1. Introduction

Olink® Metabolism is a reagent kit measuring 92 metabolism related human protein biomarkers simultaneously. The assays on this panel have been selected to focus on high-abundance proteins, and 1 µL of a 1:10 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.

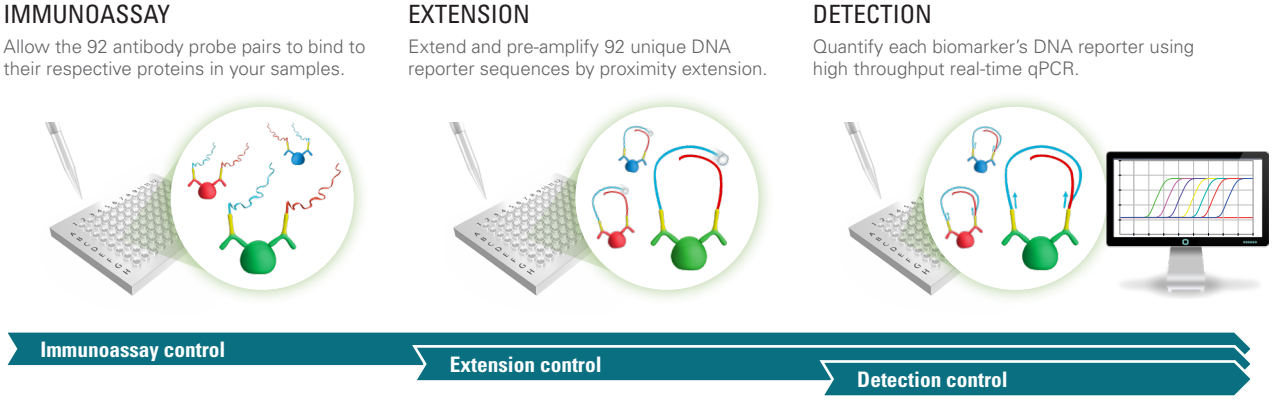

Fig 1. Olink assay procedure (above) and controls (below). The internal controls enables monitoring of the three core steps in the Olink assay and used for quality control and data normalization. Read out is performed by using the Fluidigm® Biomark™ or the Fluidigm® Biomark™ HD system.

2. Performance characteristics

2.1 SAMPLE TYPES

The ability to use different sample types was evaluated with Olink Metabolism by collecting matched serum, EDTA, acid citrate dextrose (ACD), and sodium heparin plasma samples from 4 healthy individuals. Table 1 summarizes response values for 22 normal EDTA plasma samples expressed in NPX, as well as relative differences as compared to EDTA plasma. Variations observed between responses in heparin, citrate plasma and serum, as compared to EDTA plasma, were generally small, and all assays will therefore function without limitation in these sample types.

2.2 ANALYTICAL MEASUREMENT

DETECTION LIMIT

Calibrator curves were determined for 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 Metabolism panel uses a 1:10 dilution of sample, whereas the 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 10 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 91 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 91 assays and endogenous plasma levels are shown in Figure 3. Separate calibrator curves established for each assay may be viewed at www.olink.com.

