1. Introduction

Olink® IMMUNO-ONCOLOGY is a reagent kit that measures 92 immuno-oncology related human proteins simultaneously using just 1 µL of serum, plasma or other human sample type. 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 designated target protein, if it is present in the sample. A PCR reporter sequence is formed by a proximity-dependent DNA hybridization and polymerization event. This is then measured, 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. They have been designed to monitor the technical performance of the assay, as well as the quality of individual samples. This provides information at each step of the Olink protocol (see Figure 1). The internal controls are added to each sample and include two Incubation controls, one Extension control and one Detection control. The Incubation controls (two non-human proteins) monitor all three steps starting with the immuno reaction. The Extension Control (an antibody linked to two matched oligonucleotides for immediate proximity-dependent hybridization and extension that does not require antibody binding to the target protein) monitors the extension and detection steps and is used for data normalization across samples. Finally, the Detection control (a synthetic double-stranded template) specifically monitors the detection step. If one or more of the internal control values deviate from a pre-determined range, the sample will be flagged and may be removed before statistical analysis. An external control called the inter-plate control (IPC), is included on each plate and used in a second normalization step. The IPC is made up of a pool of probes similar to the Extension control (Ext Ctrl), except that it is generated with 92 matching oligonucleotide pairs. Furthermore, the IPC improves inter-assay precision and allows for optimal comparison of data derived from multiple runs. The term "Normalized Protein eXpression (NPX)" refers to data that has been normalized as described above.

1.3 DATA ANALYSIS

The data analysis described in this document was performed using a pre-processing normalization procedure. For each sample and data point, the corresponding Cq-value for the Extension control was substracted, normalizing for technical variation within one run. Normalization between runs was 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 were set relative to a correction factor that is determined by Olink for each batch of conjugated PEA probes. The Normalized Protein eXpression (NPX) unit generated by these procedures is on a log2 scale where a higher number represents a higher level of the target in the sample, typically with the background level at around zero. Linearization of data is performed by the mathematical operation 2^NPX. Coefficient of variation (CV) calculations were performed on linearized values.

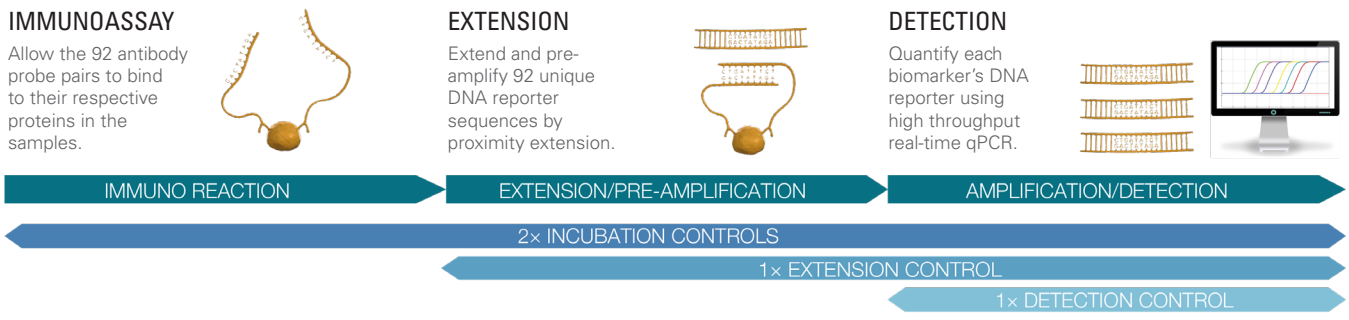

Fig 1. Olink assay procedure (above) and controls (below). The internal controls enable monitoring of the three core steps in the Olink assay and used for quality control and data normalization. Detection is performed by using the Fluidigm® Biomark™ or the Fluidigm® Biomark™ HD system.

2. Performance characteristics

2.1 SAMPLE TYPES

The ability to use different sample types with Olink IMMUNO-ONCOLOGY was evaluated by collecting matched EDTA, acid citrate dextrose (ACD), and sodium heparin plasma and serum samples from 4 healthy individuals. Table 1 summarizes response values for 32 normal EDTA plasma samples expressed in NPX, as well as the relative differences for the other sample types compared to EDTA plasma. Variations observed between responses in the different sample types tested were generally small, and all assays will therefore function without limitation in these sample types. In addition, cell lysates from 10 different cell lines were also evaluated. For more information about using cell lysates please contact support@olink.com.

2.2 ANALYTICAL MEASUREMENT

NOTE: *The technical performance data based on in vitro assays using recombinant antigen must* **NOT** *be used to derive actual concentrations of native proteins in biological samples from the relative quantification NPX data that is obtained from an Olink assay.*

DETECTION LIMIT

Calibrator curves were determined for all biomarkers, for which recombinant antigen was available, simultaneously in a multiplex format. For these assays, the Limit of detection (LOD) was defined as 3 standard deviations above background and reported in pg/mL (see Table 1 and Figure 2).

