28 april 2010

Developers Guide

Development of new instrument applications for D-dimer

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Development of new instrument applications for D-dimer

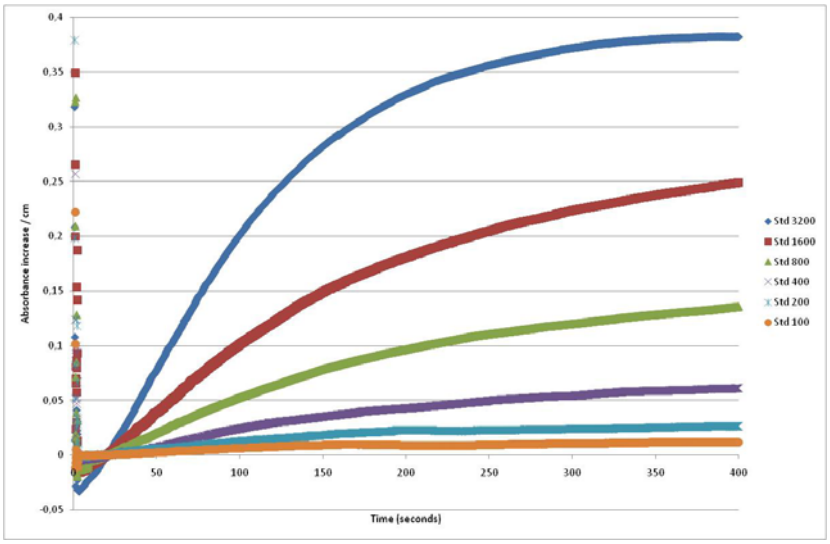
There are several things to consider, when developing a new instrument application for D-dimer. The first choice is which D-dimer kit to use. Then, before the power to the instrument is turned on, one must carefully decide the starting conditions. Even so, after carefully choosing starting conditions, one must usually optimize the application further, by changing the application parameters in an iterative process to optimize signal and linearity of the calibration curve. When the application seems ready, there might also be a number of extra performance tests to conduct (e.g. precision, prozone, correlation).

Background

MediRox's D-dimer kits are micro-particle enhanced immunoassays, generally referred to as "latex assays", which rely on turbidimetry for their function. When the Latex Reagent, having monoclonal antibody against D-dimer attached to the particles' surface, is mixed with patient sample and Reaction Buffer, the particles will agglutinate and give rise to an increase in light scattering. When this reaction is monitored in an instrument, having the necessary photometric features, it will be observed as an increase in absorbance over time. The rate of this reaction is proportional to the amount of D-dimer antigen in the sample.

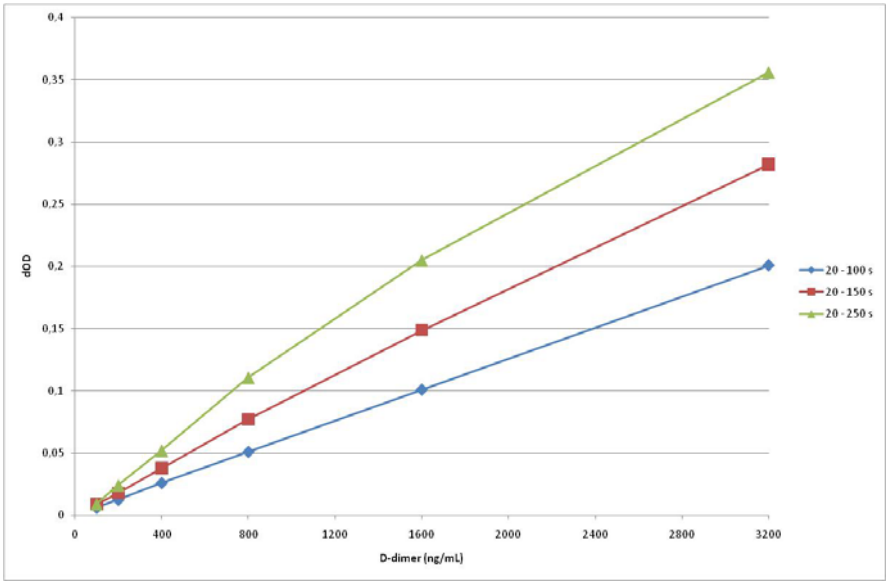
Figure 1 shows examples of such reaction curves. As can be seen by observing the upper reaction curve (3200 ng/mL), the absorbance increase over time is not linear. This is because larger and larger particles aggregates will form over time, and these have different light-scattering properties from smaller aggregates formed early in the reaction. This property of agglutination is most apparent for high D-dimer samples, although also present at low D-dimer concentrations.

