1. Introduction

Olink® CARDIOVASCULAR II is a reagent kit measuring 92 cardiovascular disease (CVD)–related human protein biomarkers simultaneously using just 1µL 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 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.

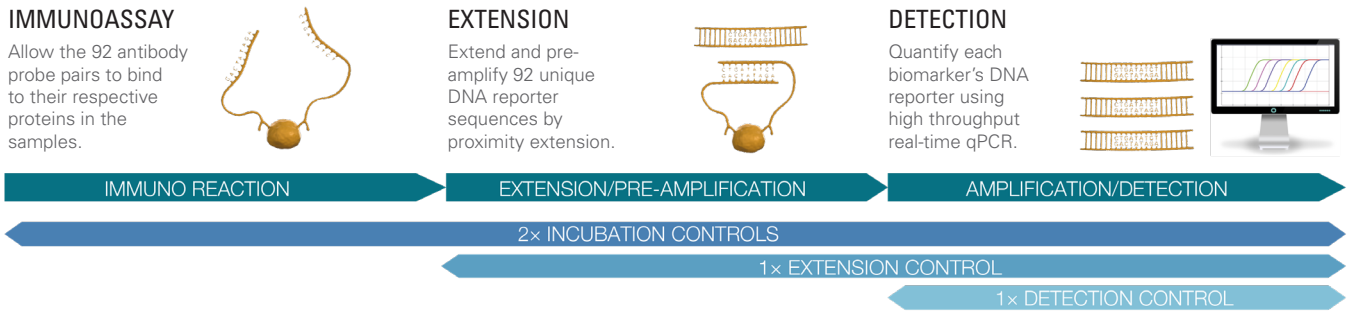

Fig 1. Olink assay procedure (above) and controls (below). The internal controls enable monitoring of the three core steps in the Olink assay and used for quality control and data normalization. Detection is performed by using the Fluidigm® Biomark™ or the Fluidigm® Biomark™ HD system.

2. Performance characteristics

2.1 SAMPLE TYPES

Performance with different sample types was evaluated for Olink CARDIOVASCULAR II by collecting matched EDTA-, acid citrate dextrose (ACD)- and sodium heparin-plasma, as well as serum samples from 4 healthy 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 20 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.

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 for 91 out of 92 biomarkers simultaneously in a multiplex format. Limit of detection (LOD) was defined as 3 standard deviations above background and reported in pg/mL for all assays for which recombinant protein antigen was available, see Table 1 and Figure 2.

HIGH DOSE HOOK EFFECT

High dose hook effect is a state of antigen excess relative to the reagent antibodies resulting in falsely lower values. In such cases a significantly lower value can be reported which leads to misinterpretation of results. Therefore, the hook effect was determined for each analyte, here reported in pg/mL for all 92 assays, 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 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 were reported in order of log10, see Table 1.

Three assays with their analytical data are exemplified in Figure 2 and the distribution of measuring ranges of 90 assays and endogenous plasma levels is shown in Figure 3. Separate calibrator curves established for each assay may be viewed at www.olink.com/cvd2.

