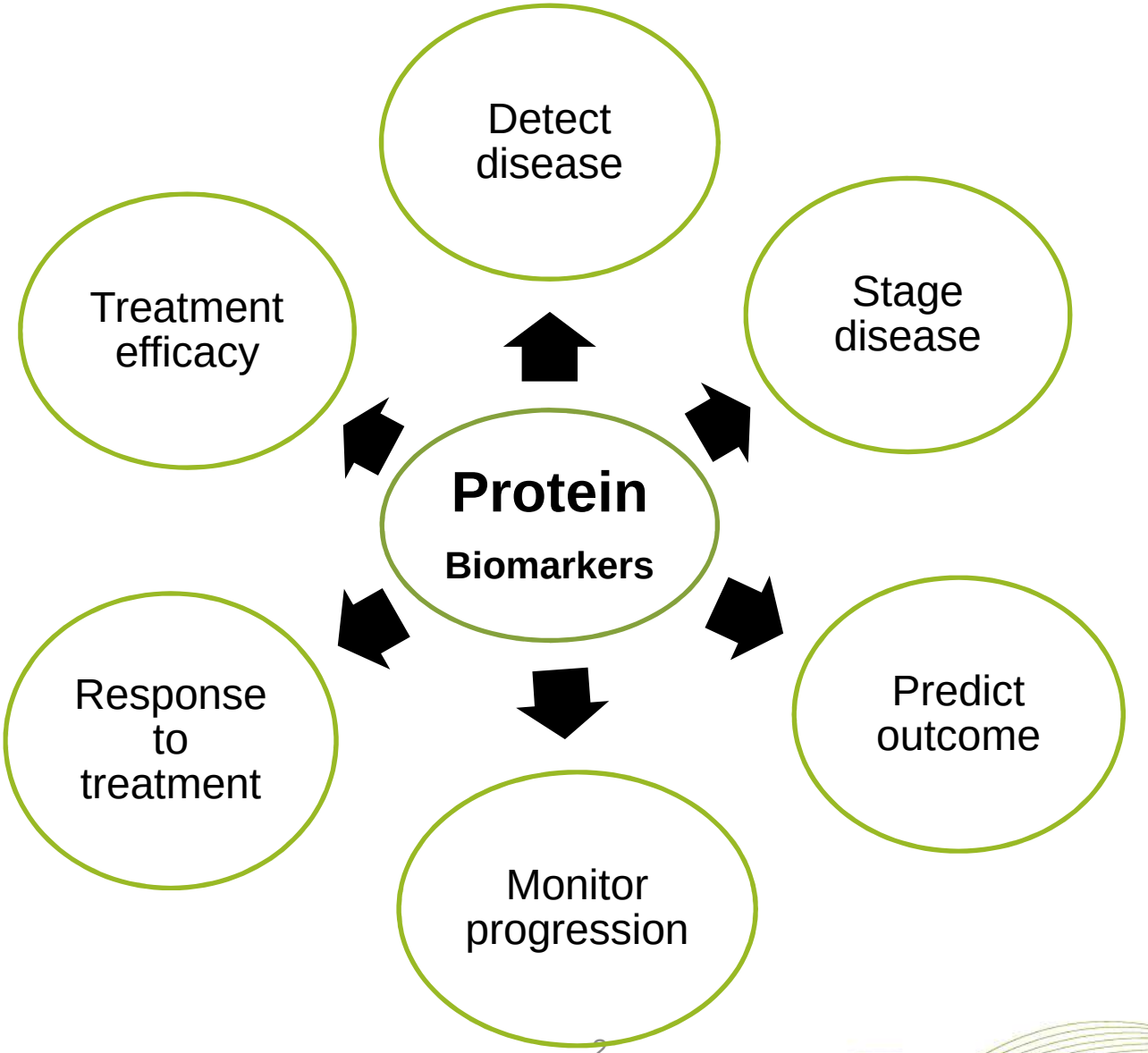
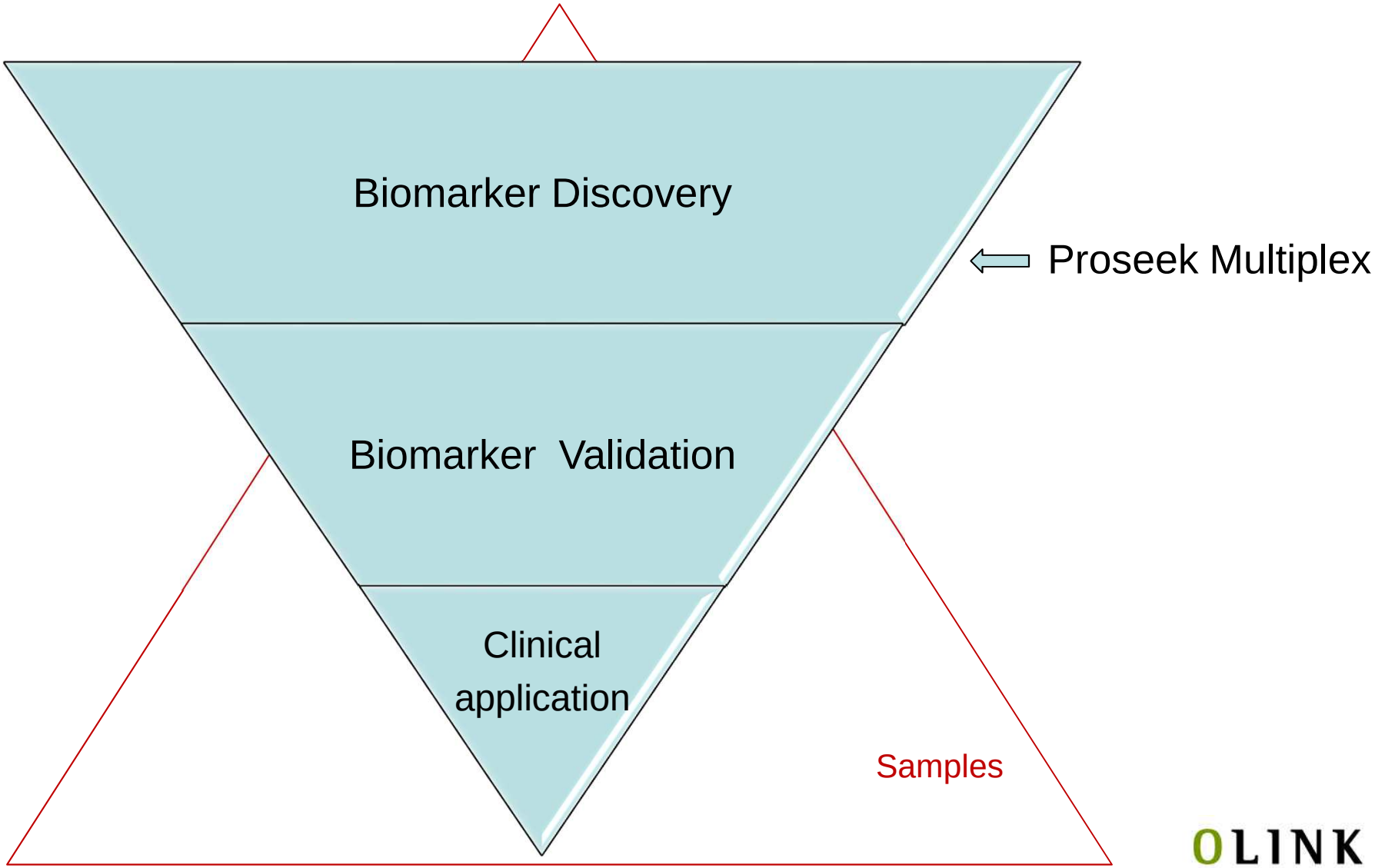
Introducing Proseek® Multiplex

Truly Scalable Immunoassays for Biomarker Research

Protein Biomarkers

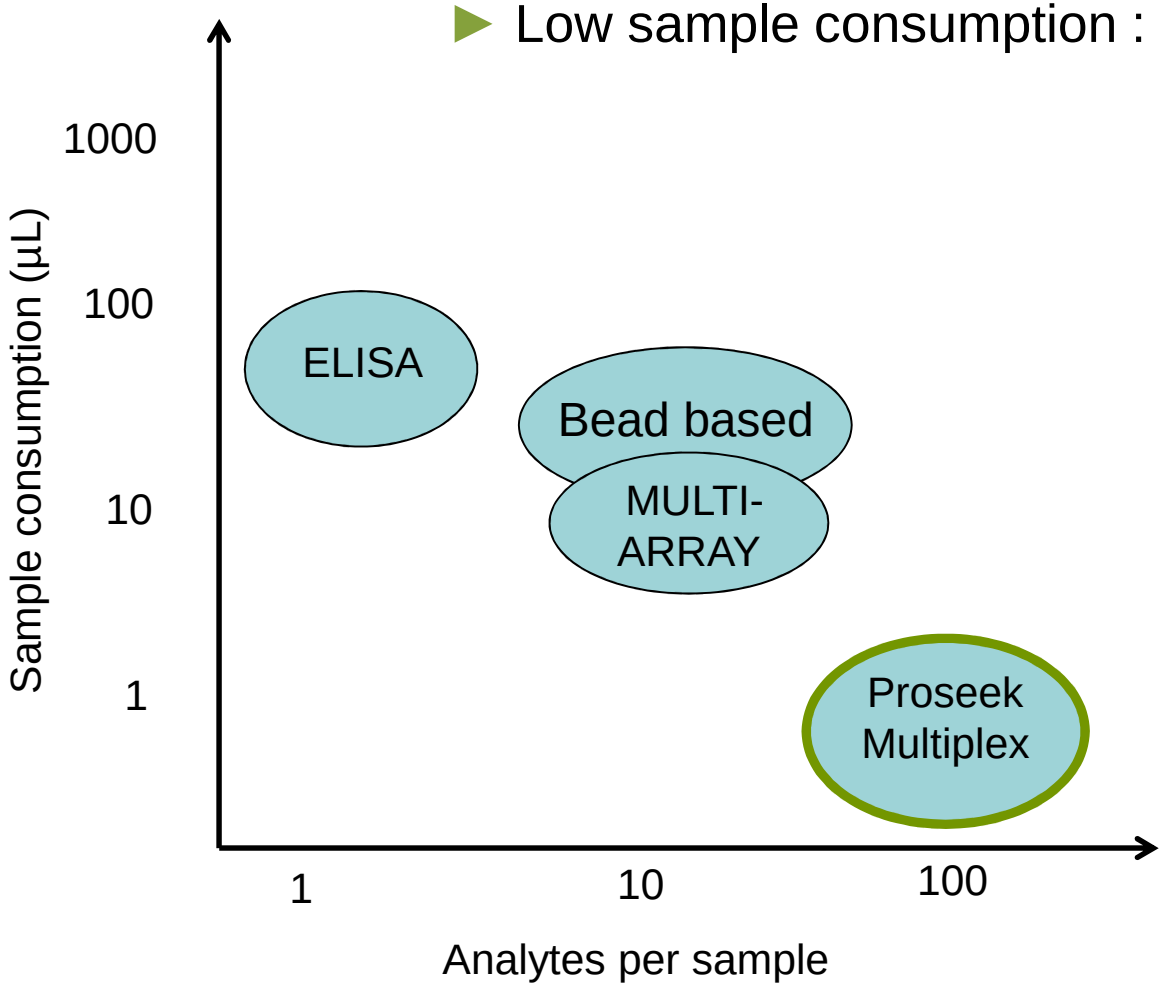


Development of new biomarkers



We address two of the major challenges

- High multiplexing: many analytes per sample : 92
- Low sample consumption : 1 uL



Why study many proteins at the same time?

- When you don't know exactly what you're looking for
  - Biomarker discovery

- Getting the complete picture
  - Proteins rarely act alone, for example in inflammation

- Discovering novel biomarker patterns for in vitro diagnostic use

Why study many proteins in small sample volumes?

- Enables access to precious biobanked serum and plasma
  - Retrospective biomarker research
  - From clinical trials, translational research
- Analysis of small tissue samples
  - Biopsies, even from needles
- Laboratory animals such as mice
  - Pharmacodynamics and toxicology studies

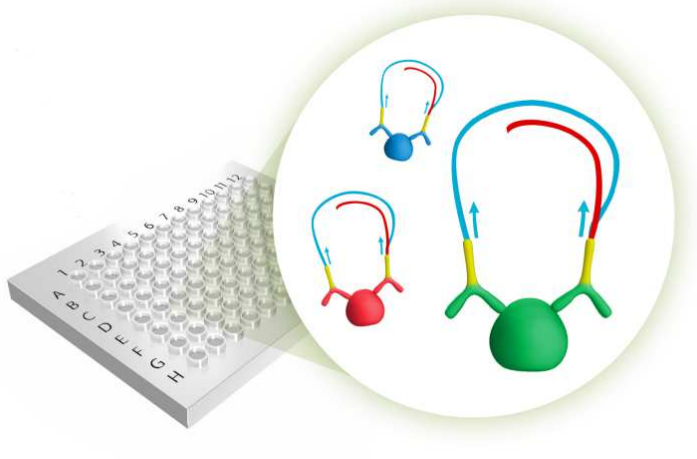
Why study many proteins in many samples?

- Getting statistical power in multivariate biomarker discovery requires many samples

- Correlating protein biomarker data to....
  - Multiple disease outcomes
  - Multiple therapeutic avenues
  - Multiple genetic blue prints (we are all different as individuals)

What does Proseek Multiplex do?

- Target multiple markers
  - 92 human proteins
- Small sample consumption
  - just one 1µL is required



- And most importantly, highly specific
  - Ensures the signal measured is from the correct protein !
  - Can not be done with competing technologies

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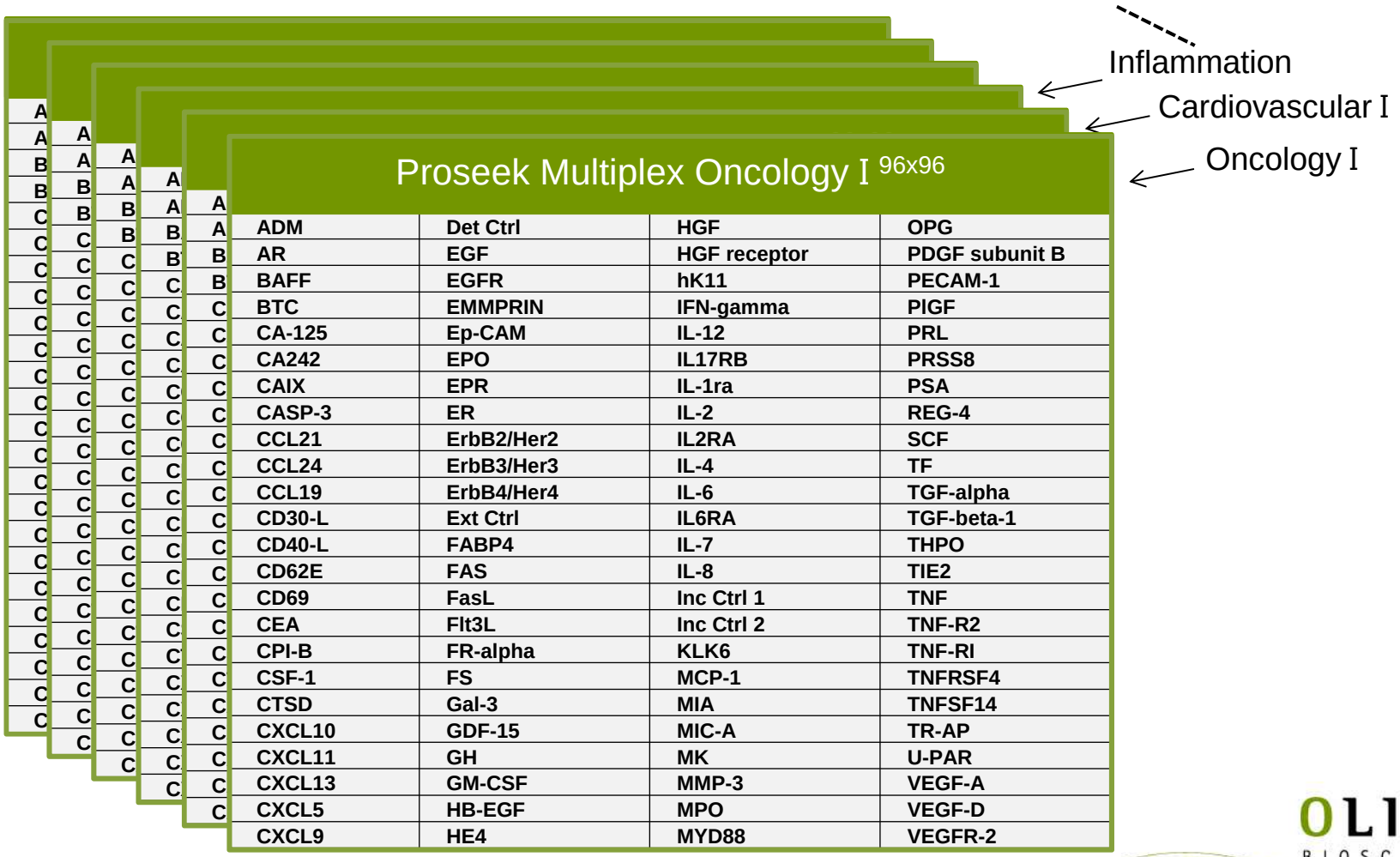
Countinous development of biomarker panels…..