Figure 1: Reaction curves MRX147 D-dimer
(20 ul sample, 100 ul Reaction Buffer, 50 ul Latex Reagent)



The calibration curves, which are derived from the reaction curves, are normally constructed by plotting the change in absorbance (dOD) in a suitable time-window of the reaction curves, against the respective D-dimer concentration for each reaction curve. For instance, if one plotted calibration curves, using the data in Figure 1, by choosing different time-windows for dOD, the signal and linearity would differ. Three such examples are displayed in Figure 2.

Figure 2: Three different calibration curves derived from the same reaction curves by using three different time frames for data collection



Selecting correct D-dimer kit

As some instruments have optical channels only at lower “Blue” wavelengths (e.g. 405 nm), whereas others only are capable of measuring only at higher “Red” wavelengths (e.g. 700 nm), MediRox has two different D-dimer kits; MRX147 -sometimes referred to as Blue, and MRX 143 -sometimes referred to as Red.

The light-scattering properties of particles are closely related to their size. The particle size in MRX143 D-dimer is suitable for instruments with optical channels in the range 600 – 900 nm. At higher wavelengths, the light scattering properties of the particles decrease, which will lower the signal. Conversely, at lower wavelengths the particles would scatter too much light, reducing the residual signal seen by the detector close to its noise level.

In an analogous manner, the particles size in MRX147 D-dimer is chosen for it to be suitable for instruments with optical channels in the range 350 – 550 nm.

There is no magical cut-off wavelength below which MRX143 D-dimer suddenly will not work, or above which MRX147 D-dimer will not work; in reality it is a compromise between cost and performance. For instance, MRX147 D-dimer could theoretically work at 700 nm, but since its light-scattering properties are poor at this wavelength, the amount of MRX147 D-dimer Latex Reagent needed in the cuvette would be high and hence also the cost. Conversely, if MRX143 D-dimer were used at 405 nm, only a slight amount of MRX143 D-dimer Latex Reagent could be added to the cuvette, to keep the light-scattering at a reasonable level, with poor performance (read, few antibodies in the reaction mixture) as a result.

Amount of Latex Regent in the cuvette

As turbidimetry relies on absorbance, and as the instruments used really are spectrophotometers, one has to consider Lambert-Beers Law. Briefly, this means that the absorbance must lie beneath 2.0; above this the light that reached the detector is too weak for a good signal-to-noise ratio. In addition, as the absorbance increases during a measurement, the initial absorbance must be even lower; a good starting point will be in the region of 1.0 – 1.3 absorbance units. As most clinical instruments don't display this value, it will have to be empirically determined in a separate experiment using a standard spectrophotometer.

For instance, MRX147 D-dimer Latex Reagent has an absorbance of 8.2 in a 1 cm cuvette at 405 nm. If one would like to run MRX147 D-dimer at 405 nm in the 0.6 cm cuvette of a Sysmex, and aim for 1.15 initial absorbance units, a dilution factor of (8.2/1.15)*0.6 = 4.3 would be suitable. Further, if the cuvette needs a minimum volume of 170 ul, the total volume of MRX147 D-dimer needed in the cuvette would be 170/4.3 = 40 ul; the rest being the samples and reaction buffer.

Amount of sample

Generally a 1/10 volume of sample is good starting point. However, the amount of sample in the cuvette is a compromise between, on one side, precision and accuracy in the lower region, and, on the other side, the upper limit of the measuring range. In other words, with a large volume of sample in the cuvette, the absorbance change during the measurement will be high even for samples with low concentration of D-dimer; however samples with high D-dimer concentrations will produce a non-linear reaction curve, reducing the upper range. Conversely, using a small volume of sample in the cuvette will render linear reaction curves even for high samples, but very small absorbance changes (dODs) for samples low samples.

