VALIDATION DATA

1. Introduction

Olink® Cardiometabolic is a reagent kit measuring 92 cardiometabolic related human protein biomarkers simultaneously. The assays on this panel have been selected to target high-abundance proteins, and 1 µL of a 1:2025 dilution of sample is used. The analytical performance of the product has been carefully validated and the results are presented below.

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 protein present in the sample. A PCR reporter sequence is formed by a proximity dependent DNA polymerization event, 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 have been designed to enable monitoring of the technical assay performance, as well as the quality of individual samples, providing 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 for immediate proximity independent of antigen binding) monitors the extension and readout steps 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 control, inter-plate control (IPC), is included on each plate and 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. Furthermore, the 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 was performed by employing a pre-processing normalization procedure. For each sample and data point, the corresponding Cq-value for the Extension control was substracted, thus 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 generated Normalized Protein eXpression (NPX) unit is 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 were performed on linearized values.

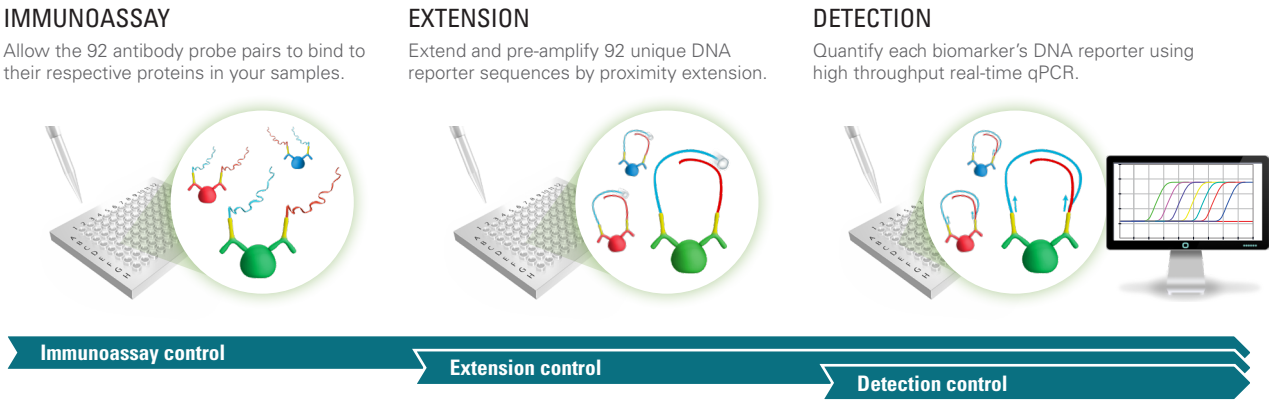

Fig 1. Olink assay procedure (above) and controls (below). The internal controls enables monitoring of the three core steps in the Olink assay and used for quality control and data normalization. Read out is performed by using the Fluidigm® Biomark™ or the Fluidigm® Biomark™ HD system.

2. Performance characteristics

2.1 SAMPLE TYPES

The ability to use different sample types was evaluated with Olink Cardiometabolic by collecting matched serum, EDTA, acid citrate dextrose (ACD), and sodium heparin plasma samples from 4 healthy individuals. Response values observed between heparin, citrate plasma or serum, are expressed as relative differences (%) compared to EDTA plasma and shown in Tabel 1 for each sample type. To evaluated the measuring range of endogenous protein levels, response values levels were assessed in 22 normal EDTA plasma samples and reported in NPX, Table 1.

2.2 ANALYTICAL MEASUREMENT

DETECTION LIMIT

Calibrator curves were determined for 90 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 where recombinant protein antigen was available, see Table 1 and Figure 2. Please note that the Cardiometabolic panel uses a 1:2025 dilution of sample, whereas our technical validation assays are performed *in vitro* using recombinant antigens. The data presented in this document are based on these *in vitro* assays and a multiplication factor of 2025 should therefore be taken in consideration when comparing the addressable biological concentration to the *in vitro* validation data.