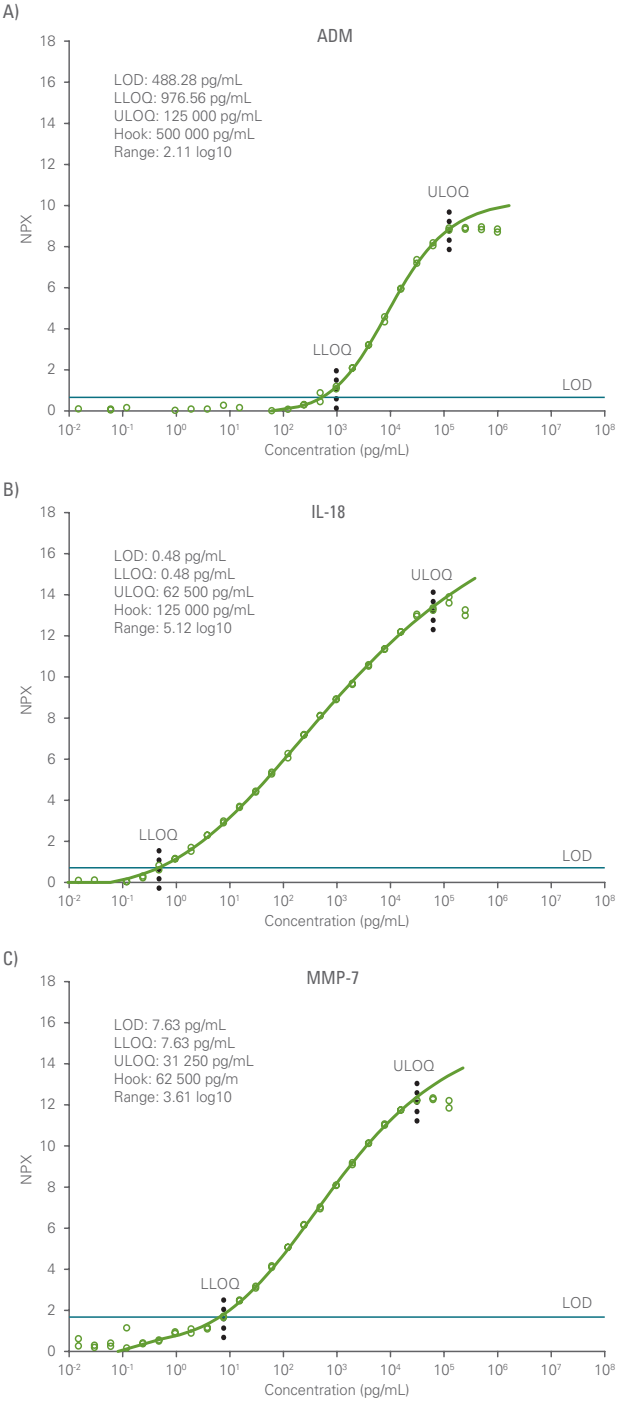

Fig 2. Calibrator curves for representative assays using a 4-parameter curve fitting model.

