Proteomics service performance - Experimental correlation across sites

Introduction

The study of proteins, or proteomics, has tremendous potential to advance human health. This potential also represents an enormous challenge for researchers, not just because of the extraordinary number of proteins that humans produce (1,2), but also because proteins can vary in composition, location, time and numbers within a cell, tissue, organ or system (3,4).
To support researchers across all disciplines, Psomagen has implemented Olink® Proximity Extension Assay (PEA) technology in its facilities. Olink's PEA technology allows the simultaneous quantitation, detection and profiling of hundreds of proteins with high sensitivity, specificity and low volume sample input (5,6). As a platinum service provider, Psomagen went through extensive training to achieve this classification, including complementary studies to characterize our performance.
In this paper, we share the high degree of correlation between samples processed by Olink's Boston laboratory and Psomagen Inc. using the Olink® Target 48 Cytokine panel. This assay detects 45 different proteins simultaneously that are useful for the analysis of inflammation-related diseases.
Our results show Psomagen's superior performance, demonstrating excellence in our ability to deliver consistent and reliable results to our partners.

Concordance analysis

Samples

Twenty human plasma samples, which had been analyzed at the Olink Boston Lab, were selected for the concordance test based on the concentration of cytokines to be tested. Samples had low (L, n=6), medium (M, n=6) and high (H, n=6) concentration for the selected cytokines, with two additional arbitrary samples (Total 20 human plasma samples). The samples were stored and maintained at -80° Celsius after the initial analysis at Olink Boston lab without any freeze/thaw cycles before shipment to Psomagen Inc. The plasma samples were shipped on dry ice and immediately placed at -80° Celsius upon arrival until the day of processing.

Statistical analysis

The data analysis performed at Psomagen Inc. included quality control determination from the internal controls. Calibrator normalized quantification values were calculated for each assay, which were further analyzed to evaluate if acceptance criteria were met.
Pair-wise quantified values for 40 assays were used to assess concordance. Pairs with one or both values missing were excluded from the analysis (n missing for Psomagen = 29, for Olink = 48, and for both = 61). Concordance was assessed using correlation coefficients. For each assay Spearman rank correlation coefficients were used to assess correlation. Intraclass correlation coefficient (ICC) was calculated using a two-way mixed effects model to assess absolute agreement (7). 95% confidence intervals were calculated for the ICC of each assay. A one-sided hypothesis test was performed to test the hypothesis that intra-class correlation was greater than 0.5.

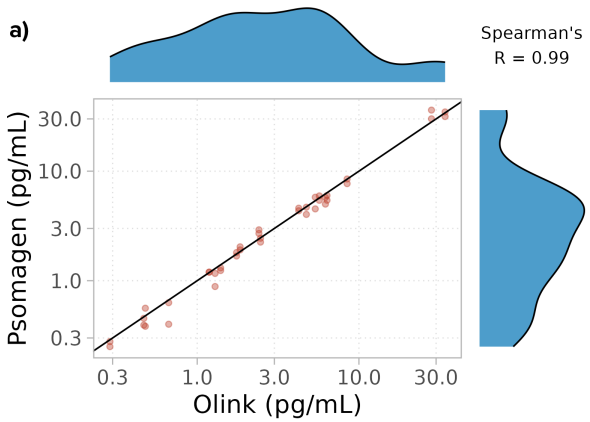
Results

Quality control results

All of the 40 assayed samples, 2 replicates for each sample, and all 45 targets passed the quality control parameters defined by Olink. Overall, run incubation and detection controls' NPX standard deviation values were below 0.2 in samples and 0.5 in external controls. Samples deviated less than 0.3 NPX from the plate median for each of the incubation and detection controls.

Correlation between samples

Two measurements of correlation were assessed. Spearman correlation and Intraclass Correlation Coefficient (ICC). The first measures the strength and direction of association between two individual observations that can be put in order.