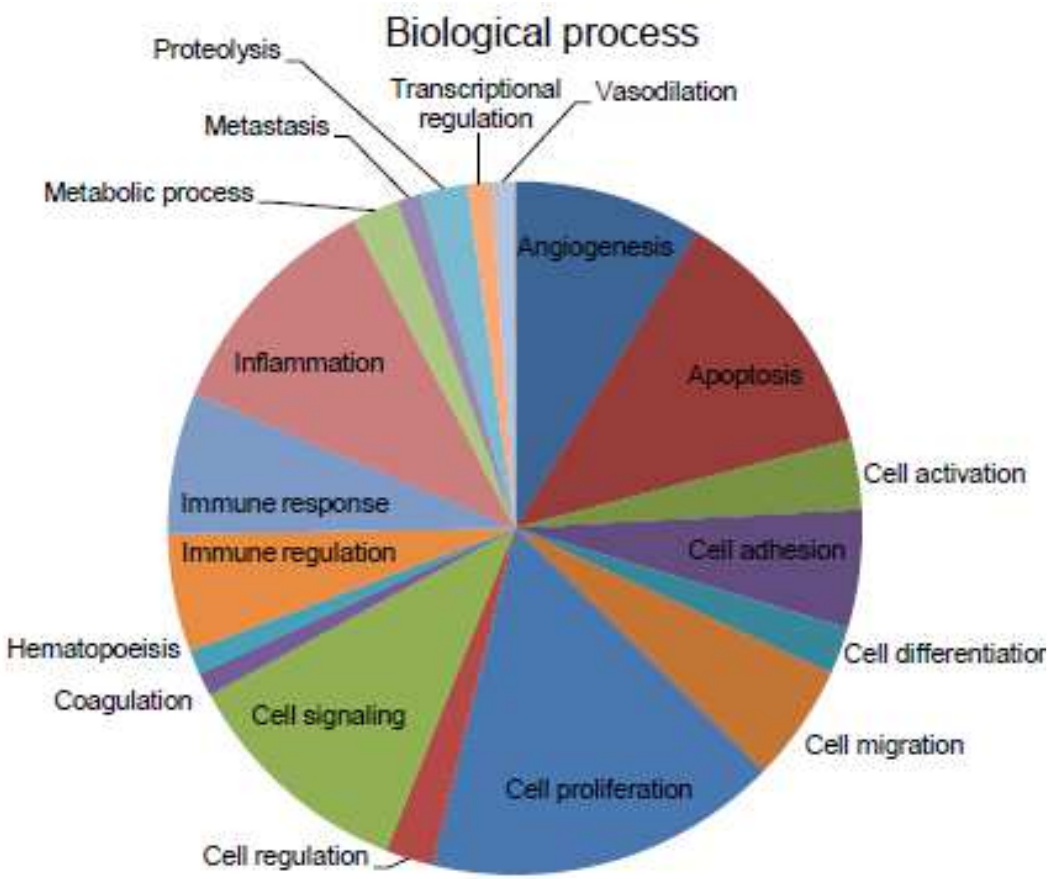


ADM	Det Ctrl	HGF	OPG
AR	EGF	HGF receptor	PDGF subunit B
BAFF	EGFR	hK11	PECAM-1
BTC	EMMPRIN	IFN-gamma	PlGF
CA-125	Ep-CAM	IL-12	PRL
CA242	EPO	IL17RB	PRSS8
CAIX	EPR	IL-1ra	PSA
CASP-3	ER	IL-2	REG-4
CCL21	ErbB2/Her2	IL2RA	SCF
CCL24	ErbB3/Her3	IL-4	TF
CCL19	ErbB4/Her4	IL-6	TGF-alpha
CD30-L	Ext Ctrl	IL6RA	TGF-beta-1
CD40-L	FABP4	IL-7	THPO
CD62E	FAS	IL-8	TIE2
CD69	FasL	Inc Ctrl 1	TNF
CEA	Flt3L	Inc Ctrl 2	TNF-R2
CPI-B	FR-alpha	KLK6	TNF-RI
CSF-1	FS	MCP-1	TNFRSF4
CTSD	Gal-3	MIA	TNFSF14
CXCL10	GDF-15	MIC-A	TR-AP
CXCL11	GH	MK	U-PAR
CXCL13	GM-CSF	MMP-3	VEGF-A
CXCL5	HB-EGF	MPO	VEGF-D
CXCL9	HE4	MYD88	VEGFR-2

Our first protein biomarker panel...of many yet to come

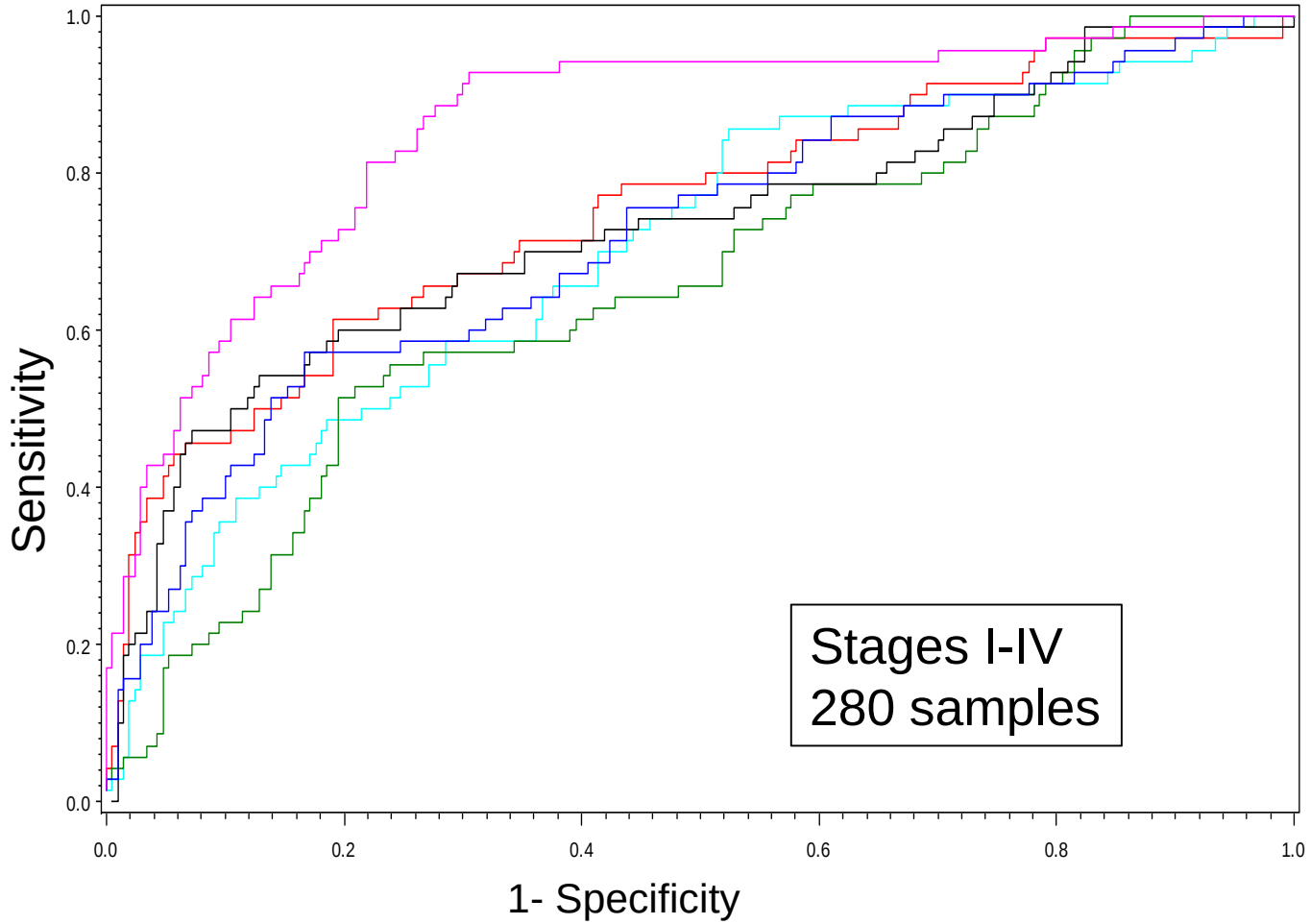
Proseek® Multiplex Oncology I 96x96

Proseek Multiplex Oncology I 96×96		
Adrenomedullin (ADM)	Fatty acid binding protein 4 adipocyte (FABP4)	Myeloperoxidase (MPO)
Amphiregulin (AR)	Fms-related tyrosine kinase 3 ligand (Flt3L)	Detection Control (Det Ctrl)
Angiopoietin-1 receptor (TIE2)	Folate receptor alpha (FR-alpha)	Osteoprotegerin (OPG)
B-cell activating factor (BAFF)	Follistatin (FS)	Ovarian cancer-related tumor marker 125 (CA-125)
Betacellulin (BTC)	Galectin-3 (Gal-3)	Incubation Control (Inc Ctrl 2)
CA242 tumor marker (CA242)	Granulocyte-macrophage colony-stimulating factor (GM-CSF)	Placenta Growth Factor (PlGF)
Carbonic Anhydrase IX (CAIX)	Incubation Control (Inc Ctrl 1)	Platelet endothelial cell adhesion molecule (PECAM-1)
Carcinoembryonic antigen (CEA)	Growth Hormone (GH)	Platelet-derived growth factor subunit B (PDGF subunit B)
Caspase-3 (CASP-3)	Growth/differentiation factor 15 (GDF-15)	Prolactin (PRL)
Cathepsin D (CTSD)	Heparin-binding EGF-like growth factor (HB-EGF)	Prostasin (PRSS8)
C-C motif chemokine 19 (CCL19)	Hepatocyte growth factor (HGF)	Prostate-specific antigen (PSA)
C-C motif chemokine 21 (CCL21)	Hepatocyte growth factor receptor (HGF receptor)	Receptor tyrosine-protein kinase ErbB-2 (ErbB2/Her2)
C-C motif chemokine 24 (CCL24)	Interferon gamma (IFN-gamma)	Receptor tyrosine-protein kinase ErbB-3 (ErbB3/Her3)
CD40 ligand (CD40-L)	Interleukin 1 receptor antagonist protein (IL-1ra)	Receptor tyrosine-protein kinase ErbB-4 (ErbB4/Her4)
C-X-C motif chemokine 10 (CXCL10)	Interleukin 12 (IL-12)	Regenerating islet-derived protein 4 (REG-4)
C-X-C motif chemokine 11 (CXCL11)	Interleukin 17 receptor B (IL17RB)	Stem cell factor (SCF)
C-X-C motif chemokine 13 (CXCL13)	Interleukin 2 (IL-2)	Tartrate-resistant acid phosphatase type 5 (TR-AP)
C-X-C motif chemokine 5 (CXCL5)	Interleukin 2 receptor subunit alpha (IL2RA)	Thrombopoietin (THPO)
C-X-C motif chemokine 9 (CXCL9)	Interleukin 4 (IL-4)	Tissue Factor (TF)
Cystatin B (CPI-B)	Interleukin 6 (IL-6)	Transforming growth factor alpha (TGF-alpha)
Early activation antigen CD69 (CD69)	Interleukin 6 receptor subunit alpha (IL6RA)	Transforming growth factor beta 1 (TGF-beta-1)
Epidermal growth factor (EGF)	Interleukin 7 (IL-7)	Tumor necrosis factor alpha (TNF)
Epidermal growth factor receptor (EGFR)	Interleukin 8 (IL-8)	Tumor necrosis factor ligand superfamily member 14 (TNFSF14)
Epididymal secretory protein E4 (HE4)	Kallikrein-11 (hK11)	Tumor necrosis factor ligand superfamily member 8 (CD30-L)
Epiregulin (EPR)	Kallikrein-6 (KLK6)	Tumor necrosis factor receptor 1 (TNF-RI)
Epithelial cell adhesion molecule (Ep-CAM)	Macrophage colony-stimulating factor 1 (CSF-1)	Tumor necrosis factor receptor 2 (TNF-R2)
Erythropoietin (EPO)	Matrix metalloproteinase-3 (MMP-3)	Tumor necrosis factor receptor superfamily member 4 (TNFRSF4)
E-selectin (CD62E)	Melanoma-derived growth regulatory protein (MIA)	Tumor necrosis factor receptor superfamily member 6 (FAS)
Estrogen receptor (ER)	MHC class I polypeptide-related sequence A (MIC-A)	Urokinase plasminogen activator surface receptor (U-PAR)
Extension Control (Ext Ctrl)	Midkine (MK)	Vascular endothelial growth factor A (VEGF-A)
Extracellular matrix metalloproteinase inducer (EMMPRIN)	Monocyte chemotactic protein-1 (MCP-1)	Vascular endothelial growth factor D (VEGF-D)
Fas antigen ligand (FasL)	Myeloid differentiation primary response protein MyD88 (MYD88)	Vascular endothelial growth factor receptor 2 (VEGFR-2)



Real life example 1

- Markers for early detection of CRC
- Study performed with Proseek Multiplex Oncology I ^{96x96}
- Thorsen et al submitted to Molecular and Cellular Proteomics



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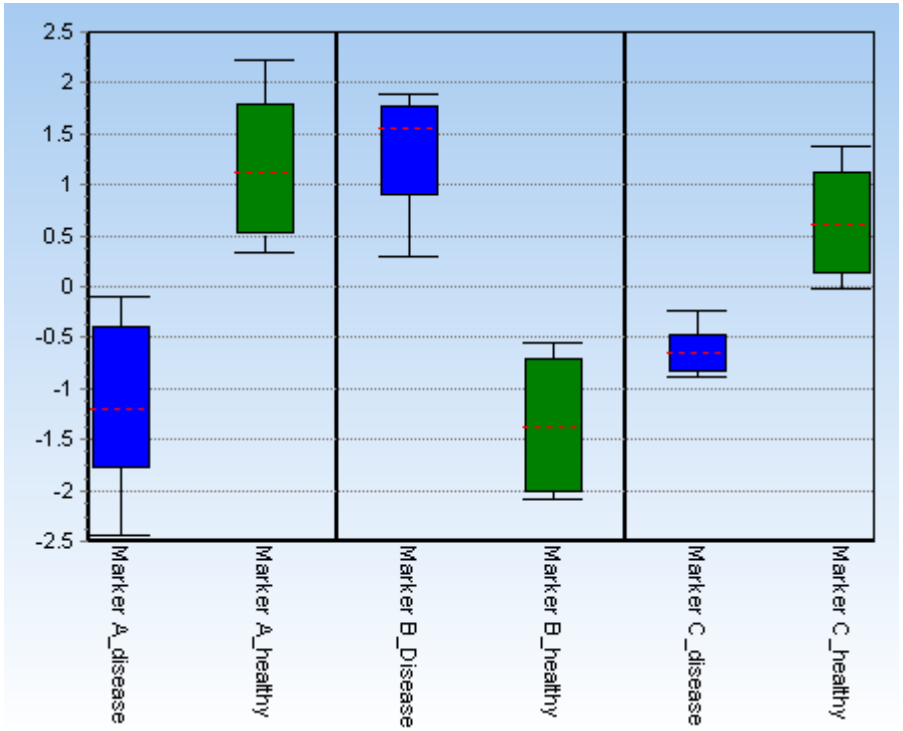
Real life example 2

