



Large Scale Investigation of Biomarkers using MesoScale Discovery Platform

Margit H. Larsen, PhD, Laboratory Manager, Copenhagen Hospital Biobank Group

Nordic Biobank Conference 2022

The Danish Blood Donor Study

- >200 donation sites – Nation wide
- Collection of data
 - Questionnaires, genetics, Socio-demography, diagnoses, medicine etc.
- ~150.000 blood donors with consent
- ~ 2.400.000 blood samples



Sample collection procedure

Donation site

Biobank Unit



Donation



Fridge



Centrifugation



Fridge



Transport



Freezer



Registration



Freezer

Sample collection procedure



Transport



Freezer facility -20°C



Procedure vary between sites

To establish a large, deeply phenotyped cohort ($N \sim 10,000$) for use:

- In large-scale omics studies
- As a national platform of reference values

Biomarker Platforms

Several quantitative assay platforms



- Resembles a regular ELISA
- Signal detection: Electrochemiluminescence
- Measure Multiple analytes in a single well
→ advanced studies
- 54 biomarkers V-Plex - distributed on 7 panels

V-PLEX Human Biomarker 54-Plex Kit

CRP	IL-12p70	IL-17D	IL-3	IL-9	PIGF	VEGF-A
Eotaxin	IL-13	IL-1 α	IL-31	IP-10	SAA	VEGF-C
Eotaxin-3	IL-15	IL-1 β	IL-4	MCP-1	TARC	VEGF-D
FGF (basic)	IL-16	IL-1RA	IL-5	MCP-4	Tie-2	GM-CSF
VEGFR-1/Fit-1	IL-17A	IL-21	IL-6	MDC	TNF- α	IFN- γ
ICAM-1	IL-17A/F	IL-22	IL-7	MIP-1 α	TNF- β	IL-2
IL-10	IL-17B	IL-23	IL-8	MIP-1 β	TSLP	
IL-12/IL-23p40	IL-17C	IL-27	IL-8 (HA)	MIP-3 α	VCAM-1	