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

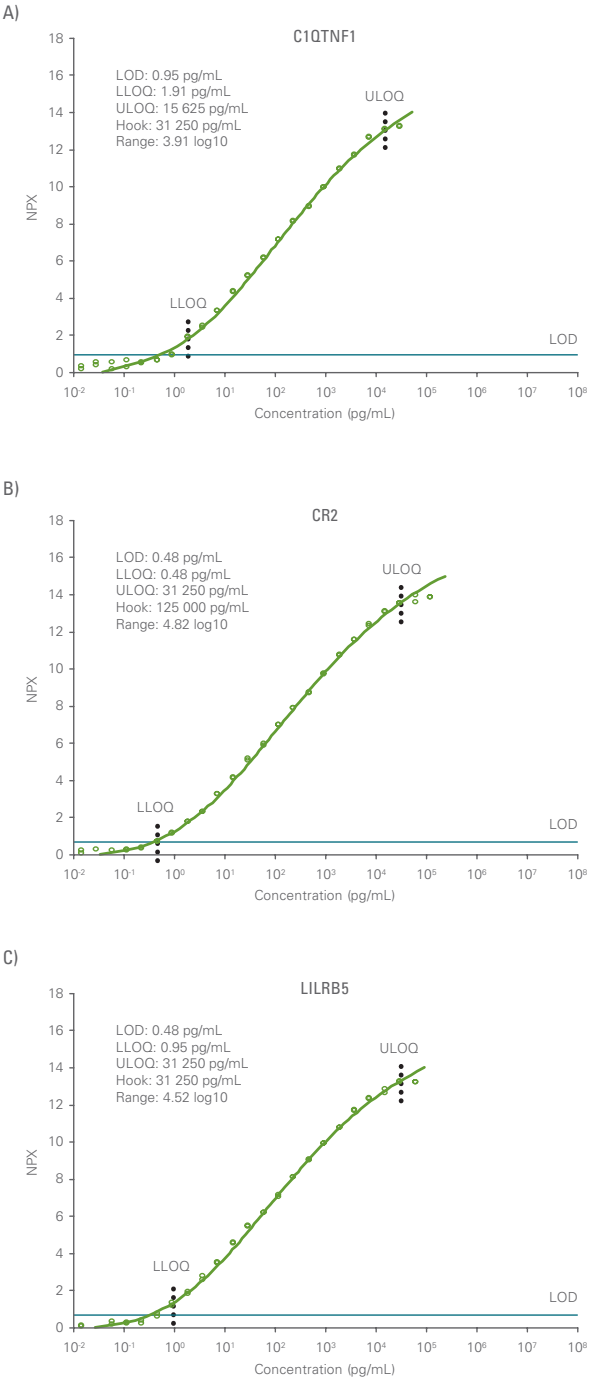

Fig 2. Calibrator curves from 3 assays and their corresponding analytical measurement data.