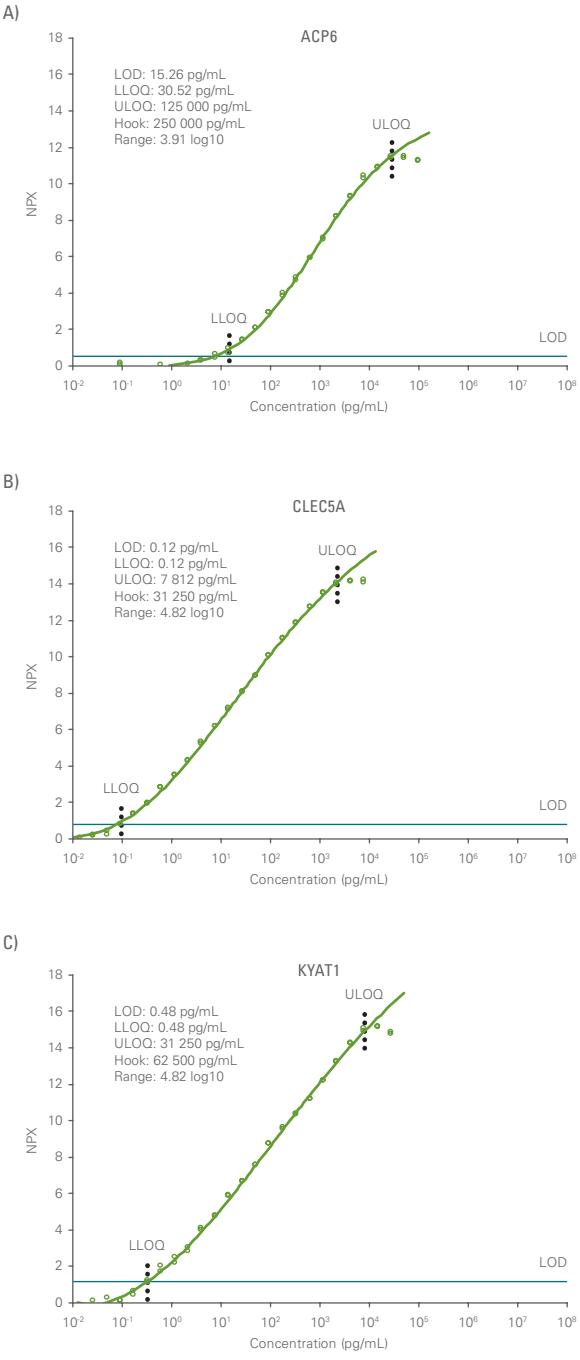

Fig 2. Calibrator curves from 3 assays and their corresponding analytical measurement data.

