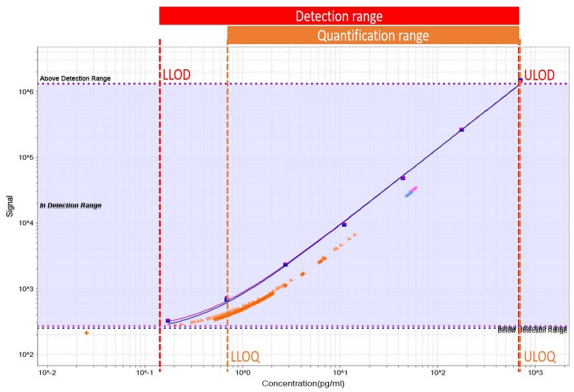
	control pool CV intraplade (median)	control pool CV interplade	median LLOD (1:10)	ULOD (1:10)	samples in LOD (n)	samples in LOD %	LLOQ (1:5)	ULOQ (1:5)	samples in LOQ (n)	samples in LOQ (%)	out of range (n)	out of range (%)
Osteopontin	26,4	27,1	422	500000	9196	99,9	8000	528979	9134	99,2	8	0,1

Ved analysering af Osteopontin panelet blev der afprøvet en del forskellige håndteringer (manuelt/ robot) og blev anvendt forskellige reagens og pladelots, pga fejlfinding omkring den relativ store CV af poolen. Konklusionen var til sidst, at antistofferne/ analysen ikke kan give en bedre CV en den fundne. 18 plader blev kørt om. Resultaterne er baseret på to forskellige sæts antistoffer/diluenter og 2 forskellige pladelots. Lablog og rådata ligger også på sharepointserveren i mappen Osteopontin/Excel files, hvis der skulle være behov for at verificere noget. Resultat Excel filen indeholder kolonnen **"RANGE"** som angiver om en prøve ligger udenfor detektionsområdet

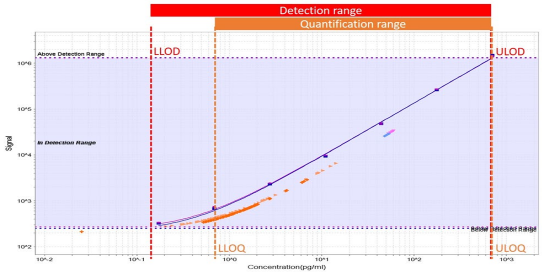
LLOD: 2.5 std. above S008 (technical blank)
ULOD: 0 std. above S001
LLOQ: lowest standard which has CV <25% and %recovery 75-125%
ULOQ: S001, if it has CV <25% and %recovery 75-125%



	control pool CV intraplade (median)	control pool CV interplade	median LLOD (1:5)	ULOD (1:5)	samples in LOD (n)	samples in LOD %	LLOQ (1:5)	ULOQ (1:5)	samples in LOQ (n)	samples in LOQ (%)	out of range (n)	out of range (%)
Ang-like 4	2,0	6,7	23,7	2500000,0	9200	99,96	602,0	2613713,7	9200	99,96	4	0,04
FGF-21	4,6	9,1	7,4	40900,0	9193	99,88	43,0	44308,5	9191	99,86	11	0,12
FGF-23	3,4	7,4	39,4	321500,0	9200	99,96	84,4	331855,3	9200	99,96	4	0,04
IL1-RA	2,8	9,4	7,5	11650,0	9199	99,95	169,0	11535,2	7293	79,24	5	0,05
Leptin	18,1	28,6	40,0	244000,0	9102	98,89	251,7	240992,9	9049	98,32	102	1,11
RAGE	3,8	12,3	5,6	125000,0	9200	99,96	124,6	119729,1	9200	99,96	4	0,04
Sclerostin	4,3	17,4	2,2	15000,0	9200	99,96	57,7	14764,2	9177	99,71	4	0,04
U-PAR	3,7	6,7	4,5	125000,0	9200	99,96	31,4	121846,9	9200	99,96	4	0,04