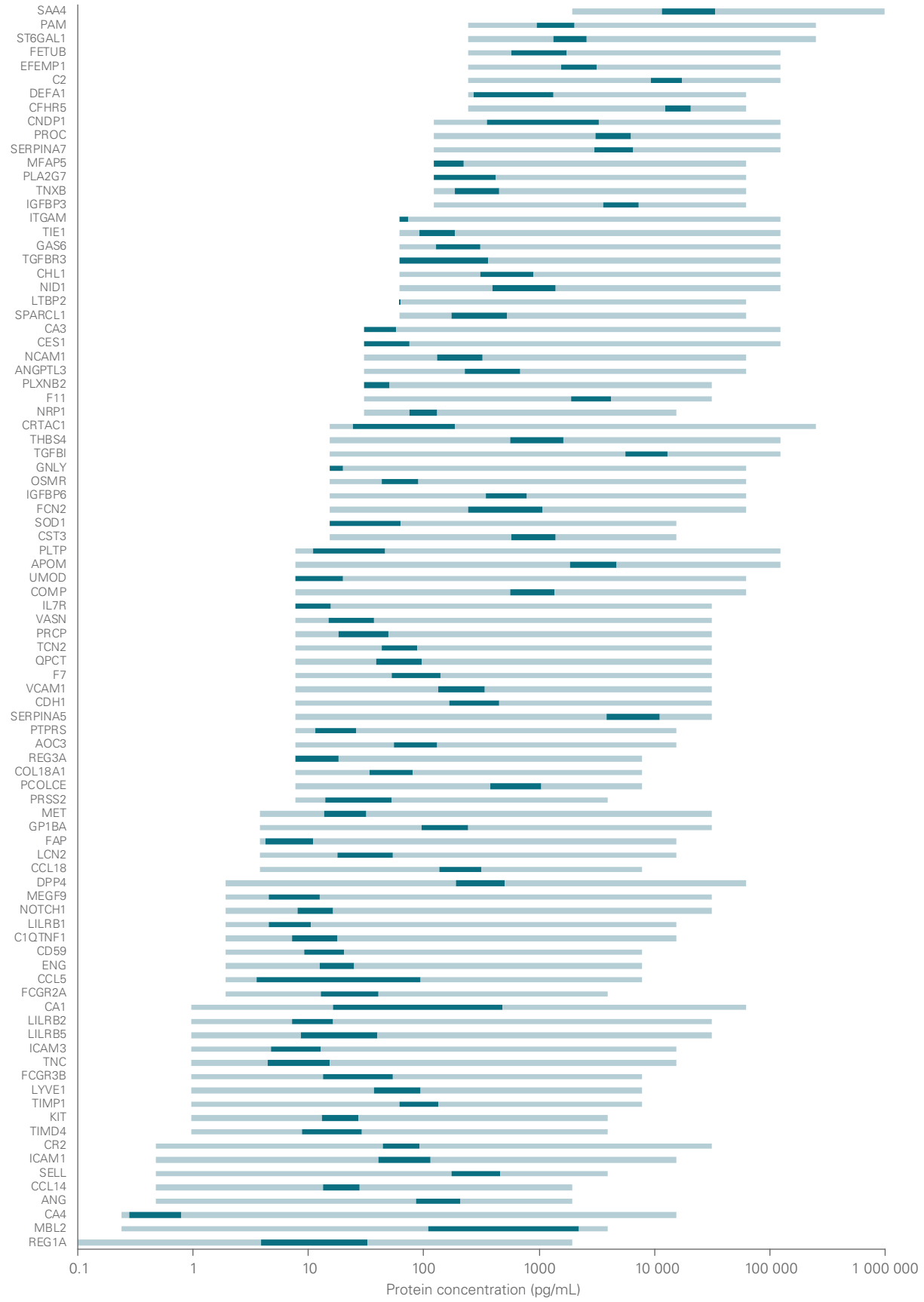

Fig 3. Distribution of analytical measuring range, defined by the lower and upper limits of quantification (LLOQ-ULOQ) in pg/mL. Normal plasma levels (dark green bars) are denoted for 90 analytes and here reported in pg/mL.

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. Please note: the Cardiometabolic panel uses a 1:2025 dilution which should be taken in consideration when comparing biological concentrations to the *in vitro* validation data.

