % less D-dimer, despite it had been stored frozen under conditions where D-dimer should be stable. In fact, this is what we ought to see, as a 10 % drop in the reactivity of the latex reagent, over a year, is normal.

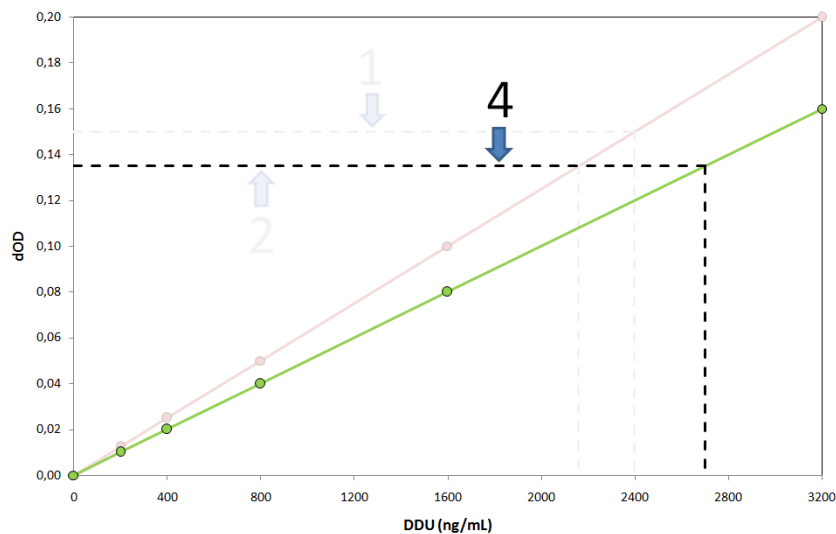
To get accurate results, the kit was re-calibrated (Figure 2, green line). As the latex reagent had lost some of its reactivity at that point, the signal (dOD) was somewhat lower for each calibration point, hence the lower slope of the calibration curve as compared to a year before. But, the important point is that kit again reported accurate D-dimer values, and the same control material as before, now was assayed as 2400 ng/mL DDU (Figure 3, arrow 3).

Figure 2: Calibration curve of an old kit, close to its date of expiry



Without a stable calibrator, the situation would have ended up as in Figure 3, where the effects of stability losses in both the reagent and the calibrator had been additive. This situation would have over-estimated the concentration of D-dimer on the control material. This is why a stable calibrator is a cornerstone in any immunoassay.

Figure 3: Calibration curve of an old kit, close to its date of expiry, with the additional problem of a calibrator having poor stability



So, expect a decrease over time in the signal/reactivity of the immunoassay. Maintain a robust quality control program to pick up any abnormalities. Re-calibrate when the controls are out of their ranges.