Comparing groups of healthy and CVD patients



Disease
Control

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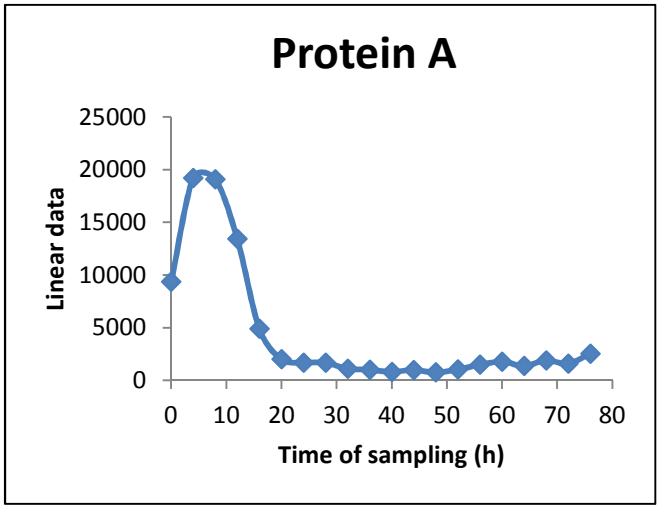
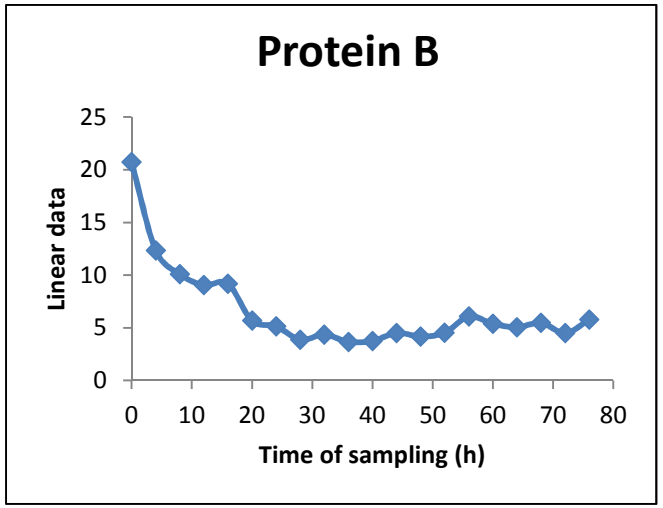
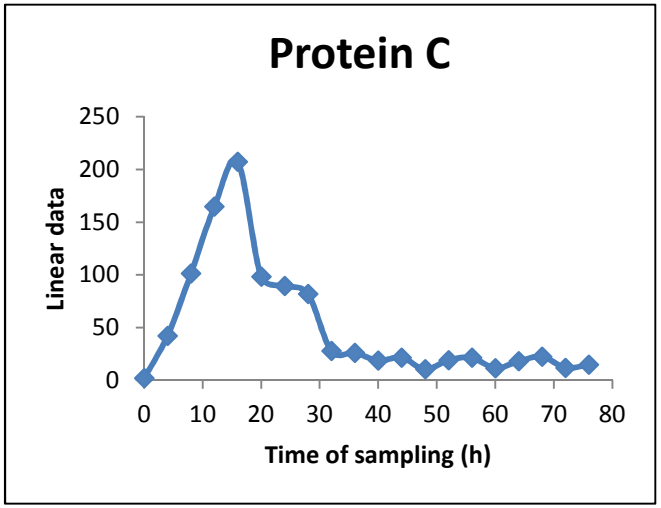
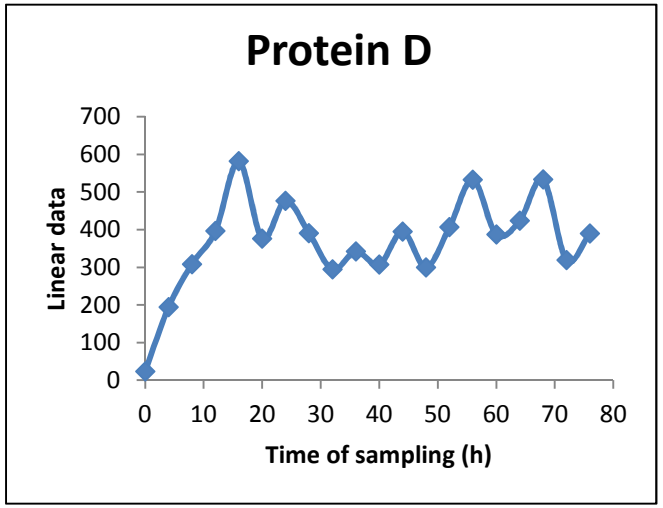
Real Life example 3

Comparing protein profiles over time in brain microdialysis fluid



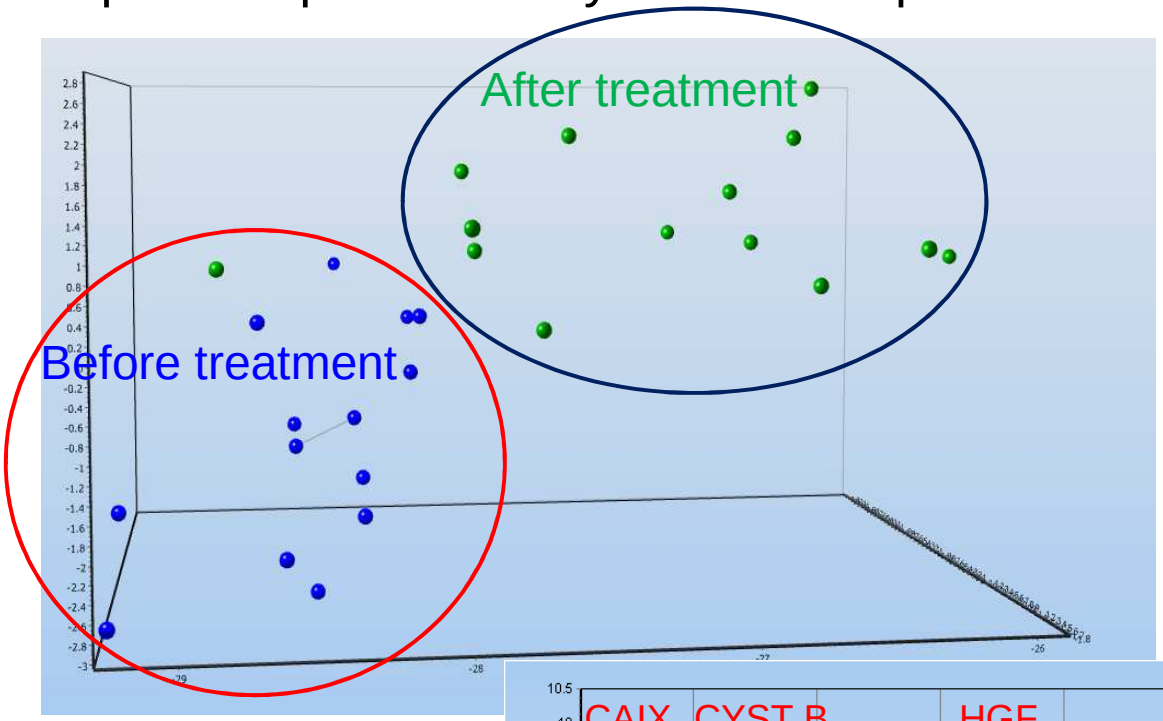


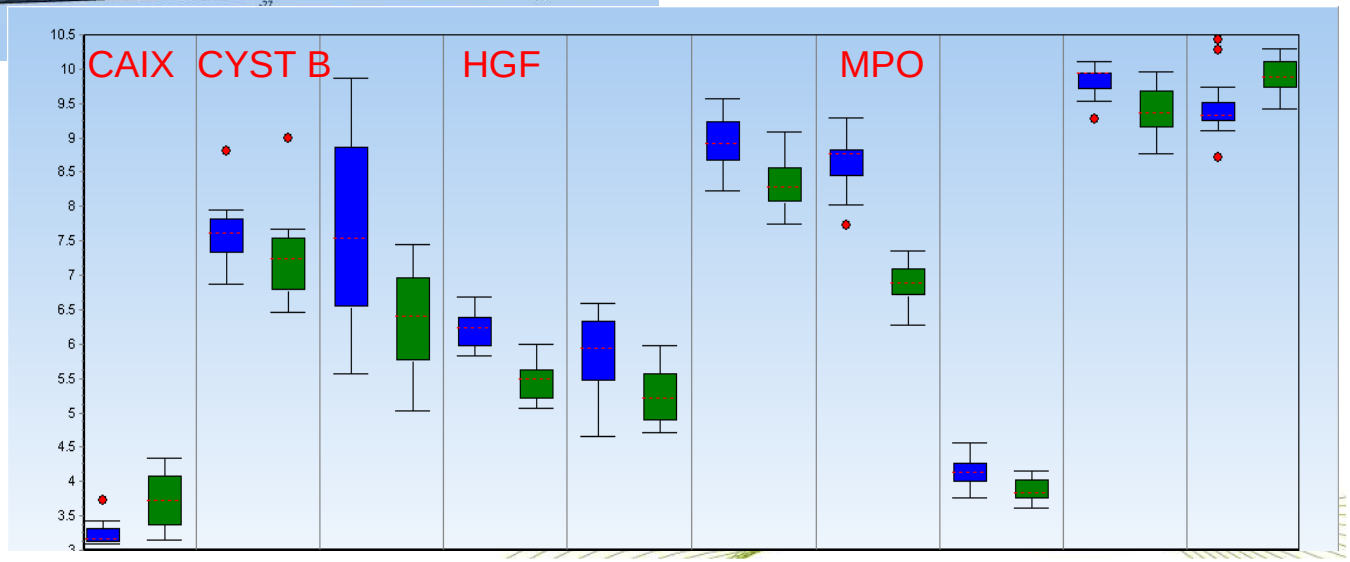




Real life example 4

Drug response biomarkers in Chronic Myelogenous Leukemia visualized with Principle Component Analysis and box plot





Data analysis software, customized for Proseek Multiplex

- Direct import of data from Biomark
- Has QC parameters and several statistical analysis methods
- http://multid.se/OlinkWizardUserGuide.pdf
  - *The only product specific analys tool for multivariate statistics in immunoassays*

GenEx multid ANALYSES

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Paired t-tests identifies putative biomarkers

(A) vs (B)	Normality Tes...	Fold change	Difference (A-...	P-Value
MPO	Passed	3.4058	1.76799	1E-8
HGF	Passed	1.64382	0.71705	6.3608761975...
IL6RA	Passed	1.49349	0.57869	9.5423780435...
E-selectin	Passed	2.70588	1.4361	3.5746934282...
VEGF-D	Passed	-1.38577	-0.47069	0.0001312826...
U-PAR	Passed	1.35344	0.43663	0.0001983101...
IL-1ra	Passed	1.49963	0.58461	0.0002112718...
Cystatin B	Passed	1.27812	0.35403	0.0004590326...
TNFSF14	Passed	1.19635	0.25864	0.0005459262...
CAIX	Passed	-1.41649	-0.50232	0.0005661618...
TNF-RI	Passed	1.25934	0.33266	0.0006719713...
PIGF	Passed	1.288	0.36513	0.0012267408...
TNF-R2	Passed	1.23019	0.29888	0.0022588399...
IL2RA	Passed	1.15688	0.21023	0.0027132323...
Stem cell factor	Passed	-1.23166	-0.3006	0.0027877186...
FR-alpha	Passed	-1.08439	-0.11689	0.0033619099...
IL-12	Passed	-1.74466	-0.80295	0.0041622586...
TGF-alpha	Passed	1.32551	0.40654	0.0047751327...
ErbB4/Her4	Passed	-1.18967	-0.25057	0.0048166776...
PECAM-1	Passed	1.1673	0.22318	0.0073897061...
Kallikrein-11	Passed	-1.19558	-0.25771	0.0115430763...
IL-8	Passed	-1.58315	-0.6628	0.0121299853...
LAP TGF-bet	Passed	1.12584	0.171	0.0150895018...

Bonferroni multiple correctio

p<0.00056

p>0.00056

GenEx multid ANALYSES

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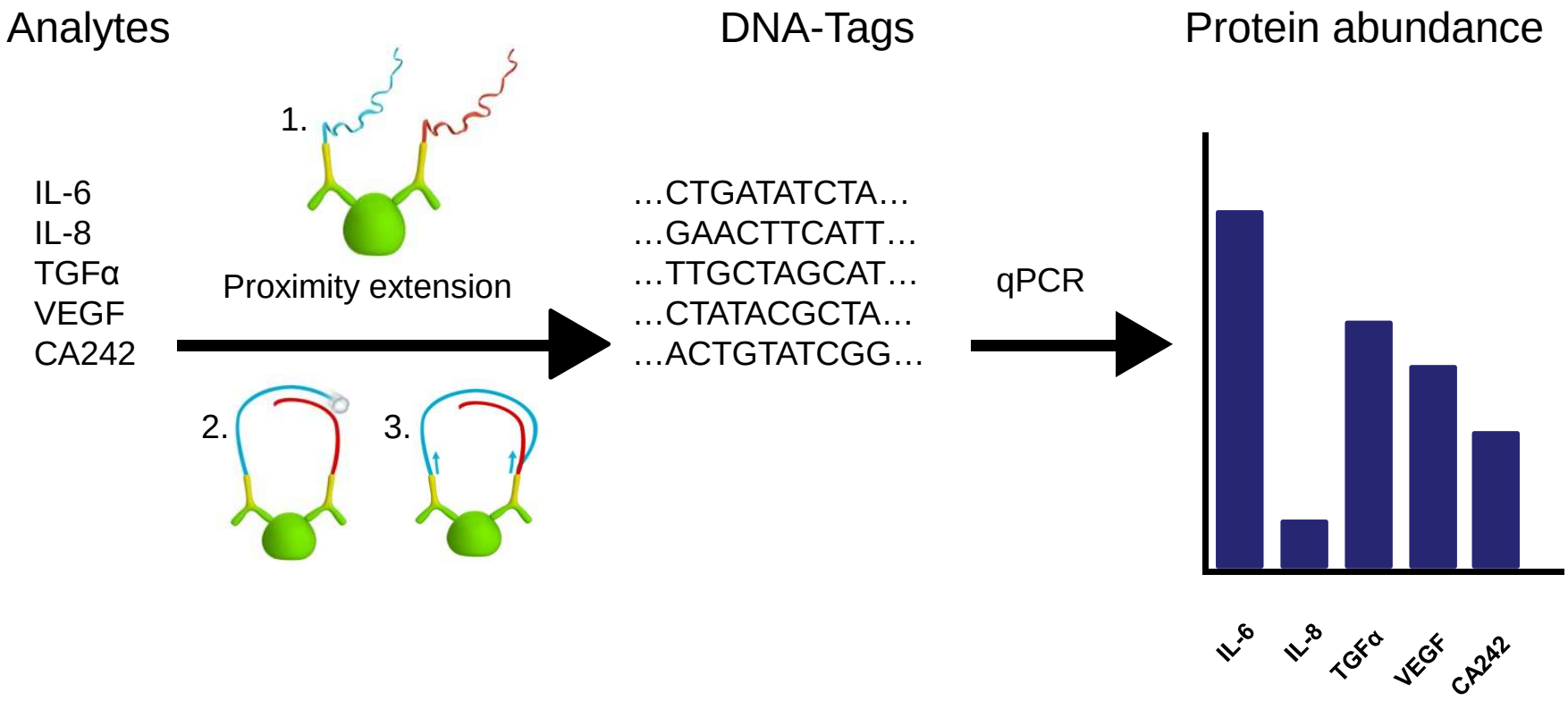
Proseek Multiplex Technology

