Application note

The compatibility of nonhuman primate plasma with Olink® PEA technology

Background

Animal models play a pivotal role in medical research especially large animal models such as dogs, pigs, and nonhuman primates (NHPs) (1, 2). Due to their close phylogenetic relationship to humans (3), NHPs are commonly suggested as the animal model for use in preclinical settings and in translational studies (1) where developing therapeutics using other animal models like rodents have failed, for example, tuberculosis, (4) or in studies aiming to improve vaccines and therapies such as with HIV (5, 6). NHPs further serve to bridge the translational research gap between small animal models and humans, and the large size of NHPs makes it easier for sample collection, running diagnostic assays, and imaging (7).

Proof of Concept

The similarity between the genome (8, 9) and exome (10, 11) of different NHPs species and humans ranges between 93 %- 98.8 %, and this similarity is translated at the protein level. Thus, antibodies targeting human proteins will potentially recognize those of NHP origin. The aim of this white paper is to examine the detectability of proteins in NHP plasma on the Olink® Explore platform, and evaluate the platform's technical performance (quality metrics) and its potential use in NHPs studies.

Olink® PEA technology

The development of Olink® Explore 3072, as well as Olink® Target 96 and 48, presents valuable tools for protein biomarker research, with improved proteome coverage and capability of identifying close to 3,000 biomarkers from small sample volumes (1 - 6 µl). Our proteomics platform leverages on the Proximity Extension Assay (PEA) (Figure 1) and combines the power of dual antibody recognition with DNA-based technologies such as Next Generation Sequencing (NGS) qPCR to enable high throughput proteomic analysis at sensitive, specific, and scalable levels. For more information on PEA technology, read the following white paper (12).

Study design

This inflammation-related study included four treatment groups of the species Macaca fascicularis, with four animals in each group. Group 1 served as a control group, whereas Groups 2-4 comprised animals treated with increasing doses of a compound. In total, 88 EDTA-plasma samples were collected before treatment and up to six time points after treatment.

To capture the best dynamic range, the samples were run neat (i.e., undiluted) and at different dilutions (1:10-1:10,000) similar to those used for human plasma. The results were compared to those obtained in a previous project using human plasma (598 samples of which 299 samples were from healthy subjects).

Results

Quality Control (QC)

The Olink QC-system consists of both internal and external controls (Figure 2) (13). The internal controls are spiked into every sample and are designed to monitor the three main steps of the Olink protocol: Immunoreaction, extension, and amplification/detection. The external controls are used to monitor technical variation (sample controls), the limit of detection (LOD) (negative controls) and to normalize each assay to compensate for potential variation between runs and plates (plate controls) (Figure 2).

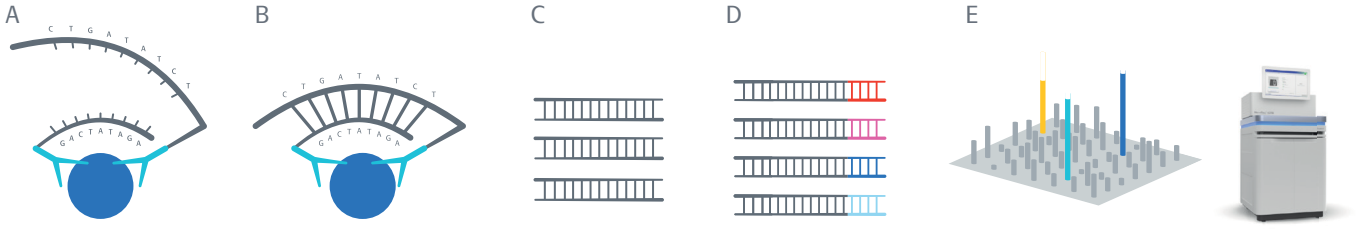

Figure 1. The main steps in Olink® Proximity Extension Assay (PEA) technology with a readout on NGS. (A) Antibody pairs with DNA oligonucleotides bind the target antigen in solution (Immunoreaction reaction). (B) Oligonucleotides in proximity hybridize and are extended by DNA polymerase. (C) The resulting DNA template contains a unique barcode pair for each protein (Extension). (D) Barcoded DNA templates are amplified by PCR, incorporating a unique index sequence to identify each sample (Amplification), and (E, Detection) the amplicons are sequenced (read) using next generation sequencing (NGS).