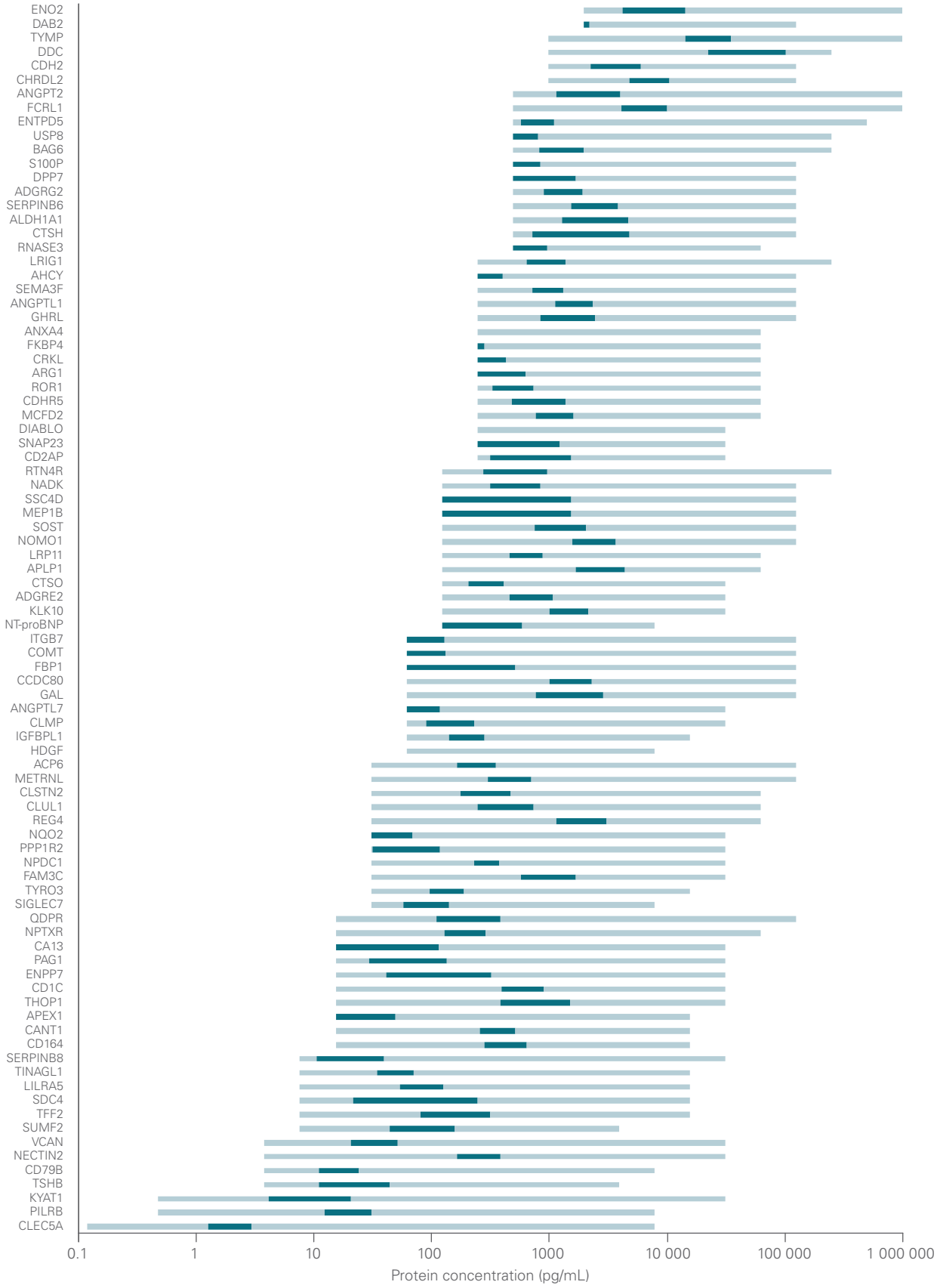

Fig 3. Distribution of analytical measuring range, defined by the lower and upper limits of quantification (LLOQ-ULOQ). Normal plasma levels (dark green bars) are denoted for 91 analytes.