HIGH DOSE HOOK EFFECT

The high dose hook effect is a state of antigen excess relative to the reagent antibodies, resulting in falsely lower values reported, which can lead to misinterpretation of results. Therefore, the hook effect was determined for each analyte where applicable, and reported in pg/mL (see Table 1).

MEASURING RANGE

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

Three selected assays with their analytical data are shown in Figure 2 and the distribution of measuring ranges of 90 assays compared to endogenous plasma levels are shown in Figure 3. Where applicable, individual calibrator curves are available on the specific biomarker page on the Olink website.

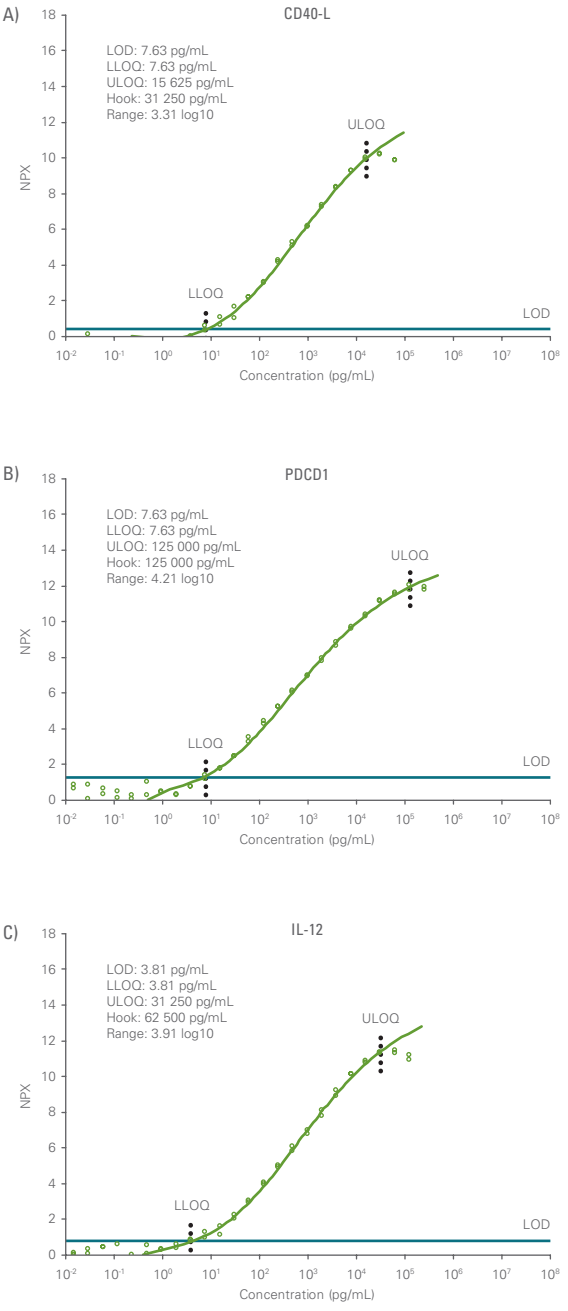

Fig 2. Calibrator curves from 3 assays and their corresponding analytical measurement data.