		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	Hemolysate	LOD	LLOQ	ULOQ	Hook	Range	Intra	Inter
Angiogenin (ANG)	P03950	7.2	8.0	8.4	96	75	90	15	0.48	0.48	1953	3906	3.6	12	10
Angiopoietin-related protein 3 (ANGPTL3)	Q9Y5C1	2.8	3.6	4.3	101	82	94	15	15	31	62500	125000	3.3	12	8
Apolipoprotein M (APOM)	O95445	6.5	7.4	7.9	98	91	101	15	7.6	7.6	125000	500000	4.2	14	10
Beta-Ala-His dipeptidase (CNDP1)	Q96KN2	1.4	2.6	3.8	366	305	301	15	122	122	125000	500000	3.0	12	10
Beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1)	P15907	3.2	3.6	4.2	99	96	131	15	244	244	250000	500000	3.0	17	14
Cadherin-1 (CDH1)	P12830	4.2	5.2	5.8	133	124	132	15	7.6	7.6	31250	62500	3.6	14	9
Carbonic anhydrase 1 (CA1)	P00915	2.5	5.8	6.7	27	17	86	0	0.95	0.95	62500	125000	4.8	14	13
Carbonic anhydrase 3 (CA3)	P07451	0.6	1.1	1.6	84	69	95	0	15	31	125000	500000	3.6	7	8
Carbonic anhydrase 4 (CA4)	P22748	1.5	2.0	2.4	104	94	105	15	0.12	0.24	15625	62500	4.8	13	7
Cartilage acidic protein 1 (CRTAC1)	Q9NQ79	1.1	2.1	3.2	136	131	136	15	15	15	250000	500000	4.2	12	10
Cartilage oligomeric matrix protein (COMP)	P49747	7.0	7.6	8.3	100	93	98	15	7.6	7.6	62500	125000	3.9	14	9
C-C motif chemokine 14 (CCL14)	Q16627	4.1	4.7	5.2	91	81	92	15	0.48	0.48	1953	7812	3.6	14	7
C-C motif chemokine 18 (CCL18)	P55774	6.3	7.1	7.9	98	74	94	15	3.8	3.8	7812	15625	3.3	16	10
C-C motif chemokine 5 (CCL5)	P13501	1.4	2.8	5.2	22	89	159	15	1.9	1.9	7812	15625	3.6	13	8
CD59 glycoprotein (CD59)	P13987	2.0	2.6	2.9	109	116	138	3.8	1.9	1.9	7812	7812	3.6	19	22
Coagulation factor VII (F7)	P08709	3.9	4.9	5.3	97	90	77	15	1.9	7.6	31250	62500	3.6	15	10
Coagulation factor XI (F11)	P03951	6.8	7.4	7.8	97	89	95	15	7.6	31	31250	62500	3.0	11	7
Collagen alpha-1(XVIII) chain (COL18A1)	P39060	2.9	3.5	4.0	102	88	99	15	7.6	7.6	7812	15625	3.0	12	7
Complement C1q tumor necrosis factor-related protein 1 (C1QTNF1)	Q9BXJ1	3.1	3.7	4.3	86	49	78	15	0.95	1.9	15625	31250	3.9	16	10
Complement C2 (C2)	P06681	4.9	5.3	5.8	99	102	132	15	122	244	125000	500000	2.7	13	8
Complement factor H-related protein 5 (CFHR5)	Q9BXR6	8.1	8.7	9.2	99	89	95	15	122	244	62500	125000	2.4	12	10
Complement receptor type 2 (CR2)	P20023	5.4	6.1	6.5	98	92	96	15	0.48	0.48	31250	125000	4.8	13	8
Cystatin-C (CST3)	P01034	6.8	7.6	8.4	95	91	96	15	15	15	15625	31250	3.0	17	11
Dipeptidyl peptidase 4 (DPP4)	P27487	4.9	5.5	6.2	100	93	104	15	1.9	1.9	62500	125000	4.5	14	8
EGF-containing fibulin-like extracellular matrix protein 1 (EFEMP1)	Q12805	3.3	3.8	4.4	108	78	82	15	244	244	125000	500000	2.7	14	9
Endoglin (ENG)	P17813	2.2	2.5	2.9	101	88	91	15	1.9	1.9	7812	15625	3.6	12	9
Fetuin-B (FETUB)	Q9UGM5	1.4	2.2	2.7	105	95	102	15	122	244	125000	125000	2.7	18	17
Ficolin-2 (FCN2)	Q15485	4.4	5.7	6.6	122	113	108	15	7.6	15	62500	125000	3.6	14	8
Glutaminyl-peptide cyclotransferase (QPCT)	Q16769	2.2	2.7	3.4	105	99	117	15	7.6	7.6	31250	31250	3.6	14	23
Granulysin (GNLY)	P22749	0.3	0.9	1.2	106	94	100	15	15	15	62500	125000	3.6	13	7
Growth arrest-specific protein 6 (GAS6)	Q14393	3.5	4.3	4.8	121	111	120	15	15	61	125000	250000	3.3	14	11
Hepatocyte growth factor receptor (MET)	P08581	2.1	2.6	2.9	97	90	97	15	3.8	3.8	31250	62500	3.9	12	7
Ig lambda-2 chain C regions (IGLC2)	P0CG05	6.2	6.7	7.5	97	91	99	15	NA	NA	NA	NA	NA	13	9
Insulin-like growth factor-binding protein 3 (IGFBP3)	P17936	4.4	5.1	5.6	103	98	106	15	122	122	62500	125000	2.7	10	7
Insulin-like growth factor-binding protein 6 (IGFBP6)	P24592	4.0	4.7	5.3	97	94	103	15	15	15	62500	125000	3.6	11	7
Integrin alpha-M (ITGAM)	P11215	0.5	1.3	2.2	101	83	91	15	31	61	125000	125000	3.3	17	14
Intercellular adhesion molecule 1 (ICAM1)	P05362	6.1	6.7	7.5	96	90	95	15	0.48	0.48	15625	31250	4.5	13	7
Intercellular adhesion molecule 3 (ICAM3)	P32942	2.5	3.0	3.6	99	90	99	15	0.95	0.95	15625	31250	4.2	11	7
Interleukin-7 receptor subunit alpha (IL7R)	P16871	1.1	1.7	2.3	103	89	101	15	3.8	7.6	31250	125000	3.6	15	14
Latent-transforming growth factor beta-binding protein 2 (LTBP2)	Q14767	NA	NA	0.8	NA	NA	NA	15	61	61	62500	125000	3.0	11	9
Leukocyte immunoglobulin-like receptor subfamily B member 1 (LILRB1)	Q8NHL6	1.5	1.9	2.2	102	91	97	15	1.9	1.9	15625	31250	3.9	10	8
Leukocyte immunoglobulin-like receptor subfamily B member 2 (LILRB2)	Q8N423	2.8	3.2	3.8	97	85	97	15	0.95	0.95	31250	31250	4.5	13	9
Leukocyte immunoglobulin-like receptor subfamily B member 5 (LILRB5)	O75023	3.5	4.8	5.6	96	90	98	15	0.48	0.95	31250	31250	4.5	12	8
Lithostathine-1-alpha (REG1A)	P05451	5.7	6.8	8.5	98	92	101	15	0.03	0.03	1953	31250	4.8	12	8