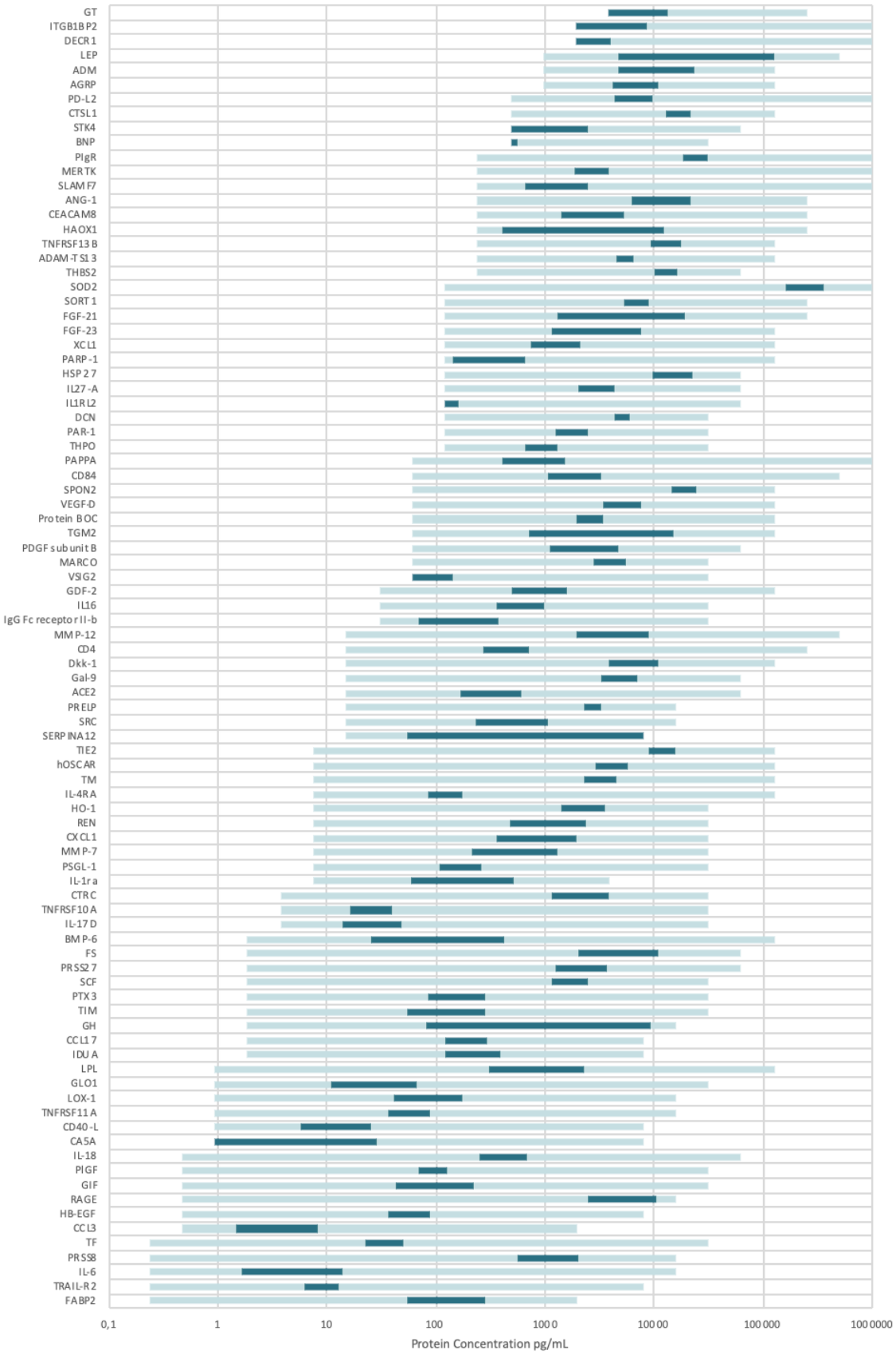

Fig 3. Distribution of analytical measuring range, defined by the lower and upper limits of quantification (LLOQ-ULOQ), and normal plasma levels where data is available (dark blue bars) for 90 out of 92 analytes.

Table 1. Sample Types; Normalized Protein eXpression (NPX), Endogenous Interference, Analytical Measurement; 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 92 analytes. Not available, NA