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. Please note: the Metabolism panel uses a 1:10 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
Adenosylhomocysteinase (AHCY)	P23526	NA	NA	1.1	77	79	112	0.2	244	244	124 999	499 999	2.7	7	11
Adhesion G protein-coupled receptor E2 (ADGRE2)	Q9UHX3	2.6	3.2	3.7	103	98	107	15	122	122	31 250	124 999	2.4	6	10
Adhesion G-protein coupled receptor G2 (ADGRG2)	Q8IZP9	1.8	2.2	2.6	102	97	107	15	244	488	124 999	250 000	2.4	6	11
Amyloid-like protein 1 (APLP1)	P51693	4.6	5.2	6.5	248	99	258	15	61	122	62 499	124 999	2.7	6	10
Angiopoietin-2 (ANGPT2)	O15123	1.3	2.1	3.1	95	82	93	15	488	488	1 000 000	1 000 000	3.3	6	12
Angiopoietin-related protein 1 (ANGPTL1)	O95841	2.5	3.1	3.6	103	102	104	15	122	244	124 999	250 000	3.0	6	12
Angiopoietin-related protein 7 (ANGPTL7)	O43827	0.9	1.2	1.7	106	98	104	15	31	61	31 250	62 499	2.7	7	7
Annexin A11 (ANXA11)	P50995	NA	NA	NA	43	28	28	7.5	3906	3906	1 000 000	1 000 000	2.4	9	10
Annexin A4 (ANXA4)	P09525	NA	NA	NA	NA	NA	NA	1.9	244	244	62 499	250 000	2.4	6	10
Appetite-regulating hormone (GHRL)	Q9UBU3	1.9	2.5	3.5	95	74	38	15	244	244	124 999	250 000	2.7	8	16
Arginase-1 (ARG1)	P05089	NA	0.5	0.9	36	59	180	0	244	244	62 499	250 000	2.4	6	11
Aromatic-L-amino-acid decarboxylase (DDC)	P20711	3.9	5.1	5.9	93	96	101	15	977	977	250 000	1 000 000	2.4	5	12
B-cell antigen receptor complex-associated protein beta chain (CD79B)	P40259	1.7	2.1	2.6	95	93	103	15	0.95	3.81	7812	15 624	3.3	5	8
Cadherin-2 (CDH2)	P19022	2.0	2.6	3.4	75	75	81	15	488	977	124 999	250 000	2.1	6	12
Cadherin-related family member 5 (CDHR5)	Q9HBB8	1.8	2.5	3.2	116	105	114	15	122	244	62 499	124 999	2.4	5	10
Calsyntenin-2 (CLSTN2)	Q9H4D0	2.3	2.9	3.6	102	93	95	15	31	31	62 499	124 999	3.3	7	10
Carbonic anhydrase 13 (CA13)	Q8N1Q1	0.4	1.2	3.0	78	78	54	15	15.26	15	31 250	124 999	3.3	7	9
Catechol O-methyltransferase (COMT)	P21964	NA	NA	2.1	84	84	85	0.5	61	61	124 999	250 000	3.3	6	12
Cathepsin O (CTSO)	P43234	1.4	1.8	2.3	95	93	101	15	122	122	31 250	124 999	2.4	6	10
CD2-associated protein (CD2AP)	Q9Y5K6	1.4	2.1	4.3	45	27	61	0.5	244	244	31 250	124 999	2.1	6	8
Chordin-like protein 2 (CHRDL2)	Q6WN34	2.5	3.3	4.1	92	85	82	15	977	977	124 999	250 000	2.1	6	10
Clusterin-like protein 1 (CLUL1)	Q15846	2.9	3.7	4.5	98	99	101	15	31	31	62 499	124 999	3.3	5	9
Coiled-coil domain-containing protein 80 (CCDC80)	Q76M96	4.2	4.9	5.7	93	81	85	15	61	61	124 999	250 000	3.3	8	11
Crk-like protein (CRKL)	P46109	NA	NA	1.3	75	53	50	0.5	244	244	62 499	124 999	2.4	7	11
C-type lectin domain family 5 member A (CLEC5A)	Q9NY25	2.9	3.3	3.9	98	101	129	15	0.12	0.12	7812	31 250	4.8	5	9
CXADR-like membrane protein (CLMP)	Q9H6B4	1.1	1.9	2.3	82	86	99	15	61	61	31 250	124 999	2.7	6	11
Diablo homolog, mitochondrial (DIABLO)	Q9NR28	NA	NA	NA	94	NA	NA	15	244	244	31 250	124 999	2.1	8	10
Dihydropteridine reductase (QDPR)	P09417	3.0	3.6	4.7	65	61	86	0	7.63	15	124 999	1 000 000	3.9	5	9
Dipeptidyl peptidase 2 (DPP7)	Q9UHL4	NA	0.4	1.5	102	104	182	15	488	488	124 999	1 000 000	2.4	7	4
Disabled homolog 2 (DAB2)	P98082	NA	NA	1.1	60	39	37	15	1953	1953	124 999	124 999	1.8	9	15
DNA-(apurinic or apyrimidinic site) lyase (APEX1)	P27695	0.2	0.8	1.8	104	145	199	3.8	15	15	15 624	62 499	3.0	6	14
Ectonucleoside triphosphate diphosphohydrolase 5 (ENTPD5)	O75356	1.5	1.9	2.3	98	91	100	15	244	488	499 999	1 000 000	3.0	9	11
Ectonucleotide pyrophosphatase/phosphodiesterase family member 7 (ENPP7)	Q6UWV6	1.9	2.9	4.4	101	97	108	15	15	15	31 250	124 999	3.3	5	11
Eosinophil cationic protein (RNASE3)	P12724	NA	0.4	1.6	96	137	872	0.9	488	488	62 499	124 999	2.1	6	18
Fc receptor-like protein 1 (FCRL1)	Q96LA6	3.3	4.0	4.6	93	97	103	15	244	488	1 000 000	1 000 000	3.3	7	10
Fructose-1,6-bisphosphatase 1 (FBP1)	P09467	NA	1.5	3.2	83	238	225	15	61	61	124 999	250 000	3.3	6	18
Galanin peptides (GAL)	P22466	3.9	5.0	6.0	94	96	94	15	31	61	124 999	250 000	3.3	9	14
Gamma-enolase (ENO2)	P09104	1.5	1.9	3.1	66	59	68	0	1953	1953	1 000 000	1 000 000	2.7	6	9
Glutaredoxin-1 (GLRX)	P35754	NA	0.4	1.5	41	63	188	0	1953	1953	1 000 000	1 000 000	2.7	9	10
GRB2-related adapter protein 2 (GRAP2)	O75791	0.3	1.2	2.9	39	24	24	7.5	3906	15625	1 000 000	1 000 000	1.8	6	5
Hepatoma-derived growth factor (HDGF)	P51858	NA	NA	0.9	36	48	137	0.5	31	61	7812	31 250	2.1	6	10
Inactive tyrosine-protein kinase transmembrane receptor ROR1 (ROR1)	Q01973	1.3	1.8	2.5	95	98	102	15	244	244	62 499	124 999	2.4	6	10
Insulin-like growth factor-binding protein-like 1 (IGFBPL1)	Q8WX77	1.6	2.0	2.4	101	97	104	15	31	61	15 624	62 499	2.4	5	8
Integrin beta-7 (ITGB7)	P26010	0.3	1.1	1.6	105	91	96	15	31	61	124 999	250 000	3.3	5	10
Kallikrein-10 (KLK10)	O43240	3.1	3.6	4.4	96	84	102	15	122	122	31 250	62 499	2.4	7	13

