1. Introduction

Olink® Inflammation is a reagent kit measuring 92 inflammation related human protein biomarkers simultaneously. The analytical performance of the product has been carefully validated and the results are presented in this document. Please note that when a new panel is developed, both the individual assays and 92-plex panel as a whole are subject to our thorough validation procedure. If individual assays are subsequently improved or one or more assays are replaced in later versions of the panel, focus is placed on thoroughly validating the individual assays in question.

1.1 TECHNOLOGY

The Olink reagents are based on the Proximity Extension Assay (PEA) technology[1-2], where 92 oligonucleotide labeled antibody probe pairs are allowed to bind to their respective target proteins, if present in the sample. A PCR reporter sequence is formed by a proximity-dependent DNA polymerization event. This is then amplified, and subsequently detected and quantified using real-time PCR. The assay is performed in a homogeneous 96-well format without any need for washing steps, see Figure 1.

1.2 QUALITY CONTROLS

Internal and external controls have been developed by Olink for data normalization and quality control purposes. These controls are designed to enable monitoring of the technical assay performance, as well as the quality of individual samples, and provide information at each step of the Olink protocol (see Figure 1). The internal controls are added to each sample and include two Immunoassay controls, one Extension control and one Detection control. The Immunoassay controls (two non-human proteins) monitor all three steps starting with the immunoreaction. The Extension Control (an antibody linked to two matched oligonucleotides) monitors the extension and readout steps independent of antigen binding, and is used for data normalization across samples. Finally, the Detection control (a synthetic double-stranded template) monitors the readout step. Samples for which one or more of the internal control values deviate from a pre-determined range will be flagged and may be removed before statistical analysis. An external inter-plate control (IPC), is included on each plate and is used in a second normalization step. This control is made up of a pool of probes similar to the Extension control (Ext Ctrl), but generated with 92 matching oligonucleotide pairs. This improves inter-assay precision and allows for optimal comparison of data derived from multiple runs. The term "Normalized Protein eXpression (NPX)" refers to normalized data as described above.

1.3 DATA ANALYSIS

Data analysis is performed by employing a pre-processing normalization procedure. For each sample and data point, the corresponding Cq-value for the Extension control is substracted, thereby normalizing for technical variation within one run. Normalization between runs is then performed for each assay by substracting the corresponding dCq-value for the Interplate Control (IPC) from the dCq-values generated. In the final step of the pre-processing procedure the values are set relative to a correction factor determined by Olink. The Normalized Protein eXpression (NPX) unit is generated on a log2 scale where a larger number represents a higher protein level in the sample, typically with the background level at around zero. Linearization of data is performed by the mathematical operation $2^{\wedge NPX}$. Coefficient of variation (CV) calculations are performed on linearized values.

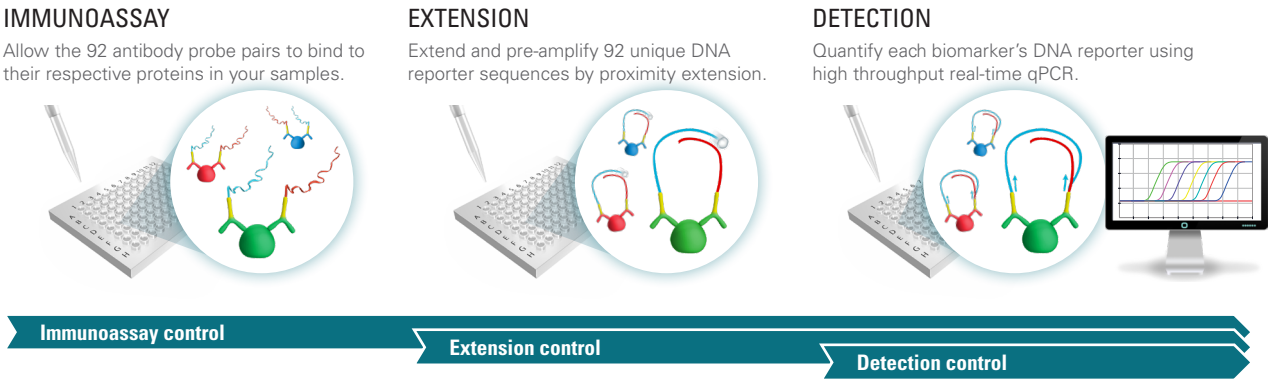


Fig 1. Olink assay procedure (above) and controls (below). The internal controls enable monitoring of the three core steps in the Olink assay and are used for quality control and data normalization. Read out is performed using the Fluidigm® Biomark™ or the Fluidigm® Biomark™ HD system.

2. Performance characteristics

2.1 SAMPLE TYPES

Performance with different sample types was evaluated for Olink INFLAMMATION by collecting matched EDTA-, acid citrate dextrose (ACD)- and sodium heparin-plasma, as well as serum samples from 5 individuals. Comparative response values between heparin plasma, citrate plasma or serum, are expressed as relative differences (%) compared to EDTA plasma and are shown in Table 1 for each sample type. To evaluate the measuring range for endogenous protein levels, response values levels were assessed in 22 normal EDTA plasma samples and reported in NPX (Table 1).
Variations observed between responses in heparin and citrate plasma, as compared to EDTA plasma, were generally small, and most of the assays will therefore function without any limitations related to the anti-coagulant used. Serum gives a higher signal compared to EDTA plasma for several assays. The results indicate that all plasma types and serum are suitable for use of this panel, but citrate and heparin plasma have not been fully validated.