Plade 1 til 15 blev analyseret med en 1:2 fortynding, alle øvrige plader med 1:5. Dermed var næsten alle Leptin prøver indenfor detektionsområdet. **Leptin assay resultater er foreløbig**, pga den høje kontrolpool CV bliver der talt med MSD om en mulig validering eller genanalyse med en Vplex assay. **Lablog og rådata** ligger også på sharepointserveren i mappen 8-plex/Excel files, hvis der skulle være behov for at verificere noget. Result Excel file contains column **"RANGE"** indicating whether a sample lies outside detection range "0", within detection range "1" (LLOD<sample<LLOQ) or within quantification range "2" (LLOQ<sample<ULOQ).

LLOD: 2.5 std. above S008 (technical blank) units for all
ULOD: 0 std. above S001
LLOQ: lowest standard which has CV <25% and %recovery 75-125%
ULOQ: S001, if it has CV <25% and %recovery 75-125%



MSD method description **2-plex Follistatin and MPO**

The concentrations of Follistatin and MPO were measured on a MESO Quickplex SQ 120 instrument (Mesocale diagnostics (MSD)), using R-plex assay kits (F21E5-3/-8, F214E-3/-8) according to the manufacturer's instructions. The assay is based on analyte specific antibody pairs, with a primary antibody that captures the analyte to one of up to 10 different spots in a 96well and a secondary antibody that generates a light signal proportional to the analyte amount upon electrical stimulation. Light signals are converted to concentrations using a standard curve, which is generated for each individual plate.

9204 samples were measured on 118 individual plates, each plate containing 78 samples in single wells, as well as 8 standards and a control pool in duplicate. The control pool was used to determine the average assay variation, both intra and inter-plate.

Samples underwent one freeze thaw cycle to be aliquoted onto 96 well plates before they were thawed in the 96 well plates for the MSD analysis. Thawed samples were spun down and diluted 1:1 with assay diluent for the analysis. All pipetting steps were performed with the BRAVO pipetting platform.

The average assay variation within and between plates was 2,7% and 7,9% for Follistatin and 3,9% and 15,8% for MPO. 99,95% and 99,98% of samples were measured in detection range of the assay for Follistatin and MPO, respectively.

Detection range was calculated for every plate based on the highest and lowest standard.

Units is given as pg/ml.

MSD method description **Adiponectin**

The concentration of Adiponectin was measured on a MESO Quickplex SQ 120 instrument (Mesocale diagnostics (MSD)), using a R-plex assay kit (# K151YTR, MSD) according to the manufacturer's instructions. The assay is based on analyte specific antibody pairs, with a primary antibody that captures the analyte to one of up to 10 different spots in a 96well and a secondary antibody that generates a light signal proportional to the analyte amount upon electrical stimulation. Light signals are converted to concentrations using a standard curve, which is generated for each individual plate.

9204 samples were measured on 118 individual plates, each plate containing 78 samples in single wells, as well as 8 standards and a control pool in duplicate. The control pool was used to determine the average assay variation, both intra and inter-plate.

Samples underwent one freeze thaw cycle to be aliquoted onto 96 well plates before they were thawed in the 96 well plates for the MSD analysis. Thawed samples were spun down and diluted 1:10.000 for the analysis. All pipetting steps were performed with the BRAVO pipetting platform, sample plates were incubated on an orbital shaker between the dilution steps.

The average assay variation within and between plates was 5,1% and 10,1%, respectively. 99,9 % of samples were measured in quantification range of the assay.

Quantification range was defined by the concentration of standards that had a CV <25% and a recovery of 75-125%.

Units is given as pg/ml.

MSD method **R-plex Osteopontin**

The concentration of Osteopontin was measured on a MESO Quickplex SQ 120 instrument (Mesocale discovery (MSD)), using a R-plex antibody set (K151YMR-2) and 96-well Smallspot Streptavidin plates (L45SA-1) according to the manufacturer's instructions. The assay is based on analyte specific antibody pairs, with a capture antibody that binds to a spot in a 96-well and a detection antibody that enables light generation, proportional to the amount of bound analyte. Light signals are converted to concentrations using a standard curve, which is generated for each individual analyte on each individual plate.