		Sample types						Endogenous Interference	Analytical measurement					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	Haemolysate	LOD	LLOQ	ULOQ	Hook	Range	Intra	Inter
2,4-dienoyl-CoA reductase, mitochondrial (DECR1)	Q16698	1.2	1.8	3.2	92	56	43	3.8	488.28	1953.12	1 000 000	1 000 000	2.7	15	15
A disintegrin and metalloproteinase with thrombospondin motifs 13 (ADAM-TS13)	Q76LX8	4.8	5.1	5.3	221	216	231	15	122.07	244.14	125 000	500 000	2.7	4	10
ADM (ADM)	P35318	3.4	5.8	6.7	78	70	49	1.9	488.28	976.56	125 000	500 000	2.1	13	11
Agouti-related protein (AGRP)	O00253	2.2	2.6	3.4	102	120	58	15	488.28	976.56	125 000	125 000	2.1	9	12
Alpha-L-iduronidase (IDUA)	P35475	3.6	4.5	5	90	98	132	7.5	0.95	1.91	7 812	31 250	3.6	6	18
Angiopoietin-1 (ANGPT1)	Q15389	5.6	6.7	7.6	15	150	260	15	61.04	244.14	250 000	500 000	3	9	9
Angiopoietin-1 receptor (TIE2)	Q02763	7.2	7.7	7.9	89	96	107	15	7.63	7.63	125 000	250 000	4.2	8	14
Angiotensin-converting enzyme 2 (ACE2)	Q9BYF1	2.3	3.1	3.8	92	91	108	15	15.26	15.26	62 500	1 000 000	3.6	8	11
Bone morphogenetic protein 6 (BMP-6)	P22004	2.1	5.1	5.4	73	22	75	7.5	1.91	1.91	125 000	125 000	4.8	21	15
Brother of CDO (BOC)	Q9BWV1	4.4	4.8	5.3	90	94	113	7.5	61.04	61.04	125 000	250 000	3.3	10	14
Carbonic anhydrase 5A, mitochondrial (CA5A)	P35218	0.8	2	4.6	93	88	112	15	0.95	0.95	7 812	31 250	3.9	9	10
Carcinoembryonic antigenrelated cell adhesion molecule 8 (CEACAM8)	P31997	2.1	2.7	4	62	133	319	3.8	244.14	244.14	250 000	1 000 000	3	11	10
Cathepsin L1 (CTSL1)	P07711	5.1	5.5	6	82	76	96	15	244.14	488.28	125 000	1 000 000	2.4	10	10
C-C motif chemokine 17 (CCL17)	Q92583	5.2	6.1	6.9	32	119	260	15	1.91	1.91	7 812	7 812	3.6	12	13
C-C motif chemokine 3 (CCL3)	P10147	1.3	1.9	3.2	73	79	130	15	0.48	0.48	1 953	3 906	3.6	9	8
CD40 ligand (CD40-L)	P29965	2	2.6	3.7	35	249	914	7.5	0.48	0.95	7 812	15 625	3.9	9	14
Chymotrypsin C (CTRC)	Q99895	8.4	9.4	10.3	97	93	109	15	3.81	3.81	31 250	31 250	3.9	10	10
C-X-C motif chemokine 1 (CXCL1)	P09341	5.9	7.3	8.9	31	167	301	1.9	7.63	7.63	31 250	31 250	3.6	10	13
Decorin (DCN)	P07585	4.8	5.1	5.4	95	132	128	15	122.07	122.07	31 250	62 500	2.4	7	11
Dickkopf-related protein 1 (Dkk-1)	O94907	6.9	7.6	8.5	36	121	267	15	15.26	15.26	125 000	125 000	3.9	11	9
Fatty acid-binding protein, intestinal (FABP2)	P12104	7.3	8.7	9.7	80	87	106	15	0.24	0.24	1 953	31 250	3.9	8	9
Fibroblast growth factor 21 (FGF-21)	Q9NSA1	3.3	5.2	8.3	77	87	80	15	122.07	122.07	250 000	500 000	3.3	12	14
Fibroblast growth factor 23 (FGF-23)	Q9GZV9	3	3.7	6.6	75	85	21	15	122.07	122.07	125 000	125 000	3	14	15