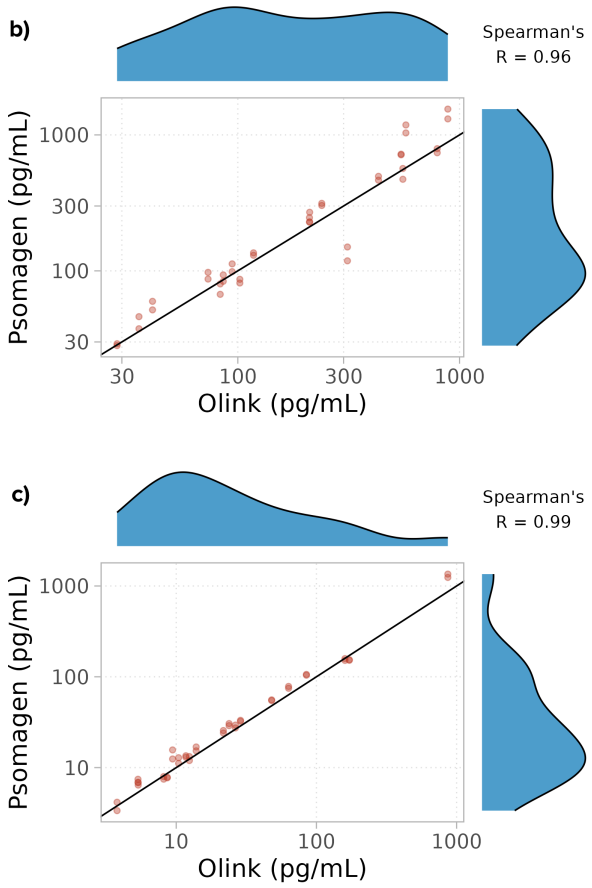


Figure 1. Spearman correlation for three representatives assays. Plots show the correlation between Olink measurements (X axis) and Psomagen measurements (Y axis). Density plots show the distribution of samples across values. A) Spearman correlation for C-C motif chemokine 7 (CCL7). B) Spearman correlation for C-X-C motif chemokine 11 (CXCL11). C) Spearman correlation for C-C motif chemokine 8 (CCL8).

The overall Spearman correlation across all assays was 0.958 (95% CI 0.954-0.962), with 6 out of 45 values (6%) between 0.75 and 0.9 indicating a strong correlation, and 35 out of 45 values (78%) over 0.9, indicating a very strong correlation. In the case of ICC, this number quantifies the similarity of pair-wise quantified values of assays from different observers. In our case, this measurement is useful to assess the consistency or reproducibility of quantitative measurements made by different observers —Psomagen and Olink— measuring the same sample. Overall, 12 out of 45 values (27%) were between 0.75 and 0.9 indicating good reliability, and 30 out of 45 values (67%) were greater than 0.90 indicating excellent reliability. Three representative assays with their correlation values data are shown in Figure 1 and the Intraclass Correlation Coefficient of 45 targets are shown in Table 1.

Table 1. Intraclass Correlation Coefficient for all 45 assays. Included is the p-value and the upper and lower confidence intervals for each assayed protein.