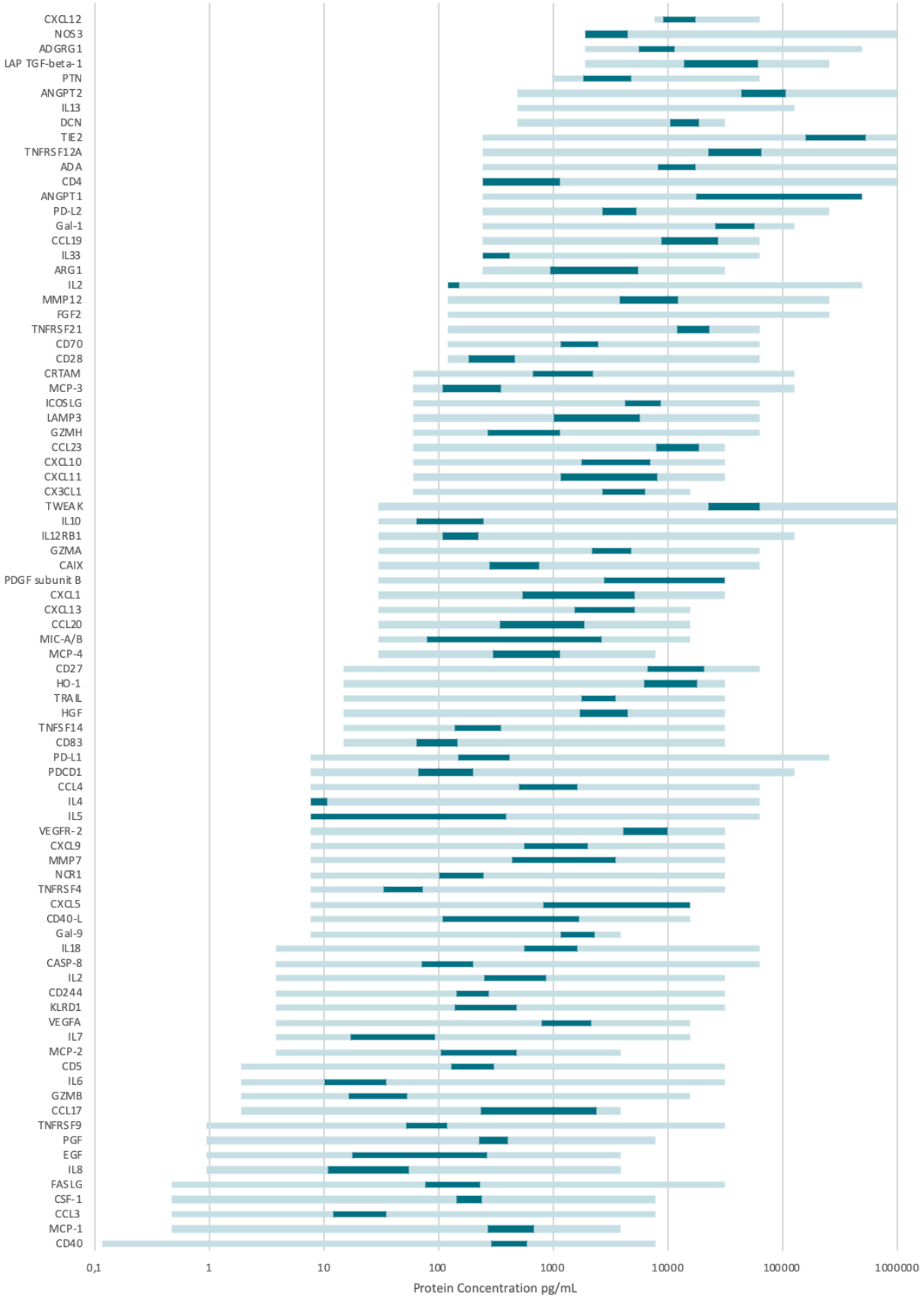

Fig 3. Distribution of analytical measuring range (light blue bars), defined by the lower and upper limits of quantification (LLOQ-ULOQ), and normal plasma levels (dark blue bars) for all assays with currently available data.

Table 1. Assay performance parameters. Sample Types; Normalized Protein eXpression (NPX), Endogenous Interference, Analytical rRnge; Limit of Detection (LOD), Lower Limit of Quantification (LLOQ), Upper Limit of Quantification (ULOQ), High Dose Effect (Hook), Range and Precision indicative of assay performance are shown for the analytes where applicable. Not available, NA