		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
Kynurenine--oxoglutarate transaminase 1 (KYAT1)	Q16773	3.2	3.9	5.1	69	72	85	0	0.48	0.48	31 250	62 499	4.8	6	9
Large proline-rich protein BAG6 (BAG6)	P46379	1.1	1.4	2.0	101	97	98	0.9	244	488	250 000	499 999	2.7	7	12
Leucine-rich repeats and immunoglobulin-like domains protein 1 (LRIG1)	Q96JA1	2.7	3.2	3.8	97	95	107	15	122	244	250 000	1 000 000	3.0	7	10
Leukocyte immunoglobulin-like receptor subfamily A member 5 (LILRA5)	A6NI73	3.4	4.0	4.6	97	98	110	15	3.81	7.63	15 624	62 499	3.3	5	8
Low-density lipoprotein receptor-related protein 11 (LRP11)	Q86VZ4	3.1	3.7	4.3	92	98	88	15	122	122	62 499	250 000	2.7	6	11
Lysophosphatidic acid phosphatase type 6 (ACP6)	Q9NPH0	2.3	2.9	3.3	96	93	93	15	15	31	124 999	250 000	3.6	6	10
Meprin A subunit beta (MEP1B)	Q16820	NA	1.7	3.1	85	77	84	15	122	122	124 999	124 999	3.0	6	8
Meteorin-like protein (METRNL)	Q641Q3	2.4	3.1	3.6	105	93	102	15	31	31	124 999	250 000	3.6	5	8
Multiple coagulation factor deficiency protein 2 (MCFD2)	Q8NI22	2.0	2.5	3.2	88	97	107	15	244	244	62 499	250 000	2.4	5	9
NAD kinase (NADK)	O95544	2.3	2.9	3.5	90	123	227	7.5	61	122	124 999	250 000	3.0	5	10
Nectin-2 (NECTIN2)	Q92692	5.3	6.0	6.6	99	99	112	15	3.81	3.81	31 250	124 999	3.9	5	13
Neural proliferation differentiation and control protein 1 (NPDC1)	Q9NQX5	3.8	4.3	4.7	96	99	91	15	31	31	31 250	62 499	3.0	6	12
Neuronal pentraxin receptor (NPTXR)	O95502	2.4	3.0	3.5	98	89	101	15	15	15	62 499	124 999	3.6	6	12
Nodal modulator 1 (NOMO1)	Q15155	3.2	4.0	4.5	95	96	108	15	122	122	124 999	250 000	3.0	6	12
N-terminal prohormone of brain natriuretic peptide (NT-proBNP)	NA	0.7	2.5	3.9	101	75	71	15	122	122	7812	15 624	1.8	4	6
Paired immunoglobulin-like type 2 receptor beta (PILRB)	Q9UKJ0	4.0	4.6	5.4	95	98	117	15	0.48	0.48	7812	31 250	4.2	5	10
Peptidyl-prolyl cis-trans isomerase FKBP4 (FKBP4)	Q02790	NA	0.3	1.6	50	62	110	0.5	244	244	62 499	62 499	2.4	8	11
Phosphoprotein associated with glycosphingolipid-enriched microdomains 1 (PAG1)	Q9NWQ8	1.0	1.6	2.6	77	54	49	15	15	15	31 250	124 999	3.3	7	7
Pro-cathepsin H (CTSH)	P09668	0.6	2.3	3.3	92	102	111	15	488	488	124 999	499 999	2.4	5	11
Protein FAM3C (FAM3C)	Q92520	4.8	5.6	6.6	85	104	131	15	15	31	31 250	124 999	3.0	6	12
Protein phosphatase inhibitor 2 (PPP1R2)	P41236	0.5	1.5	2.3	75	64	70	1.9	31	31	31 250	62 499	3.0	6	8
Protein S100-P (S100P)	P25815	NA	0.2	0.9	97	114	176	7.5	488	488	124 999	1 000 000	2.4	5	9
Regenerating islet-derived protein 4 (REG4)	Q9BYZ8	5.9	6.8	7.7	89	73	95	15	31	31	62 499	124 999	3.3	6	12