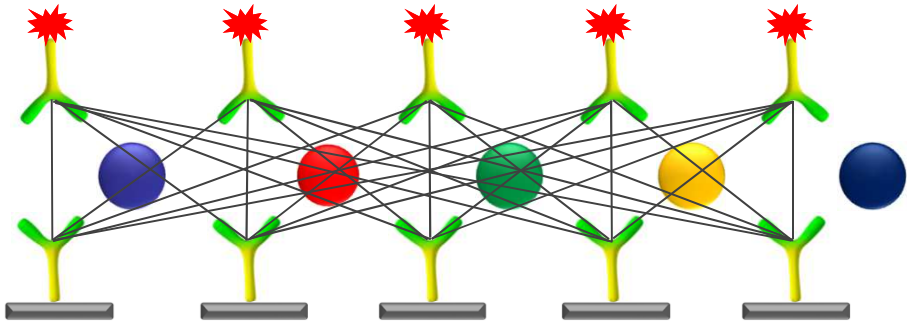
The technology is based on Proximity Extension Assay (PEA)



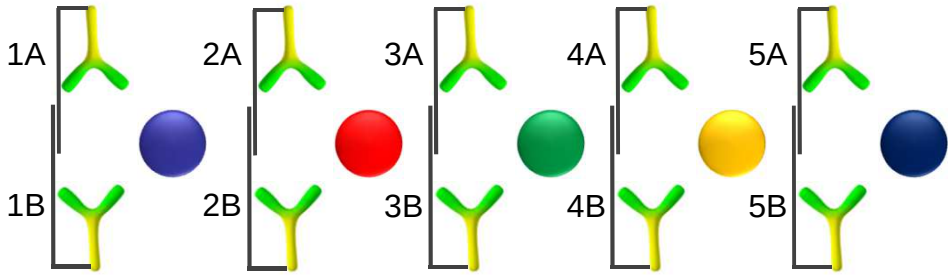
"A cDNA synthesis for proteins"

Multiplexing often means specificity problems

Problem: Multiplex sandwich immunoassays → cross reactivity



Solution: Only matched pairs detected through DNA-barcoding



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It works for different biological materials
- and only in 1 µl

- Biobanked material
- Precious samples
- Limited sample volume

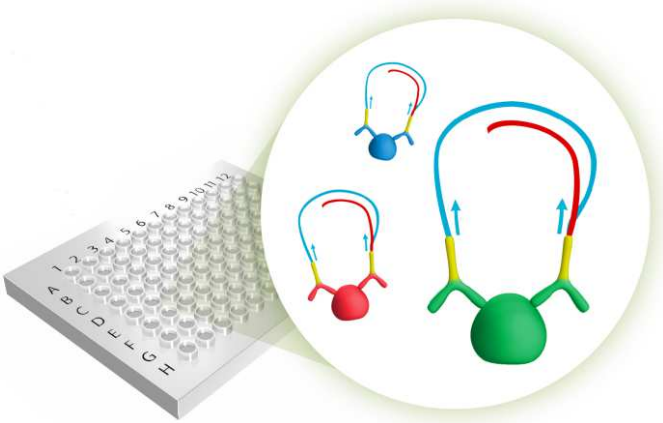
- Serum and plasma
- Tissue lysate
- Cell lysate
- Fine Needle Biopsy
- Microdialysis fluid
- Cell culture media
- Serum from xenograft mice

”I really think that one of the best advantages of your platform is the low amount of sample required. This is a huge positive since many of our samples are quite precious.”
Sharon Pitteri, Stanford, Canary foundation

How does this sound?

- True scalability
- Small sample consumption
- No cross-reactive events detected
- Many different sample types

……there is more



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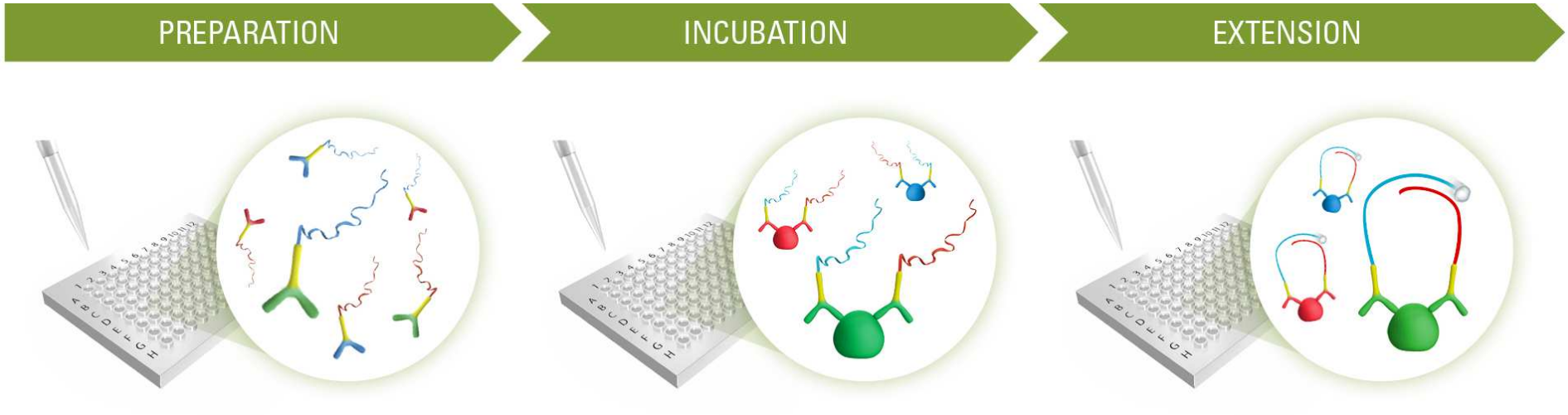
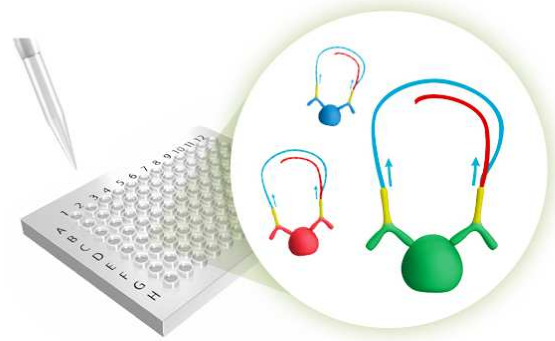
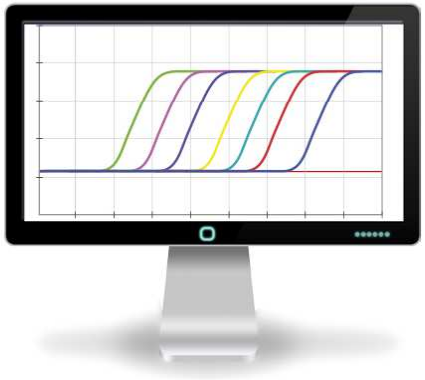
It is not even complicated



DETECTION

SAMPLE ANALYSIS





Hands-on-time: less than 3 hours

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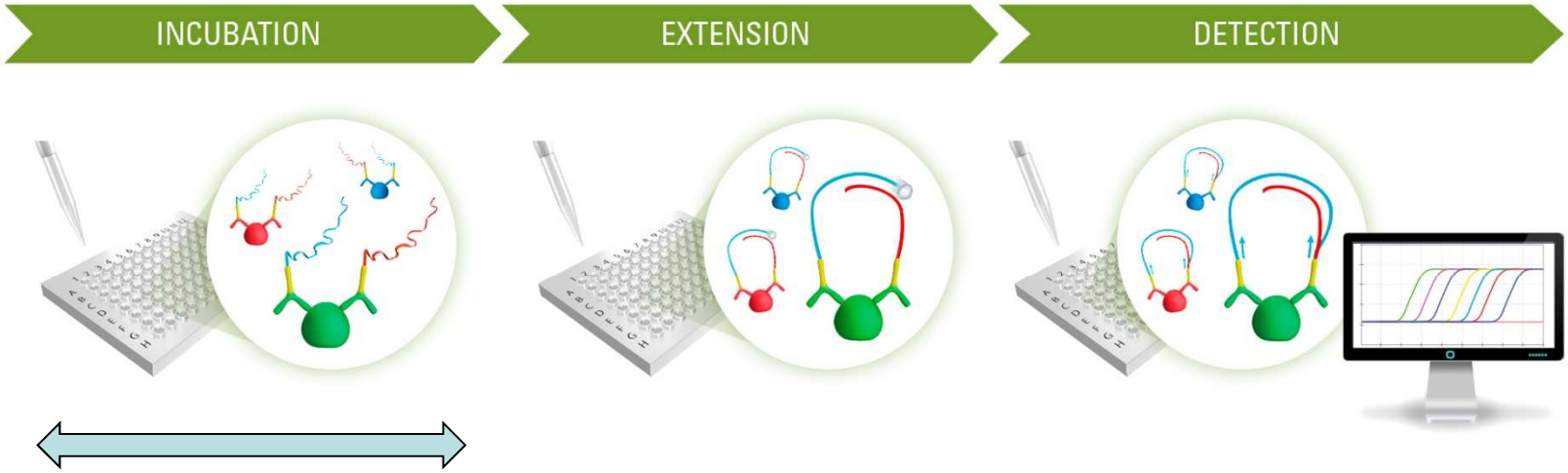
Four internal controls are included in every assay



2 Incubation Controls

Spiked antigen measuring variation in the incubation step

Extension Control

Monitors PEA, compensates for PEA, PCR, and qPCR variations between samples

Detection Control

Monitors PCR and qPCR steps, used for detection of outliers and technical controls

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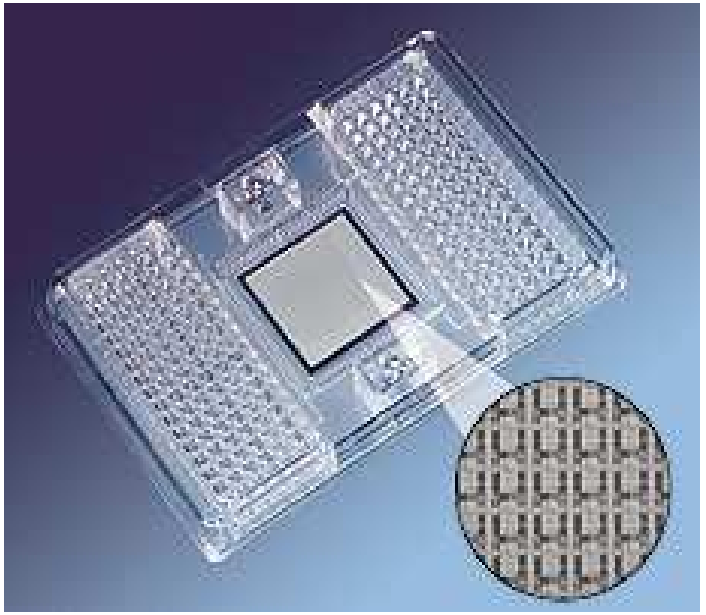
We use high throughput real time PCR



- Microfluidic system – Fluidigm Biomark system
- 96.96 Dynamic Array ™ chip measuring 96x96 reactions
- 9,216 data points per run in 7 nL reaction chambers