		Sample types						Endogenous interference	Analytical range					Precision	
		Normal plasma levels (NPX)			Relative to EDTA plasma (%)			(mg/mL)	pg/mL				log10	% CV	
Target	UniProt No	10th %tile	Median	90th %tile	ACD	Heparin	Serum	Hemolysate	LOD	LLOQ	ULOQ	Hook	Range	Intra	Inter
Adenosine deaminase (ADA)	P00813	3.3	3.8	4.5	94	90	103	0.2	244.1	244.1	1000000	1000000	3.6	5.8	12.7
Adhesion G-protein coupled receptor G1 (ADGRG1)	Q9Y653	NA	1.5	1.9	94	86	120	15	1953.1	1953.1	500000	1000000	2.4	8.0	14.5
Angiopoietin-1 (ANGPT1)	Q15389	5.9	7.4	10.4	16	157	274	15	122.1	244.1	500000	500000	3.3	7.4	8.2
Angiopoietin-1 receptor (TIE2)	Q02763	7.6	8.1	8.5	97	92	105	15	244.1	244.1	1000000	1000000	3.6	7.2	8.4
Angiopoietin-2 (ANGPT2)	O15123	4.7	5.3	6.1	90	84	107	15.0	488.3	488.3	1000000	1000000	3.3	7.4	12.4
Arginase-1 (ARG1)	P05089	NA	1.6	4.6	33	52	163	0.0	244.1	244.1	31250	250000	2.1	6.5	7.0
Carbonic anhydrase 9 (CAIX)	Q16790	3.3	4.1	4.8	90	100	110	15	15.3	30.5	62500	125000	3.3	7.7	7.3
Caspase-8 (CASP-8)	Q14790	4.2	4.8	5.7	72	130	174	0.2	3.8	3.8	62500	62500	4.2	7.2	9.7
C-C motif chemokine 13 (MCP-4)	Q99616	6.1	7.2	8.8	57	120	227	15	7.6	30.5	7812	7812	2.4	8.3	13.1
C-C motif chemokine 17 (CCL17)	Q92583	5.7	7.4	9.4	37	134	276	15	1.9	1.9	3906	7812	3.3	8.7	13.9
C-C motif chemokine 19 (CCL19)	Q99731	7.9	8.6	10.3	98	87	108	15	122.1	244.1	62500	125000	2.4	7.7	11.4
C-C motif chemokine 2 (MCP-1)	P13500	9.2	9.8	10.6	100	99	122	15.0	0.5	0.5	3906	3906	3.9	8.0	11.9
C-C motif chemokine 20 (CCL20)	P78556	5.1	6.2	8.2	102	75	74	15	15.3	30.5	15625	31250	2.7	8.5	16.0
C-C motif chemokine 23 (CCL23)	P55773	8.7	9.4	10.6	95	85	90	15	30.5	61.0	31250	62500	2.7	8.2	14.1
C-C motif chemokine 3 (CCL3)	P10147	4.6	5.2	6.4	70	98	145	15	0.5	0.5	7812	3906	4.2	8.2	14.5
C-C motif chemokine 4 (CCL4)	P13236	6.3	7.1	8.3	66	99	134	15.0	7.6	7.6	62500.0	125000	3.9	8.5	15.7
C-C motif chemokine 7 (MCP-3)	P80098	1.6	2.3	3.0	85	111	127	7.5	30.5	61.0	125000.0	1000000	3.3	8.7	10.2
C-C motif chemokine 8 (MCP-2)	P80075	5.4	6.9	8.3	62	92	173	15	1.9	3.8	3906	3906	3.0	7.6	12.7
CD27 antigen (CD27)	P26842	7.6	8.2	8.7	92	100	108	15	7.6	15.3	62500	125000	3.6	6.7	7.2
CD40 ligand (CD40-L)	P29965	2.9	4.3	7.0	39	292	1042	15	7.6	7.6	15625	31250	3.3	7.7	9.4
CD40L receptor (CD40)	P25942	10.2	10.7	11.2	95	105	126	15.0	0.1	0.1	7812	31250	4.8	7.3	10.0
CD70 antigen (CD70)	P32970	3.4	4.0	4.7	92	97	169	15	122.1	122.1	62500	125000	2.7	8.0	11.2