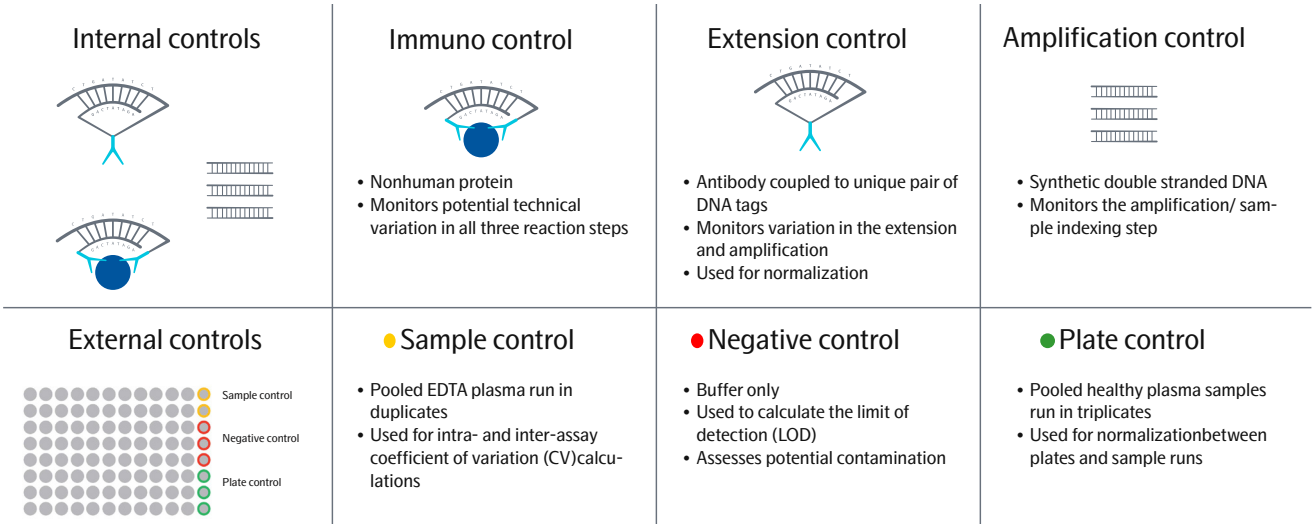

Figure 2. Internal and external controls incorporated into the Olink® Explore platform workflow.

Internal controls

Counts of barcoded NGS reads are translated into our relative quantification unit, Normalized Protein eXpression (NPX). Since the internal controls (Figure 2) are spiked into every sample at the same concentration, their signal is expected to be the same across the plate. If either the incubation or amplification control differ by more than ± 0.3 NPX from the median of all samples, the sample has not passed QC. Such deviations can be caused by factors such as pipetting errors or pre-analytical variations in the samples affecting the performance (e.g., matrix effects).

Definition

NPX is an arbitrary value that denotes Normalized Protein eXpression, and is expressed in Log2 scale. NPX is used by Olink to intuitively reflect protein abundance in the sample. The use of NPX allows users to identify changes in the level of each individual protein and compare those changes across samples within a project, enabling the establishment or identification of study-, disease-, or treatment-specific protein signatures.

Figure 3 shows NPX deviations from the corresponding medians of incubation and amplification controls in the NHP samples. The dashed lines indicate the ±0.3 NPX threshold, and samples with internal controls outside this threshold failed QC on the corresponding panel. The 88 NHP plasma samples generated a total of ~256K NPX data points, out of which 6,801 (2.6 %) failed QC. In comparison, the 598 human samples generated a total of ~1.72M datapoints, out of which ~43K (2.4 %) failed QC. These results show that the quality metrics from internal controls were similar in both cohorts regardless of the sample origin. This also illustrates technical consistency in the sample analysis workflow and the absence of interference in the NHP plasma samples.

External controls

The plate controls consist of pooled healthy human EDTA plasma samples that are run in triplicate on each plate and are used to normalize the NPX values in every Olink run. The NPX values of all other samples on the plate are defined relative to the plate controls, with negative NPX values indicating lower levels of proteins compared to controls and vice versa for positive NPX values.

For healthy human plasma, the average NPX value of any protein is therefore expected to be close to zero.