9204 samples were measured on 118 individual plates and each plate contained 78 samples in single wells, as well as 8 standards and a control pool in duplicate. The control pool was used to determine assay variation, both intra and inter-plate.

Samples underwent one freeze thaw cycle to be aliquoted onto 96 well plates before they were thawed in the 96 well plates for the MSD analysis. Thawed samples were spun down and diluted 1:10. All pipetting steps were conducted with the BRAVO pipetting platform.

Samples from x plates were fitted with the standard curve from another plate.

The average assay variation within and between plates was: x % of samples were measured in detection range of the assay.

Detection range was calculated for every plate based on the highest and lowest standard and quantification range was given by the supplier (V-plex) / was defined as the standards that had a CV <25 and a recovery of 75-125%.

	control pool CV intraplade (median (averaį	control pool CV interplade	median LLOD		ULOD	samples in LOD (n)	samples in LOD %	LLOQ	ULOQ	samples in LOQ (n)	samples in LOQ (%)	out of range (n)	out of range (%)
Osteocalcin-1 (alle)	4,6 (8,9)	11,3	74,9		200000,0	9203,0	99,99	220,0	190370,0	9203,0	99,99	1,0	0,0
Osteocalcin-1 (coat 1h)	4,2 (4,3)	10,0	69,9		200000,0	9203,0	99,99	220,0	190370,0	6084,0	100,00	0,0	0,0
Osteocalcin-1 (coat 24h eller batch)	7,8 (17,9)	13,6	90,4		200000,0	9203,0	99,99	726,0	13478,0	529,0	16,95	0,0	0,0

Plade 1 til 5 blev analyseret med en 1:50 fortynding, alle øvrige plader med 1:10. **Plader 6-45 er coated mellem 24h og en uge før forsøg, det resulterede i højere CVer. Derefter er alle plader coated samme dag som analysen. Måske giver det mening at behandle data separat, det er tydeligt at der er højere CVer på 24h / batch coating pladerne.** Lablog og rådata ligger også på sharepointserveren i mappen Osteocalcin/Excel files, hvis der skulle være behov for at verificere noget. Result Excel file indholder en kolonne "RANGE" som viser om en prøve ligger udenfor detection range "0", indenfor detection range "1" (LLOD<sample<LLOQ) eller indenfor quantification range "2" (LLOQ<sample<ULOQ), den er angivet for det samlede set (Osteopontin-1 alle).

E-mail from Jens 06.02.2023

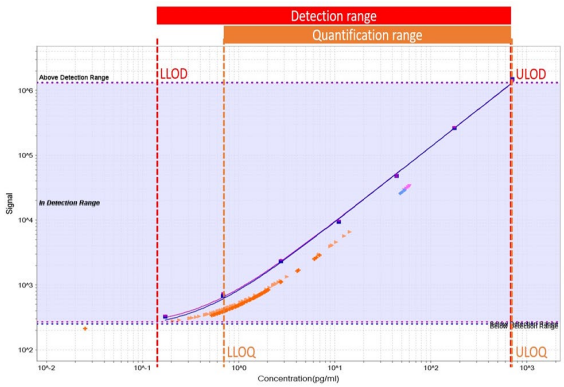
Så er der nye data fra MSD biomarkør platformen til DD2. Det er denne gang Osteocalcin som skal adderes til DD2 datasætte. Du skal bruge fanen " osteocalcin sample data". Der er et par ting du skal være obs på og de kommer her:

- Nogle (ganske få) prøver er out-of-range de skal markeres (kollone E)
- Nogle prøver er within detection range de skal også markers (kollone E)
- Nogle lang de fleste prøver er within detection range de behøver ikke nogen markering (kollone E)
- Nogle prøver er coated >72h (kollone h) de skal markeres med højt da de har højere CV end de som er coated op til og med 24h.
- Sample ID er det ID du skal bruge for at koble til specifikke personer i DD2 – helt lige som de øvrige biomarkører.
- Det er kolonne C og D som er resultatet på analysen

	control pool CV intraplade (median)	control pool CV interplade	median LLOD (1:10000)	ULOD (1:10000)	samples in LOD (n)	samples in LOD %	LLOQ (1:10000)	ULOQ (1:10000)	samples in LOQ (n)	samples in LOQ (%)	out of range (n)	out of range (%)
Adiponectin	5,1	10,1	53047	20	9202	100,0	48918	200399910	9202	100,0	2	0,02

Adiponectin blev analyseret med en 1:10000 fortynding af plasma prøverne. Fortyndingsprotokollen var ikke helt optimalt for plade 1-10, hvor der er en højere CV på pool og lavere gennemsnitlige prøvekoncentrationer. Derefter var både intra og inter plade CVer meget fine. 8 pladeomkørsler blev fortaget med et andet antistoflot, men baseret på den genfundne poolkoncentration blev det vurderet at data kunne indgå i den samlede analyse. Lablog og rådata ligger også på sharepointserveren i mappen Adiponectin/Excel files, hvis der skulle være behov for at verificere noget. Resultat Excel filen indeholder kolonnen **"RANGE"** som angiver om en prøve ligger udenfor detektionsområdet

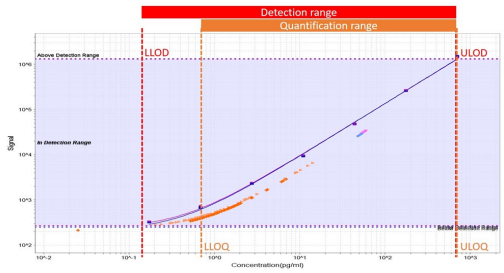
LLOD: 2.5 std. above S008 (technical blank)
ULOD: 0 std. above S001
LLOQ: lowest standard which has CV <25% and %recovery 75-125%
ULOQ: S001, if it has CV <25% and %recovery 75-125%



	control pool CV intraplade (median)	control pool CV interplade (rerun)	median LLOD (1:100)	ULOD (1:100)	samples in LOD (n)	samples in LOD %	LLOQ (1:100)	ULOQ (1:100)	samples in LOQ (n)	samples in LOQ (%)	out of range (n)	out of range (%)
CD163	6,5	16,9 (6,4)	14392,8	50000000,0	9045	100,0	52368,3	48546696,8	9045	100,0	3	0,0
Galectin-3	10,6	21,5 (18,1)	6878,7	300000,0	8354	92,3	68030,3	318492,5	1062	11,7	694	7,7
GDF-15	7,2	18,7 (1,6)	4,8	50000,0	9045	100,0	12,4	50321,2	9045	100,0	3	0,0
NT-proBNP	11,1	25,6 (17,6)	23,9	50000,0	8763	96,9	204,9	54114,2	3835	42,4	285	3,1
Resistin	7,6	21,8 (15,2)	11,2	250000,0	9041	99,9	239,7	250587,6	9036	99,9	7	0,1
Serpin	6,5	21,8 (27,5)	175,6	1100000,0	9045	100,0	1048,8	1113216,9	9045	100,0	3	0,0
YKL-40	6,3	18,0 (7,8)	46,3	516000,0	8992	99,4	2028,1	519016,2	8992	99,4	56	0,6

Plade 17-20 og 22-25 blev analyseret med en anden pool end de andre fordi de blev genanalyseret. **Der mangler data fra plade 21 og 38** pga. mislykket analyse og ingen mulighed for at genanalysere. Prøvenavnene fra plade 21 og 38 ligger i en separat fane. Pool CVer for Galectin-3 og NT-proBNP er sandsynligvis lidt højere end i prøvepopulationen, fordi poolen lå lavere end prøvegennemsnit og ikke på den lineare del af standardkurven. **Lablog og rådata** ligger på sharepointserveren i mappen 7-plex/Excel files, hvis der skulle være behov for at verificere noget. Result Excel file contains column **"RANGE"** indicating whether a sample lies outside detection range "0", within detection range "1" (LLOD<sample<LLOQ) or within quantification range "2" (LLOQ<sample<ULOQ). Der er en word dokument med metodebeskrivelse i 7plex mappen. Alle **koncentrationer er pg/mL**