Figure 1 shows an example of a rather balanced situation. The reaction curve for 3200 ng/mL is linear up to about 100 seconds, and a sample with 100 ng/mL D-dimer still produces a decent signal-to-noise (although it is difficult to appreciate this in the scale of the figure). This situation could produce a linear calibration curve, depending on the reading window used to calculate dOD (see below and Figure 2).

If the instrument is capable of fitting the calibration data to a non-linear calibration curve, or point-to-point calibration curve, this gives some more flexibility to the whole situation. That is, by using a larger sample volume, one will get a higher slope of the calibration curve in the lower region (where precision and accuracy is most important, close to the cut-off!); even if the slope of the calibration curve is lower in the upper measuring range, it is still positive and probably good enough (i.e. even if the accuracy is poorer than in the linear case, usually the clinician would not bother if the assay results is 2300 or 2500; it is still way above the clinical cut-off!).

Amount of Reaction Buffer

This will simply be the volume of Latex Reagent and sample subtracted from the cuvette volume.

Time frame for data collection

The reaction curves in the figure were produced by first pipetting 20 ul sample and 100 ul Reaction Buffer in the cuvette. The reaction was then started up adding 50 ul of Latex Reagent to the cuvette. The first few seconds are very noisy, as the pipett is inserted into the light-path of the cuvette, after which is takes a further few seconds to settle. Even if the instrument at hand, does not insert a pipett into the light beam (as in this case), it is often best to wait a moment, typically 10 – 20 seconds, before the sample has settled down and the first data is collected.

Most instruments calculate the dOD as the difference in absorbance between this first time-point and a second time-point. When shall the second time point be? Just like for the discussion around sample volumes (above), it is a matter of how linear (if at all) the calibration curve must be. For the data in the figure the second time point should be somewhere between 100 – 150 seconds for a linear calibration curve; shorter data collection time will yield poor signal-to-noise, and longer times will make the upper calibration point deviate from linearity (Figure 2).

Trying the application on the instrument

After the starting conditions above have been determined, the application has to be tested for real on the target instrument. As the optics of each instrument is different, one really must try and optimize in an iterative way. If the instrument is really modern, it may come with extra software where the actual reaction curves (that is dOD against time just like in Figure 1) can be visualized. In such a fortunate case, the iterative process may be significantly shorter with the valuable visual feed-back of the reaction curves.

By continuing the Sysmex example above, 40 ul of MRX147 D-dimer micro-particle reagent seems to be suitable for a 0.6 cm path-length. 20 ul sample might be a bit high, as the preliminary reaction curves on the test-instrument made the high sample (3200 ng/mL) hooked a bit early. So let's try 15 ul. As this Sysmex have a pre-dilution feature, and as small volumes are difficult to handle (even for a robot), we will take 55 ul sample, dilute it with 55 ul Dilution Buffer, and, finally, make the instrument take 30 ul of this dilution; in effect 15 ul of sample. 100 ul of reaction buffer will make a total reaction volume of 170 ul.

Sample (pre-diluted 1:1)	30 ul
Reaction Buffer	100 ul
MRX147 D-dimer Micro-particle Reagent	40 ul
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Total reaction volume	170 ul

By trying this application above on the final instrument (in this case a Sysmex CA-1500), and by running several calibration curves, each with a somewhat different setting (varying one-by-one the different volumes and the dOD calculation time-window), we can find good settings for the instrument.

As it turned out, for the Sysmex, the volumes above where OK. The final time frame for dOD-collection chosen was 15 – 175 seconds.

Summary

1. Pick your reagent by looking at which optical wavelength the instrument operates at.
2. Find out the optical path-length of the cuvette, and calculate the dilution ratio needed for the micro-particle reagent.
3. Find out the minimal cuvette volume, and calculate how much micro-particle reagent is needed.
4. Use a sample volume that is approximately 1/10 of the total reaction volume.
5. Calculate how much Reaction Buffer that is needed (= total - latex reagent – sample).
6. Program the preliminary application on the new instrument.
7. Run a first calibration curve with data collection in the approximate interval 20 – 150 seconds.
8. Observe the results, and, by using reasoning, change one of the parameters that you think will make the reaction curve better (more linear, if that is the goal, or higher slope if the precision in the lower region is prioritized, etc).
9. Repeat 8 until the standard curve is good.
10. Consider which performance tests to test using the final application.