2.2 ANALYTICAL MEASUREMENT

DETECTION LIMIT

Calibrator curves were determined for 90 out of 92 biomarkers simultaneously in a multiplex format. In cases where no suitable antigen was available, no calibrator data is presented. Limit of detection (LOD) was defined as 3 standard deviations above background, and reported in pg/mL, see Table 1.

HIGH DOSE HOOK EFFECT

The high dose hook effect is seen when there is an antigen excess relative to the reagent antibodies, resulting in falsely lower values. In such cases, a significantly lower value can be reported that can lead to misinterpretation of results. Therefore, the hook threshold was determined for each analyte and reported in pg/mL, see Table 1.

MEASURING RANGES

The analytical measuring range was defined by the lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) and reported in pg/mL. Quantification limits of LLOQ and ULOQ were calculated with the following trueness and precision criteria; relative error ≤ 30% and CV ≤ 30%, of back-calculated values, respectively. Measuring ranges are presented in Table 1, ordered by LLOQ and displayed on a log10 scale..

Example calibrator curves showing the measuring ranges for selected representative assays are shown in Figure 2. The overall distribution of measuring ranges for the assays with available recombinant antigens is shown in Figure 3.. Separate calibrator curves established for each assay may be viewed at www.olink.com.

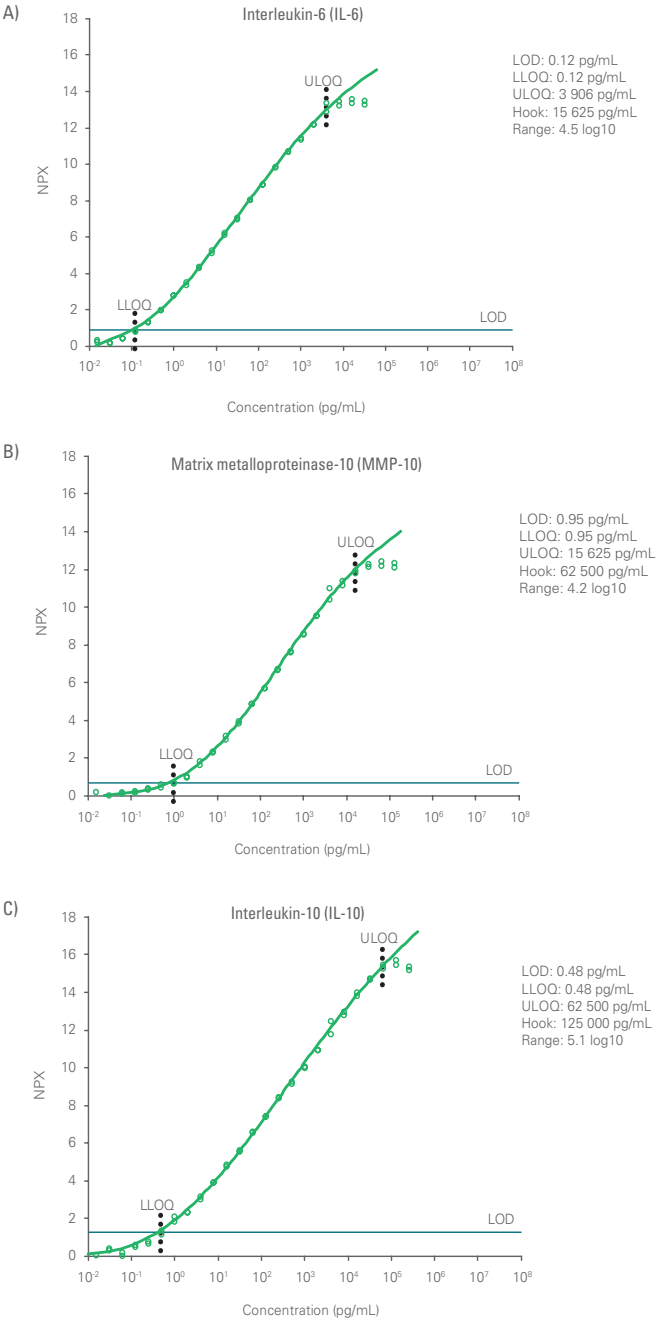


Fig 2. Calibrator curves for representative assays using a 4-parameter curve fitting model.