Multiarray plate



Good platform for biobank research

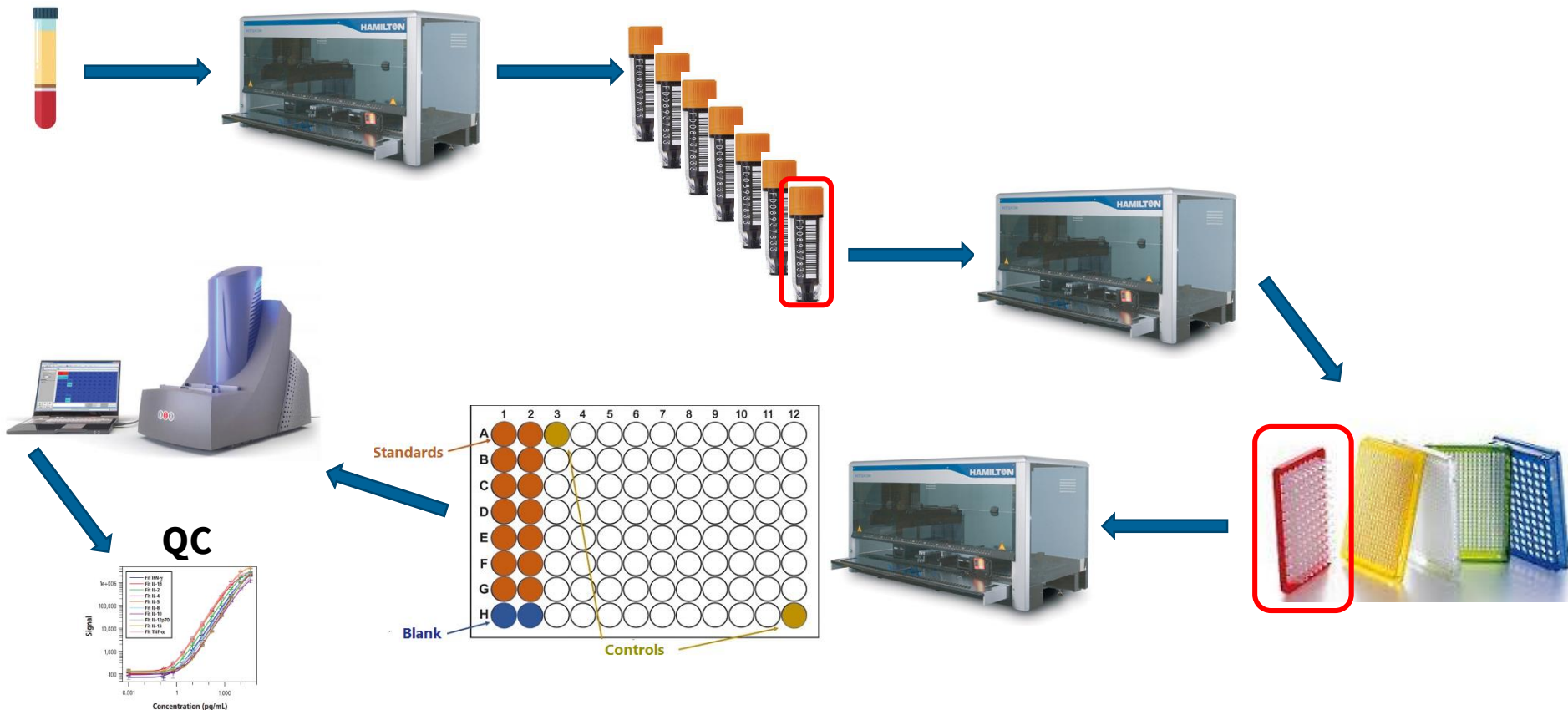


Look into the quality of:

- Reproducibility?
- Storage time?
- Regional differences?