Follistatin (FS)	P19883	9.6	10.8	11.7	92	24	103	15	0.95	1.91	62 500	125 000	4.5	9	15
Galectin-9 (Gal-9)	O00182	6.1	6.5	7.1	105	104	116	0.5	15.26	15.26	62 500	125 000	3.6	5	13
Gastric intrinsic factor (GIF)	P27352	3.9	4.7	6	80	78	98	15	0.48	0.48	31 250	125 000	4.8	11	15
Gastrotropin (GT)	P51161	0.8	1.1	2.3	98	91	99	15	488.28	3906.25	250 000	1 000 000	1.8	16	15
Growth hormone (GH)	P01241	4.4	7.4	11.2	89	90	108	15	1.91	1.91	15 625	31 250	3.9	7	9
Growth/differentiation factor 2 (GDF-2)	Q9UK05	4.5	5.8	6.5	217	61	136	15	7.63	30.52	125 000	125 000	3.6	9	11
Heat shock 27 kDa protein (HSP 27)	P04792	7.7	8.4	9.5	54	43	49	0	122.07	122.07	62 500	250 000	2.7	11	12
Heme oxygenase 1 (HO-1)	P09601	9.8	10.4	11.2	94	98	110	15	1.91	7.63	31 250	125 000	3.6	8	10
Hydroxyacid oxidase 1 (HAOX1)	Q9UJM8	2	3.8	7.3	83	94	114	15	122.07	244.14	250 000	500 000	3	9	9
Interleukin-1 receptor antagonist protein (IL-1ra)	P18510	2.8	3.6	5.4	80	102	144	15	3.81	7.63	3 906	7 812	2.7	12	36
Interleukin-1 receptor-like 2 (IL1RL2)	Q9HB29	3.3	3.9	4.6	82	92	108	15	122.07	122.07	62 500	125 000	5.1	10	14
Interleukin-17D (IL-17D)	Q8TAD2	1.4	1.8	2.3	86	71	80	15	3.81	3.81	31 250	125 000	3.9	13	12
Interleukin-18 (IL-18)	Q14116	7.2	7.9	8.5	75	83	97	7.5	0.48	0.48	62 500	125 000	5.1	11	11
Interleukin-27 (IL-27)	Q8NEV9, Q14213	3.1	3.4	4.2	106	69	84	7.5	122.07	122.07	62 500	125 000	2.7	7	11
Interleukin-4 receptor subunit alpha (IL-4RA)	P24394	2	2.3	2.8	87	91	105	15	7.63	7.63	125 000	125 000	4.2	9	15
Interleukin-6 (IL-6)	P05231	1.9	3.1	4.3	99	107	126	7.5	0.24	0.24	15 625	31 250	4.8	9	9
Kidney injury molecule 1 (KIM1)	Q96D42	6.4	7.8	9	90	95	108	15	0.48	1.91	31 250	125 000	4.2	11	9
Lactoylglutathione lyase (GLO1)	Q04760	2.8	3.5	5.2	125	127	299	0	0.95	0.95	31 250	125 000	4.5	8	11
Lectin-like oxidized LDL receptor 1 (LOX-1)	P78380	4.9	5.6	7.1	97	210	436	0.9	0.95	0.95	15 625	15 625	4.2	9	11
Leptin (LEP)	P41159	3.6	6.9	8.2	107	122	135	15	61.04	61.04	62 500	250 000	3	6	10
Lipoprotein lipase (LPL)	P06858	7.7	9	9.6	95	81	79	15	0.48	0.95	125 000	1 000 000	5.1	7	8
Low affinity immunoglobulin gamma Fc region receptor II-b (IgG Fc receptor II-b)	P31994	1.1	2	2.8	106	82	113	15	15.26	30.52	31 250	125 000	3	9	15
Lymphotactin (XCL1)	P47992	3.4	4.3	5.3	94	84	108	15	61.04	122.07	125 000	125 000	3	10	10
Macrophage receptor MARCO (MARCO)	Q9UEW3	6.3	6.5	7.1	101	89	107	15	30.52	61.04	31 250	125 000	2.7	6	9
Matrix metalloproteinase-12 (MMP12)	P39900	5.8	6.7	8	134	118	135	15	15.26	15.26	500 000	1 000 000	4.5	11	10