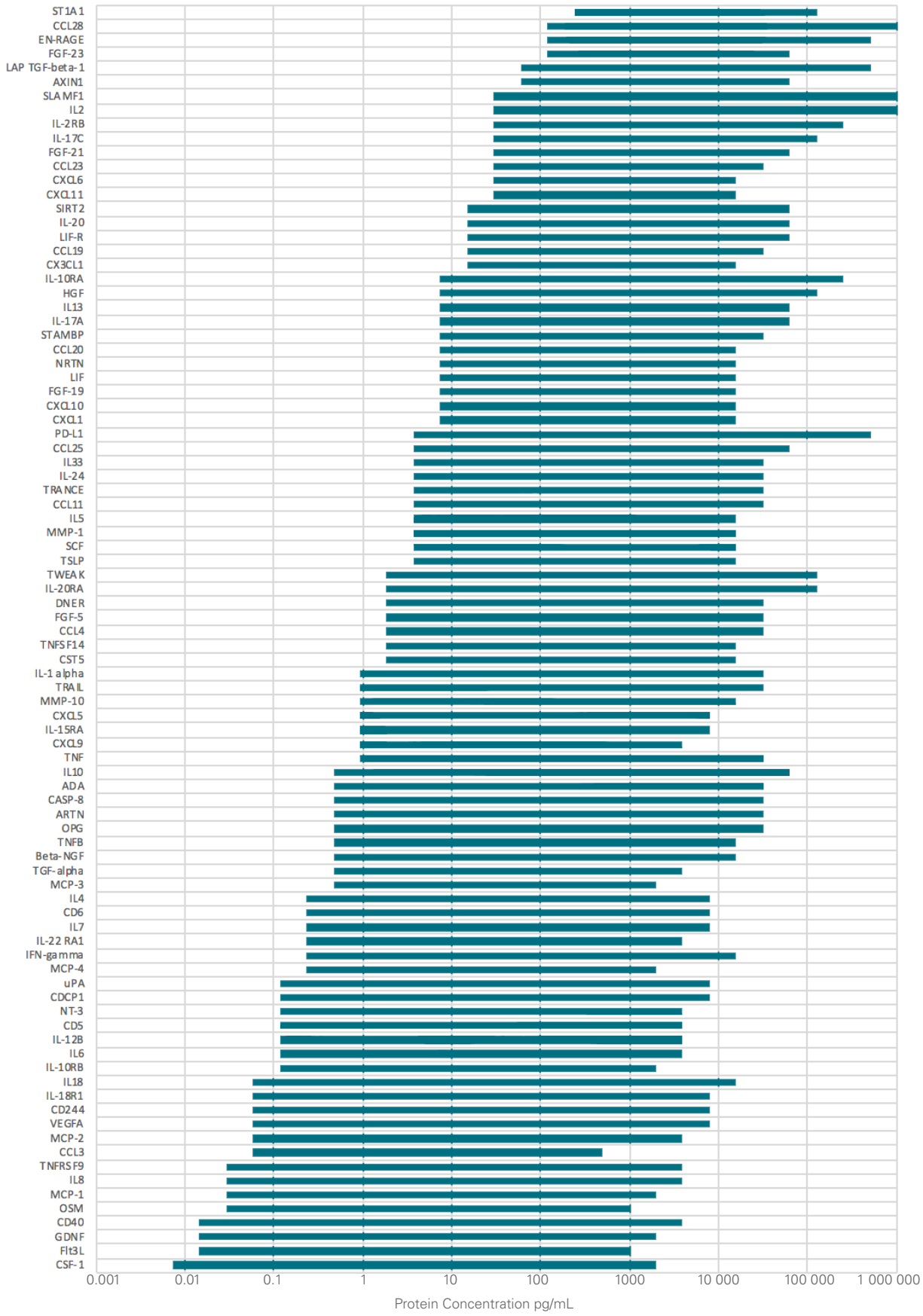


Fig 3. Distribution of analytical measuring range, defined by the limits of quantification LLOQ-ULOQ, for 90 out of 92 analytes.

Table 1. Sample Types; acid citrate dextrose plasma (ACD), ethylenediaminetetraacetic acid plasma (EDTA), sodium heparin plasma (heparin) and serum, Analytical Measurement; Limit of Detection (LOD), Lower/Upper Limit of Quantification (LLOQ/ULOQ), High Dose Effect (Hook), Range and Precision indicative of assay performance are shown for 92 analytes. Values below LOD were not reported (NR). Values where data was not available are denoted as NA. Endogenous interference was performed by addition of hemolysate (Hemo), lipids and bilirubin (Bili) in serum and EDTA plasma matrix. The values stated are the highest tested concentrations without impact on assay performance in either serum or EDTA plasma.