LLOD: 2.5 std. above S008 (technical blank)
ULOD: 0 std. above S001
LLOQ: lowest standard which has CV <25% and %recovery 75-125%
ULOQ: S001, if it has CV <25% and %recovery 75-125%



E-mail from Jens 12.04.2023

Så har jeg kigget på data fra MSD analyserne (7plax asay) på CD163, Galectin 3, GDF-15, Nt-proBNP, Resistin, Serpin og YLK-40. De er alle inkluderet i arket.

Kolonnen "Sample" i hvert under ark er som i tidligere data set = projektID fra DD2 inkl nummer på det rør som er anvend til analysen (sidste ciffer: 12 (for langt de fleste prøver)). De første 6 numre i sample kan derfor linkes direkte til et cpr nummer hos jer.

Der er for hver analyse markeret om målingen er Within detection Range: 1, outside detection range: 0, eller Within quantification range: 2. De data synes jeg skal med på forskermaskinerne (se figuren i det første ark)

Måleenheden er for alle analyser pg/ml

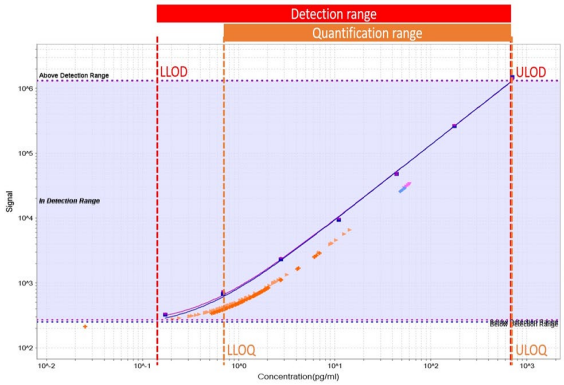
Da SDCC havde problemer i lab er der kun data fra 116 og ikke som tidligere 118 plader. Derfor er n lidt lavere for nogle af analyserne. Det må vi desværre leve med.

Hvis vi på et senere tidspunkt skal bruge en metode beskrivelse så har jeg en sådan liggende.

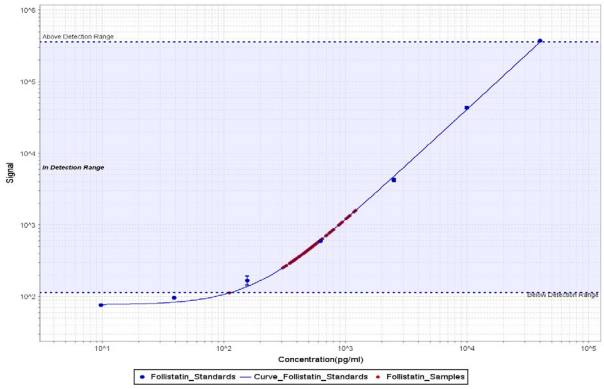
	control pool CV intraplade (median)	control pool CV interplade	median LLOD	ULOD	samples in LOD (n)	samples in LOD %	LLOQ	ULOQ	samples in LOQ (n)	samples in LOQ (%)	out of range (n)	out of range (%)
Follistatin	2,7	7,9	99	40000	9199	99,95	612	41427	1735	18,9	5	0,05
MPO	3,9	15,8	26	200000	9202	99,98	182	49850	9151	99,42	2	0,02