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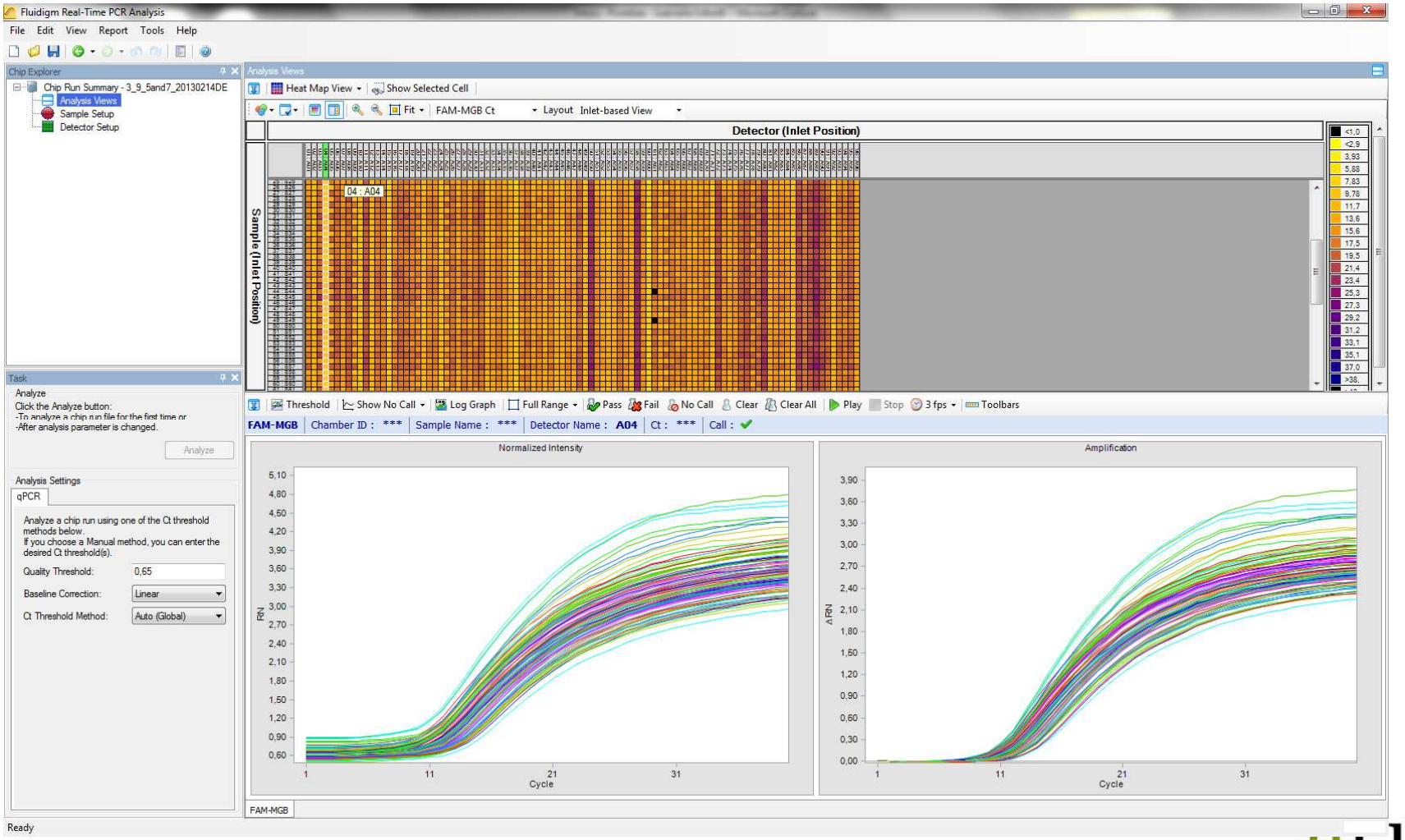
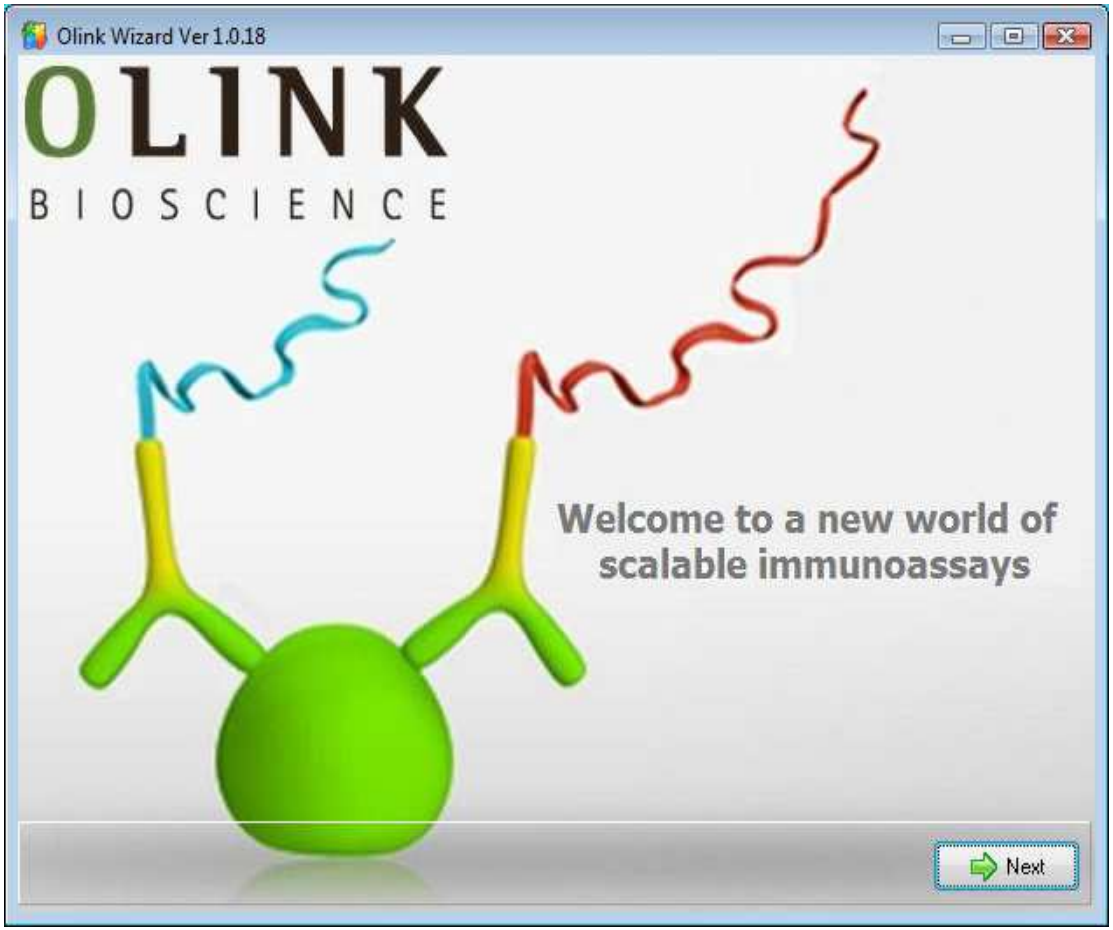
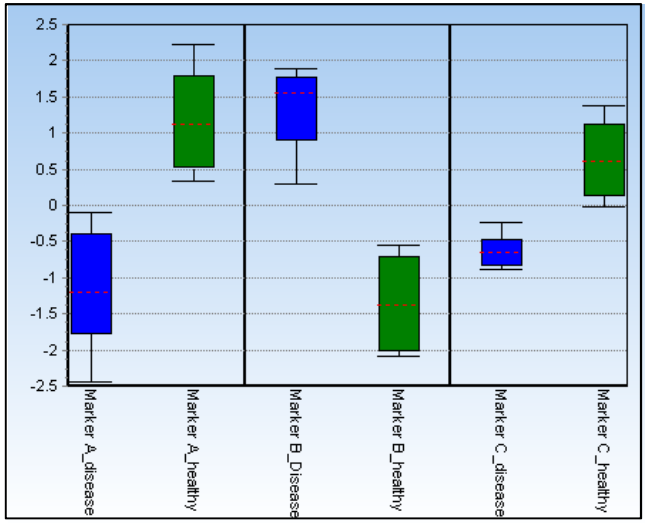
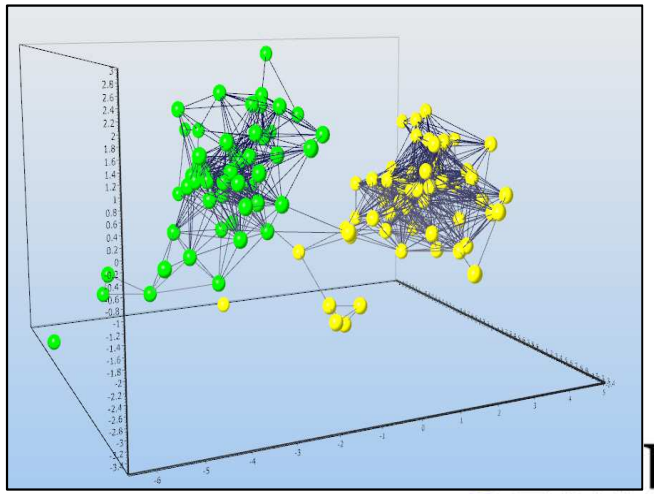
Typical data readout



Olink Wizard in GenEx helps to analyze data







The product

- Proseek Multiplex Probe Kit 96x96
- Proseek Multiplex Detection Kit 96x96
- Proseek Multiplex Controls

www.olink.com

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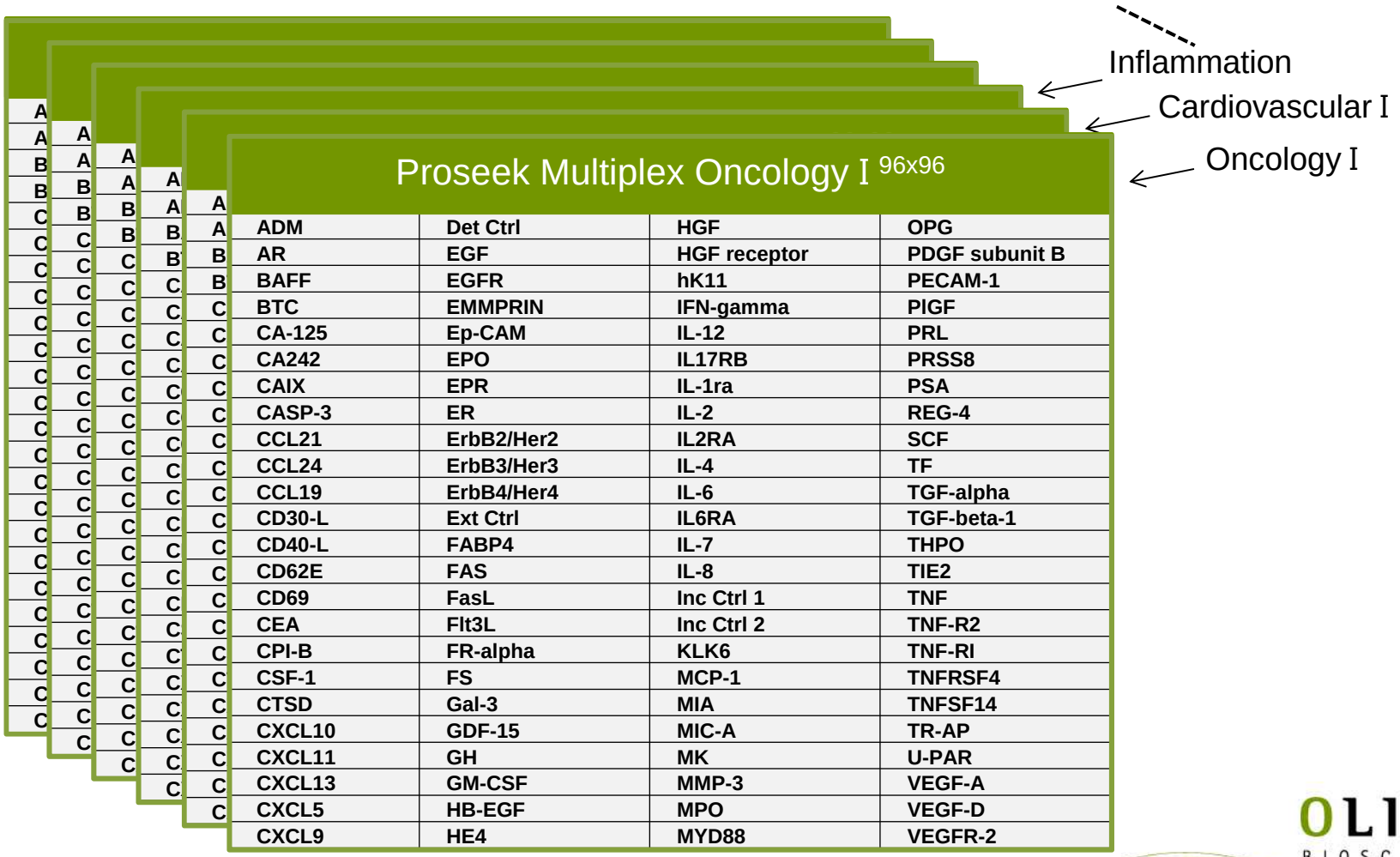
Coming next…..



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CTSD	Gal-3	MIA	TNFSF14
CXCL10	GDF-15	MIC-A	TR-AP
CXCL11	GH	MK	U-PAR
CXCL13	GM-CSF	MMP-3	VEGF-A
CXCL5	HB-EGF	MPO	VEGF-D
CXCL9	HE4	MYD88	VEGFR-2

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Cardio 96x96 (coming in October)

Adrenomodulin
AgRP
Aminopeptidase P2
bFGF
bNGF
BNP
CA125
Cathepsin D
Cathepsin L
Cathepsin S
CCL3
CCL4
CD40
CD40L
CX3CL1
CXCL-16
Cystatin B
DKK-1
DKK-1
ECP
EGF
Endoglin
EPO
E-Selectin/CD62E
ESM-1
FABP3
FABP4
Fas
FGF-23
Follistatin
F-Spondin
Galanin
Galectin-3
GDF-15
GH
HB-EGF
HGF
HSP27
IL-10
IL-17
IL-1alpha
IL-1beta
IL-1RA
IL-2
IL-25
IL-4
IL-6
IL-6R
IL-8
Kallikrein 11
Kallikrein-6
LIGHT
MCP-1
M-CSF
MMP-1
MMP-10
MMP-12
MMP-3
MMP-7
MMP-9
MPO
Myoglobin
NT-pro-BNP
Osteoprotegerin
PaPPA
PDGF-BB
Pecam-1/CD31
PLA2G7
PlGF
Prolactin
PSGL-1
PTX3
RAGE
Renin
Resistin
SCF
ST2
Thrombomodulin
Tie-2
TIM-1
Tissue Factor
TNF-R1
TNF-R2
tPA
TRAIL
TRANCE
U-PAR
VEGF
VEGF D
YKL-40

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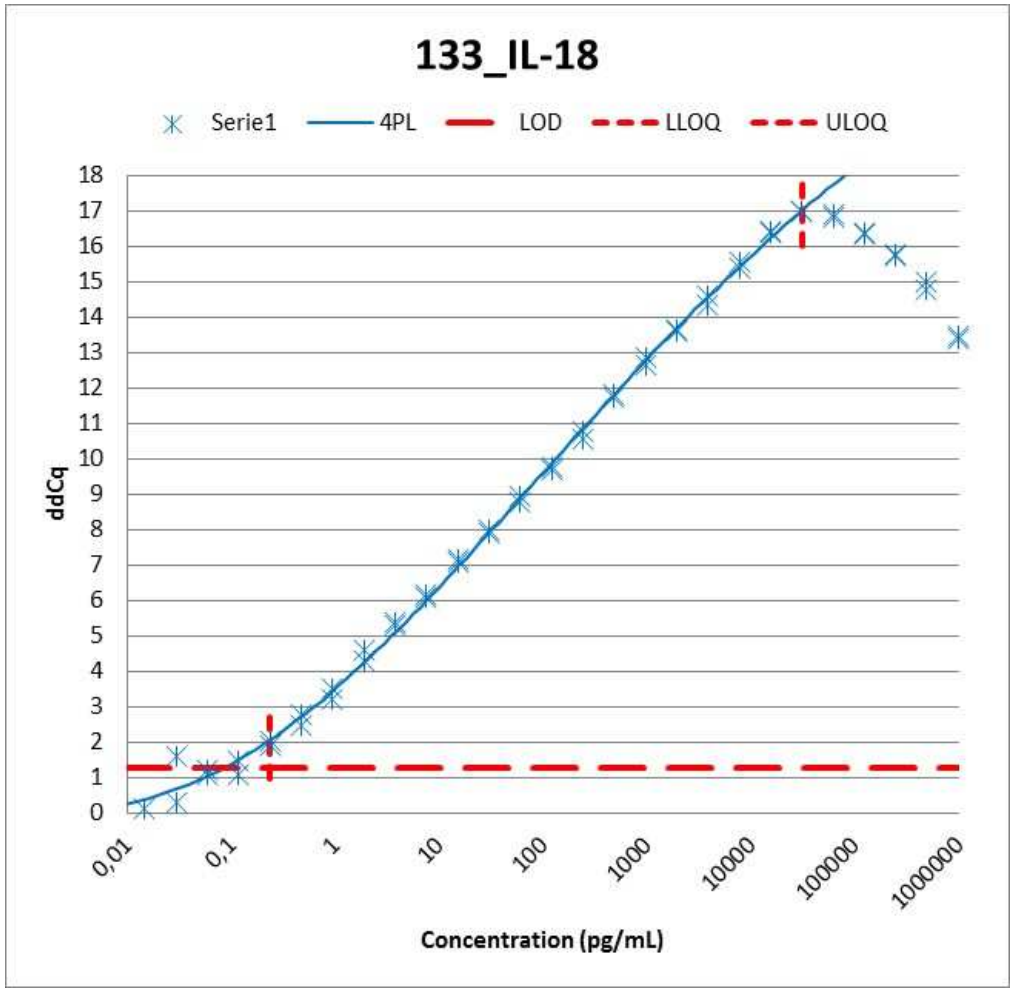
IL-18