		Sample types							Analytical measurement					Precision		Endogenous interference		
		Signal-to-background (2^{NPX})				Relative 2^{NPX} to EDTA			pg/mL				log10			g/L	mg/mL	µg/mL
Target	UniProt No	ACD	EDTA	Heparin	Serum	ACD	Heparin	Serum	LOD	LLOQ	ULOQ	Hook	Range	Intra	Inter	Hemo	Lipids	Bili
Adenosine Deaminase (ADA)	P00813	45	58	47	58	77%	80%	99%	0.48	0.48	31250	125000	4.8	5%	29%	15	20	630
Artemin (ARTN)	Q5T4W7	NR	1	NR	1	NR	NR	102%	0.24	0.48	31250	62500	4.8	7%	18%	15	20	630
Axin-1 (AXIN1)	O15169	4	9	3	4	50%	30%	42%	61	61	62500	250000	3.0	6%	19%	15	20	630
Beta-nerve growth factor (Beta-NGF)	P01138	3	3	2	4	95%	81%	123%	0.48	0.48	15625	31250	4.5	6%	14%	15	20	630
Caspase 8 (CASP-8)	Q14790	3	3	3	5	84%	105%	154%	0.48	0.48	31250	62500	4.8	7%	22%	15	20	630
C-C motif chemokine 4 (CCL4)	P13236	37	68	56	99	55%	82%	146%	1.9	1.9	31250	62500	4.2	6%	17%	7.5	20	315
C-C motif chemokine 19 (CCL19)	Q99731	1014	1128	870	1249	90%	77%	111%	15	15	31250	62500	3.3	8%	15%	7.5	20	315
C-C motif chemokine 20 (CCL20)	P78556	169	184	132	139	91%	72%	75%	7.6	7.6	15625	15625	3.3	7%	13%	15	20	158
C-C motif chemokine 23 (CCL23)	P55773	322	384	313	358	84%	81%	93%	31	31	31250	62500	3.0	6%	13%	15	20	315
C-C motif chemokine 25 (CCL25)	O15444	71	82	73	95	86%	89%	115%	3.8	3.8	62500	125000	4.2	6%	18%	15	10	158
C-C motif chemokine 28 (CCL28)	Q9NRJ3	3	4	2	4	75%	60%	111%	61	122	1000000	1000000	3.9	7%	14%	15	20	630
CD40L receptor (CD40)	P25942	399	463	452	646	86%	98%	140%	0.01	0.01	3906	15625	5.4	5%	21%	15	20	630
CUB domain-containing protein 1 (CDCP1)	Q9H5V8	7	8	7	9	81%	87%	107%	0.12	0.12	7812	31250	4.8	6%	24%	7.5	10	315
C-X-C motif chemokine 1 (CXCL1)	P09341	135	422	713	1301	32%	169%	308%	3.8	7.6	15625	15625	3.3	6%	15%	15	10	79
C-X-C motif chemokine 5 (CXCL5)	P42830	336	3011	5114	9171	11%	170%	305%	0.95	0.95	7812	15625	3.9	7%	13%	15	10	315
C-X-C motif chemokine 6 (CXCL6)	P80162	50	177	409	840	28%	231%	475%	7.6	31	15625	31250	2.7	8%	14%	15	20	315
C-X-C motif chemokine 9 (CXCL9)	Q07325	54	68	59	68	80%	87%	100%	0.95	0.95	3906	7812	3.6	6%	12%	15	20	315
C-X-C motif chemokine 10 (CXCL10)	P02778	378	505	378	546	75%	75%	108%	7.6	7.6	15625	31250	3.3	7%	11%	15	20	315
C-X-C motif chemokine 11 (CXCL11)	O14625	94	308	578	850	31%	188%	276%	7.6	31	15625	15625	2.7	7%	14%	15	20	158
Cystatin D (CST5)	P28325	86	97	89	101	88%	91%	104%	1.9	1.9	15625	31250	3.9	5%	21%	15	20	315
Delta and Notch-like epidermal growth factor-related recep (DNER)	Q8NFT8	181	201	181	220	90%	90%	109%	0.95	1.9	31250	62500	4.2	5%	26%	15	20	630
Eotaxin-1 (CCL11)	P51671	247	294	307	318	84%	104%	108%	3.8	3.8	31250	62500	3.9	5%	14%	15	20	630
Eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1)	Q13541	14	102	12	61	14%	12%	59%	NA	NA	NA	NA	NA	6%	23%	15	10	158
Fibroblast growth factor 5 (FGF-5)	P12034	2	2	2	2	90%	76%	104%	1.9	1.9	31250	125000	4.2	7%	14%	15	20	630
Fibroblast growth factor 19 (FGF-19)	O95750	387	477	394	503	81%	83%	105%	7.6	7.6	15625	31250	3.3	6%	19%	15	20	315
Fibroblast growth factor 21 (FGF-21)	Q9NSA1	30	39	35	35	76%	89%	89%	31	31	62500	500000	3.3	8%	21%	3.8	5	158
Fibroblast growth factor 23 (FGF-23)	Q9GZV9	27	38	33	9	71%	86%	22%	122	122	62500	62500	2.7	9%	26%	7.5	10	315
Fms-related tyrosine kinase 3 ligand (Flt3L)	P49771	405	493	410	532	82%	83%	108%	0.01	0.01	977	3906	4.8	6%	15%	15	10	315
Fractalkine (CX3CL1)	P78423	58	70	64	101	84%	92%	145%	15.3	15.3	15625	31250	3.0	7%	24%	15	20	630
Glial cell line-derived neurotrophic factor (GDNF)	P39905	5	5	3	4	92%	51%	85%	0.01	0.01	1953	3906	5.1	7%	12%	15	20	630
Hepatocyte growth factor (HGF)	P14210	85	130	67	198	66%	52%	153%	7.6	7.6	125000	125000	3.9	6%	16%	7.5	10	315
Interferon gamma (IFN-gamma)	P01579	22	25	24	26	91%	100%	108%	0.24	0.24	15625	31250	4.8	7%	24%	15	NA	NA
Interleukin-1 alpha (IL-1 alpha)	P01583	NR	NR	3	2	NR	NR	NR	0.48	0.95	31250	125000	4.5	7%	18%	15	20	630
Interleukin-2 (IL-2)	P60568	NR	NR	NR	2	NR	NR	NR	30.5	30.5	1000000	1000000	4.5	9%	16%	15	20	630
Interleukin-2 receptor subunit beta (IL-2RB)	P14784	NR	2	2	2	NR	96%	101%	15	31	250000	1000000	3.9	7%	19%	15	20	630
Interleukin-4 (IL-4)	P05112	NR	2	NR	2	NR	NR	115%	0.24	0.24	7812	15625	4.5	7%	16%	15	20	630
Interleukin-5 (IL-5)	P05113	4	2	3	2	175%	126%	105%	3.8	3.8	15625	62500	3.6	7%	17%	15	20	630
Interleukin-6 (IL-6)	P05231	28	31	31	40	90%	99%	128%	0.12	0.12	3906	15625	4.5	6%	8%	15	20	315
Interleukin-7 (IL-7)	P13232	3	7	4	19	45%	64%	283%	0.24	0.24	7812	15625	4.5	6%	18%	15	20	315
Interleukin-8 (IL-8)	P10145	41	69	73	129	60%	106%	188%	0.03	0.03	3906	7812	5.1	6%	15%	15	20	79
Interleukin-10 (IL-10)	P22301	7	10	8	10	75%	77%	105%	0.48	0.48	62500	125000	5.1	7%	12%	15	20	630
Interleukin-10 receptor subunit alpha (IL-10RA)	Q13651	3	2	2	2	137%	94%	105%	3.8	7.6	250000	500000	4.5	6%	19%	15	20	630
Interleukin-10 receptor subunit beta (IL-10RB)	Q08334	61	75	71	79	81%	95%	105%	0.12	0.12	1953	3906	4.2	5%	31%	7.5	20	630
Interleukin-12 subunit beta (IL-12B)	P29460	12	14	10	16	87%	74%	111%	0.12	0.12	3906	3906	4.5	6%	16%	15	20	630
Interleukin-13 (IL-13)	P35225	NR	NR	NR	NR	NR	NR	NR	7.6	7.6	62500	500000	3.9	14%	26%	15	20	630
Interleukin-15 receptor subunit alpha (IL-15RA)	Q13261	2	2	2	2	93%	84%	112%	0.95	0.95	7812	15625	3.9	6%	20%	15	20	630