Follistatin og MPO blev analyseret uden fortynding af plasma prøverne. Næsten alle prøver ligger indenfor detektionsområdet, men for Follistatin ligger kun 18,9% indenfor kvantificeringsområdet. Det skyldes at prøverne ligger rundt omkring den sidste standard, som havde en lav CV for dobbeltbestemmelsen og lå pænt på regressionskurven. De allerfleste prøver ligger alligevel pænt på den lineare del af kurven, se eksempel nedenunder. Lablog og rådata ligger også på sharepointserveren i mappen 2plex/Excel files, hvis der skulle være behov for at verificere noget. Resultat Excel filen indeholder kolonnen **"RANGE"** som angiver om en prøve ligger udenfor detektionsområdet "0", indenfor detektionsområdet "1" (LLOD<sample<LLOQ) eller indenfor kvantificeringsområdet "2" (LLOQ<sample<ULOQ).

LLOD: 2.5 std. above S008 (technical blank)
ULOD: 0 std. above S001
LLOQ: lowest standard which has CV <25% and %recovery 75-125%
ULOQ: S001, if it has CV <25% and %recovery 75-125%



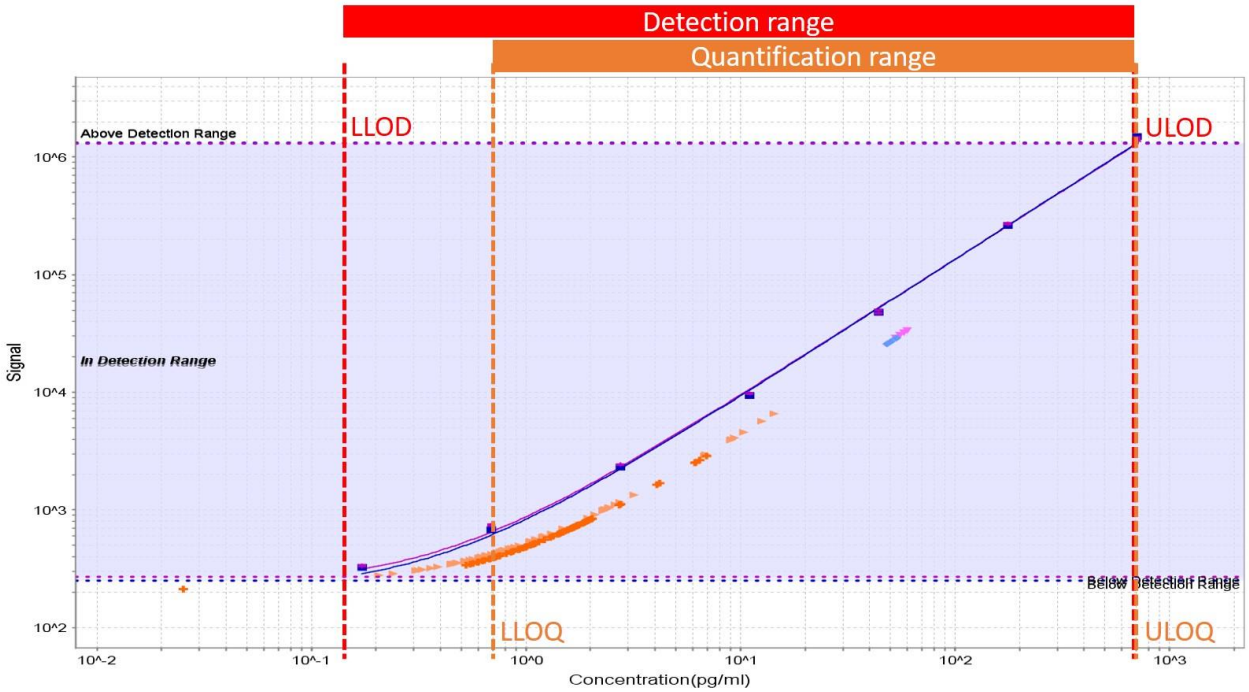
FOLLISTATIN standard og prøver_ eksempel



General information on assay/data quality:

Definitions:

LLOD: 2.5 std. above S008 (technical blank)
ULOD: 0 std. above S001
LLOQ: lowest standard which has CV <25% and %recovery 75-125%
ULOQ: S001, if it has CV <25% and %recovery 75-125%



Result Excel file contains column “RANGE” indicating whether a sample lies outside detection range “0”, within detection range “1” (LLOD<sample<LLOQ) or within quantification range “2” (LLOQ<sample<ULOQ).

