Do it once—do it right

Can you trust your protein assays?

Have you ever considered whether you can trust the results you get after running a multiplex protein assay? How can you identify human errors like pipetting problems or changes in incubation time? There might be technical issues with instruments or temperature variation that affect the results. Also, how can multi-center studies detect sample variablities? Many technologies deal with this by requiring the samples to be run in replicates, but how can you decide which one gives the correct result?

Olink goes in another direction. We do not recommend replicates since we want to minimize the amount of sample needed to obtain the required data. Despite our measurements being based on a single sample run, we still get trustworthy results. In such cases, how can we trust the data? The answer lies in the internal controls used in Olink's Proximity Extension Assay (PEA) technology, and our high reproducibility.

Olink's internal controls

Olink's specifically engineered internal controls are added to each sample to give full control of each step of the assay run. Since they are added at the same concentration in each well of the plate, they are expected to give the same result. If they don't, it means that something has happened in that specific sample that might affect the result. The internal controls comprise one or two controls for the immuno reaction depending on the readout platform, one for the extension and one for the amplification step (Figure 1). Together, these internal controls reveal if any sample should be excluded from further statistical analysis. A sample does not pass the QC when one or several internal controls deviate for that specific sample. The behavior of the internal controls makes it possible to understand why a sample fails sample QC (Figure 2). Reasons for deviating controls may be due to:

- Different sample matrices or sample types that make the immuno reaction slightly more or less efficient
- Sample volume or concentration is too high or too low
- Sample quality is inadequate, for example after evaporation because the sample has been stored in a freezer for long periods of time
- Operational issues during analysis, such as inconsistencies in

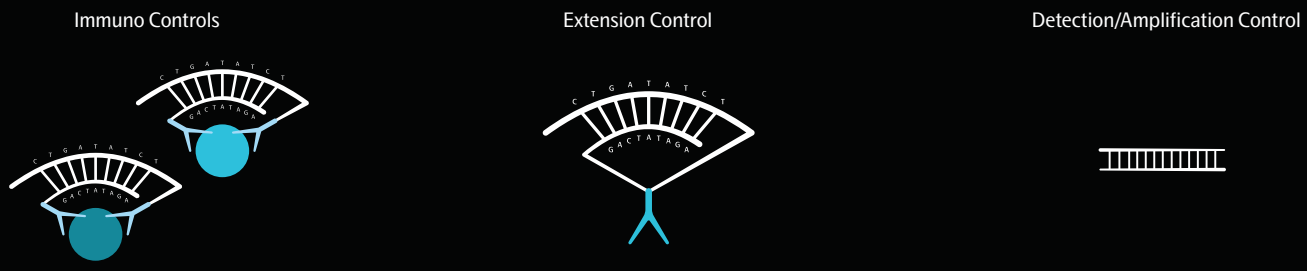

Figure 1. Internal controls for PEA. The Immuno Controls are PEA assays for non-human proteins which monitor potential variation across all steps, and are used in data QC. The Extension Control is an antibody with both DNA-oligos attached and therefore is always in proximity. This controls for the extension, library preparation and sequencing steps and is also used for data normalization purposes. The Detection/Amplification Control is a piece of synthetic double-stranded DNA that is used in the data quality control. This control can also help evaluate potential issues in the final amplification and detection steps.

Figure 2. Data analysis example of deviating internal controls. The circled sample to the left has a deviating Incubation Control. The circled sample to the right has deviating Detection Control and Extension Control.

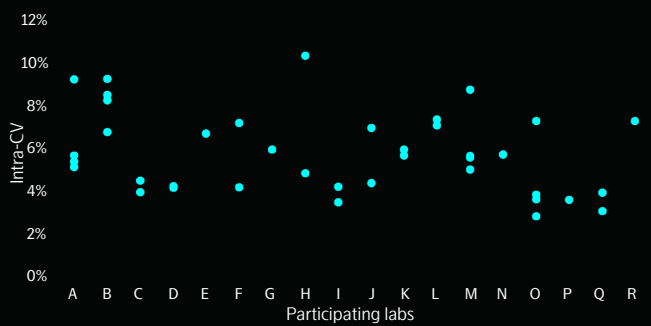

Figure 3. Average intra-CV per operator. The lowest calculated average intra-CV was 3% and the highest was 10%.

pipetting or vortexing

The advantages of internal controls are many and at Olink they are used to:

- normalize data
- detect sample abnormalities
- monitor assay performance
- improve statistical data

Lab performance test

We have assembled evidence for reproducability from both internal and external studies.

All certified Olink core labs, including Olink's Analysis Service labs, were asked to run the same pooled EDTA plasma sample on the Olink® Target 96 Neurology panel. Quality control was performed individually per operator and intra- and inter-CVs were reported.

TERMINOLOGY

Intra-CV is a measure of the variance between data points within a plate.

Inter-CV is a measure of the variance between different plates.

Average intra- and inter-CV per assay correspond nicely to values from the Olink Target 96 Neurology panel validation data for the panel, and as an example, the intra-CVs are presented in Figure 3.

Customer example

The data presented in this section was supplied courtesy of a major pharma company client and have since been used as a basis for several of their continuing Olink studies. The customer wanted to assess the performance of multiplex Olink PEA in comparison with other technologies and to understand whether PEA could be utilized for their future clinical trials.

Plasma samples from 10 normal subjects and 10 patients were analyzed in triplicates by two different operators, and among other parameters, intra- and inter-CV were measured. The intra-CV results are listed in Table 1.

The results of the pilot study were:

- 96% of the samples passed quality control
- 87% of the proteins were detected in more than 75% of the samples
- Average intra-CV (by the same operator) was 4%
- Average inter-CV (between operators and plates) was 6%

The CVs are well within accepted industry standards for other methodologies using replicate runs, even though Olink has a higher multiplex grade.

The major pharma company concluded that Olink PEA showed excellent precision and linearity. They stated that the sensitivity of Olink's high-multiplex panels was fairly comparable to low-plex platforms, and decided to use Olink PEA in their clinical trials.

Table 1. Intra-CV per operator calculated from triplicate samples.

Sample	Operator 1	Operator 2
1	8%	7%
2	5%	4%
3	9%	8%
4	5%	4%
5	5%	4%
6	5%	3%
7	5%	4%
8	11%	10%
9	11%	6%
10	7%	4%
11	8%	6%
12	5%	4%
13	11%	8%
14	10%	7%
15	7%	6%
16	7%	6%
17	9%	8%
18	10%	7%
19	9%	7%
20	7%	5%