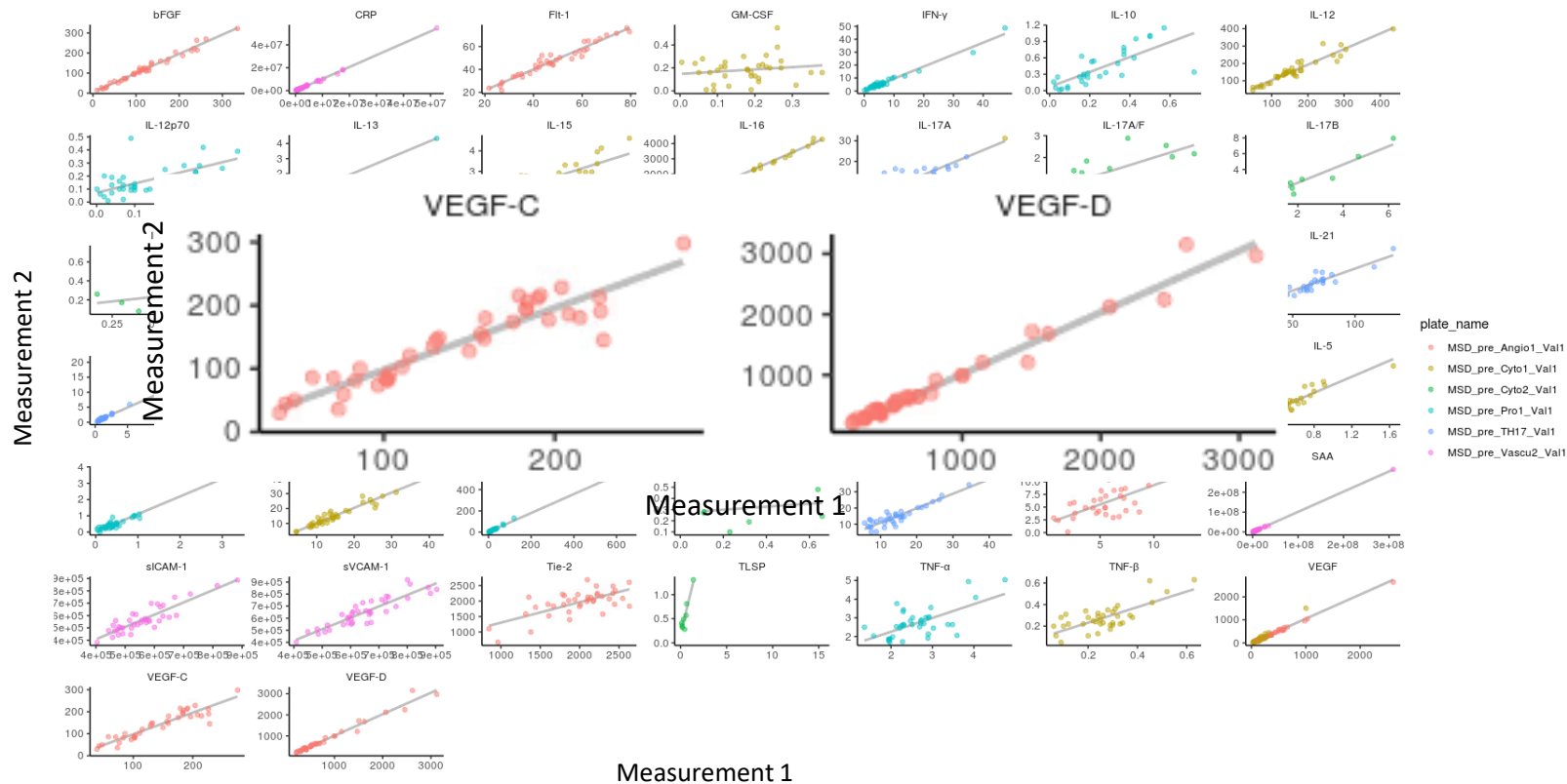
- Find 1,000 samples in freezers
- Optimize Laboratory flow to handle all samples