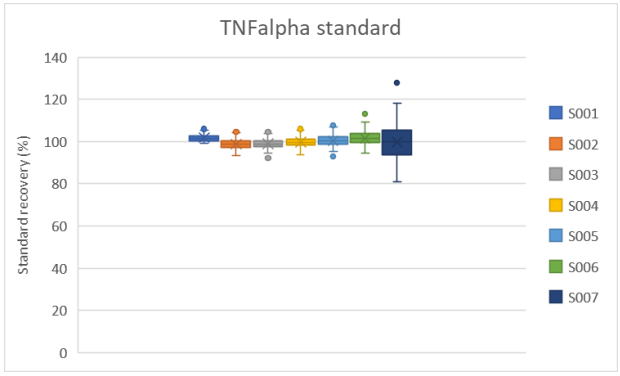
Overview Vplex panel IL6 and TNFα in DD2 cohort

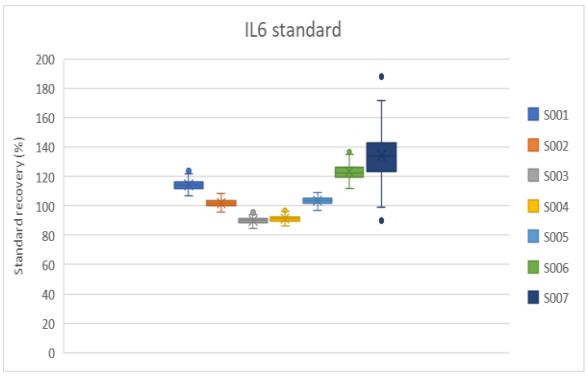
TNFα and IL6 were detected with a Vplex assay, which stands for **v**alidated assay. This type of assay undergoes extensive testing and quality control by the manufacturer, which results in higher reproducibility, broader dynamic range, higher sensitivity, precision, recovery and linearity, compared to the other available assay types.
The TNFα assay was very stable and almost all samples were within the detection range, about 90% in the quantification range.

IL6 assay showed more variability, standards in the lower end of the curve had slightly elevated recovery values, which means that the measured signal/concentration was above the calculated standard curve. Due to this higher variation in the lower end (and of course the biologically given distribution of IL6 concentrations), fewer samples are within detection range and even fewer in quantification range.
With very stringent criteria the lower limit of the quantification range should be between standard 5 and 6, since the criterion for lower quantification limit is only met in 67% of the measured 118 plates.

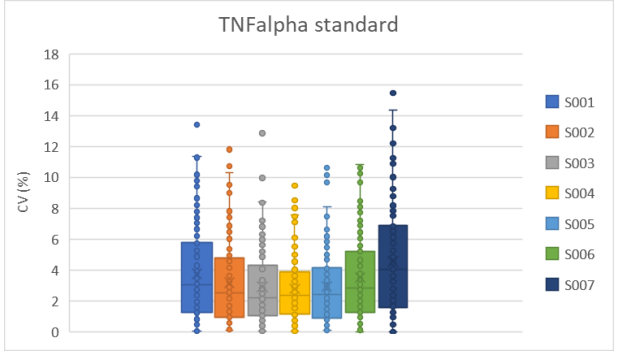
The reason for this higher standard recovery% seems to arise from slightly different standard preparation/dilution, apparent as overall lower standard signal in the plates where recovery is elevated. This is supported by TNFalpha standard signal values, but the assay seems to be more robust since recovery % is not affected here.