CD83 antigen (CD83)	Q01151	2.5	3.0	3.5	88	83	104	15	3.8	15.3	31250	62500	3.3	7.8	11.0
C-X-C motif chemokine 1 (CXCL1)	P09341	7.2	8.7	10.8	37	157	285	7.5	7.6	30.5	31250	31250	3.0	7.2	11.8
C-X-C motif chemokine 10 (CXCL10)	P02778	6.7	7.5	9.5	88	87	112	15.0	30.5	61.0	31250	31250	2.7	11.5	14.3
C-X-C motif chemokine 11 (CXCL11)	O14625	4.6	5.8	8.9	29	147	281	0.5	30.5	61.0	31250	31250	2.7	8.8	12.2
C-X-C motif chemokine 13 (CXCL13)	O43927	8.1	8.8	9.8	106	81	131	15	30.5	30.5	15625	15625	2.7	6.2	10.6
C-X-C motif chemokine 5 (CXCL5)	P42830	7.8	10.2	13.1	13	190	271	7.5	7.6	7.6	15625	31250	3.3	7.9	11.5
C-X-C motif chemokine 9 (CXCL9)	Q07325	6.2	6.9	8.2	89	94	95	15	1.9	7.6	31250	31250	3.6	9.8	13.6
Cytotoxic and regulatory T-cell molecule (CRTAM)	O95727	4.1	4.8	5.8	90	105	127	15.0	30.5	61.0	125000	125000	3.3	7.2	15.1
Decorin (DCN)	P07585	4.7	5.0	5.4	99	120	118	15.0	488.3	488.3	31250	1000000	1.8	8.2	8.2
Fibroblast growth factor 2 (FGF2)	P09038	NA	NA	2.0	95	56	89	15	30.5	122.1	250000	1000000	3.3	6.3	9.0
Fractalkine (CX3CL1)	P78423	6.1	6.6	7.1	94	110	143	15	30.5	61.0	15625	1000000	2.4	7.1	7.5
Galectin-1 (Gal-1)	P09382	6.4	6.7	7.2	94	102	111	15	244.1	244.1	125000.0	250000	2.7	6.0	8.0
Galectin-9 (Gal-9)	O00182	7.7	8.1	8.5	102	102	110	0.5	3.8	7.6	3906	15625	2.7	5.3	9.1
Granzyme A (GZMA)	P12544	5.1	5.5	6.2	90	88	109	7.5	30.5	30.5	62500	125000	3.3	9.9	13.4
Granzyme B (GZMB)	P10144	2.2	3.0	3.6	89	76	88	0.5	1.9	1.9	15625	31250	3.9	8.1	13.4
Granzyme H (GZMH)	P20718	3.5	4.5	5.7	104	103	116	0.5	30.5	61.0	62500	125000	3.0	8.5	12.1
Heme oxygenase 1 (HO-1)	P09601	11.5	12.2	12.8	94	92	98	15.0	15.3	15.3	31250	31250	3.3	7.3	13.1
Hepatocyte growth factor (HGF)	P14210	6.9	7.5	8.3	74	73	153	15	3.8	15.3	31250	250000	3.3	8.4	10.0
ICOS ligand (ICOSLG)	O75144	5.3	5.7	6.0	95	144	146	15	61.0	61.0	62500	1000000	3.0	6.6	7.4
Interferon gamma (IFN-gamma)	P01579	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*
Interleukin-1 alpha (IL-1 alpha)	P01583	NA	NA	NA	88	81	69	15.0	NA	NA	NA	NA	NA	19.6	NA
Interleukin-10 (IL10)	P22301	2.1	2.6	3.5	87	85	111	15	15.3	30.5	1000000	1000000	4.5	9.7	12.0
Interleukin-12 (IL12)	P29459,P29460	4.9	5.8	6.8	84	75	109	15.0	3.8	3.8	31250	62500	3.9	7.5	11.4
Interleukin-12 receptor subunit beta-1 (IL12RB1)	P42701	2.0	2.3	2.8	91	84	98	15	30.5	30.5	125000	125000	3.6	7.1	11.1
Interleukin-13 (IL13)	P35225	NA	NA	0.4	93	64	110	15	244.1	488.3	125000	1000000	2.4	8.9	6.6
Interleukin-15 (IL15)	P40933	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*