Sample flow – semi automation



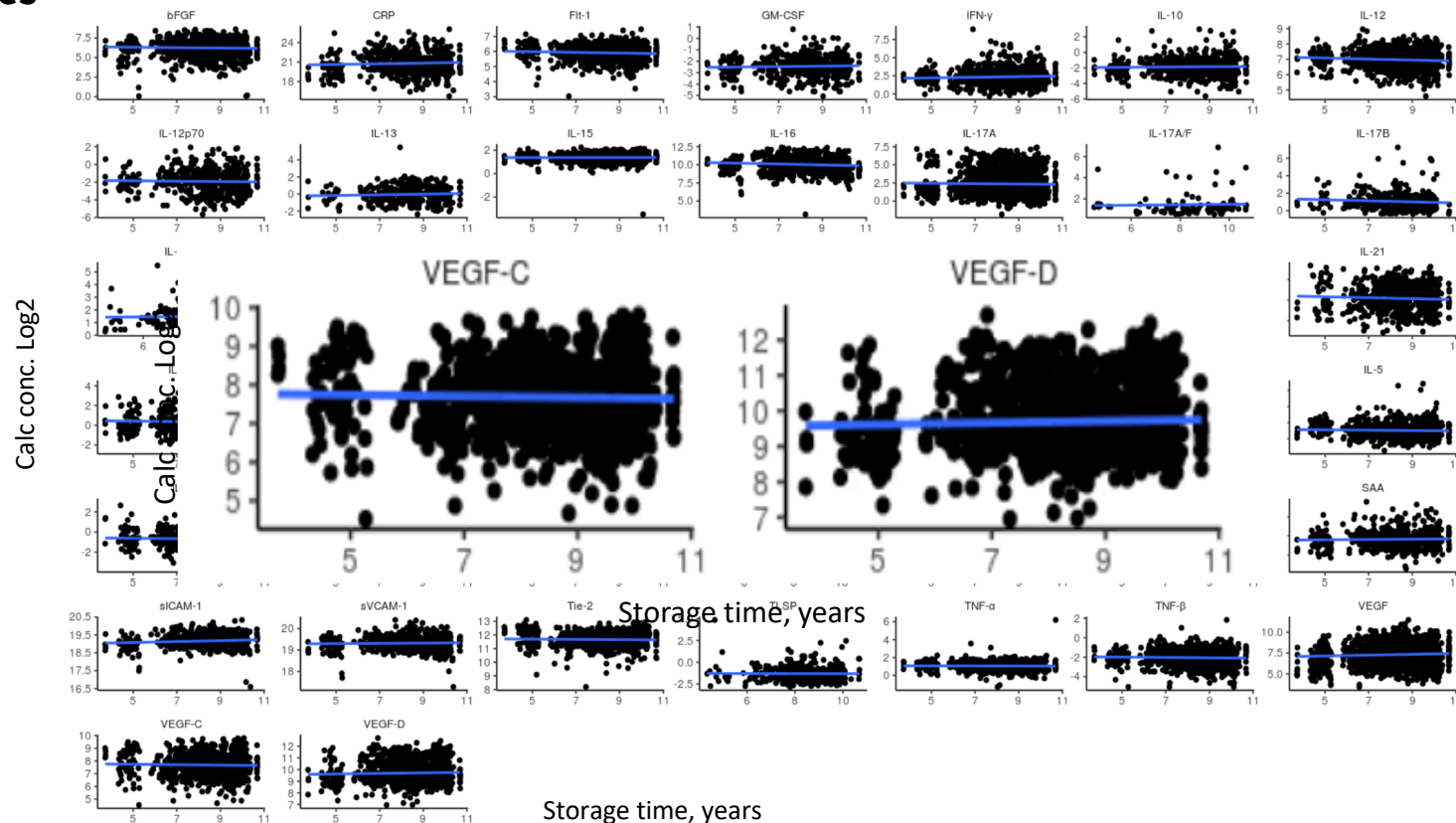
Pilot Study Results - Reproducibility

Correlation between repeated measurement 1 and 2



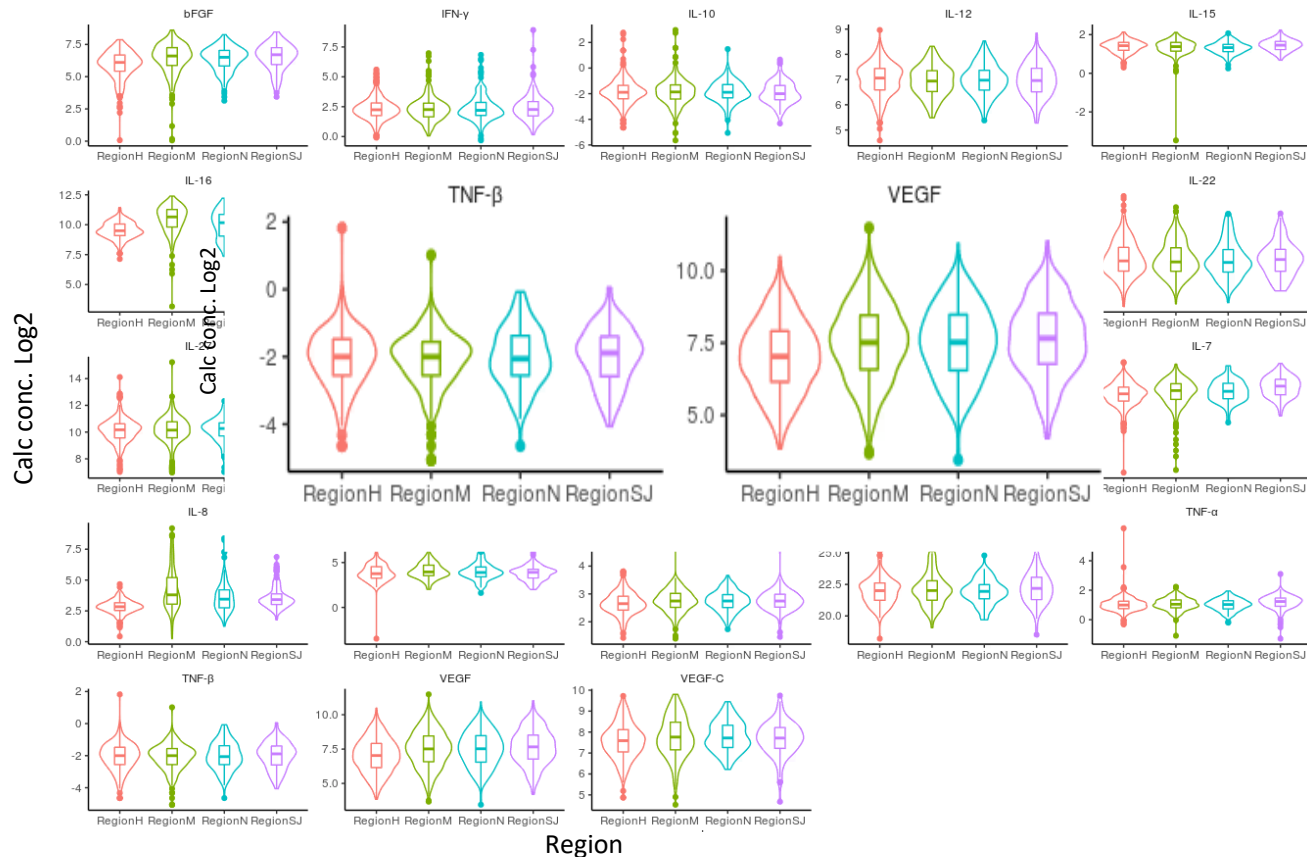
Pilot Study Results – Storage time

All analytes



Pilot Study Results – Regional differences

Violin plot



Next step

After the pilot study we expanded the number of samples

10,000 samples * distributed on 7 panels → ~ 900 plates

Results – biomarker levels

~10,000 cohort samples

- Most of the markers within range
- Some markers have many measurements below range (IL-1B, IL-3, IL-12p70, IL-2, IL-4, IL-9 IL-13, IL-17A/F)

Value	Count	Below range	% below
Proinflammatory			
CRP (mg/L)	9323	<5	0
SAA (mg/L)	9323	<5	0
TNF-A	9876	<5	0
TNF-B	9784	166	1.7
IL-1A	9785	24	0.2
IL-1B	9874	4603	46.6