Reticulon-4 receptor (RTN4R)	Q9BZR6	0.9	1.3	2.0	105	94	102	15	122	122	250 000	1 000 000	3.3	6	10
Retinal dehydrogenase 1 (ALDH1A1)	P00352	1.3	2.1	2.9	46	63	103	0	488	488	124 999	250 000	2.4	6	10
Ribosyldihydronicotinamide dehydrogenase [quinone] (NQO2)	P16083	NA	1.2	2.4	31	32	39	15	15	31	31 250	124 999	3.0	7	12
Scavenger receptor cysteine-rich domain-containing group B protein (SSC4D)	Q8WTU2	NA	2.0	4.9	119	101	106	15	61	122	124 999	250 000	3.0	9	14
Sclerostin (SOST)	Q9BQB4	3.2	3.8	4.8	103	104	64	15	61	122	124 999	124 999	3.0	7	15
Semaphorin-3F (SEMA3F)	Q13275	1.8	2.2	2.7	97	85	102	15	15	244	124 999	124 999	2.7	6	11
Serpin B6 (SERPINB6)	P35237	2.2	2.5	3.3	86	80	99	15	488	488	124 999	250 000	2.4	6	16
Serpin B8 (SERPINB8)	P50452	0.7	1.0	2.6	110	105	131	15	3.81	7.63	31 250	124 999	3.6	5	14
Sialic acid-binding Ig-like lectin 7 (SIGLEC7)	Q9Y286	2.0	2.5	3.0	102	100	106	15	7.63	31	7812	62 499	2.4	4	11
Sialomucin core protein 24 (CD164)	Q04900	3.4	3.8	4.4	85	114	198	15	7.63	15	15 624	62 499	3.0	4	10
Soluble calcium-activated nucleotidase 1 (CANT1)	Q8WVQ1	3.6	3.9	4.5	91	106	121	15	15	15	15 624	124 999	3.0	5	9
Sulfatase-modifying factor 2 (SUMF2)	Q8NBJ7	2.3	3.3	4.3	82	96	135	15	7.63	7.63	3906	31 250	2.7	4	8
Synaptosomal-associated protein 23 (SNAP23)	O00161	0.3	1.2	2.8	42	21	27	3.8	244	244	31 250	62 499	2.1	6	6
Syndecan-4 (SDC4)	P31431	1.5	2.3	5.3	90	207	319	15	7.63	7.63	15 624	124 999	3.3	6	9
T-cell surface glycoprotein CD1c (CD1C)	P29017	3.6	4.1	4.7	92	96	105	15	15	15	31 250	62 499	3.3	6	10
Thimet oligopeptidase (THOP1)	P52888	3.8	4.6	5.7	101	104	122	0.2	15	15	31 250	124 999	3.3	5	10
Thioredoxin domain-containing protein 5 (TXNDC5)	Q8NBS9	NA	1.3	1.8	92	85	95	1.9	15625	31250	1 000 000	1 000 000	1.5	7	10
Thymidine phosphorylase (TYMP)	P19971	5.2	5.9	6.8	91	105	108	15	977	977	1 000 000	1 000 000	3.0	9	13
Thyrotropin subunit beta (TSHB)	P01222	1.6	2.4	3.3	96	100	105	15	3.81	3.81	3906	7812	3.0	5	6
Trefoil factor 2 (TFF2)	Q03403	3.8	4.6	6.0	95	99	106	15	3.81	7.63	15 624	62 499	3.3	5	10
Tubulointerstitial nephritis antigen-like (TINAGL1)	Q9GZM7	2.8	3.1	3.7	100	96	102	15	3.81	7.63	15 624	31 250	3.3	6	11
Tyrosine-protein kinase receptor TYRO3 (TYRO3)	Q06418	1.2	1.4	1.7	109	93	105	15	31	31	15 624	31 250	2.7	5	9
Ubiquitin carboxyl-terminal hydrolase 8 (USP8)	P40818	NA	NA	1.0	58	58	75	3.8	244	488	250 000	1 000 000	2.7	9	11
Versican core protein (VCAN)	P13611	2.3	2.8	3.4	100	101	121	15	1.91	3.81	31 250	62 499	3.9	5	11