		Sample types							Analytical measurement					Precision		Endogenous interference		
		Signal-to-background (2^{NPX})				Relative 2^{NPX} to EDTA			pg/mL				log10			g/L	mg/mL	µg/mL
Target	UniProt No	ACD	EDTA	Heparin	Serum	ACD	Heparin	Serum	LOD	LLOQ	ULOQ	Hook	Range	Intra	Inter	Hemo	Lipids	Bili
Interleukin-17A (IL-17A)	Q16552	NR	2	2	2	NR	93%	109%	3.8	7.6	62500	62500	3.9	8%	17%	15	20	630
Interleukin-17C (IL-17C)	Q9P0M4	4	5	4	5	81%	81%	110%	31	31	125000	500000	3.3	8%	18%	15	20	630
Interleukin-18 (IL-18)	Q14116	124	153	133	164	81%	87%	108%	0.06	0.06	15625	15625	5.4	6%	19%	15	10	315
Interleukin-18 receptor 1 (IL-18R1)	Q13478	93	115	102	132	81%	89%	115%	0.06	0.06	7812	15625	5.1	5%	26%	15	20	630
Interleukin-20 (IL-20)	Q9NYY1	2	2	1	2	97%	79%	103%	7.6	15	62500	125000	3.6	7%	22%	15	20	630
Interleukin-20 receptor subunit alpha (IL-20RA)	Q9UHF4	NR	2	NR	2	NR	NR	99%	1.9	1.9	125000	125000	4.8	6%	22%	15	20	630
Interleukin-22 receptor subunit alpha-1 (IL-22 RA1)	Q8N6P7	NR	4	3	3	NR	79%	87%	0.24	0.24	3906	15625	4.2	7%	23%	15	20	630
Interleukin-24 (IL-24)	Q13007	NR	2	2	2	NR	116%	108%	1.9	3.8	31250	125000	3.9	6%	29%	15	20	630
Interleukin-33 (IL-33)	O95760	NR	2	NR	2	NR	NR	97%	3.8	3.8	31250	125000	3.9	9%	26%	15	20	630
Latency-associated peptide transforming growth factor beta 1 (LAP TGF-beta-1)	P01137	81	183	190	352	44%	104%	192%	61	61	500000	500000	3.9	7%	24%	7.5	20	315
Leukemia inhibitory factor (LIF)	P15018	48	38	60	22	126%	158%	57%	3.8	7.6	15625	31250	3.3	7%	18%	15	20	630
Leukemia inhibitory factor receptor (LIF-R)	P42702	6	8	7	9	75%	83%	107%	30.5	15.3	62500	250000	3.3	7%	26%	15	20	630
Macrophage colony-stimulating factor 1 (CSF-1)	P09603	235	264	227	297	89%	86%	113%	0.004	0.01	1953	3906	5.4	5%	25%	15	20	630
Macrophage inflammatory protein 1-alpha (CCL3)	P10147	5	7	5	9	71%	74%	129%	0.06	0.06	488	977	3.9	6%	14%	15	20	315
Matrix metalloproteinase-1 (MMP-1)	P03956	3	9	6	9	28%	68%	93%	1.9	3.8	15625	31250	3.6	5%	19%	15	20	630
Matrix metalloproteinase-10 (MMP-10)	P09238	104	103	117	145	101%	114%	141%	0.95	0.95	15625	62500	4.2	5%	28%	15	20	315
Monocyte chemotactic protein 1 (MCP-1)	P13500	1064	1213	1063	1494	88%	88%	123%	0.03	0.03	1953	3906	4.8	6%	13%	15	20	315
Monocyte chemotactic protein 2 (MCP-2)	P80075	363	610	488	982	60%	80%	161%	0.06	0.06	3906	7812	4.8	6%	8%	15	20	315
Monocyte chemotactic protein 3 (MCP-3)	P80098	4	5	6	6	78%	110%	120%	0.48	0.48	1953	3906	3.6	7%	17%	15	20	315
Monocyte chemotactic protein 4 (MCP-4)	Q99616	3330	5647	7379	12228	58%	130%	220%	0.24	0.24	1953	7812	3.9	6%	24%	15	20	315
Natural killer cell receptor 2B4 (CD244)	Q9BZW8	60	75	67	81	81%	90%	108%	0.06	0.06	7812	15625	5.1	5%	24%	15	20	630
Neurotrophin-3 (NT-3)	P20783	4	5	2	4	75%	37%	78%	0.12	0.12	3906	7812	4.5	6%	13%	15	20	630
Neurturin (NRTN)	Q99748	NR	2	NR	2	NR	NR	109%	3.8	7.6	15625	62500	3.3	9%	15%	15	20	630
Oncostatin-M (OSM)	P13725	6	14	12	37	46%	86%	272%	0.03	0.03	977	3906	4.5	5%	12%	15	20	79
Osteoprotegerin (OPG)	O00300	1205	1385	1103	1520	87%	80%	110%	0.24	0.48	31250	62500	4.8	6%	12%	15	20	315
Programmed cell death 1 ligand 1 (PD-L1)	Q9NZQ7	6	5	5	6	135%	99%	124%	3.8	3.8	500000	1000000	5.1	9%	25%	15	20	630
Protein S100-A12 (EN-RAGE)	P80511	8	4	12	35	196%	274%	825%	122	122	500000	1000000	3.6	8%	17%	15	20	630
Signaling lymphocytic activation molecule (SLAMF1)	Q13291	6	7	6	8	82%	89%	113%	31	31	1000000	1000000	4.5	9%	21%	15	20	630
SIR2-like protein 2 (SIRT2)	Q8IXJ6	8	36	8	20	23%	21%	57%	7.6	15.3	62500	250000	3.6	8%	22%	15	20	630
STAM-binding protein (STAMPB)	O95630	10	25	9	17	39%	37%	67%	7.6	7.6	31250	62500	3.6	5%	27%	15	20	630
Stem cell factor (SCF)	P21583	334	368	356	418	91%	97%	113%	1.9	3.8	15625	31250	4	5%	20%	15	20	630
Sulfotransferase 1A1 (ST1A1)	P50225	3	2	5	5	122%	228%	193%	244	244	125000	500000	2.7	6%	25%	1.88	20	630
T-cell surface glycoprotein CD5 (CD5)	P06127	21	23	20	24	90%	89%	104%	0.06	0.12	3906	15625	4.5	5%	22%	15	20	630
T-cell surface glycoprotein CD6 isoform (CD6)	P30203	19	23	21	22	84%	91%	98%	0.24	0.24	7812	31250	4.2	6%	23%	15	20	630
T-cell surface glycoprotein CD8 alpha chain (CD8A)	P01732	1196	921	879	1178	146%	108%	153%	NA	NA	NA	NA	NA	9%	10%	15	NA	NA
Thymic stromal lymphopoietin (TSLP)	Q969D9	NR	2	NR	2	NR	NR	94%	3.8	3.8	15625	62500	3.6	6%	20%	15	20	630
TNF-beta (TNFB)	P01374	13	14	13	16	93%	91%	114%	0.24	0.48	15625	15625	4.5	6%	22%	15	20	630
TNF-related activation-induced cytokine (TRANCE)	O14788	23	27	23	29	83%	84%	106%	3.8	3.8	31250	125000	3.9	7%	24%	15	20	630
TNF-related apoptosis-inducing ligand (TRAIL)	P50591	383	425	382	484	90%	90%	114%	0.95	0.95	31250	31250	4.5	5%	17%	15	20	630
Transforming growth factor alpha (TGF-alpha)	P01135	3	3	3	11	90%	81%	342%	0.48	0.48	3906	31250	3.9	6%	27%	15	20	158
Tumor necrosis factor (Ligand) superfamily, member 12 (TWEAK)	O43508	410	537	432	669	76%	80%	124%	1.9	1.9	125000	500000	4.8	6%	11%	15	20	315
Tumor necrosis factor (TNF)	P01375	7	8	8	8	81%	85%	87%	0.95	0.95	31250	500000	4.5	6%	18%	15	NA	NA
Tumor necrosis factor ligand superfamily member 14 (TNFSF14)	O43557	5	6	7	23	74%	119%	376%	0.95	1.9	15625	31250	3.9	6%	15%	15	20	315
Tumor necrosis factor receptor superfamily member 9 (TNFRSF9)	Q07011	74	84	76	95	88%	91%	114%	0.03	0.03	3906	3906	5.1	5%	21%	15	20	315
Urokinase-type plasminogen activator (uPA)	P00749	1227	1408	1306	1346	87%	93%	96%	0.12	0.12	7812	15625	4.8	5%	11%	7.5	20	315
Vascular endothelial growth factor A (VEGF-A)	P15692	1218	1909	1608	3004	64%	84%	157%	0.06	0.06	7812	15625	5.1	6%	8%	15	20	630