		Sample types						Endogenous interference	Analytical range					Precision	
		Normal plasma levels (NPX)			Relative to EDTA plasma (%)			(mg/mL)	pg/mL				log10	% CV	
Target	UniProt No	10th %tile	Median	90th %tile	ACD	Heparin	Serum	Hemolysate	LOD	LLOQ	ULOQ	Hook	Range	Intra	Inter
Interleukin-18 (IL18)	Q14116	8.0	8.9	9.6	87	92	108	3.8	1.0	3.8	62500	62500	4.2	8.0	12.5
Interleukin-2 (IL2)	P60568	NA	NA	NA	107	60	122	15	122.1	122.1	500000	1000000	3.6	11.8	7.2
Interleukin-33 (IL33)	O95760	NA	NA	0.8	102	77	112	15	244.1	244.1	62500	125000	2.4	7.3	9.8
Interleukin-4 (IL4)	P05112	NA	NA	NA	100	76	105	15	7.6	7.6	62500	62500	3.9	7.1	13.3
Interleukin-5 (IL5)	P05113	NA	0.9	5.2	94	94	102	15.0	7.6	7.6	62500	125000	3.9	11.9	15.4
Interleukin-6 (IL6)	P05231	3.4	4.1	5.2	106	106	119	15.0	1.0	1.9	31250	31250	4.2	7.3	10.8
Interleukin-7 (IL7)	P13232	3.3	4.2	6.1	45	102	307	15.0	1.9	3.8	15625	31250	3.6	7.6	10.9
Interleukin-8 (IL8)	P10145	4.5	5.3	7.1	71	116	175	7.5	0.5	1.0	3906	7812	3.6	7.9	13.5
Killer cell immunoglobulin-like receptor 3DL1 (KIR3DL1)	P43629	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*
Latency-associated peptide transforming growth factor beta-1 (LAP TGF-beta-1)	P01137	1.6	1.9	3.1	77	102	168	15.0	1953.1	1953.1	250000	250000	2.1	6.4	13.3
Lymphocyte activation gene 3 protein (LAG3)	P18627	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*
Lysosome-associated membrane glycoprotein 3 (LAMP3)	Q9UQV4	4.2	5.9	6.7	90	94	111	15	30.5	61.0	62500	250000	3.0	7.2	9.6
Macrophage colony-stimulating factor 1 (CSF-1)	P09603	7.9	8.2	8.5	94	92	111	15	0.2	0.5	7812	31250	4.2	7.0	11.3
Macrophage metalloproteinase-12 (MMP12)	P39900	6.1	7.1	8.0	138	117	125	15	30.5	122.1	250000	500000	3.3	8.1	9.8
Matrix metalloproteinase-7 (MMP7)	P09237	6.7	8.4	9.8	442	421	472	15.0	7.6	7.6	31250	62500	3.6	7.0	11.3
MHC class I polypeptide-related sequence A/B (MIC-A/B)	Q29983,Q29980	NA	4.6	5.5	88	95	109	15.0	30.5	30.5	15625	1000000	2.7	6.5	10.0
Mucin-16 (MUC-16)	Q8WXI7	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*
Natural cytotoxicity triggering receptor (NCR1)	O76036	3.7	4.2	4.9	90	97	118	15	7.6	7.6	31250	62500	3.6	7.3	8.9
Natural killer cell receptor 2B4 (CD244)	Q9BZW8	5.7	6.1	6.6	86	97	113	15.0	1.9	3.8	31250	31250	3.9	7.4	9.8
Natural killer cells antigen CD94 (KLRD1)	Q13241	4.7	5.6	6.8	84	94	109	15	3.8	3.8	31250	31250	3.9	6.9	10.0
Nitric oxide synthase, endothelial (NOS3)	P29474	NA	0.9	2.4	128	59	76	15.0	976.6	1953.1	1000000	1000000	2.7	18.7	19.5
Placenta growth factor (PGF)	P49763	7.9	8.3	8.8	88	95	109	15.0	1.0	1.0	7812	31250	3.9	7.7	11.3
Platelet-derived growth factor subunit B (PDGF subunit B)	P01127	7.6	9.1	11.2	22	102	179	15	15.3	30.5	31250	62500	3.0	8.2	13.3
Pleiotrophin (PTN)	P21246	NA	1.6	2.7	73	22	42	15	488.3	976.6	62500	125000	1.8	8.4	17.6
Pro-epidermal growth factor (EGF)	P01133	3.9	5.4	8.4	28	145	710	15	1.0	1.0	3906	3906	3.6	8.0	9.6
Programmed cell death 1 ligand 1 (PD-L1)	Q9NZQ7	4.3	4.9	5.6	69	91	108	15	7.6	7.6	250000	1000000	4.5	8.9	10.6
Programmed cell death 1 ligand 2 (PD-L2)	Q9BQ51	2.2	2.6	3.0	93	92	111	15	244.1	244.1	250000	500000	3.0	6.4	11.1
Programmed cell death protein 1 (PDCD1)	Q15116	3.3	4.0	4.7	89	98	111	15	7.6	7.6	125000	125000	4.2	9.7	12.9
Stromal cell-derived factor 1 (CXCL12)	P48061	NA	0.6	1.8	88	60	76	15.0	7812.5	7812.5	62500	125000	0.9	7.6	13.1
T-cell surface glycoprotein CD4 (CD4)	P01730	NA	NA	NA	109	88	81	15.0	244.1	244.1	1000000	1000000	3.6	7.1	9.6
T-cell surface glycoprotein CD5 (CD5)	P06127	4.6	5.3	5.9	93	94	105	15	1.9	1.9	31250	31250	4.2	6.9	16.9
T-cell surface glycoprotein CD8 alpha chain (CD8A)	P01732	8.4	9.6	10.5	100	86	89	15.0	NA	NA	NA	NA	NA	10.5	10.3
T-cell-specific surface glycoprotein CD28 (CD28)	P10747	1.2	1.5	2.0	97	81	106	15	61.0	122.1	62500	125000	2.7	5.8	12.9
TNF-related apoptosis-inducing ligand (TRAIL)	P50591	7.7	8.3	8.8	100	99	114	15.0	7.6	15.3	31250	31250	3.3	7.6	8.9
Tumor necrosis factor (TNF)	P01375	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*	NA*
Tumor necrosis factor ligand superfamily member 12 (TWEAK)	O43508	7.6	8.3	9.0	81	92	129	15.0	30.5	30.5	1000000	1000000	4.5	12.5	11.0
Tumor necrosis factor ligand superfamily member 14 (TNFSF14)	O43557	3.1	3.8	4.6	71	125	341	7.5	15.3	15.3	31250	31250	3.3	7.8	8.9
Tumor necrosis factor ligand superfamily member 6 (FASLG)	P48023	6.1	6.7	7.7	109	100	109	15.0	0.5	0.5	31250	31250	4.8	7.7	11.2
Tumor necrosis factor receptor superfamily member 12A (TNFRSF12A)	Q9NP84	5.8	6.5	7.3	96	98	92	15	244.1	244.1	1000000	1000000	3.6	9.2	13.1
Tumor necrosis factor receptor superfamily member 21 (TNFRSF21)	O75509	7.7	8.1	8.6	85	90	115	15	30.5	122.1	62500	125000	2.7	7.4	10.7
Tumor necrosis factor receptor superfamily member 4 (TNFRSF4)	P43489	2.9	3.4	4.0	90	97	124	15.0	3.8	7.6	31250	31250	3.6	7.1	12.2
Tumor necrosis factor receptor superfamily member 9 (TNFRSF9)	Q07011	5.4	5.9	6.6	96	96	110	15	1.0	1.0	31250	31250	4.5	7.5	10.3
Vascular endothelial growth factor A (VEGFA)	P15692	7.9	8.4	9.2	75	92	142	15.0	3.8	3.8	15625	31250	3.6	7.8	10.1
Vascular endothelial growth factor receptor 2 (VEGFR-2)	P35968	7.1	7.7	8.1	100	96	111	15.0	7.6	7.6	31250	31250	3.6	6.9	12.2

