Olink®

White paper

Reliable and reproducible data at any scale with PEA™

Introduction

The Olink platform is flexible and scalable, helping researchers at every stage of their protein biomarker research. Solutions are available to measure thousands of proteins down to just five, all using the same Proximity Extension Assay (PEA™) technology (1). This gives researchers the ability to uncover real biological insights that can transform how people think about health and wellness over time.

Regardless of study scale, researchers must be able to trust the data they generate. This need is especially important given the reproducibility crisis in scientific research, where many published findings cannot be reliably repeated (2). A major challenge is the lack of consistency across platforms, technologies and laboratories, making it difficult to build on previous work with confidence.

Reliable and reproducible data and biomarkers are critical for diagnostic, prognostic, and therapeutic applications. In protein biomarker research, reproducibility refers to the ability to consistently replicate experimental results under the same conditions. Reproducibility can be measured by comparing concordance between labs and products to ensure that results are consistent, accurate, and reliable.

High concordance is essential for smooth transitions throughout the entire research process, from large-scale exploratory studies to clinical applications. This allows scientists to build on their data with confidence, knowing that each step is supported by a stable, high-quality foundation.

This white paper provides an overview of how we evaluate the Olink PEA technology with regards to reproducibility, and presents evidence supporting the reliability of Olink data.

Evaluating reproducibility

Reproducibility is a vital part of scientific research. It allows results to be checked and confirmed by others, which is crucial for expanding knowledge and preventing errors and misinterpretations.

Olink® Concordance Test

To enable customers to be confident in their results and to ensure the highest level of data accuracy and reliability, we have developed the Olink® Concordance Test. This gives our customers a chance to verify their performance and demonstrate adherence to Olink-approved data and quality standards. The test is designed for all users of Olink technology who have the Olink Target, Reveal or Explore platforms installed in-house.

The test itself is a 96-well plate containing 50 selected plasma samples. The samples are a combination of healthy plasma, replicates of a healthy plasma pool and samples of various diseases within the fields of inflammation, neurology, cardiovascular diseases and oncology. The plasma plate has been run with all Olink product lines except Focus to generate reference files with Olink-approved data. When a customer runs the Concordance Test with a selected panel, the data are compared to the reference file from that same panel. The data comparison is presented to the customer in a Concordance Test report where various statistical methods are used.

Statistical methods used in Olink Concordance Test

When comparing data between labs using our Concordance Test, several statistical and quality control methods are used to ensure that lab results are consistent, accurate, and reliable. The results are summarized in the Concordance Test report. The following section provides a summary of each statistical and quality control method included in the Olink Concordance Test.

Regression analysis

Regression analysis is performed to provide a statistical overview of how well a model fits a dataset, often summarized by the coefficient of determination (R^2). A high R^2 value indicates a strong agreement between datasets, indicating high reliability and comparability of their data. It also helps identify outlying data points that deviate significantly from the fitted model.

By applying regression analysis to Concordance Test data, and plotting results from different laboratories, we can assess wether their results follow a consistent pattern.

Regression analysis can be used to compare labs by modeling their lab-specific baseline shifts and trends. In the Concordance Test, all assays above LOD are used to create a linear regression model assessing the relationship between the results from the external lab and the Olink-approved dataset.

Correlations

Correlation measures the strength and direction of the relationship between two datasets. In lab comparisons, high correlation indicates that the results are consistent between labs, which is crucial for ensuring reproducibility.

In the Concordance Test, assays above LOD are used to calculate the median and mean Pearson correlation coefficients. A high correlation confirms a strong agreement between two datasets, which supports the reliability of the data.

Coefficient of Variation

The Coefficient of Variation (CV) metric is particularly useful in lab comparisons because it allows for the comparison of variability between datasets. A lower CV indicates higher precision and repeatability of lab results. In the Concordance Test, the CV within each dataset (intra-CV) and between datasets (inter-CV) is calculated using the results from replicates of a pool of healthy plasma samples. Recurrent measuring of CV can help in monitoring the performance of a lab over time, ensuring consistent quality.

Quality Control warnings

As part of the regular workflow in the Olink data analysis software programs, irregularities in the data or runs will be highlighted during Quality Control (QC). Monitoring and evaluating QC warnings are essential for maintaining the integrity of lab results. QC warnings alert lab personnel to potential errors or deviations from standard lab or technical procedures, and help maintain high standards of lab practice by identifying and correcting issues promptly. In the Concordance Test, we base the assessment on samples that pass QC, ensuring that lab results are accurate and reliable.

Addressing challenges in protein biomarker analysis

As always, some caution is needed when analyzing results, especially when evaluating measures. In particular, when assessing correlations, both biological and technical factors can influence correlation values.