		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	Hemolysate	LOD	LLOQ	ULOQ	Hook	Range	Intra	Inter
Liver carboxylesterase 1 (CES1)	P23141	1.6	2.2	3.1	143	459	107	15	15	31	125000	125000	3.6	17	12
Low affinity immunoglobulin gamma Fc region receptor II-a (FCGR2A)	P12318	2.9	3.5	4.4	98	93	101	15	1.9	1.9	3906	15625	3.3	12	9
Low affinity immunoglobulin gamma Fc region receptor III-B (FCGR3B)	O75015	3.5	4.4	5.4	95	90	97	15	0.95	0.95	7812	31250	3.9	12	8
L-selectin (SELL)	P14151	7.7	8.5	9.0	98	94	97	15	0.48	0.48	3906	15625	3.9	13	7
Lymphatic vessel endothelial hyaluronic acid receptor 1 (LYVE1)	Q9Y5Y7	4.8	5.4	6.1	99	94	100	15	0.95	0.95	7812	15625	3.9	12	9
Lysosomal Pro-X carboxypeptidase (PRCP)	P42785	0.6	1.8	2.2	NA	NA	NA	15	7.6	7.6	31250	62500	3.6	13	14
Mannose-binding protein C (MBL2)	P11226	7.8	9.9	11	98	90	92	15	0.24	0.24	3906	7812	4.2	12	10
Mast/stem cell growth factor receptor Kit (KIT)	P10721	4.5	4.9	5.4	97	92	101	15	0.95	0.95	3906	7812	3.6	11	9
Membrane cofactor protein (CD46)	P15529	2.3	2.8	3.2	98	91	104	15	NA	NA	NA	NA	NA	16	11
Membrane primary amine oxidase (AOC3)	Q16853	2.9	3.6	4.0	97	91	97	15	3.8	7.6	15625	15625	3.3	10	5
Metalloproteinase inhibitor 1 (TIMP1)	P01033	5.0	5.7	6.2	86	104	167	15	0.95	0.95	7812	15625	3.9	13	8
Microfibrillar-associated protein 5 (MFAP5)	Q13361	1.5	2.0	2.5	96	82	89	15	61	122	62500	125000	2.7	13	10
Multiple epidermal growth factor-like domains protein 9 (MEGF9)	Q9H1U4	2.3	3.0	3.4	97	89	92	15	0.95	1.9	31250	62500	4.2	12	9
Neural cell adhesion molecule 1 (NCAM1)	P13591	2.8	3.4	3.9	101	93	99	15	15	31	62500	125000	3.3	12	9
Neural cell adhesion molecule L1-like protein (CHL1)	O00533	3.3	4.0	4.7	100	93	99	15	61	61	125000	125000	3.3	12	8
Neurogenic locus notch homolog protein 1 (NOTCH1)	P46531	2.9	3.4	3.8	101	97	100	15	0.95	1.9	31250	31250	4.2	11	7
Neuropilin-1 (NRP1)	O14786	1.8	2.1	2.5	100	91	99	15	15	31	15625	31250	2.7	9	7
Neutrophil defensin 1 (DEFA1)	P59665	0.5	1.1	2.1	118	101	166	15	244	244	62500	125000	2.4	19	28
Neutrophil gelatinase-associated lipocalin (LCN2)	P80188	1.6	2.4	3.0	NA	NA	NA	15	3.8	3.8	15625	62500	3.6	18	11
Nidogen-1 (NID1)	P14543	3.1	3.7	4.8	75	92	102	15	31	61	125000	125000	3.3	12	9
Oncostatin-M-specific receptor subunit beta (OSMR)	Q99650	1.6	2.0	2.4	98	88	97	15	7.6	15	62500	62500	3.6	10	7
Peptidyl-glycine alpha-amidating monooxygenase (PAM)	P19021	2.1	2.6	3.1	109	97	106	15	244	244	250000	500000	3.0	11	8
Phospholipid transfer protein (PLTP)	P55058	1.2	1.7	2.3	94	86	98	15	7.6	7.6	125000	500000	4.2	15	12