		Sample types						Endogenous Interference	Analytical measurement					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	Haemolysate	LOD	LLOQ	ULOQ	Hook	Range	Intra	Inter
Matrix metalloproteinase-7 (MMP7)	P09237	5.8	7.7	8.6	425	412	487	15	7.63	7.63	31 250	62 500	3.6	9	9
Melusin (ITGB1BP2)	Q9UKP3	1.2	1.6	3.4	36	22	13	15	976.56	1953.13	1 000 000	1 000 000	2.7	11	11
Natriuretic peptides B (BNP)	P16860	NA	NA	NA	NA	NA	NA	NA	488.28	488.28	31 250	1 000 000	1.8	NA	NA
NF-kappa-B essential modulator (NEMO)	Q9Y6K9	2.7	3.6	4.9	68	49	87	0.2	NA	NA	NA	NA	NA	9	9
Osteoclast-associated immunoglobulin-like receptor (hOSCAR)	Q8IYS5	9.6	10	10.2	101	94	118	15	1.91	7.63	125 000	125 000	4.2	5	10
Pappalysin-1 (PAPPA)	Q13219	2	2.7	3.4	82	NA	104	15	61.04	61.04	1 000 000	1 000 000	4.2	13	12
Pentraxin-related protein PTX3 (PTX3)	P26022	2.5	3.3	4.1	41	46	90	15	1.91	1.91	31 250	31 250	4.2	8	10
Placenta growth factor (PGF)	P49763	6.3	6.5	7.1	84	87	110	15	0.48	0.48	31 250	31 250	4.8	12	13
Platelet-derived growth factor subunit B (PDGF subunit B)	P01127	5.4	6.9	7.8	17	89	233	15	15.26	61.04	62 500	125 000	3	11	12
Poly [ADP-ribose] polymerase 1 (PARP-1)	P09874	1.7	2.4	3.9	NA	NA	31	3.8	61.04	122.07	125 000	125 000	3	9	11
Polymeric immunoglobulin receptor (PIgR)	P01833	5.9	6.2	6.6	110	107	115	7.5	122.07	244.14	1 000 000	1 000 000	3.6	3	14
Programmed cell death 1 ligand 2 (PD-L2)	Q9BQ51	2.3	2.8	3.2	85	87	108	15	488.28	488.28	1 000 000	1 000 000	3.3	9	10
Proheparin-binding EGF-like growth factor (HB-EGF)	Q99075	4.7	5.5	6.1	62	55	208	15	0.48	0.48	7 812	31 250	4.2	8	10
Pro-interleukin-16 (IL16)	Q14005	3.8	4.5	5.4	81	98	127	7.5	3.81	30.52	31 250	125 000	3	11	12
Prolargin (PRELP)	P51888	6.2	6.4	6.7	104	90	108	15	15.26	15.26	15 625	62 500	3	7	8
Prostasin (PRSS8)	Q16651	8.1	8.5	9.5	100	102	117	15	0.24	0.24	15 625	31 250	4.8	8	11
Protein AMBP (AMBP)	P02760	6.3	6.6	7.1	103	99	114	15	1953.12	1953.12	125 000	1 000 000	1.8	6	7
Proteinase-activated receptor 1 (PAR-1)	P25116	5.7	6.3	6.9	78	108	9	15	30.52	122.07	31 250	125 000	2.4	9	12
Protein-glutamine gamma-glutamyltransferase 2 (TGM2)	P21980	3.8	4.8	8.6	20	7	52	0	61.04	61.04	125 000	125 000	3.3	8	12
Proto-oncogene tyrosine-protein kinase Src (SRC)	P12931	2.6	3.2	5.2	63	38	31	15	15.26	15.26	15 625	15 625	3	10	12
P-selectin glycoprotein ligand 1 (PSGL-1)	Q14242	4	4.9	5.2	111	88	93	15	1.91	7.63	31 250	125 000	3.6	6	10
Receptor for advanced glycosylation end products (RAGE)	Q15109	11.9	12.8	13.6	92	76	111	15	0.48	0.48	15 625	31 250	4.5	9	11
Renin (REN)	P00797	5.1	6.7	7.3	89	106	117	15	7.63	7.63	31 250	125 000	3.6	8	12
Serine protease 27 (PRSS27)	Q9BQR3	7	7.5	8.5	79	110	150	15	1.91	1.91	62 500	125 000	4.5	9	13
Serine/threonine-protein kinase 4 (STK4)	Q13043	0.6	1	2.3	41	19	23	3.8	488.28	488.28	62 500	125 000	2.1	7	10
Serpin A12 (SERPINA12)	Q8IW75	1.8	3.1	8.3	83	82	111	15	15.26	15.26	7 812	31 250	2.7	10	22
SLAM family member 5 (CD84)	Q9UIB8	3.7	4.5	5.3	94	143	304	15	61.04	61.04	500 000	1 000 000	3.9	9	12
SLAM family member 7 (SLAMF7)	Q9NQ25	1.5	2	2.8	73	84	107	15	244.14	244.14	1 000 000	1 000 000	3.6	11	9
Sortilin (SORT1)	Q99523	5.5	5.8	6.2	87	118	176	15	122.07	122.07	250 000	1 000 000	3.3	8	12
Spondin-2 (SPON2)	Q9BUD6	8.4	8.7	9	122	129	134	15	30.52	61.04	125 000	500 000	3.3	5	11
Stem cell factor (SCF)	P21583	8.6	9.1	9.7	97	105	118	15	1.91	1.91	31 250	125 000	4.2	7	12
Superoxide dismutase [Mn], mitochondrial (SOD2)	P04179	8.3	8.5	8.9	105	103	114	15	15.26	122.07	1 000 000	1 000 000	3.9	6	9
T-cell surface glycoprotein CD4 (CD4)	P01730	3.1	3.7	4.4	106	91	84	7.5	15.26	15.26	250 000	1 000 000	4.2	10	9
Thrombomodulin (TM)	P07204	7.7	8.2	8.6	84	92	111	15	7.63	7.63	125 000	125 000	4.2	11	10
Thrombopoietin (THPO)	P40225	1.9	2.2	2.6	73	102	159	15	122.07	122.07	31 250	125 000	2.4	9	11
Thrombospondin-2 (THBS2)	P35442	5.4	5.6	5.9	111	108	122	15	61.04	244.14	62 500	125 000	2.4	5	8
Tissue factor (TF)	P13726	4.8	5.2	5.9	84	94	98	15	0.24	0.24	31 250	125 000	5.1	8	13
TNF-related apoptosis-inducing ligand receptor 2 (TRAIL-R2)	O14763	3.9	4.3	4.8	85	95	114	15	0.24	0.24	7 812	15 625	4.5	10	12
Tumor necrosis factor receptor superfamily member 10A (TNFRSF10A)	O00220	2.3	2.9	3.4	84	92	105	15	0.95	3.81	31 250	31 250	3.9	11	11
Tumor necrosis factor receptor superfamily member 11A (TNFRSF11A)	Q9Y6Q6	3.7	4.2	4.9	88	108	133	15	0.95	0.95	15 625	31 250	4.2	10	13
Tumor necrosis factor receptor superfamily member 13B (TNFRSF13B)	O14836	7.4	7.8	8.5	93	92	104	15	61.04	244.14	125 000	500 000	2.7	10	10
Tyrosine-protein kinase Mer (MERTK)	Q12866	3	3.5	4.1	75	82	111	15	244.14	244.14	1 000 000	1 000 000	3.6	10	10
Vascular endothelial growth factor D (VEGFD)	O43915	6.4	6.9	7.7	102	107	127	15	61.04	61.04	125 000	125 000	3.3	7	10
V-set and immunoglobulin domain-containing protein 2 (VSIG2)	Q96IQ7	2.5	3.2	3.7	85	87	104	15	30.52	61.04	31 250	1 000 000	2.7	8	10