*These recently updated assays were subject to rigorous validation and QC during their development, but final validation data in full-panel context is not yet available. This will be updated as soon as possible.

2.3 PRECISION

REPEATABILITY

Intra-assay variation (within-run) was calculated as the mean %CV for 6 different samples, run in triplicate, in 8 separate runs during the validation studies. Inter-assay variation (between runs) was calculated between experiments with the same operator. The reported inter-assay %CV is the average %CV for three different operators. These calculations were performed on linearized values for all analytes where response levels could be measured in serum and normal plasma, see Table 1.

Across all 92 assays, the mean intra-assay and inter-assay variations were observed to be 8.3% and 11.5%, respectively. The distribution of both intra-assay and inter-assay variations are shown in Figure 4.

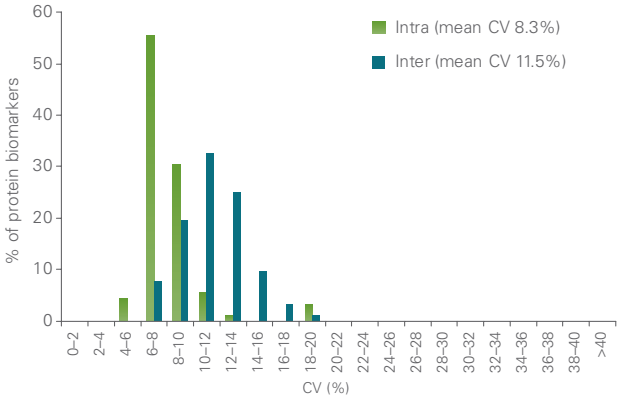

Fig 4. Distribution of intra-assay and inter-assay variations of Olink IMMUNO-ONCOLOGY

REPRODUCIBILITY

Variations due to different operators in different laboratories using different equipment are another potential source of assay variation. Olink has Analysis Service labs in Sweden and the USA, and in addition there are many Olink-certified core laboratories around the world running the Olink platform (see www.olink.com/service for details). Our experience over several years is that inter-site reproducibility is very good providing that operators are properly trained. For more information please contact support@olink.com.

2.4 ANALYTICAL SPECIFICITY

ASSAY SPECIFICITY

The antibodies selected for use in Olink IMMUNO-ONCOLOGY have previously been evaluated against the 92 panel-specific proteins as well as against an additional 107 proteins. In principle, the specificity is tested by creating a test sample consisting of a pool of antigens, which is then incubated with all 92 antibody probe pairs from the panel. Only if there is a correct match will a reporter sequence be created and serve as a template for subsequent real-time qPCR. Ten sub-pools of antigen are evaluated to cover the 92 assays, see Figure 5.