Recovery:91%

No interference from:
Lipid, Haemolysate, Bilirubin

LOD	0,12	pg/mL
LLOQ	0,24	pg/mL
ULOQ	31250	pg/mL
Range	5,12	log10

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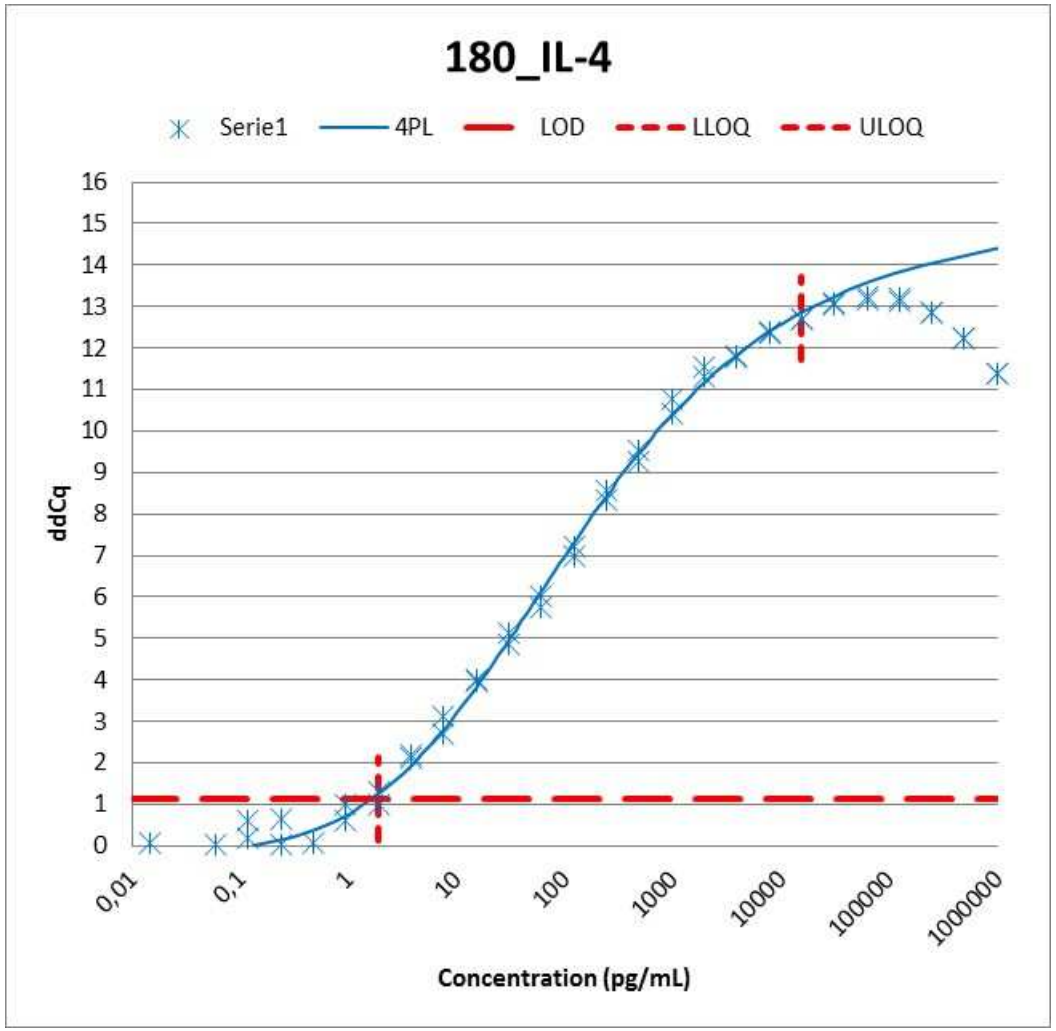
IL-4



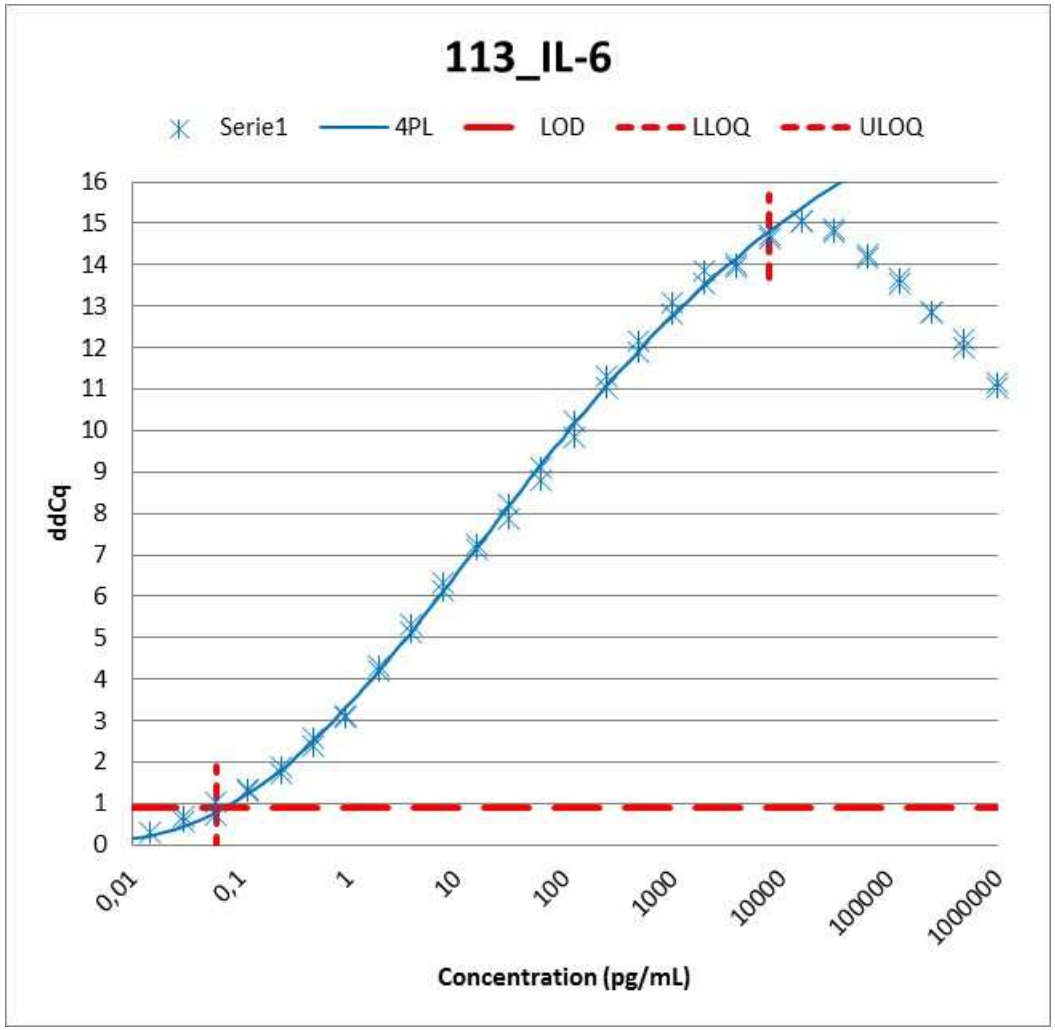
Recovery:105%

No interference from:
Lipid, Haemolysate, Bilirubin

LOD	1,91	pg/mL
LLOQ	1,91	pg/mL
ULOQ	15630	pg/mL
Range	3,91	log10

OLINK BIOSCIENCE

IL-6



Recovery:108%

No interference from:
Lipid, Haemolysate, Bilirubin

LOD	0,06	pg/mL
LLOQ	0,06	pg/mL
ULOQ	7810	pg/mL
Range	5,12	log10

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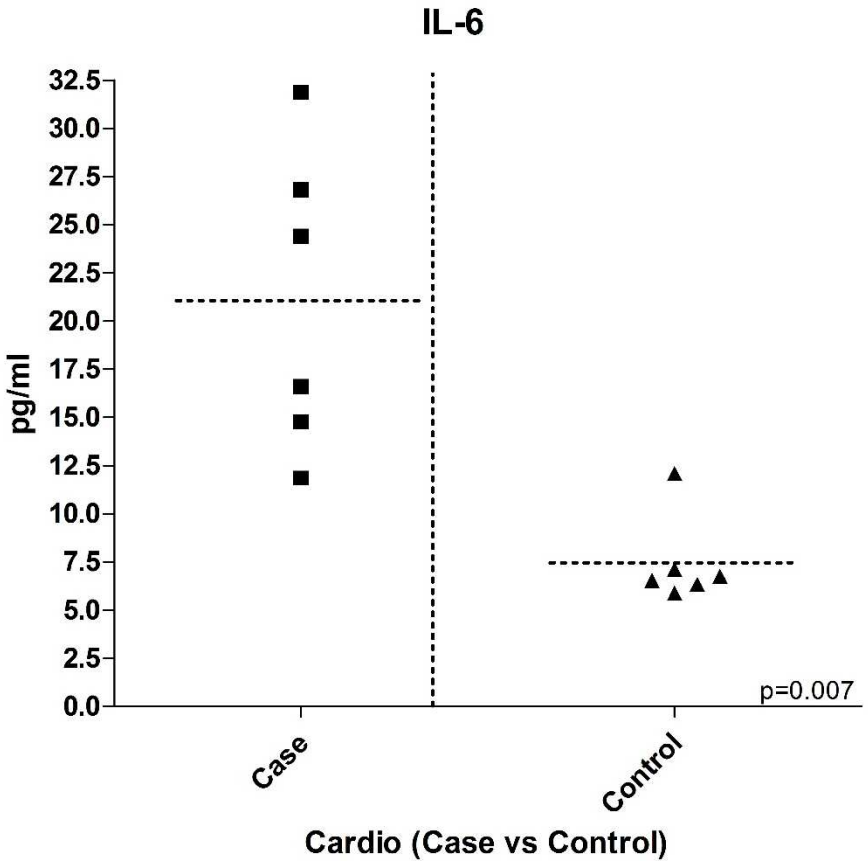
IL-6