2.3 PRECISION

REPEATABILITY

Intra-assay variation (within-run) was calculated as the mean CV for 7 individual samples, within each of 10 separate runs during the validation studies. Inter-assay variation (between-run) was calculated as the mean CV, for the same 7 individual samples, among 6 separate runs during the validation studies. Variation calculations were assessed on linearized values for all 92 analytes, see Table 1.

Across all 92 assays, the mean intra-assay and inter-assay variations observed were 7% and 18%, respectively. The distributions of intra-assay and inter-assay variations iare shown in Figure 4.

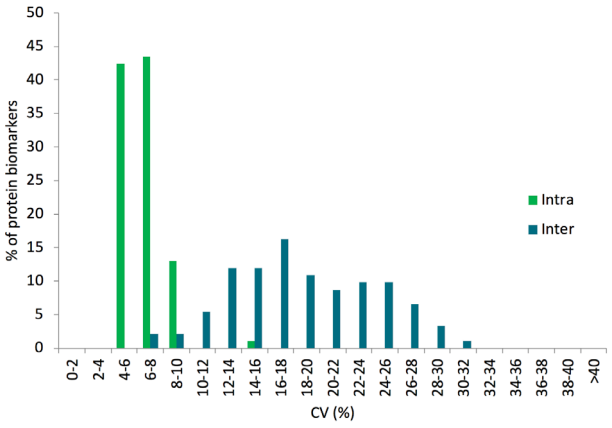


Fig 4. Distribution of intra-assay and inter-assay variations for all assays in the panel.