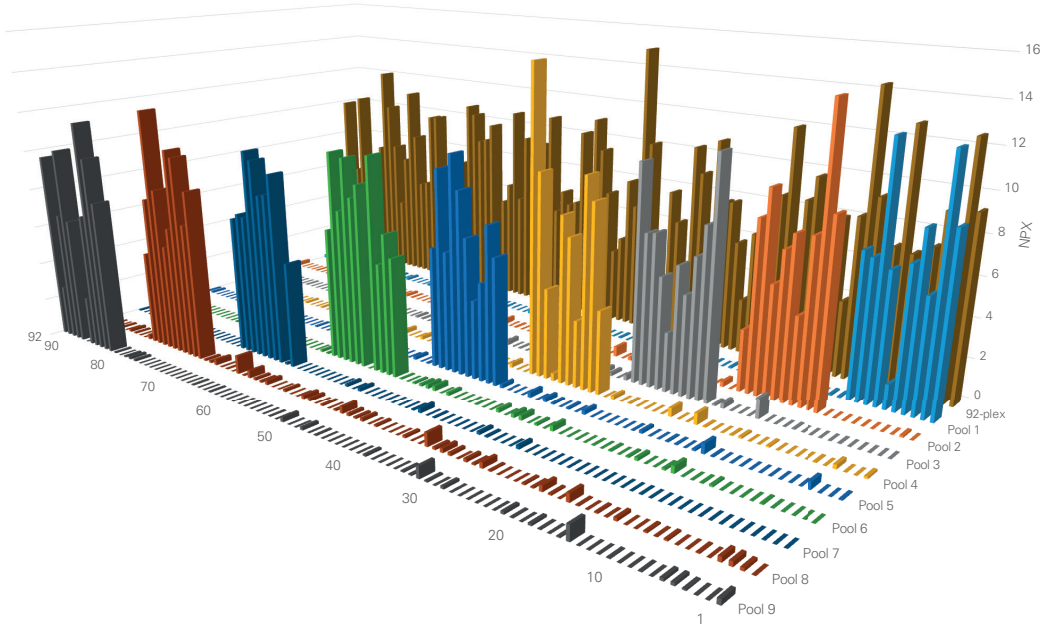

Fig 5. Assay readout specificity of the Olink platform. For each assay, specificity is confirmed by testing antigen sub-pools against the complete 92-plex pool.

ENDOGENOUS INTERFERENCE

Endogenous interference from heterophilic antibodies, e.g. human anti-mouse antibody (HAMA), and rheumatoid factor is known to cause problems in some immunoassays. Evaluation of the potential impact of this specific interference was investigated during the validation of previous panels. No interference due to HAMA or RF was detected for any of the samples in previously tested panels, indicating sufficient blocking of these agents (data not shown).

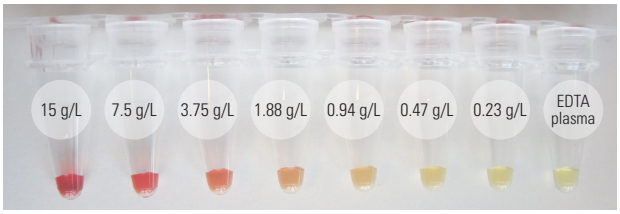

Fig 6. Endogenous interference. Levels tested for hemolysate were 0.23-15 g/L hemoglobin. The highest hemolysate concentration translates to about 10% hemolysis.

Bilirubin, lipids and hemolysate, are plasma and serum components that are known to interfere with some analytical assays. These were evaluted for potential impact on the Olink assays at different added concentrations. An example of the hemolysate levels tested is shown in Figure 6. These additions represent different patient health conditions and/or sample collection irregularities. In 14 out of 92 assays, altered signal was observed by the addition of hemolysate. The reason is most likely due to the specific analytes leaking out of the disrupted blood cells. A concentration of 15 g/L of hemolysate represents 10% hemolysis of a sample. Table 1 reports the highest concentration of hemolysate that does not have an impact on assay performance.

Interference by bilirubin and lipids has previously been evaluated, and disturbance was only observed at extreme levels corresponding to 8 or 10 times normal [3, 4] values. This test was not therefore repeated for Olink IMMUNO-ONCOLOGY.

2.5 SCALABILITY

Assay performance has been previously evaluated with regard to scalability, meaning the capability of the Olink technology to maintain the same quality of performance irrespective of multiplex level. Previously, we have shown that a step-wise increase of multiplex grade (8, 24, 48, 72 and 96) does not compromise assay performance (data not shown). To further strengthen that Olink provides consistent results, single-plex assays for Growth Hormone (GH) and Matrix Metalloproteinase (MMP-7) were compared when run in a full 96-plex reaction. The results for each assay and their observed dCq-values were plotted against the entire 96-plex reaction. The square of the correlation coefficient (R^2) value was generated by linear regression.

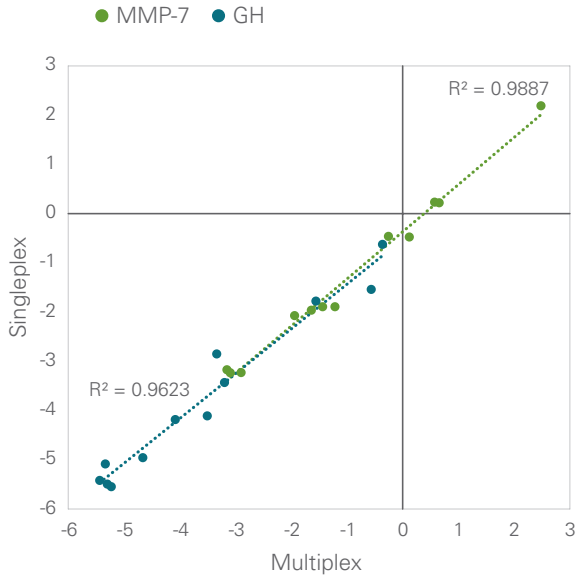

Fig 7. Scalability of the Olink technology platform. The experiment was performed using the Olink CARDIOVASCULAR II panel. Human plasma samples were analyzed in singleplex for Growth Hormone (GH) and Matrix Metalloproteinase (MMP-7) with the equivalent assays performed in a full 96-plex reaction. The 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

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