Plasma serine protease inhibitor (SERPINA5)	P05154	8.0	8.7	9.3	97	155	171	15	7.6	7.6	31250	62500	3.6	11	7
Platelet glycoprotein Ib alpha chain (GP1BA)	P07359	4.1	4.9	5.4	98	99	126	15	1.9	3.8	31250	125000	3.9	14	9
Platelet-activating factor acetylhydrolase (PLA2G7)	Q13093	0.2	1.5	1.9	101	88	98	15	122	122	62500	500000	2.7	13	8
Plexin-B2 (PLXNB2)	O15031	1.3	1.7	2.0	104	97	101	15	15	31	31250	62500	3.0	11	9
Procollagen C-endopeptidase enhancer 1 (PCOLCE)	Q15113	5.1	6.2	6.6	96	74	91	15	7.6	7.6	7812	31250	3.0	15	8
Prolyl endopeptidase FAP (FAP)	Q12884	1.3	1.8	2.2	97	88	94	15	1.9	3.8	15625	31250	3.6	11	8
Receptor-type tyrosine-protein phosphatase S (PTPRS)	Q13332	1.3	1.6	1.9	102	90	95	15	3.8	7.6	15625	62500	3.3	10	7
Regenerating islet-derived protein 3-alpha (REG3A)	Q06141	NA	0.7	1.2	96	89	87	15	7.6	7.6	7812	15625	3.0	10	6
Serum amyloid A-4 protein (SAA4)	P35542	3.6	4.5	5.3	86	88	97	15	1953	1953	1000000	1000000	2.7	21	11
SPARC-like protein 1 (SPARCL1)	Q14515	2.0	2.6	3.2	102	80	83	15	31	61	62500	125000	3.0	12	9
Superoxide dismutase [Cu-Zn] (SOD1)	P00441	NA	NA	1.5	NA	NA	NA	0	15	15	15625	125000	3.0	10	7
T-cell immunoglobulin and mucin domain-containing protein 4 (TIMD4)	Q96H15	3.5	4.4	5.1	99	92	98	15	0.95	0.95	3906	7812	3.6	12	9
Tenascin (TNC)	P24821	2.2	2.8	3.6	96	81	98	15	0.95	0.95	15625	31250	4.2	12	8
Tenascin-X (TNXB)	P22105	1.0	1.3	1.7	99	88	100	15	122	122	62500	125000	2.7	9	6
Thrombospondin-4 (THBS4)	P35443	4.2	5.2	5.9	104	87	96	15	15	15	125000	250000	3.9	12	9
Thyroxine-binding globulin (SERPINA7)	P05543	4.6	5.3	5.8	96	92	97	15	122	122	125000	500000	3.0	12	8
Transcobalamin-2 (TCN2)	P20062	3.7	4.2	4.7	100	89	96	15	1.9	7.6	31250	62500	3.6	13	12
Transforming growth factor beta receptor type 3 (TGFBR3)	Q03167	1.4	3.2	3.6	96	51	51	15	61	61	125000	125000	3.3	14	11
Transforming growth factor-beta-induced protein ig-h3 (TGFBI)	Q15582	7.7	8.5	9.2	94	85	91	15	15	15	125000	125000	3.9	14	8
Trypsin-2 (PRSS2)	P07478	1.9	2.9	3.7	100	90	95	15	7.6	7.6	3906	31250	2.7	12	8
Tyrosine-protein kinase receptor Tie-1 (TIE1)	P35590	1.6	2.0	2.3	99	90	96	15	31	61	125000	125000	3.3	9	7
Uromodulin (UMOD)	P07911	NA	1.1	1.5	105	91	100	15	7.6	7.6	62500	125000	3.9	11	7
Vascular cell adhesion protein 1 (VCAM1)	P19320	4.3	4.9	5.5	99	92	96	15	7.6	7.6	31250	125000	3.6	12	8
Vasorin (VASN)	Q6EMK4	2.1	2.7	3.1	97	87	98	15	3.8	7.6	31250	62500	3.6	12	9
Vitamin K-dependent protein C (PROC)	P04070	4.3	5.1	5.6	99	93	97	15	122	122	125000	125000	3.0	15	12