*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 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 were observed to be 6.6% and 10.9%, respectively. The distribution of both intra-assay and inter-assay variations are shown in Figure 4.

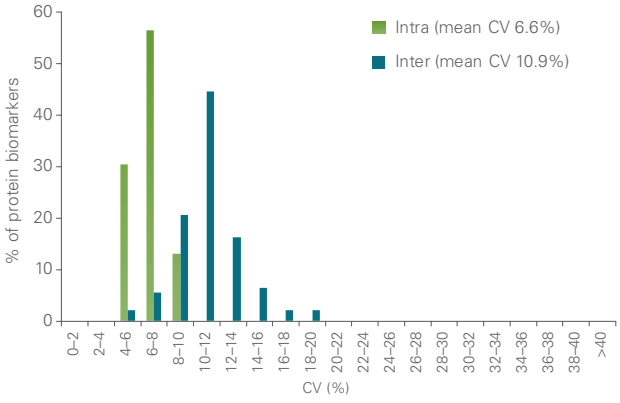

Fig 4. Distribution of intra-assay and inter-assay variations of Olink Metabolism.

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 10 fold pre-dilution step of samples prior to running the Olink Metabolism assay protocol. Six individuals samples were distributed to two laboratories together with Olink Metabolism 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.

The intra-assay mean CV value results for beta-site 1 and 2 was 8.5% and 7.6%, and the mean inter-assay CV was 7.8% and 6.3%, respecitvely. Overall, a very good reproducibility and repeatability was observed with an avarage global inter-site CV of 10%.

2.4 ANALYTICAL SPECIFICITY

ASSAY SPECIFICITY

The antibodies used in Olink Metabolism 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.