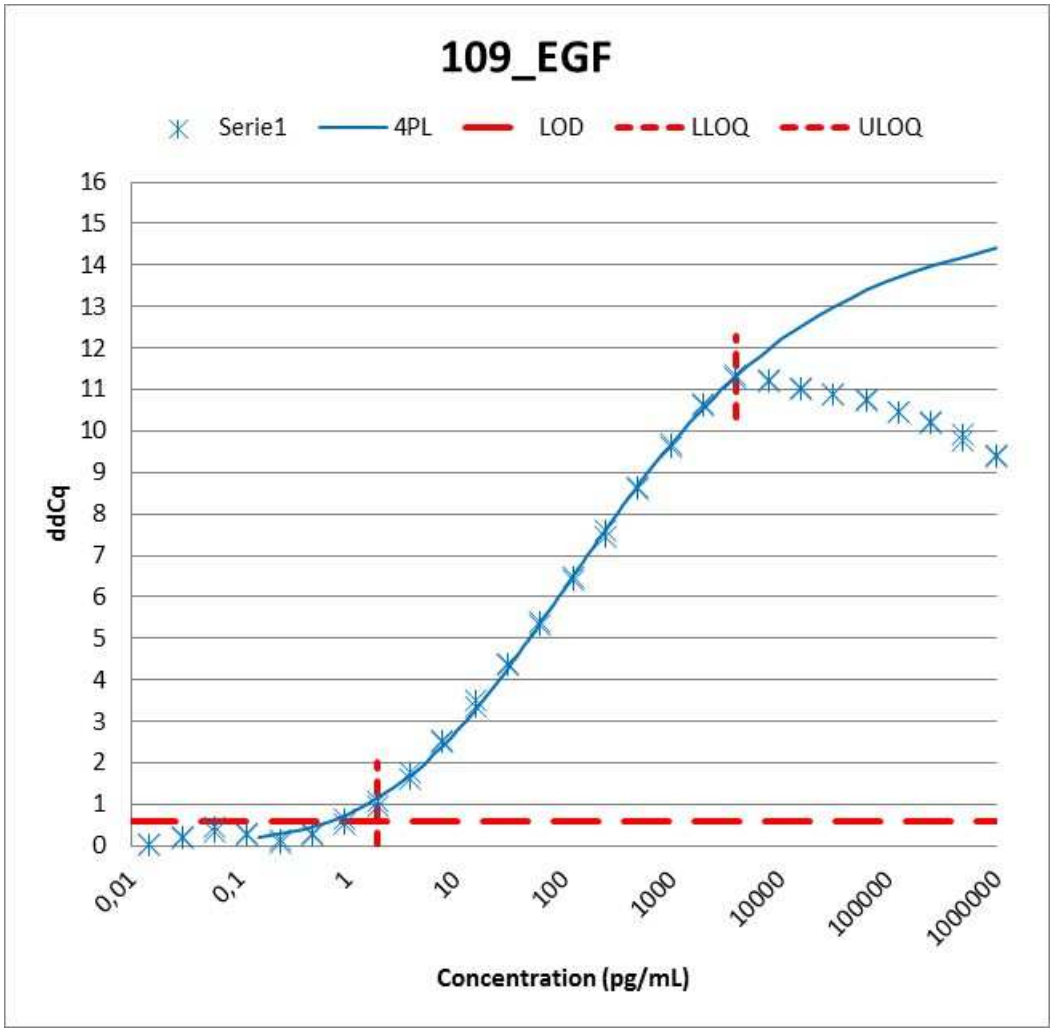
Recovery:108%

No interference from:
Lipid, Haemolysate, Bilirubin

LOD	0,06	pg/mL
LLOQ	0,06	pg/mL
ULOQ	7810	pg/mL
Range	5,12	log10

OLINK BIOSCIENCE

EGF



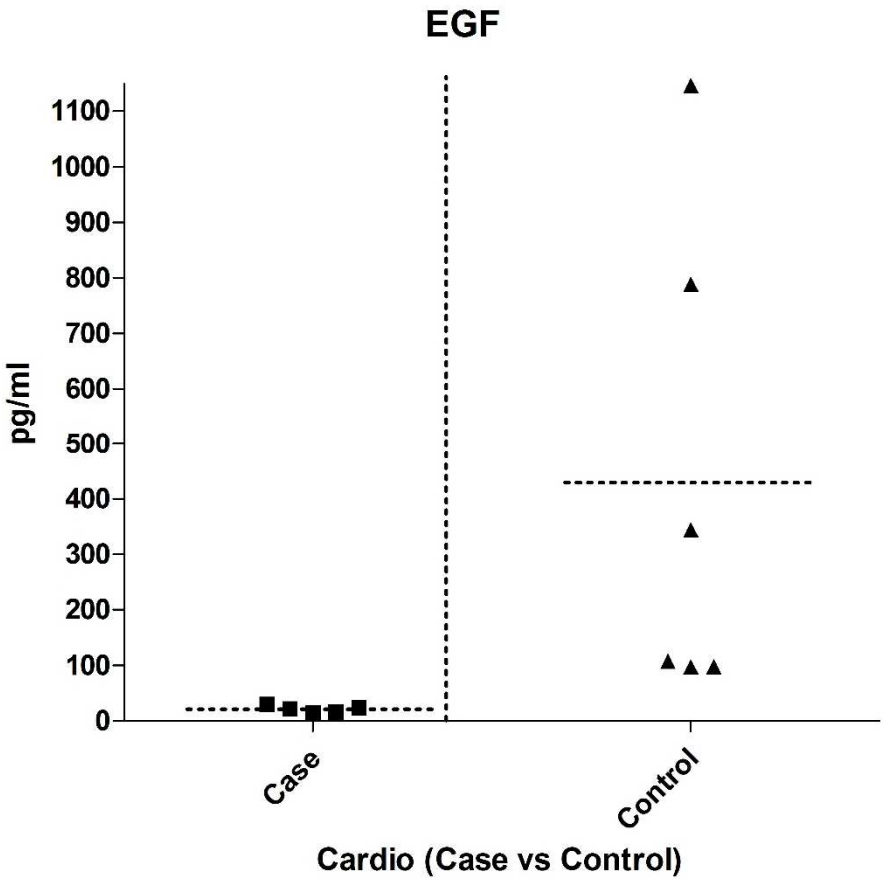
Recovery:100%

No interference from:
Lipid, Haemolysate, Bilirubin

LOD	0,95	pg/mL
LLOQ	1,91	pg/mL
ULOQ	3910	pg/mL
Range	3,31	log10

OLINK
BIOSCIENCE

EGF



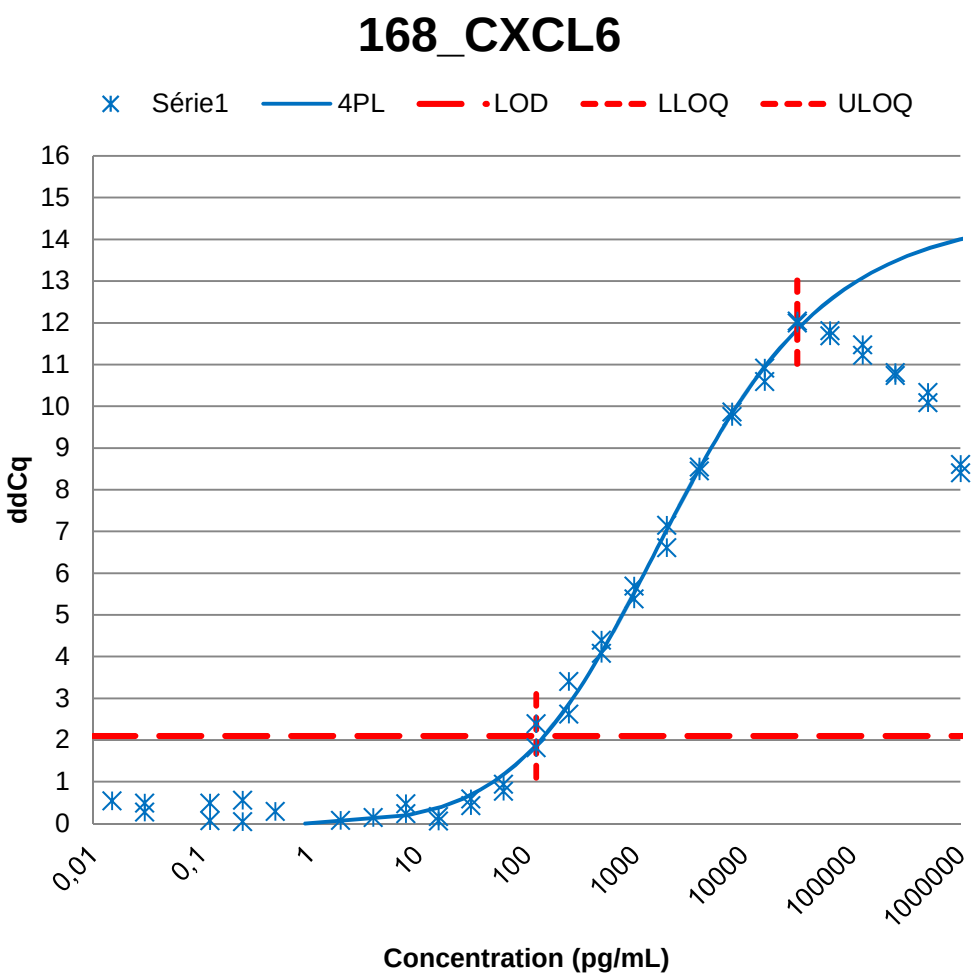
Recovery:100%

No interference from:
Lipid, Haemolysate, Bilirubin

LOD	0,95	pg/mL
LLOQ	1,91	pg/mL
ULOQ	3910	pg/mL
Range	3,31	log10

OLINK
BIOSCIENCE

CXCL6



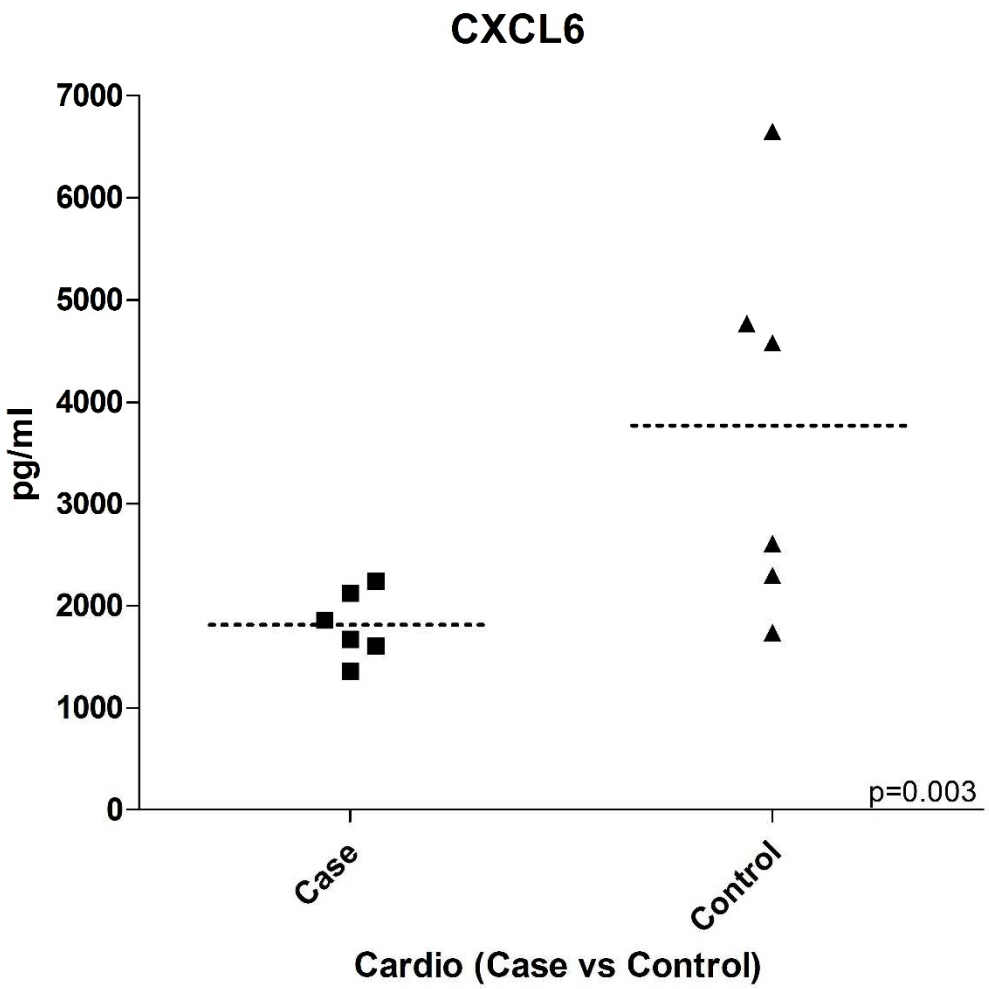
Recovery:95%

No interference from:
Lipid, Haemolysate, Bilirubin

LOD	122,07	pg/mL
LLOQ	122,07	pg/mL
ULOQ	31250	pg/mL
Range	2,41	log10

OLINK BIOSCIENCE

CXCL6



Recovery:95%

No interference from:
Lipid, Haemolysate, Bilirubin

LOD	122,07	pg/mL
LLOQ	122,07	pg/mL
ULOQ	31250	pg/mL
Range	2,41	log10

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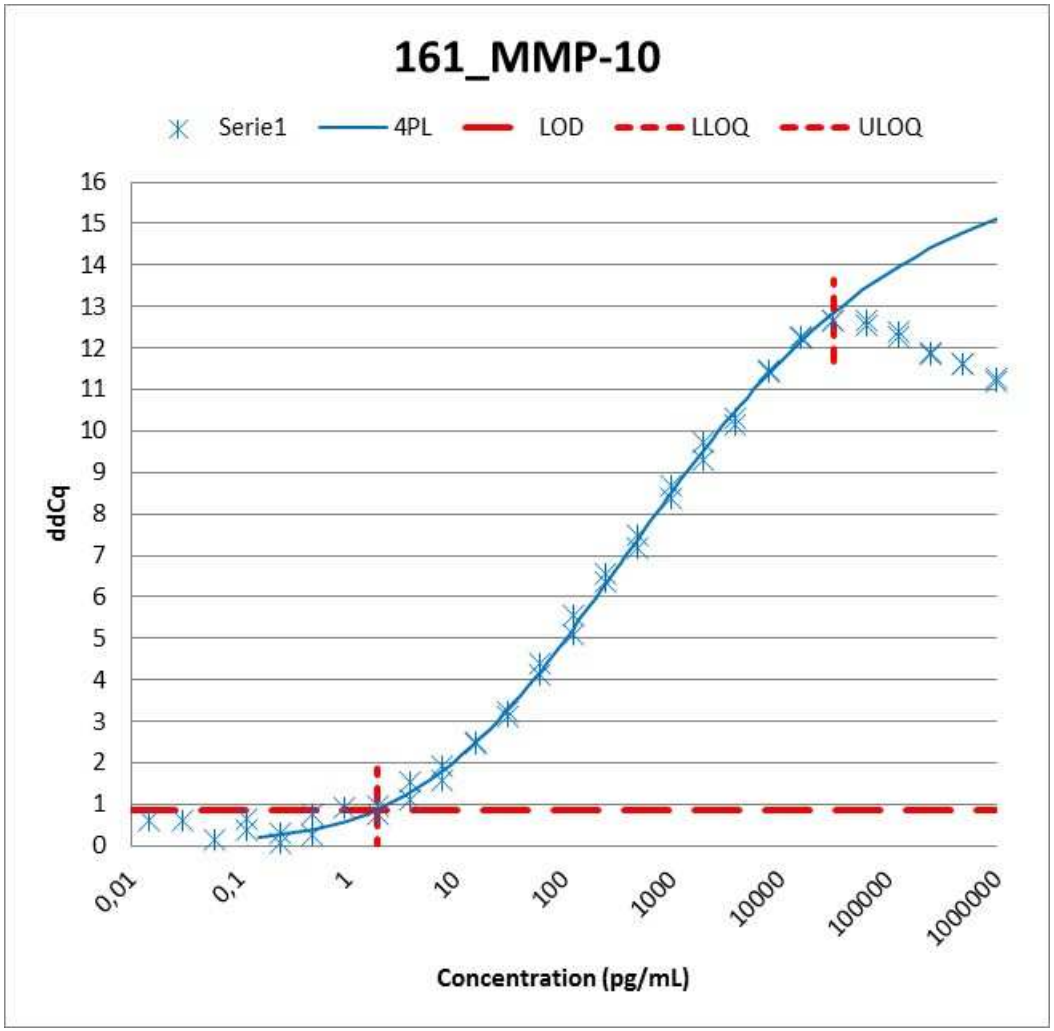
MMP-10



Recovery:100%

No interference from:
Lipid, Haemolysate, Bilirubin

LOD	1,91	pg/mL
LLOQ	1,91	pg/mL
ULOQ	31250	pg/mL
Range	4,21	log10

OLINK BIOSCIENCE

Who we are

- Founded in 2004
- Headquartered in Uppsala, Sweden
- Privately held
- Serving the top 10 global pharma
- Propriety immunoassay platform
- Proseek® & Duolink® Product Lines

  (> 1000 users worldwide, > 35 patents and > 500 scientific publications)

www.olink.com

OLINK BIOSCIENCE

Proseek Multiplex
-Scalable Immunoassays

Available at:

Olink Bioscience

Contact for Europe
Mats Bergström
mats.bergstrom@olink.com

Phone : +46 18 444 39 70
E-mail : order@olink.com

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Performance

http://www.olink.com/products/proseek-multiplex/downloads/data-packages

Performance – Sample material

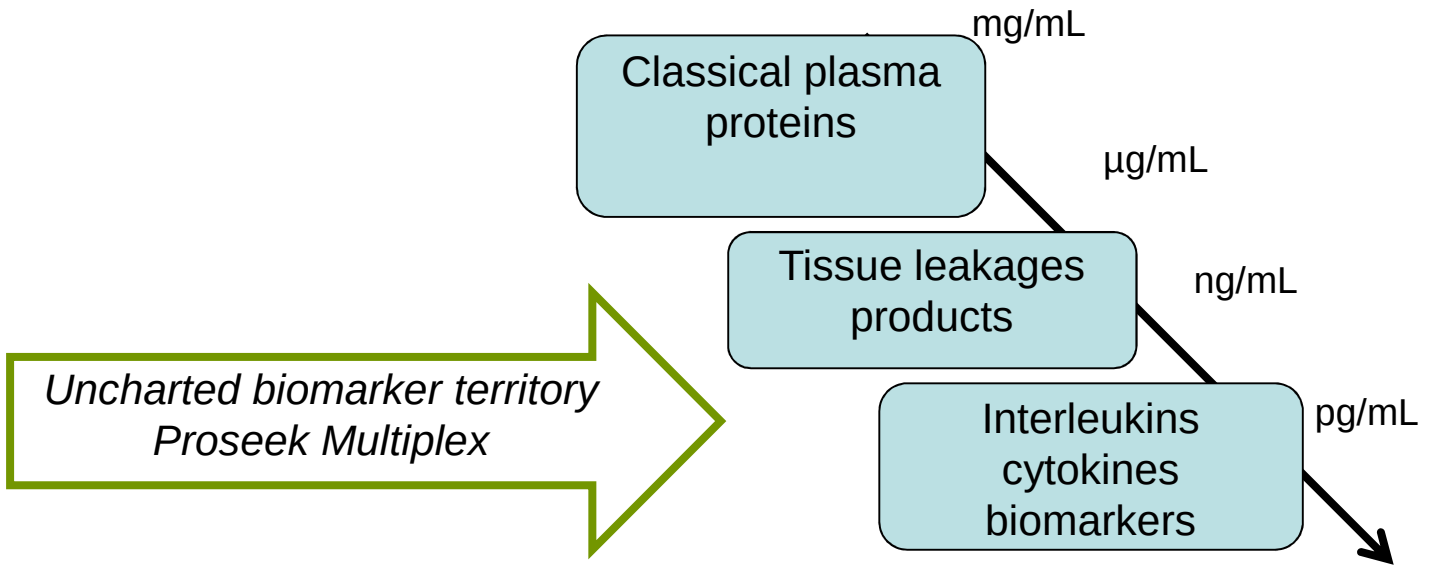
- **Complex biological samples**
  - Serum
  - Plasma (EDTA, Citrate, Heparin)

- **Additional biological samples tested as part of proof of principle studies**
  - Fine needle aspiration biopsies
  - Micro dialysis fluid
  - Tissue lysate
  - Cell culture media
  - Cell lysate
  - Serum from xenograft mice

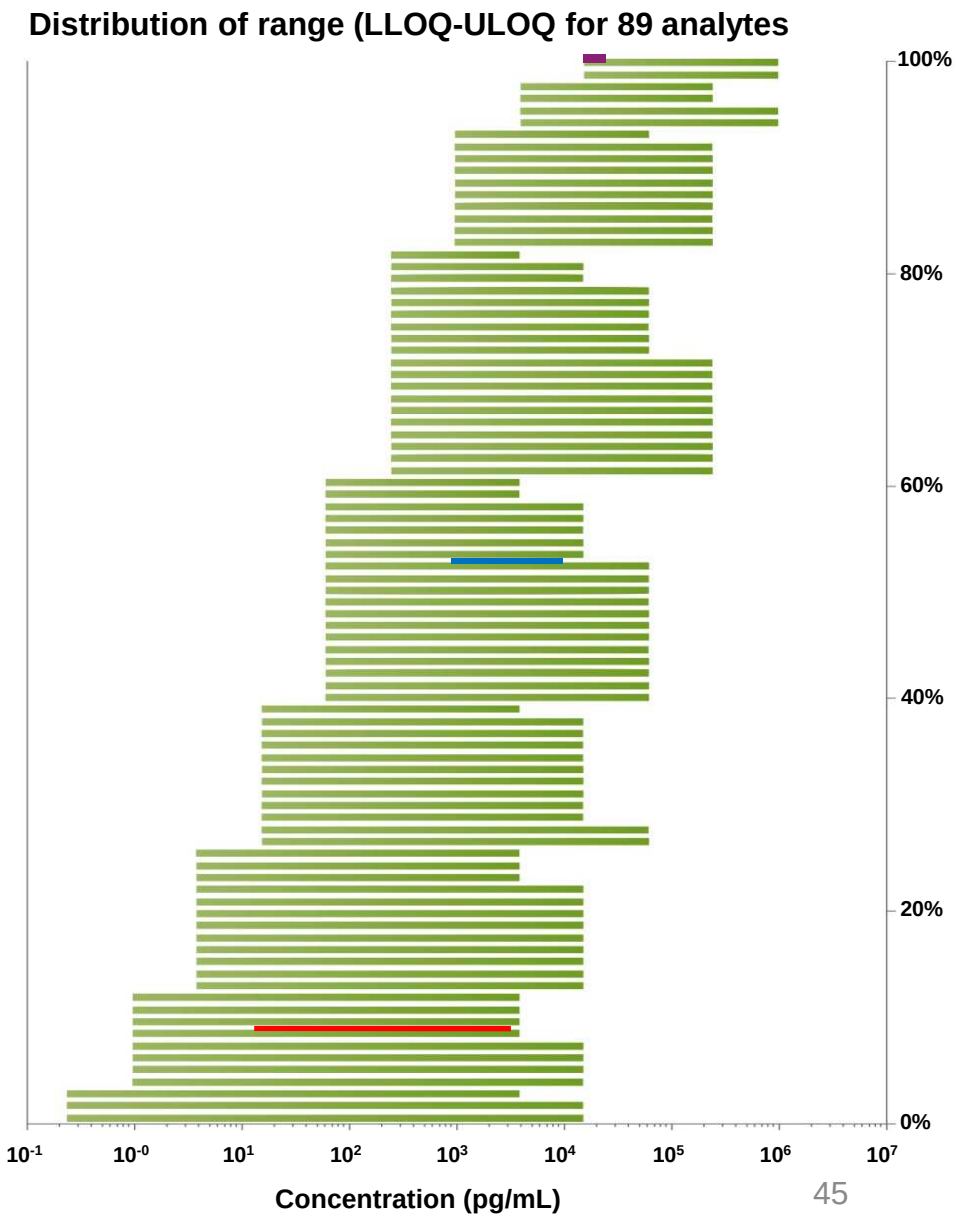
Biological material is complex

For example blood:

- Over 100 000 different proteins
- 22 most abundant proteins = 99% of the content



Performance – Dynamic range

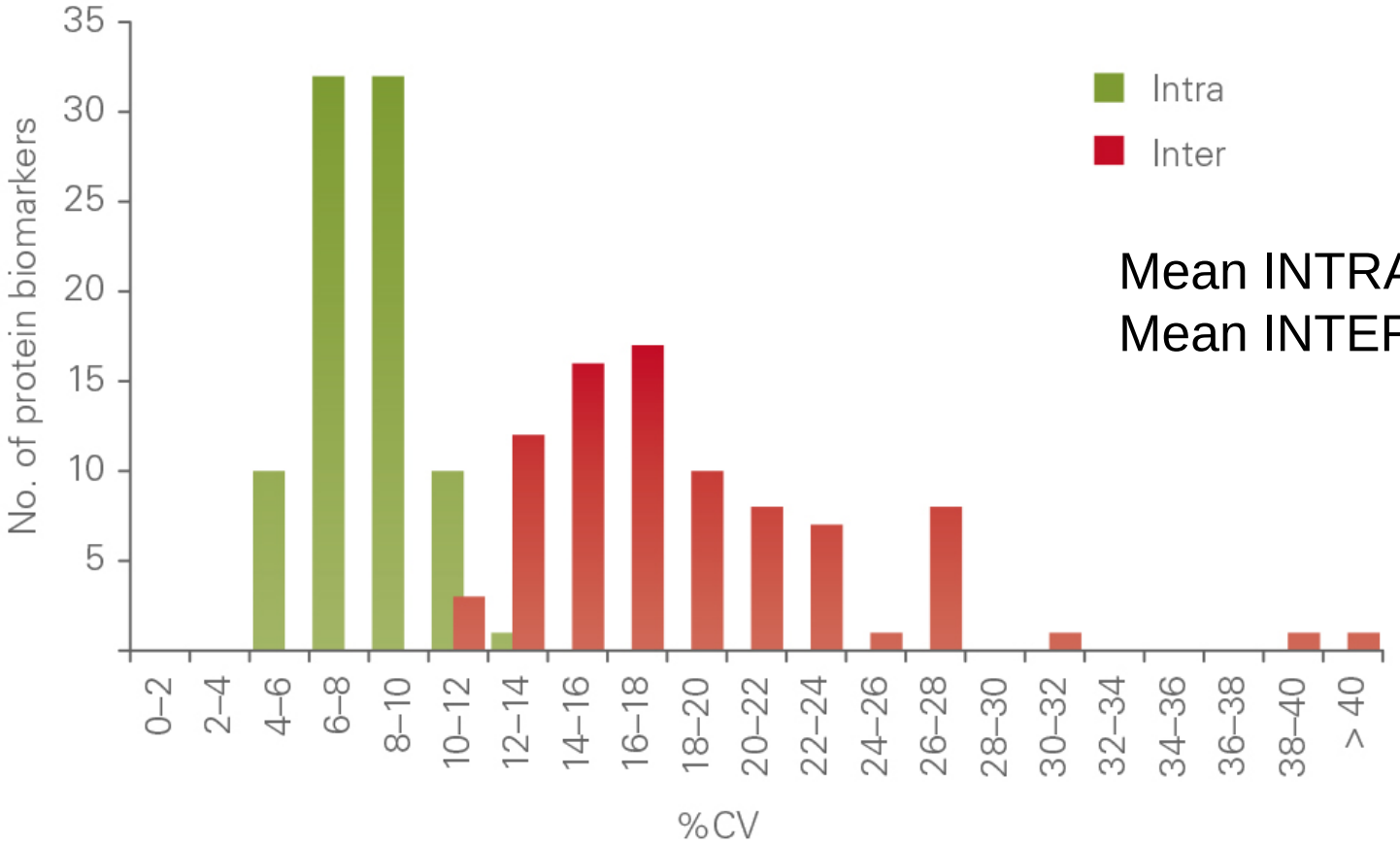


Target	LLOQ (pg/mL)	ULOQ (pg/mL)
Galectin 3	15 625	1 000 000
CEA	61	62 500
Growth Hormone	0.95	15 625

LOD: Limit of detection
LLOQ: Lower limit of quantification
ULOQ: Upper limit of quantification

Performance - Precision

Repeatability



Mean INTRA-plate 8 %CV
Mean INTER-plate19 %CV

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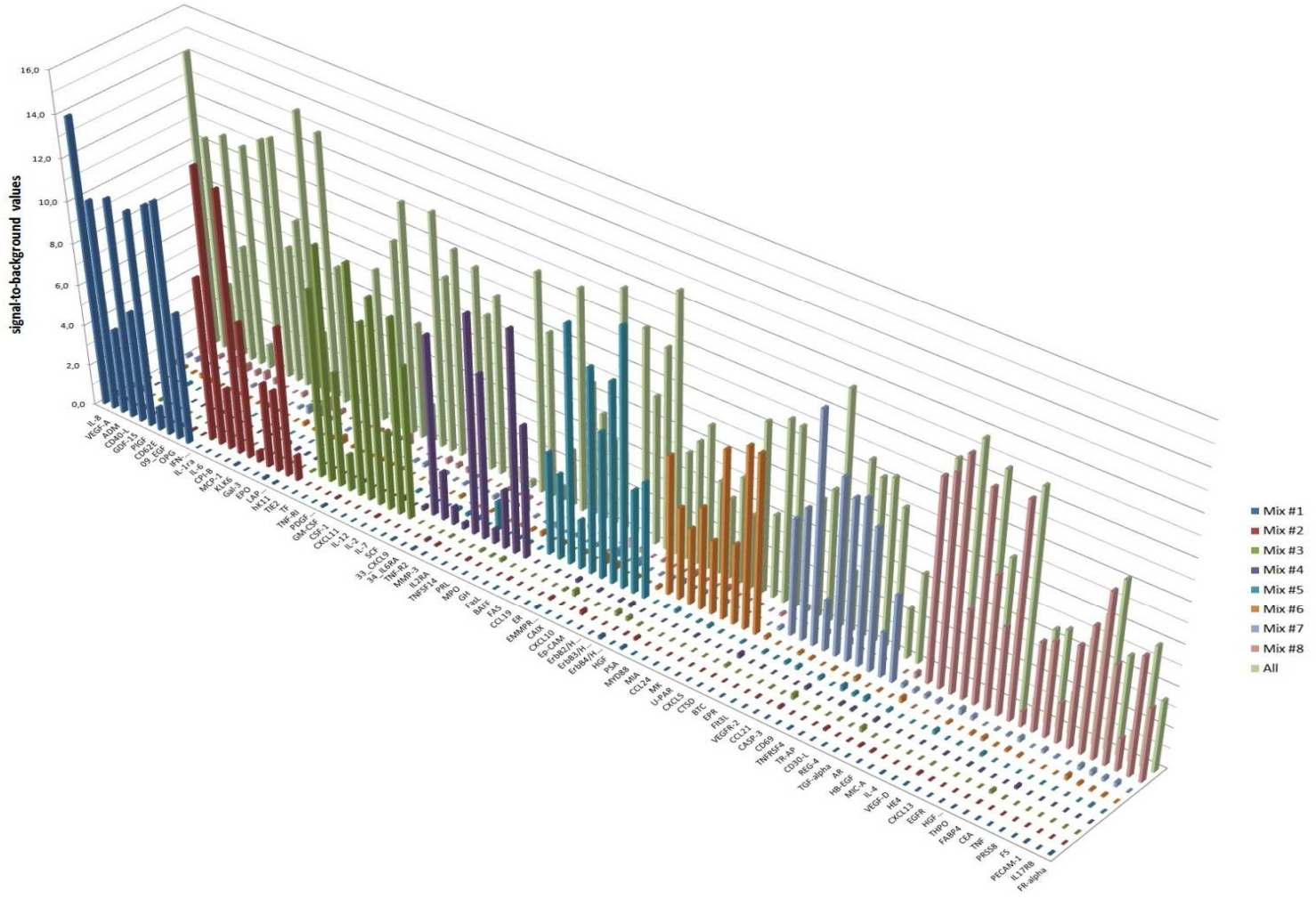
Sensitivity of Proseek Multiplex

- Some assays are very sensitivity compared to Luminex and ELISA
  - e.g. IL8, IL6, and VEGF (sub pg/mL)
- Others have medium sensitivity
  - e.g. TNFa, IFNg
- Sensitivity depends on the antibodies that we have been able to source for that specific analyte.
- All detailed data for every assay is available in online
  - http://www.olink.com/products/proseek-multiplex/downloads/data-packages

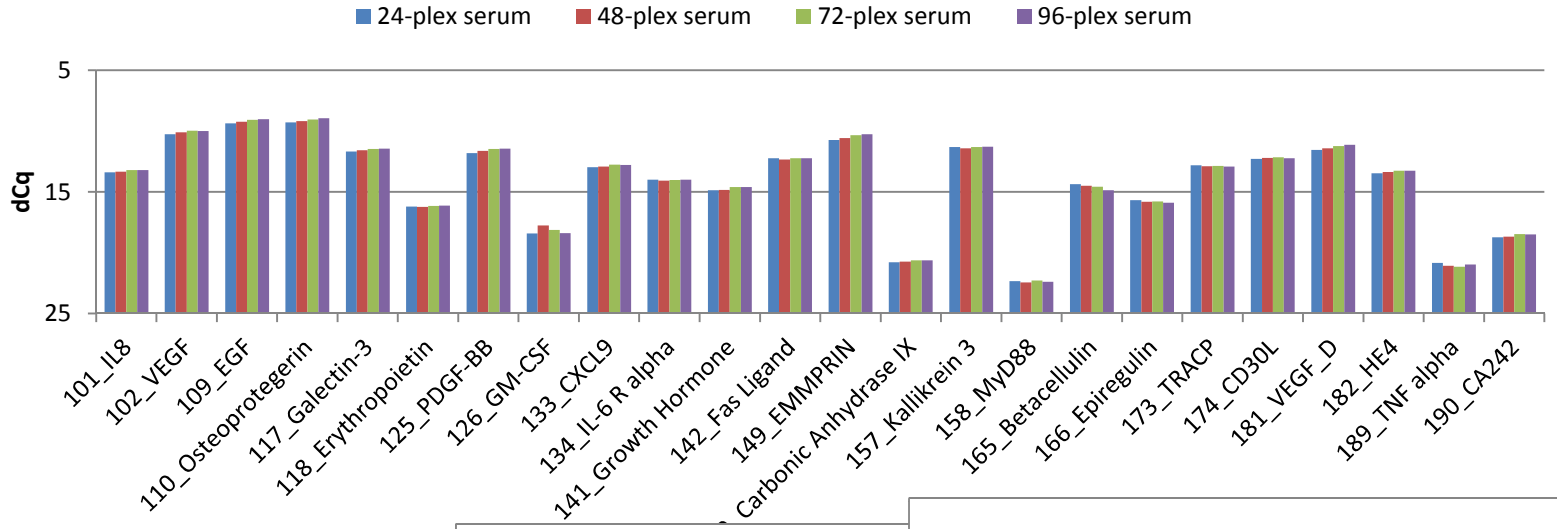
Performance – analytical specificity

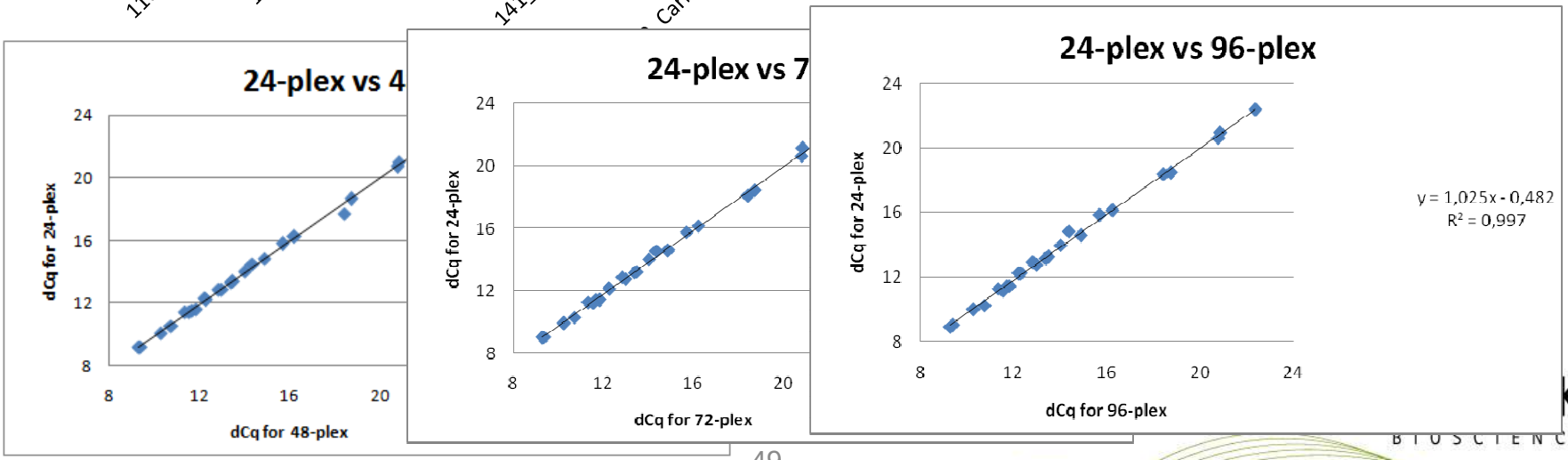
- 8 blocks of recombinant proteins vs the complete reporter system
- Only specific reporter primers generate detectable signals



Scalability

No effect seen on individual assays regardless if running 24, 48, 72 or 96 markers at the same time.





Proseek Multiplex® in a nutshell

- Sample
  - 1 µL
  - Plasma, Serum, Fine Needle Biopsy, Tissue lysate, Cell lysate, Cell culture media, Serum from xenograft mice, and Microdialysis fluid
- Capacity
  - 92 biomarkers in 96 samples simultaneously
  - 9,216 data points per run
- Analytical performance
  - High specificity
  - Sensitivity from sub pg/mL to ng/mL
  - Precision mean CV <10% (intra), <20% (inter)
  - Scalable
- Easy to run protocol
  - Complete reagent kit
  - Homogenous assay

This product is for research use only. Not for use in human diagnostic or therapeutic procedures.

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Proseek® Multiplex

-Scalable Immunoassays

Available from:

Olink Bioscience

Phone : +46 18 444 39 70

E-mail : order@olink.com

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