*U/µl

2.3 PRECISION

REPEATABILITY

Intra-assay variation (within-run) was calculated as the mean %CV for 6 individual samples run in triplicates within each of 10 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 of three operators' %CV. Variation calculations were performed on linearized values for 92 analytes for which 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 12.9% and 9.5%, respectively. The distribution of both intra-assay and inter-assay variations are shown in Figure 4.

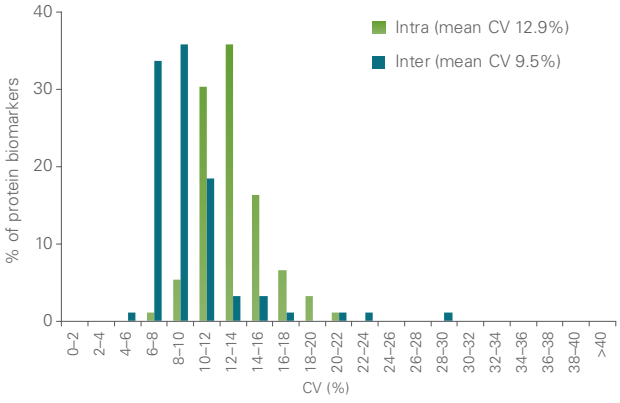

Fig 4. Distribution of intra-assay and inter-assay variations of Olink Cardiometabolic.

REPRODUCIBILITY

Inter-site variations (between-site) was also investigated during the validation in a beta-site study to estimate the expected increase in variation values produced by introducing a 2025 fold pre-dilution step of samples prior to running the Olink Cardiometabolic assay protocol. Six individuals samples were distributed to two laboratories together with Olink Cardiometabolic reagent kits. Each site was instructed to perform the analysis of the 6 individual samples according to the same run design and asked to perform two independent runs. In this document the results from beta-site 2 were excluded due to technical failure which was indpendent of the performance of the reagent kit. The intra-assay mean CV value results for beta-site 1 was 9.9% and the mean inter-assay CV was 5.4%. Overall, a very good reproducibility and repeatability was observed with an avarage global inter-site CV of 22.9%.

2.4 ANALYTICAL SPECIFICITY

ASSAY SPECIFICITY

The antibodies used in Olink Cardiometabolic were all specific for their respective targets. 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 in Olink, see Figure 5.

