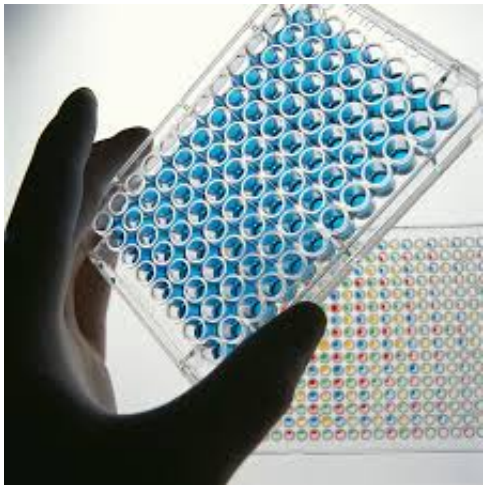
ARP blog – ELISA Troubleshooting tips – High CV

Author : Dan Souw



This is the third in a series of blog posts looking at troubleshooting problems with ELISAs. This one is going to focus on the possible causes of high coefficient of variance in your ELISA.

Sometimes it's obvious that duplicate wells have completely different optical density, and sometimes it's not until you analyse your data that you can tell that they differ by more than, say 20%.

Sometimes it might just be a handful of samples effected, other times it can be seen across the plate.

The magnitude of the problem may depend on where you're seeing a high CV. For example, if it's only present on a single calibrator/standard level, then you may be able to mask this calibrator in your analysis software without adversely affecting your curve fitting.

If you run QC samples with each ELISA, then you'll be able to be confident whether this has affected your curve fit or not. The only real exceptions are removing the top or bottom calibrator levels where the uncertainty would be highest. In this case, you would have less confidence in any sample falling above the level of the highest/lowest QC sample.

The most obvious cause of high CVs is often pipetting, but it's not always the cause. Errors in sample preparation, washing or general preparation of reagents can all lead to high CVs. So, in this post, we're going to look at some of the most common possible causes of high CV in an ELISA.

First up, look at your plate or your raw data to see if there anything to indicate a systematic problem. For example, if a multi-channel pipette has been used, you might see the same error all

down one row or column of your plate, indicating a pipette channel was either under or over dispensing. This might be obvious to the eye, or only from the data.

In this instance, you could double check the calibration of each channel of a multichannel pipette used using water and a microbalance, for example.

Below we'll list a few other possibilities:

If we're confident in our tools (the pipette), then let's consider some of the other steps:

Sample preparation.

If you are using diluted samples, be sure to vortex or pipette mix them well before adding them to a plate. If you have a lot of samples to dilute, it can be easy to miss one.

Poorly mixed diluted samples could result in one well of a duplicate receiving more of the analyte of interest than another.

Reagent preparation

Depending on the ELISA you're performing, it's possible that poor mixing of reagents, such as capture or detection antibodies or substrates, could all result in different wells receiving different levels of the reagent, leading to a higher CV. In this case, you'd expect to see it high CVs scattered almost randomly across the plate.

Plate washing

Be sure to wash all wells equally and thoroughly. For example, if using an automated plate washer make sure all dispensing heads are unblocked and dispensing the correct volume of wash buffer.

Again, this would probably introduce a systematic error seen across multiple plates, so if you've run more than one plate, or a colleague has also run a plate, check the data between the two to see if the problem is unique to one or two specific well locations. If so, the plate washer may be the culprit.

Bubbles in well

Bubbles can be introduced into wells when pipetting and can, depending on the sample type, be hard to avoid without care. Sometimes this can affect the binding of analyte or reagents.

However, bubbles present in the assay wells when being read on a plate reader can be especially

problematic, so be sure to pop any visible bubbles (with a needle, for example) if they are present before reading a plate.

This can be especially true when using a detection reagent like ABTS which is ‘stopped’ with a 1% SDS solution.

Edge effects

‘Edge effects’ are unexpectedly higher or lower optical densities/luminance in wells on the periphery of the plate compared to those in the centre or neighbouring wells.

Generally, most ELISA plates are very well made and consistent nowadays, so usually the plate itself is rarely to blame. Although, many years ago, one would also question the manufacturing consistency of the plates themselves (e.g. thicker well sides/bottoms at the edges of the plate vs the middle affecting the optics of the plate).

Nowadays edge effects are mostly only seen where there is, or has been, a temperature differential across the plate affecting the kinetics of the assay in those wells.

Try to ensure all reagents are well mixed and at room temperature (or the ELISA manufacturers specified temperature) prior to use.

For incubation steps, try to control the temperature, even for ‘room temperature’ incubations using an incubator.

Hopefully, if you’ve been seeing some anomalously high CVs in your ELISA this little guide will give you some good tips to troubleshoot the problem. As always, try to use well-calibrated pipettes and prepare all reagents freshly if possible before repeating an assay – the last thing you want is the same problem when you’ve got dwindling volumes of sample.