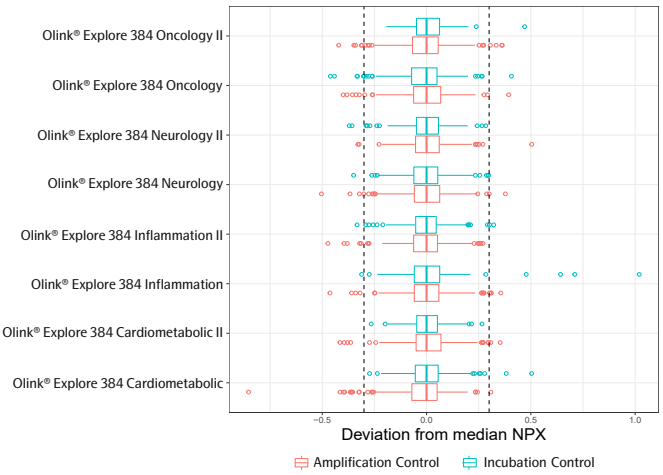

Figure 3. The biomarkers targeted by the Olink Explore platform are distributed over eight panels. The boxplot shows the deviation of NPX values of internal controls (amplification (red) and incubation (blue)) from the median NPX of all NHP plasma samples in each of the Olink Explore panels.

Figure 4 shows an overlay of the median NPX values per protein in the 88 NHP samples and 299 healthy human samples. In both populations, most proteins have a median NPX close to zero, indicating that their levels on average resemble the levels observed in healthy human plasma. A larger fraction of proteins in the NHP samples show negative NPX values, indicating that some proteins are expressed at lower levels in NHPs compared to humans. The NHP plasma also contains both control and treatment samples, potentially impacting on the median expression level of proteins when compared to healthy human plasma.

EDTA plasma samples are also run in duplicates (i.e., sample control) on every plate to monitor the technical variation in the signal of every protein. If the median intra-plate Coefficient of Variation (CV) is less than 15 %, the run is considered successful. In both the human and NHP sample runs, the intra-plate CVs were less than 15 % indicating high technical precision (Table 1).

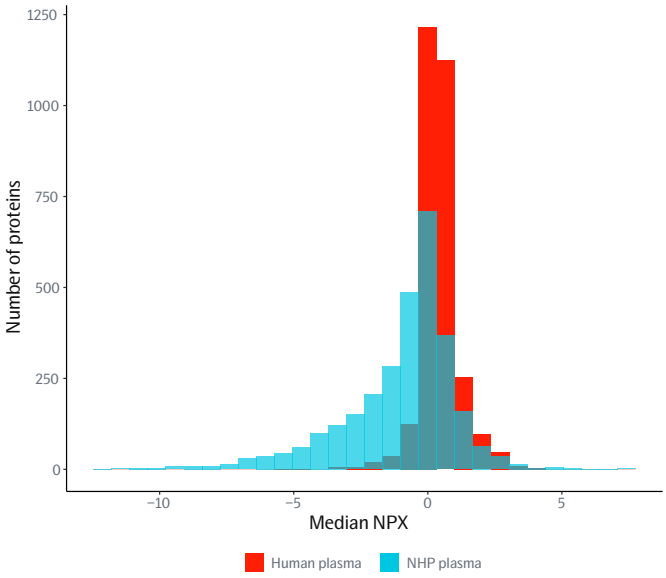

Figure 4. Histogram showing the distribution of median NPX values per protein in the Olink Explore platform. Median values correspond to either the 88 NHP samples (blue), or the 299 healthy human samples (red).

Table 1. Comparison of the median intra-assay CVs in human and NHP plasma sample runs.

Panel	Median CV	
	Human Plasma	NHP Plasma
Olink® Explore 384 Oncology II	8.3 %	8.0 %
Olink® Explore 384 Oncology	6.7 %	6.5 %
Olink® Explore 384 Neurology II	8.7 %	8.7 %
Olink® Explore 384 Neurology	5.9 %	7.5 %
Olink® Explore 384 Inflammation II	5.3 %	4.8 %
Olink® Explore 384 Inflammation	5.4 %	5.4 %
Olink® Explore 384 Cardiometabolic II	6.7 %	7.8 %
Olink® Explore 384 Cardiometabolic	4.8 %	3.4 %

Detectability of proteins in NHP plasma on Olink® Explore 3072

Table 2 shows the detectability (NPX above LOD in more than 50 % of the samples) per panel in NHP plasma compared to human plasma. The detectability is similar in both studies, although slightly higher in the human samples. This demonstrates that NHP plasma achieves good detectability on the Olink Explore platform compared to the expected detectability in human plasma. The results also show higher detectability for the Cardiometabolic, Neurology, Oncology, and Inflammation panels compared to their counterpart expansion panels (Cardiometabolic II, Neurology II, Oncology II, Inflammation II). This is expected since the expansion panels target more proteins of intracellular origin. The slight variation in detectability between NHP and human plasma may be due to differences in protein expression between the two sample types or species, experimental setup, or the lack of antibody affinity to some NHP proteins.