REPRODUCIBILITY

Inter-site variation (between-site) was also investigated during the validation in a beta-site study, to estimate the expected variations in values between different laboratories, with different operators and using different equipment. Seven individual samples were distributed to each of two sites together with the panel reagent kits. Each site was instructed to perform the analysis of the seven individual samples according to the same run design. Each site was also asked to perform two independent runs.

Together with data obtained at the Olink Proteomics lab, this enabled the estimation of intra- and inter-assay variations across three different sites (Figure 5).

As shown in Figure 5, the mean intra-assay CVs ranged from 5% to 12%. The mean inter-assay CV ranged from 11% to 21%. When the results of the beta sites were each compared with those from Olink Proteomics, the CVs were 11% and 13%.

Overall, the panel showed very good reproducibility and repeatability with an average inter-site CV of 17%.

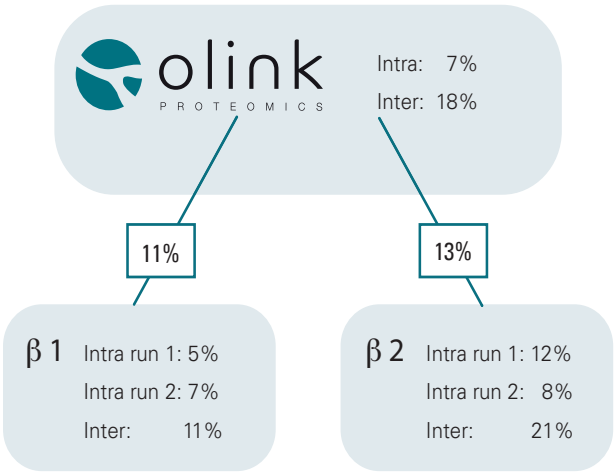


Fig 5. Validation of the panel at two (β1-β2) different laboratories. Larger boxes show intra-assay and inter-assay variations for each site and small boxes represent the inter-site run variations in direct comparison to Olink Proteomics.

2.4 ANALYTICAL SPECIFICITY

ASSAY SPECIFICITY

To test the target-protein specificity of the PEA probes used in the panel, all of the antibodies used were tested for cross-reactivity against all of the recombinant proteins used during assay validation. The probes were also checked for cross-reactivity to more than 100 additional proteins (data not shown). This was caried out by creating a test sample consisting of a pool of antigens, which was then incubated with all 92 antibody probe pairs from the panel. To optimize this analysis, 10 sub-pools of antigen were evaluated to cover the 92 assays (see Figure 6).

The lack of significant signal from these tests indicates that each probe pair is specific for its target antigen, demonstrating the readout specificty of the PEA method.

ENDOGENOUS INTERFERENCE

Endogenous interference from heterophilic antibodies, e.g. human anti-mouse antibodies (HAMA), and rheumatoid factor are known to cause problems in immunoassays.

To evaluate the potential impact of this specific interference, a special "mismatch" system was designed. The only way to generate a signal in this system is to bring antibody probe pairs into proximity, by cross-binding substances other than antigens, e.g. heterophilic antibodies or rheumatoid factor. A total of 69 different "mismatched" probe pairs of varying host species origin were designed and evaluated with samples from the Heterophilic Assessment panel from Scantibodies laboratory Inc. (part. No. 3KG027) with HAMA concentration (<3-3641 ng/ml) and another set