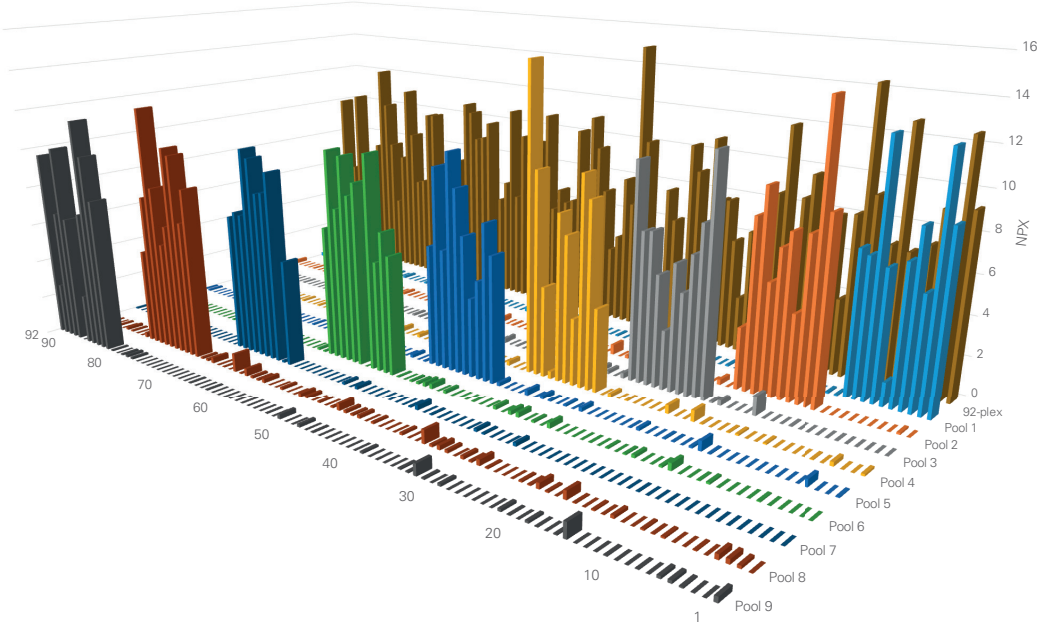

Fig 5. Assay readout specificity of the Olink platform. For each assay, specificity is confirmed by testing antigen sub-pools against the complete 92-plex pool as to each sub-mix.

ENDOGENOUS INTERFERENCE

Endogenous interference from heterophilic antibodies, e.g. human anti-mouse antibody (HAMA), and rheumatoid factor are 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 could be detected for any of the samples in previously tested panels, indicating sufficient blocking of these agents (data not shown).

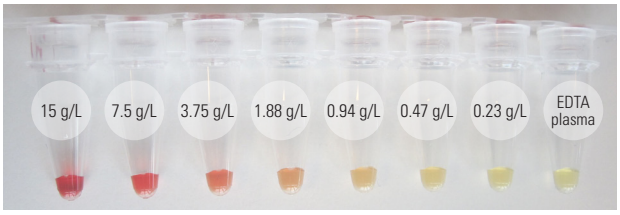

Fig 6. Endogenous interference. Levels tested for hemolysate were 0.23-15 g/L hemoglobin. The highest hemolysate concentration translates to about 10% hemolysis.

The potential impact of bilirubin, lipids and hemolysate, known interfering plasma and serum components, were evaluted at different added concentrations. An example of hemolysate levels tested is shown in Figure 6. These additions represent different patient health conditions and/or sample collection irregularities. Interferens by bilirubin and lipids has previously been evaluated, and disturbance has only been observed at extrem levels corresponding to 8 or 10 times normal[3, 4] values and therefore not performed for Olink Cardiometabolic. In 4 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 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 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.

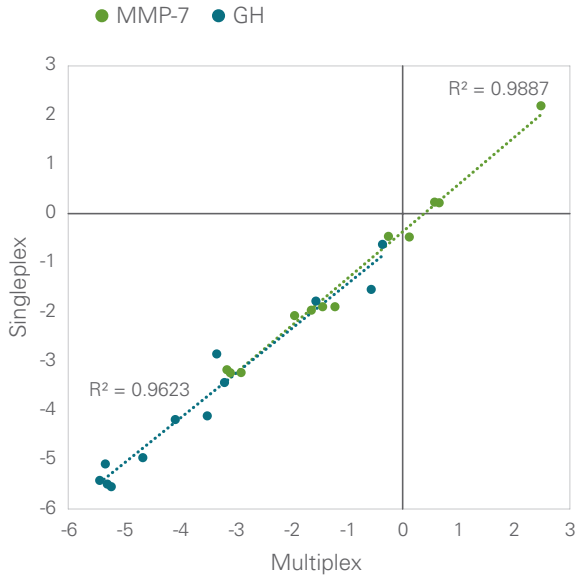

Fig 7. Scalability of the Olink technology platform. The experiment was performed using the Olink CVD II $^{96\times96}$ panel. Human plasma samples were analyzed in singleplex for Growth Hormone (GH) and Matrix Metalloproteinase (MMP-7) with the equivalent assays performed in a full 96-plex reaction. 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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