2.3 PRECISION

REPEATABILITY

Intra-assay variation (within-run) was calculated as the mean %CV for 6 individual samples run, within each of 10 separate runs during the validation studies. Inter-assay variation (between-run) was calculated between experiments with the same operator. The reported inter-assay mean %CV is the average of three operators' %CV. Variation calculations were performed on linearized values for 91 analytes for which response levels could be measured in serum and normal plasma, see Table 1.

Across all 91 assays, the mean intra-assay and inter-assay variations observed were 9.1% and 11.7%, respectively. The distributions of intra-assay and inter-assay variations are shown in Figure 4.

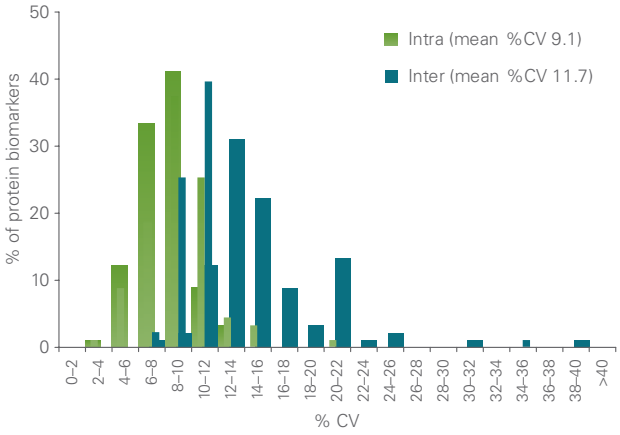

Fig 4. Distribution of intra-assay and inter-assay variations of Olink CARDIOVASCULAR II

REPRODUCIBILITY

Inter-site variations (between-site) were investigated during the validation of previous panels in beta-site studies to estimate the expected variations in values between different laboratories, with different operators and using different equipment. The beta-site studies have shown reproducibility and repeatability in line with Olink results. For more information, download our Data Validation documents at www.olink.com/data-validation

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

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 carried 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 5).