IL-1RA	9813	<5	0
IL-3	9810	7028	71.6
IL-6	9876	438	4.4
IL-12	9690	<5	0
IL-12p70	9873	3254	33
IL-15	9785	<5	0
T cell derived			
IFN-G	9876	<5	0
IL-2	9876	2243	22.7
IL-4	9874	3148	31.9
IL-5	9785	24	0.2
IL-9	9813	1908	19.4
IL-10	9875	148	1.5
IL-13	9873	4464	45.2
IL-16	9785	0	0
IL-17A	9785	94	1
IL-17A/F	9809	1683	17.2
IL-17B	9811	651	
IL-17C	9812	1325	13.5
IL-17D	9812	564	5.7
Chemokines			
Eotaxin	9447	<5	0
Eotaxin-3	9447	8	0.1
IL-8	9876	<5	0
IP-10	9447	<5	0
MCP-1	9447	<5	0
MCP-4	9446	<5	0
MDC	9446	5	0.1
MIP-1A	9447	95	1
MIP-1B	9447	5	0.1
TARC	9447	<5	0
Growth factors and vascular			
bFGF	9876	6	0.1
Flt-1	9876	<5	0
Tie-2	9876	8	0.1
IL-7	9785	<5	0
PIGF	9876	8	0.1
VEGF	9876	<5	0
VEGF-C	9876	13	0.1
VEGF-D	9876	8	0.1
TSLP	9812	1216	12.4
GM-CSF	9785	1154	11.8
sICAM-1 (ng/mL)	9322	<5	0
sVCAM-1 (ng/mL)	9322	<5	0