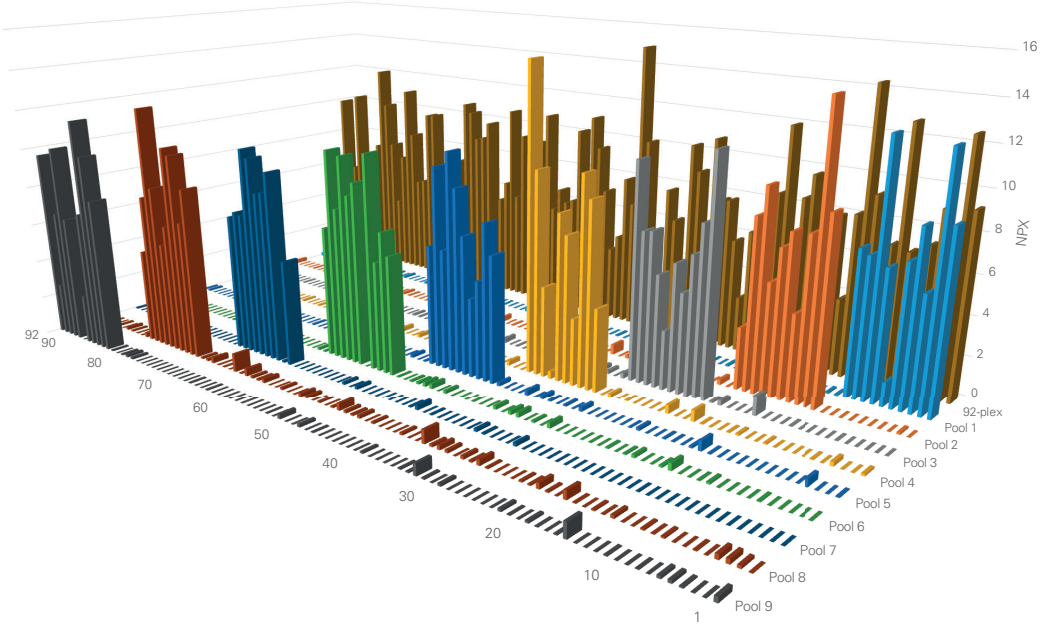

Fig 5. Assay readout specificity of the Olink platform. For each assay, specificity is confirmed by testing antigen sub-pools against the complete 92-plex pool as to each sub-mix.

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).

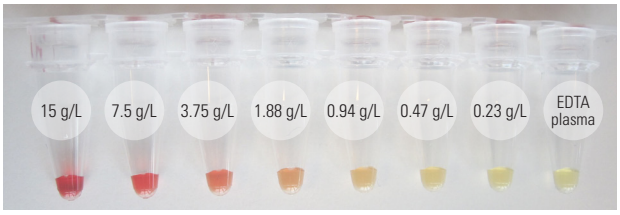

Fig 6. Endogenous interference. Levels tested for hemolysate were 0.23-15 g/L hemoglobin. The highest hemolysate concentration translates to about 10% hemolysis.

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 Metabolism. In 25 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.

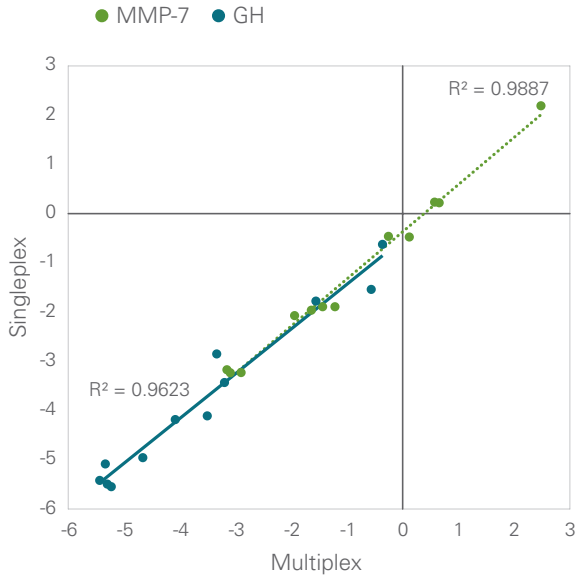

Fig 7. Scalability of the Olink technology platform. The experiment was performed using the Olink CVD II $^{96\times96}$ panel. Human plasma samples were analyzed in singleplex for Growth Hormone (GH) and Matrix Metalloproteinase (MMP-7) with the equivalent assays performed in a full 96-plex reaction. 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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1056, v2.0, 2018-02-13

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