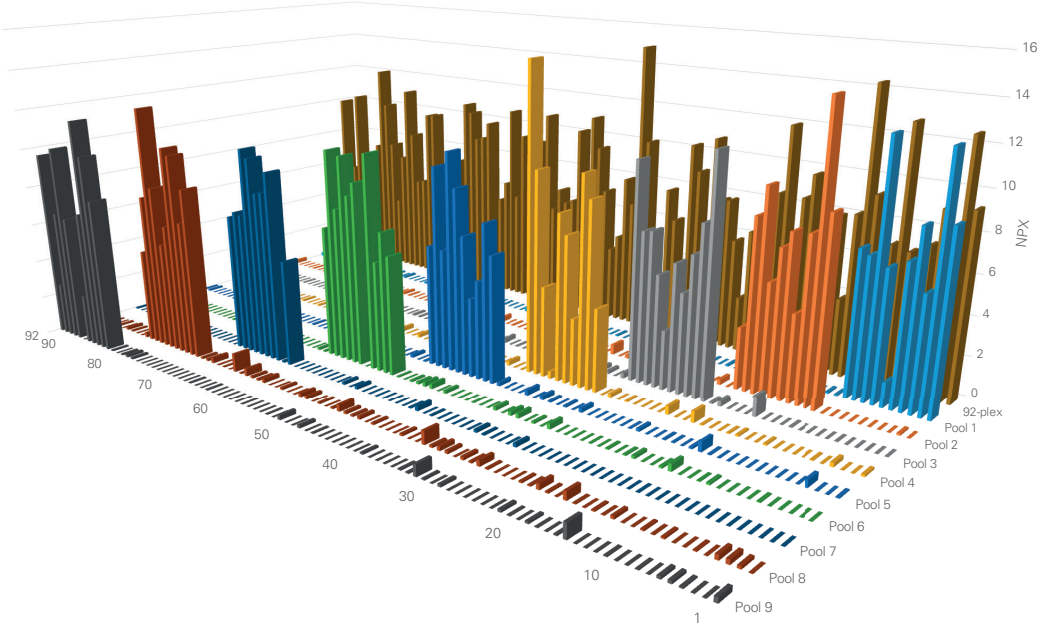

Fig 5. Assay readout specificity of the Olink platform. For each assay, specificity is confirmed by testing antigen sub-pools against the complete 92-plex pool as to each sub-mix.

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 antibody (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. No interference due to HAMA or RF could be detected for any of the samples in any of the previously tested panels, indicating sufficient blocking of these agents (data not shown).

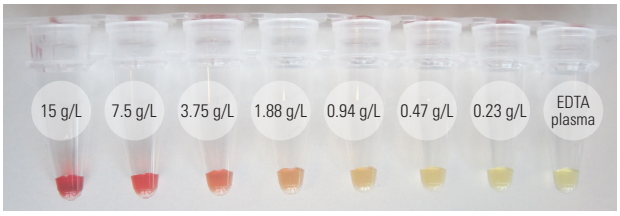

Fig 6. Endogenous interference. Levels tested for hemolysate were 0.23-15 g/L hemoglobin. The highest hemolysate concentration translates to about 10% hemolysis.

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

An example of hemolysate levels tested is shown in Figure 6. These additions represent different patient health conditions and/or sample collection irregularities. For all assays, bilirubin and lipids could be added to concentrations corresponding to at least 8 or 10 times normal values 3, 4, respectively, without disturbing assay performance (data not shown). In 22 out of 92 assays, altered signal was observed by the addition of hemolysate. The reason is most likely due to actual analyte 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.

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. 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 assays for Growth Hormone (GH) and Matrix Metalloproteinase (MMP-7) were compared when run in the full Olink CVD II panel. 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.

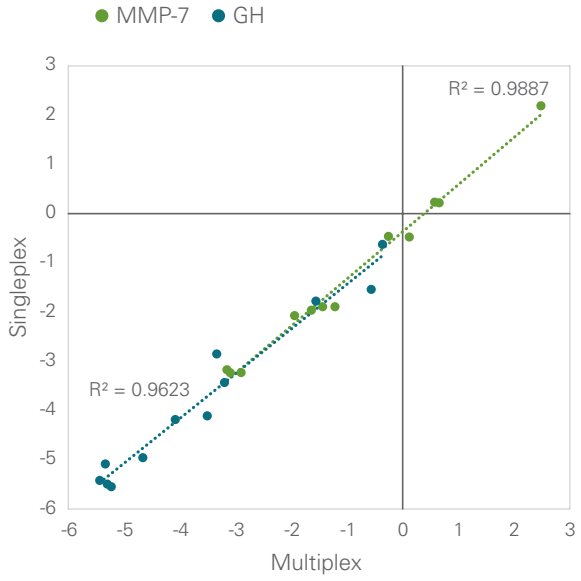

Fig 7. Scalability of the Olink technology platform. This experiment was performed using the Olink CVD II panel. Human plasma samples were analyzed in singleplex for Growth Hormone (GH) and Matrix Metalloproteinase (MMP-7) with the equivalent assays performed in a full 96-plex reaction. The observed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

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