Table 2. Comparison of panel detectability obtained in NHP plasma versus human plasma analyzed on Olink Explore 3072.

*Based on unpublished Olink data.

Panel	Detectability		
	Human Plasma	NHP Plasma	Expected Range (Human)*
Olink® Explore 384 Oncology II	49%	38%	45 – 55%
Olink® Explore 384 Oncology	90%	80%	80 – 90%
Olink® Explore 384 Neurology II	51%	44%	45 – 55%
Olink® Explore 384 Neurology	85%	81%	80 – 90%
Olink® Explore 384 Inflammation II	78%	53%	70 – 80%
Olink® Explore 384 Inflammation	88%	77%	80 – 90%
Olink® Explore 384 Cardiometabolic II	66%	56%	60 – 70%
Olink® Explore 384 Cardiometabolic	93%	85%	85 – 95%

Treatment effects on protein levels in NHP plasma

To assess whether significant changes within the NHP plasma proteome can be identified in response to treatment, each protein was analyzed using a linear mixed model, including timepoint, treatment group, and the interaction between the two as fixed effects. A random effect per animal was also included to account for potential differences in the NPX baseline of each animal. In total, 998 proteins were significantly differentiated across either timepoints or treatment groups (multiple testing adjusted P-value <0.05).

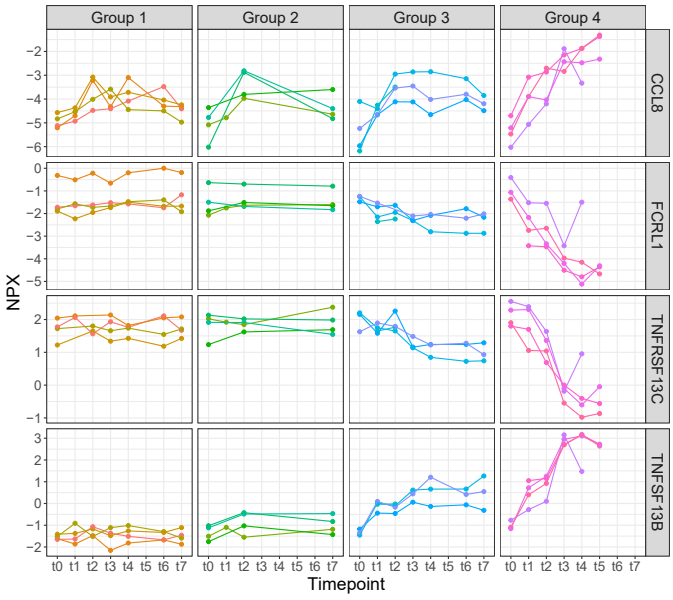

Figure 5. NPX levels (y-axis) per time point (x-axis) of the top four most significant proteins in a linear mixed model analysis. Each row corresponds to one protein, and each column to a treatment group. Each point is one NPX observation, with lines connecting observations belonging to the same animal. Time points are denoted T0-T7.

Figure 5 shows the trajectories of the four most significant proteins in the analysis. For these proteins, levels largely remain stable in the control (group 1) and low dose treatment (group 2) groups, however, among the animals treated with higher doses (groups 3 and 4), there was a highly significant treatment response. The response was especially pronounced in group 4, with some proteins showing an average change of ~4 NPX units upon treatment corresponding to a ~2^4 (i.e., 16-fold) increase in protein concentration. This illustrates how PEA technology can be used to detect treatment effects in the plasma proteome of NHP samples and its usability in NHP studies.

Summary

In this work, we analyzed NHP plasma samples on the Olink Explore platform to assess the compatibility of NHP plasma with the Olink® PEA technology. We investigated various quality metrics and compared them to the results obtained from a previous study with human plasma. The results showed similar quality metrics in NHP and human plasma with no identified interference effects from the NHP plasma. For the majority of panels in Olink Explore 3072, the detectability observed was similar to the ranges seen in human cohorts. Strong treatment effects were also observed for approximately 1,000 proteins in the NHP plasma proteome, signifying that the Olink Explore platform is able to accurately measure the effects of treatment on protein levels in NHPs. Overall, the results indicate that NHP plasma is compatible with Olink Explore 3072 and may be compatible with Olink® Target panels (data not shown, for more information please contact support@olink.com), and can yield reliable results in studies with NHPs.

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