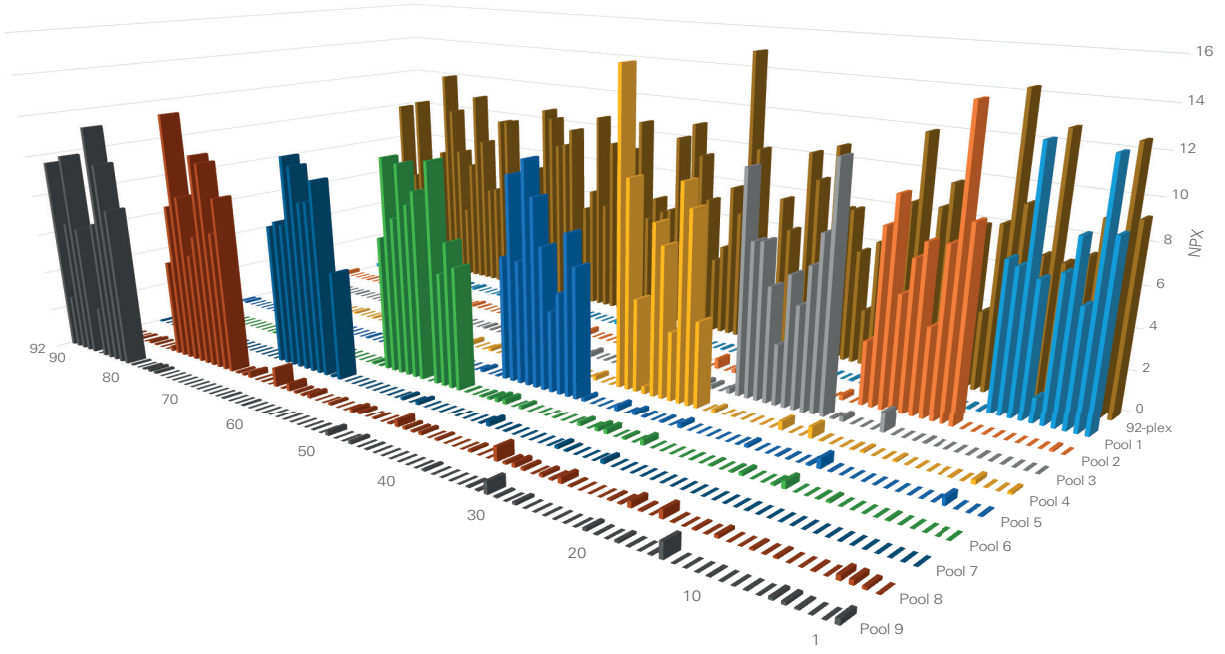


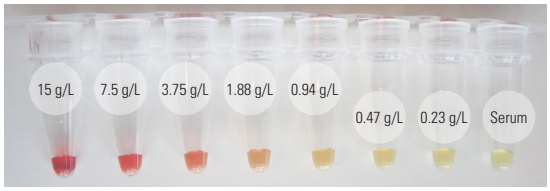
Fig 6. Assay readout specificity of the Olink platform. For each assay, specificity is confirmed by testing antigen sub-pools against the complete 92-plex pool as to each sub-mix.

of samples known to contain rheumatoid factor (<20-1190 IU/ml). No interference (i.e. signal above LOD) due to HAMA or RF could be detected for any of the samples (data not shown).

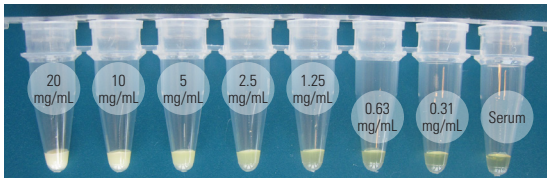
The potential impact of some known interfering serum and plasma components was evaluated using serial dilutions of hemolysate, lipids and bilirubin, respectively in EDTA plasma and serum, as shown in Figure 7.

These additions simulate different patient health conditions and/or sample collection irregularities. Table 1 lists the highest concentration of each substance that did not impact on assay performance. In 10 out of 92 assays, altered values were recorded after the addition of hemolysate. The reason is most likely due to more of the measured analyte leaking out of the disrupted blood cells. A concentration of 15 g/L of hemolysate represents 10% hemolysis of a sample. Also, in 10 assays, interference was observed after addition of lipids ≥5 mg/mL, which would correspond to very high serum triglyceride levels[3]. Addition of bilirubin altered 37 out of 92 assays at ≥79 µg/mL, which is more than 4 times the normal total bilirubin levels[4].

A) Hemolysate



B) Lipids



C) Bilirubin

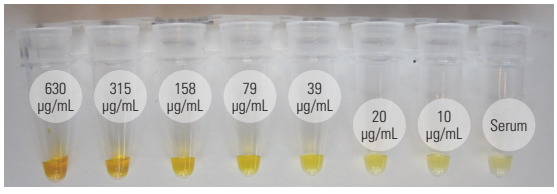


Fig 7. Endogenous interference. Levels tested for hemolysate were 0.23 - 15 g/L hemoglobin, lipids 0.3 - 20 mg/mL and bilirubin 10 - 630 µg/mL. The highest hemolysate concentration translates to about 10% hemolysis.

2.5 SCALABILITY

Assay performance was further evaluated with regard to scalability, meaning the capability of the Olink technology to maintain the same quality of performance irrespective of multiplex grade. A step-wise increase of multiplex grade (24, 48, 72 and 96) was performed and the observed dCq values for the 24-plex were plotted against the 48-plex, 72-plex and 96-plex for each analyte. The correlation coefficient R^2 value generated by linear regression analysis reflects the correlation between the multiplex assays. The R^2 values were >0.99 for the different multiplex blocks, as shown in Figure 8, demonstrating the scalability of the system.

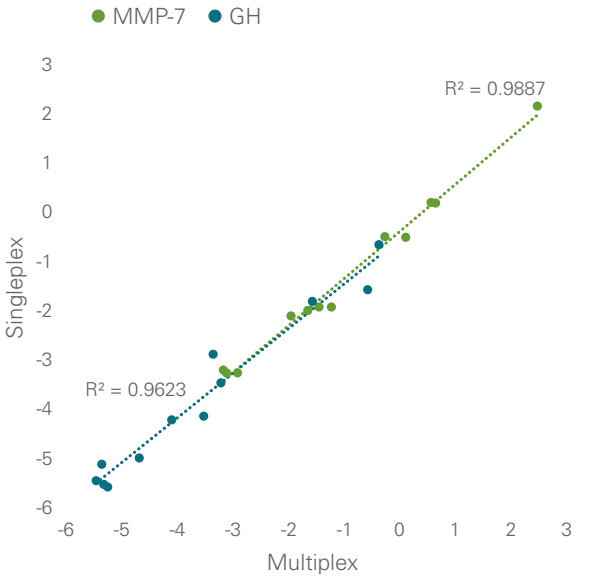


Fig 8. Scalability of the Olink technology platform. This experiment was performed using the Olink Oncology I panel. Human serum samples were analyzed with a 24-plex, 48-plex and 72-plex assay and the complete Olink Oncology I panel. The observed dCq (log2) values were plotted, and the correlation coefficient R^2 value was generated by linear regression.

3. References

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TECHNICAL SUPPORT

For technical support, please contact us at support@olink.com or +46 18 444 3970

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