Unpublished data

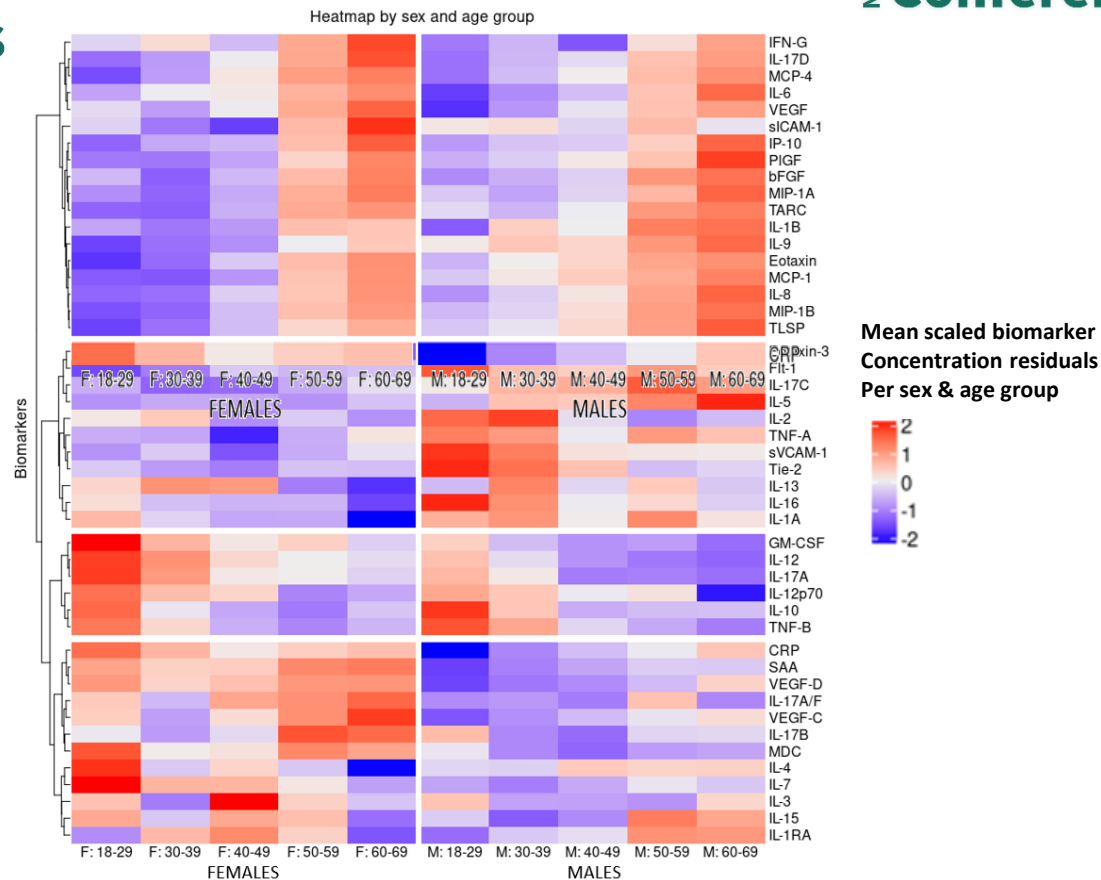
Results – biomarker levels

~10,000 cohort samples

Heatmap

Demographic differences
per sex and age group

Unpublished data

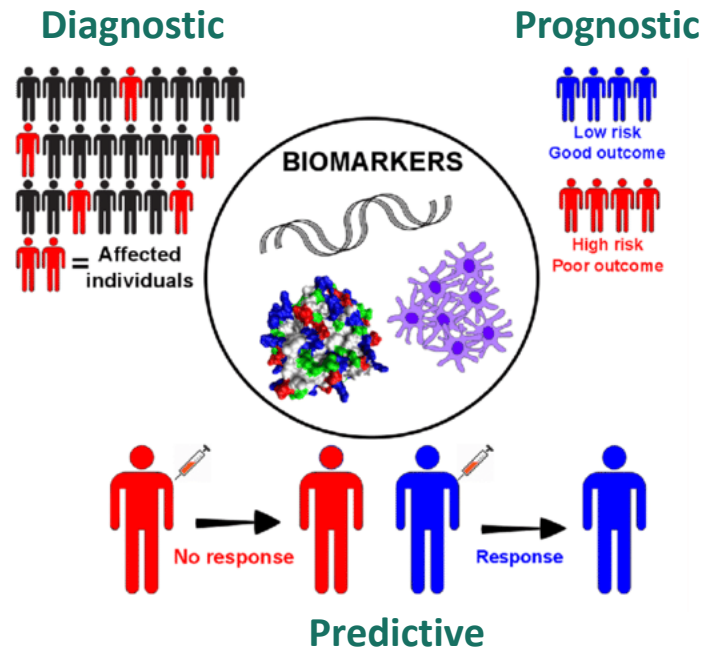


What now...