Assay	ICC	Lower 95% CI	Upper 95% CI	p-value
CCL11	0.996	0.993	0.998	7.84E-34
CCL13	0.856	0.720	0.925	9.72E-05
CCL19	0.871	0.222	0.961	8.66E-02
CCL2	0.955	0.890	0.979	2.86E-07
CCL3	0.991	0.982	0.995	1.63E-26
CCL4	0.876	0.775	0.933	1.46E-06
CCL7	0.988	0.977	0.994	1.63E-24
CCL8	0.983	0.957	0.993	1.04E-09
CSF1	0.774	0.242	0.913	1.19E-01
CSF2	0.998	0.996	0.999	1.77E-39
CSF3	0.797	0.649	0.887	4.76E-04
CXCL10	0.919	0.821	0.961	4.38E-06
CXCL11	0.843	0.707	0.917	9.78E-05
CXCL12	0.590	0.315	0.768	2.40E-01
CXCL8	0.918	0.850	0.956	1.87E-09
CXCL9	0.978	0.957	0.989	5.70E-18
EGF	0.791	0.575	0.894	7.43E-03
FLT3LG	0.976	0.954	0.987	1.46E-17
HGF	0.998	0.996	0.999	2.57E-39
IFNG	0.977	0.855	0.992	8.41E-04
IL10	0.994	0.987	0.997	2.08E-24
IL13	0.937	0.883	0.966	5.57E-11
IL15	0.978	0.959	0.988	1.28E-19
IL17A	0.956	0.918	0.977	2.11E-13
IL17C	0.815	0.636	0.905	1.74E-03
IL17F	0.934	0.873	0.965	8.04E-10
IL18	0.989	0.980	0.994	1.32E-25
IL1B	0.836	0.684	0.915	3.45E-04
IL2	0.980	0.962	0.989	2.18E-20
IL27	0.807	-0.027	0.948	2.24E-01
IL33	0.713	0.497	0.843	2.63E-02
IL4	0.804	0.658	0.892	3.59E-04
IL6	0.977	0.890	0.992	1.65E-04

IL7	0.962	0.922	0.981	4.09E-11
LTA	0.946	0.897	0.971	1.55E-11
MMP1	0.957	0.913	0.979	2.12E-11
MMP12	0.993	0.985	0.996	4.04E-19
OLR1	0.977	0.958	0.988	1.11E-19
OSM	0.998	0.996	0.999	3.62E-40
TGFA	0.998	0.997	0.999	4.10E-35
TNF	0.997	0.995	0.999	2.37E-33
TNFSF10	0.927	0.867	0.961	2.33E-10
TNFSF12	0.908	0.833	0.950	8.90E-09
TSLP	0.703	0.498	0.834	2.61E-02
VEGFA	0.791	0.544	0.898	1.38E-02

Summary

Biomarker discovery and validation is a critical component for the development of new therapies, diagnosis and evaluation of outcomes. Our results show that Psomagen proteomics services, and in particular, Olink Target 48 Cytokine assay, is performed with a high degree of quality. Researchers can be assured that Psomagen's proteomic platform can deliver accurate and reproducible results for research, development and commercial purposes.

References

1. Aebersold R, et al. "*How many human proteoforms are there?*" Nature Chemical Biology, vol 14(3), pp 206-214. Feb 2018. https://doi.org/10.1038/nchembio.2576
2. Uhlén M, Fagerberg L, Hallström BM, et al. Proteomics. "*Tissue-based map of the human proteome*" Science, vol 347(6220), pp 1260419, Jan 2015. https://doi.org/10.1126/science.1260419
3. Kim MS, Pinto SM, Getnet D, et al. "*A draft map of the human proteome*" Nature, vol 509(7502), pp 575-581, May 2014. https://doi.org/10.1038/nature13302
4. Wang D, Eraslan B, Wieland T, et al. "*A deep proteome and transcriptome abundance atlas of 29 healthy human tissues*" Molecular Systems Biology, vol 15(2), pp e8503, Feb 2010. https://doi.org/10.15252/msb.20188503
5. Gullberg, Mats, et al. "*Cytokine Detection by Antibody-Based Proximity Ligation*" Proceedings of the National Academy of Sciences, vol 101(22), pp. 8420–24, June 2004. https://doi.org/10.1073/pnas.0400552101
6. Lundberg, Martin, et al. "*Homogeneous Antibody-Based Proximity Extension Assays Provide Sensitive and Specific Detection of Low-Abundant Proteins in Human Blood*" Nucleic Acids Research, vol 39(15), pp. e102–e102, Aug 2011. https://doi.org/10.1093/nar/gkr424.
7. Shrout, P. E., & Fleiss, J. L. "*Intraclass correlations: Uses in assessing rater reliability*" Psychological Bulletin, vol 86(2), pp 420–428, 1979. https://doi.org/10.1037/0033-2909.86.2.420