Here follow some important aspects to keep in mind when analyzing the data from any biomarker analysis.

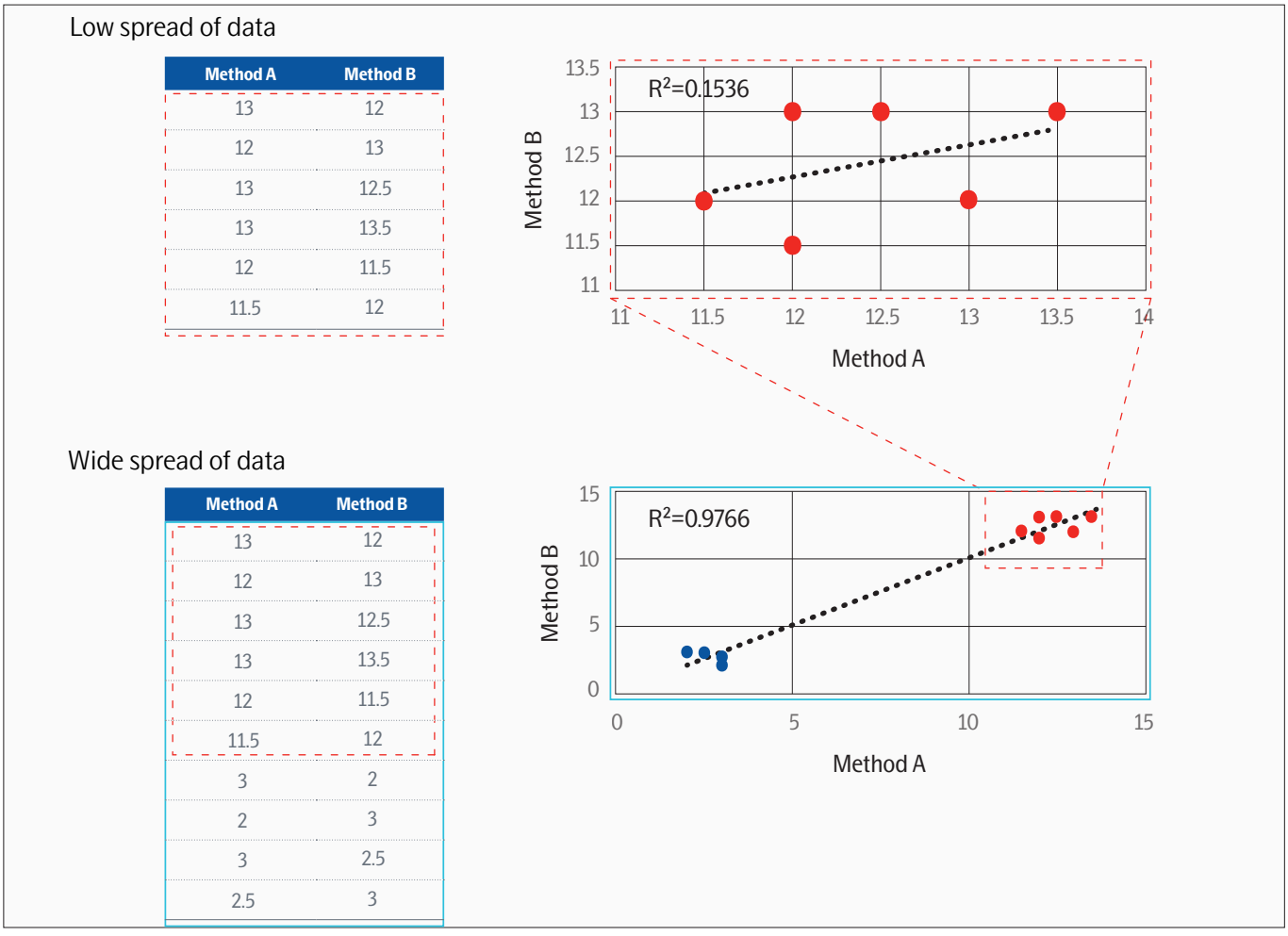

Figure 2 Correlation values derived from data with a low spread are rarely meaningful. For technical evaluations, a diverse sample set is preferable, as it allows measures to be compared and enables correlation values to offer valuable insights into method performance.

Reasons for lower correlation

Protein levels are dynamic, and comparisons of datasets obtained from different platforms or measurements can show a lower correlation than expected for multiple reasons. Biological differences and environmental conditions naturally introduce variation and can lead to lower correlation in certain cases.

Technical factors such as instruments, different operators, or experimental conditions can also influence results. See the Olink white paper, *Pre-analytical variation in protein biomarker research* for more information (3).