We now have a large biomarker dataset

Reference material for researchers and clinicians

Will help us understand the underlying biology



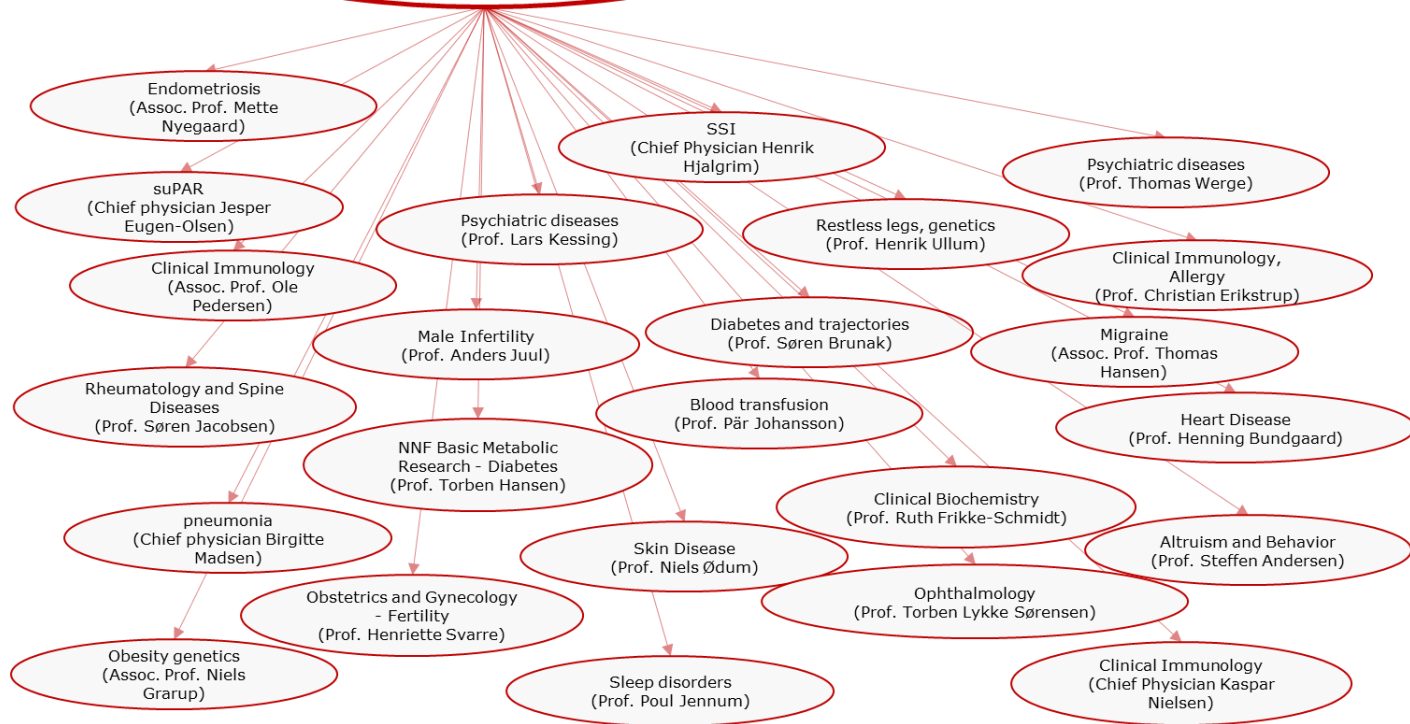
Collaborators



The Danish Blood Donor Study

Established scientific
collaborators

NORDIC Biobank
Conference



First manuscripts in preparation..

Thank you!

Contact information:

Margit.anita.hoerup.larsen@regionh.dk

Conflict of interest

No conflicts of interest.

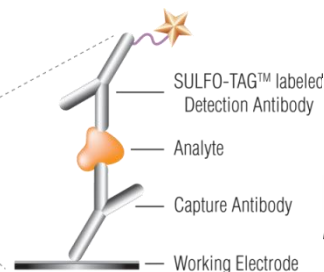
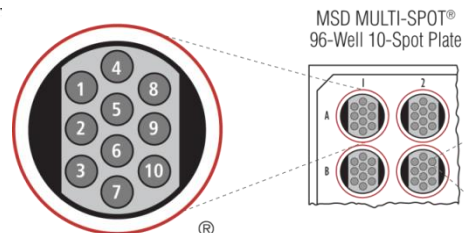
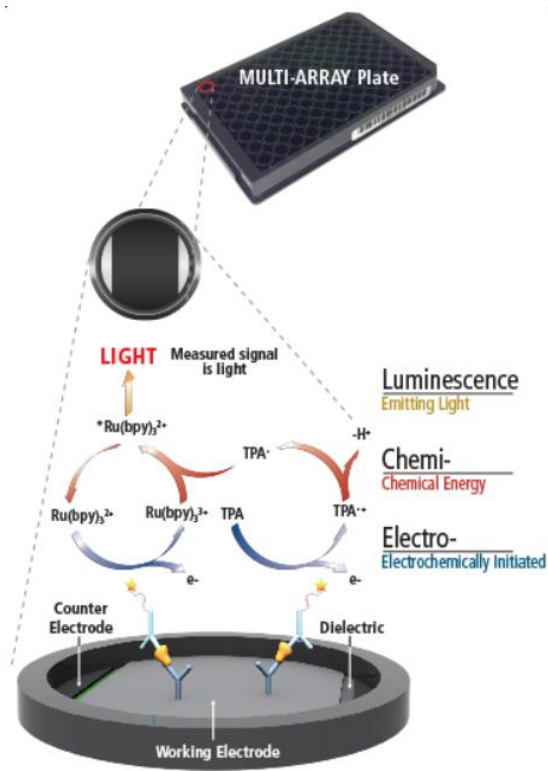
Based on the pilot study, **acceptance criteria were defined** for each biomarker (on calculated concentrations):

- Mean acceptance criteria of calibration curve: 80-120%
- CV of calibration curve: $\leq 20\%$
- Internal control: within $\pm 3 \times \text{SD}$ mean concentration

Importantly, we demonstrated acceptable long-term stability of samples with freezing times within 10 years at 20°C.

Meso Scale Discovery (MSD)

NORDIC Biobank
Conference



MSD®
Meso Scale Discovery
A division of Meso Scale Diagnostics, LLC.
www.mesoscale.com