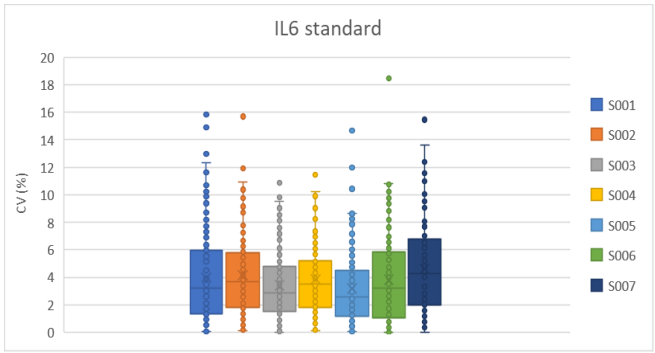
Standard recovery





CV on standard measurements





IL6

Detection limits (pg/ml)

- Median calculated LLOD 0,151 (min 0,10 max 0,35)
- ULOD 706

Quantification limits

Kit

- $LLOQ_{KIT}$ **0,633**
- $ULOQ_{KIT}$ **488**

Variation on internal control measurements

- Interplate CV on control pool concentration: 12,34
- Median of Intraplate CV on control pool concentration: 6,08
- Control recovery on the individual plates, relative to median control pool concentration (80,4-148,4%)

Number of samples within range

within detection range (according to limits on the respective plate)
9060

within quantification limits (*Kit*) (0,633< samples<488)
8015

% detected (I intervallet LLOD-ULOD) 98,5
% quantifiable (I intervallet LLOQ-ULOQ KIT) 87,1
% not detected (under LLOD) 1,5

Distribution of calculated concentration values (pg/ml):

MAX	191,9
MIN	0,04
AVERAGE	1,8
MEDIAN	1,2

TNFalpha

Detection limits (pg/ml)

Median calculated LLOD **0,032** (min 0,02 max 0,10)
ULOD **358**

Quantification limits (pg/ml)

	KIT
Kit LLOQ	0,690
Kit ULOQ	248

Variation on internal control measurements

Interplate CV on control pool concentration: 14,4%
Median of Intraplate CV on control pool concentration: 4,98%

Control recovery on the individual plates, relative to median control pool concentration (72,8-135,1%)

Number of samples within range

within detection range according to limits on the respective plate
9201 (2 below curve fit, including the empty sample well, 1 below detection range)

within quantification limits (KIT) (0,69< samples<248)
8229

- % detected (I intervallet LLOD-ULOD) 99,99%
- % quantifiable (I intervallet LLOQ-$ULOQ_{KIT}$) 89,4%
- % not detected (under LLOD) 0,01%

Distribution of calculated concentration values:

MAX	86,4
MIN	0,04
AVERAGE	1,1
MEDIAN	0,98

MSD method **R-plex Osteopontin**

The concentration of Osteopontin was measured on a MESO Quickplex SQ 120 instrument (Mesocale discovery (MSD)), using a R-plex antibody set (K151YMR-2) and 96-well Smallspot Streptavidin plates (L45SA-1) according to the manufacturer's instructions. The assay is based on analyte specific antibody pairs, with a capture antibody that binds to a spot in a 96-well and a detection antibody that enables light generation, proportional to the amount of bound analyte. Light signals are converted to concentrations using a standard curve, which is generated for each individual analyte on each individual plate.

9204 samples were measured on 118 individual plates and each plate contained 78 samples in single wells, as well as 8 standards and a control pool in duplicate. The control pool was used to determine assay variation, both intra and inter-plate.

Samples underwent one freeze thaw cycle to be aliquoted onto 96 well plates before they were thawed in the 96 well plates for the MSD analysis. Thawed samples were spun down and diluted 1:10. All pipetting steps were conducted with the BRAVO pipetting platform.

Samples from x plates were fitted with the standard curve from another plate.

The average assay variation within and between plates was: x % of samples were measured in detection range of the assay.

Detection range was calculated for every plate based on the highest and lowest standard and quantification range was given by the supplier (V-plex) / was defined as the standards that had a CV <25 and a recovery of 75-125%.

(this was page 2 in the document from adiponectin)