In datasets with low biological variation, it is difficult to achieve high correlation because small differences between samples can be hard to distinguish from random noise, due to how correlation is mathematically defined (4). In contrast, a wide spread of samples can make it easier to detect trends. As seen in Figure 2, adding a few samples to create a a wider spread of the data points results in a stronger correlation and a higher R^2 value, meaning the data give a clearer pattern. In part, this can be managed by a proper study plan and by making sure that a diverse sample set is included in the repeatability test. This is why the Concordance Test sample set is designed to include a combination of healthy, as well as series of disease samples to capture the diverse range across healthy and diseased samples. However, in some cases where there is minimal biological difference between the measured samples, a high correlation will be difficult to achieve.

Difference in measured levels in serum vs. plasma

Serum is the liquid part of blood after it has been allowed to coagulate fully for 30-60 minutes at room temperature. Serum is free of clotting proteins but contains the clotting metabolites that result from the clotting process.

Plasma is the liquid part of blood that has been treated with anti-coagulants, after cells have been removed by centrifugation. Since plasma has been prevented from clotting it is reflective of the blood as it circulates in the body.

Plasma and serum are both derived from whole blood but a difference in measured levels can be expected for many proteins (4). For consistency, all samples in a study should be of the same matrix. While this is not an issue for the Olink Concordance test where all included samples are plasma samples, it is an important factor that is sometimes overlooked in larger studies or in comparisons between larger studies.

Evaluation results

In a large evaluation experiment, Olink Concordance Test was used to assess the performance between three labs and the data were used to compare different Olink products.

Study design

The Concordance Test was run with several of the Olink kit products for head-to-head comparisons. There were three replicate runs for each product and all Concordance Test samples were used to generate the data.

The study was performed at three different sites comparing Olink® Explore HT, Olink® Explore 3072 and Olink® Reveal using NGS readout as well as five Olink Target® 96 panels (Target 96 Inflammation, Target 96 Neurology, Target 96 CVD II, Target 96 CVD III and Target 96 Immuno-Oncology),and two Olink® Target 48 panels (Target 48 Cytokine and Target 48 Immune Surveillance) using qPCR readout.

Performance assessment between sites

To assess the performance between the sites, a comparison of regression, correlation and CV was included in the evaluation, in line with the methods used to conduct a Concordance Test report. After evaluation of the results, all labs passed the Concordance Test.

Correlation accross product lines

Plots showing the correlation of all assays available on more than one panel were generated between the different sites and product lines. A large number of assays showed high correlation between different products. Table 1 presents a summary of all assays with a correlation value (R-value) of R≥0.8. For the assays available in all product lines additional graphs were generated. The median NPX value of the 6 replicate runs from two sites were plotted for each assay. An example of IL-6 is shown in Figure 3, where correlation between all products is presented in a single figure.

In this product-to-product comparison, we did not filter out data points below LOD or exclude sample warnings when calculating correlation and CV.

Table 1 Number of overlapping assays in all Olink products used in the comparison. Number of assays with R≥ 0.8 in the concordance study with combined data from all sites.

Products	No. of overlapping proteins	No. of proteins with R≥0.8
Explore HT vs Explore 3072	2831	2219 (78%)
Explore HT vs Reveal	1033	879 (85%)
Explore HT vs Target 96	227	202 (89%)
Explore HT vs Target 48	85	71 (84%)
Explore 3072 vs Reveal	852	689 (81%)
Explore 3072 vs Target 96	229	208 (91%)
Explore 3072 vs Target 48	85	74 (87%)
Reveal vs Target 96	117	96 (82%)
Reveal vs Target 48	66	49 (74%)
Target 96 vs Target 48	43	37 (86%)

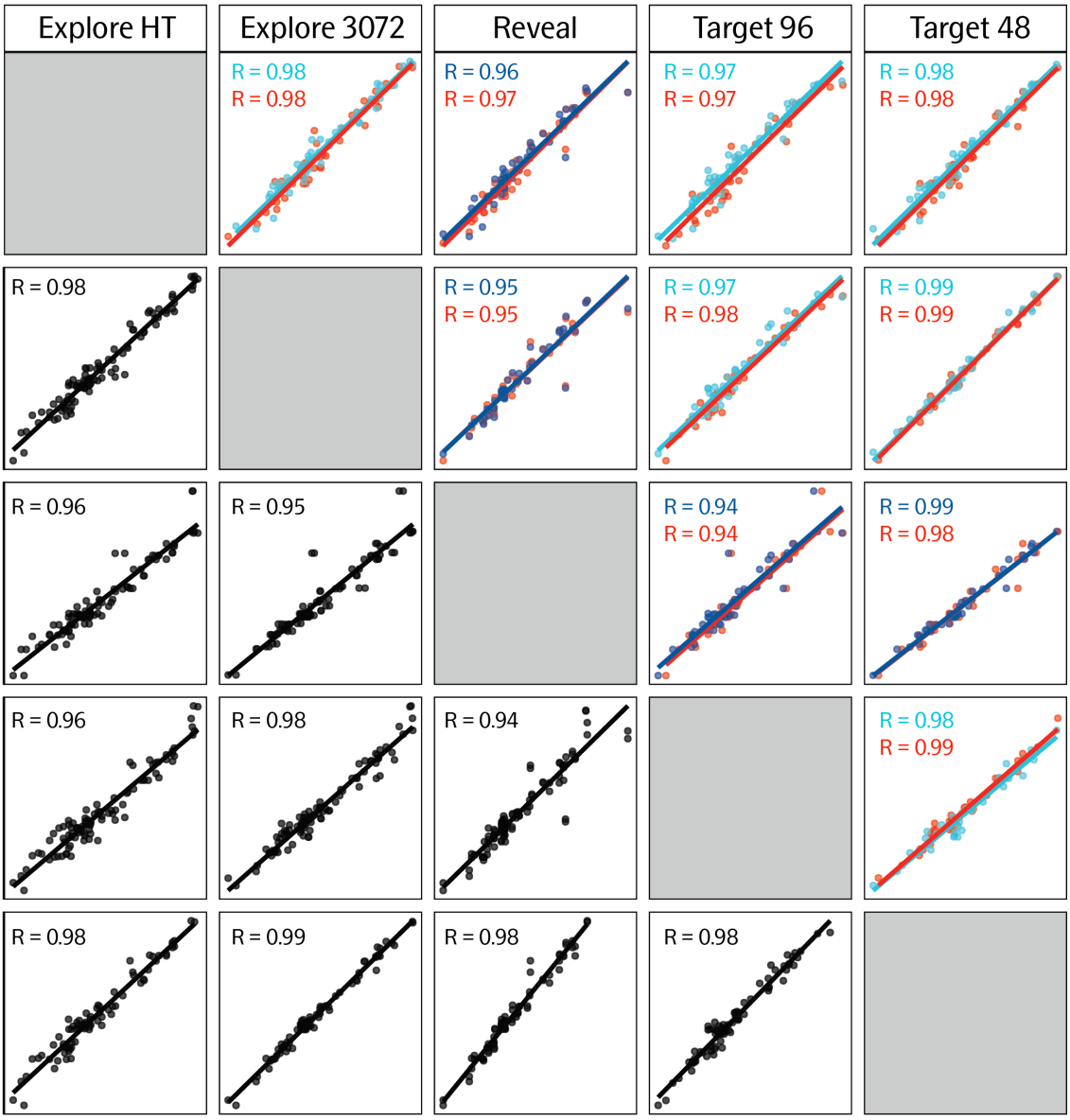

Figure 3 Correlation of IL-6 NPX values across Olink products and laboratories. Regression analyses were performed across five Olink products comparing data from three different laboratories (three replicates each). The lower left panels display regression plots for the combined data from the three laboratories, while the upper right panels show regression plots separated by laboratory. Each plot includes the Pearson correlation coefficient (R). Dot and R colors indicate the data source: black for combined data, light blue for site A, red for site B, and dark blue for site C.

The data generated in the evaluation experiment was extensive, and cannot be presented in its entirety in this white paper, however, Olink Support can provide additional data on request.

Conclusions

In this white paper, we presented results demonstrating that Olink is a reproducible solution from low to high multiplexing levels.

To demonstrate that Olink provides consistent and reliable results across different labs we developed the Olink Concordance test and performed a concordance study showing that the same protein biomarkers on different Olink panels are highly correlated, independent of the multiplex degree or readout method used (qPCR or NGS). In most comparisons, over 80% of overlapping proteins had strong correlation values. This shows that neither the readout technology nor product line significantly influenced the end results.

Reproducibility is a key part of scientific progress. High concordance means that researchers can rely on their data at every stage. When results are reliable, data from early studies can be used with confidence in larger clinical research, and scientists can build on previous work without the risk of introducing variability that could affect their conclusions.

Using the Concordance Test, the Olink offering becomes scalable and customers can expect consistent data quality, whether samples are run at Olink Analysis Services or through any of our Service Providers across the global network.

Such confidence empowers scientists to smoothly transition through all phases of their research using one reliable, high-quality technology, supporting any scale of study from routine measurements of a few proteins, to transformative, population-scale initiatives.

References

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