

From Chip to Bead

PROTAGEN[®]



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Diagnostics
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- Introduction to Protagen AG
- Autoantibodies as biomarkers
- UNlarray[®] projects
- Beads based arrays



Established in 1997
Private Stock Corporation
50 Employees

1500 m² Modern Lab Space in the
Technology Center Dortmund
Otto-Hahn-Str.15
44227 Dortmund
Germany



Protein Services

GMP-Compliant Protein Analysis

- Complete protein characterization and release testing
- Glycans & PTM
- Impurities & HCP
- Bioassays

Customized method development

Biomarker & target discovery

Customized Bio-IT Solutions

- IT support for Protein analytics and Protein Mass Spectrometry
- Array data analysis
- Statistical methods for biomarker discovery
- Modiro® – The PTM Explorer
- ProteinScape®

Diagnostics

Protagen Diagnostics is dedicated to the discovery, development and commercialization of high-value, multiplex *in-vitro* diagnostics based on autoantibody profiling in blood.

R&D programs in oncology, neurology, auto-immune and metabolic diseases.

Co-development option with existing biomarker programs at Protagen Diagnostics and partners.

UNlarray[®]

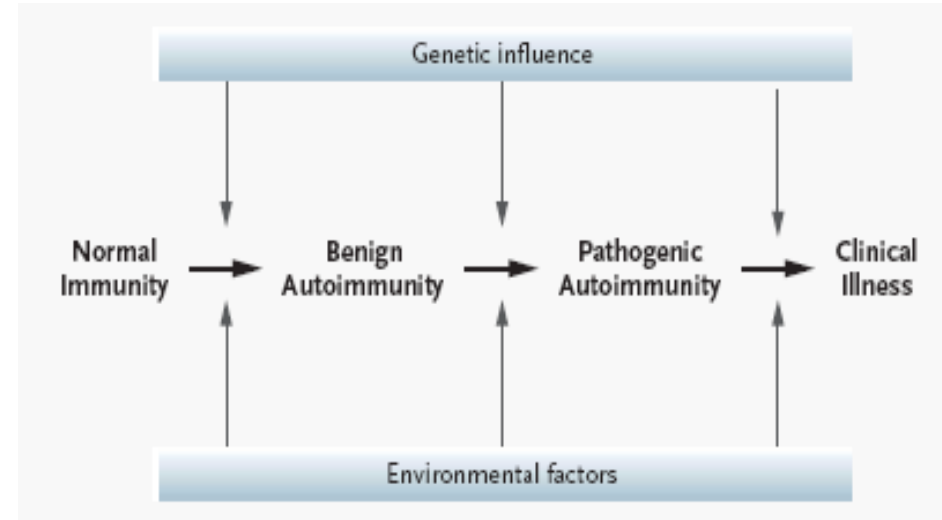
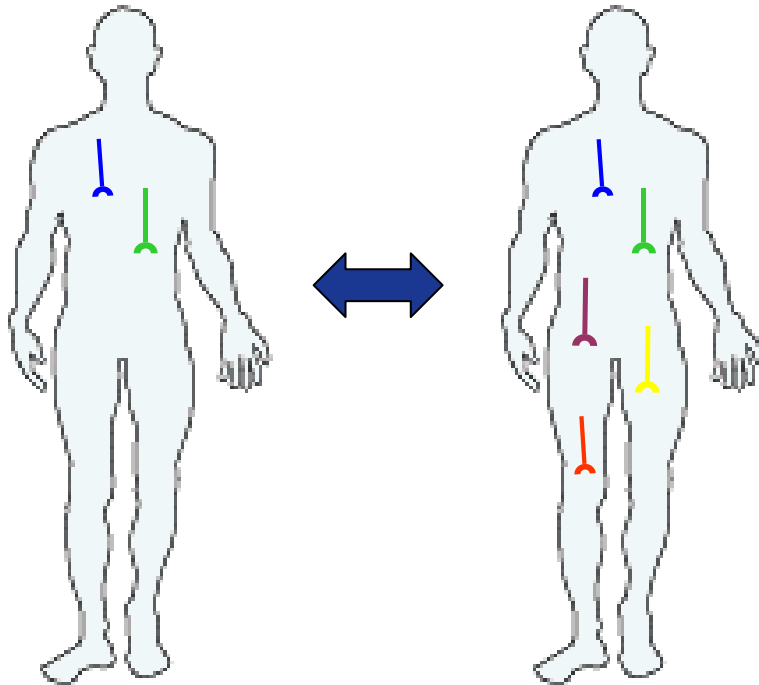
- High throughput protein expression & production for multiplex analysis (protein-microarray, bead based arrays)
- Broad application in discovery and validation of biomarkers for
 - diagnosis / prognosis
 - treatment stratification
 - treatment monitoring

UNlchip[®]

- Protein microarray analysis of antibody-antigen interaction for
 - diagnostic purposes
 - antibody performance prediction (crossreactivity and stability)

Non-disease

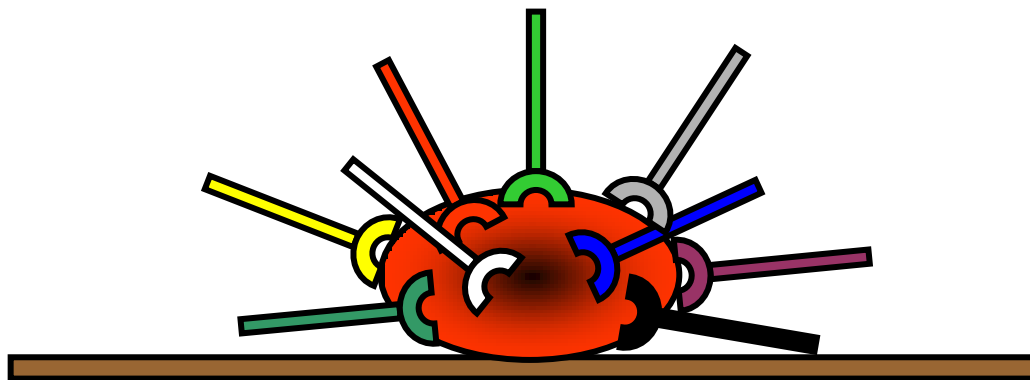
Disease



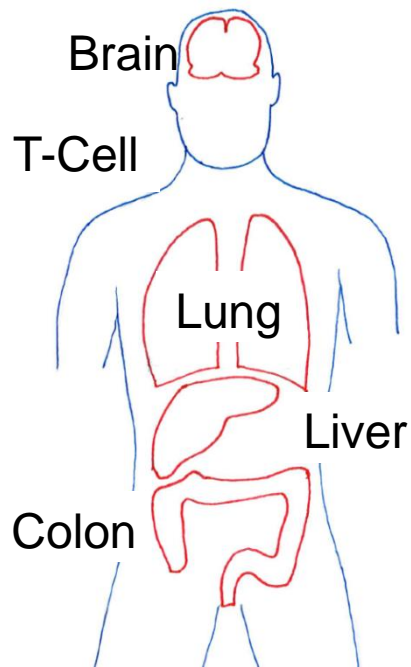
Arbuckle *et al.*, 2003

- Specific autoantibodies are present in patients
- Autoantibodies and their corresponding antigens may serve as biomarkers for diagnosis

- result from a specific polyclonal immune response directed against immunogenic antigens
- are dynamic components of the immune system
- change specifically during pathogenesis and treatment
- are used for diagnostic purposes
- are attractive molecules for the development of novel serum based diagnostics
- enable retrospective/prospective studies



Polyclonal Antibody-Pool



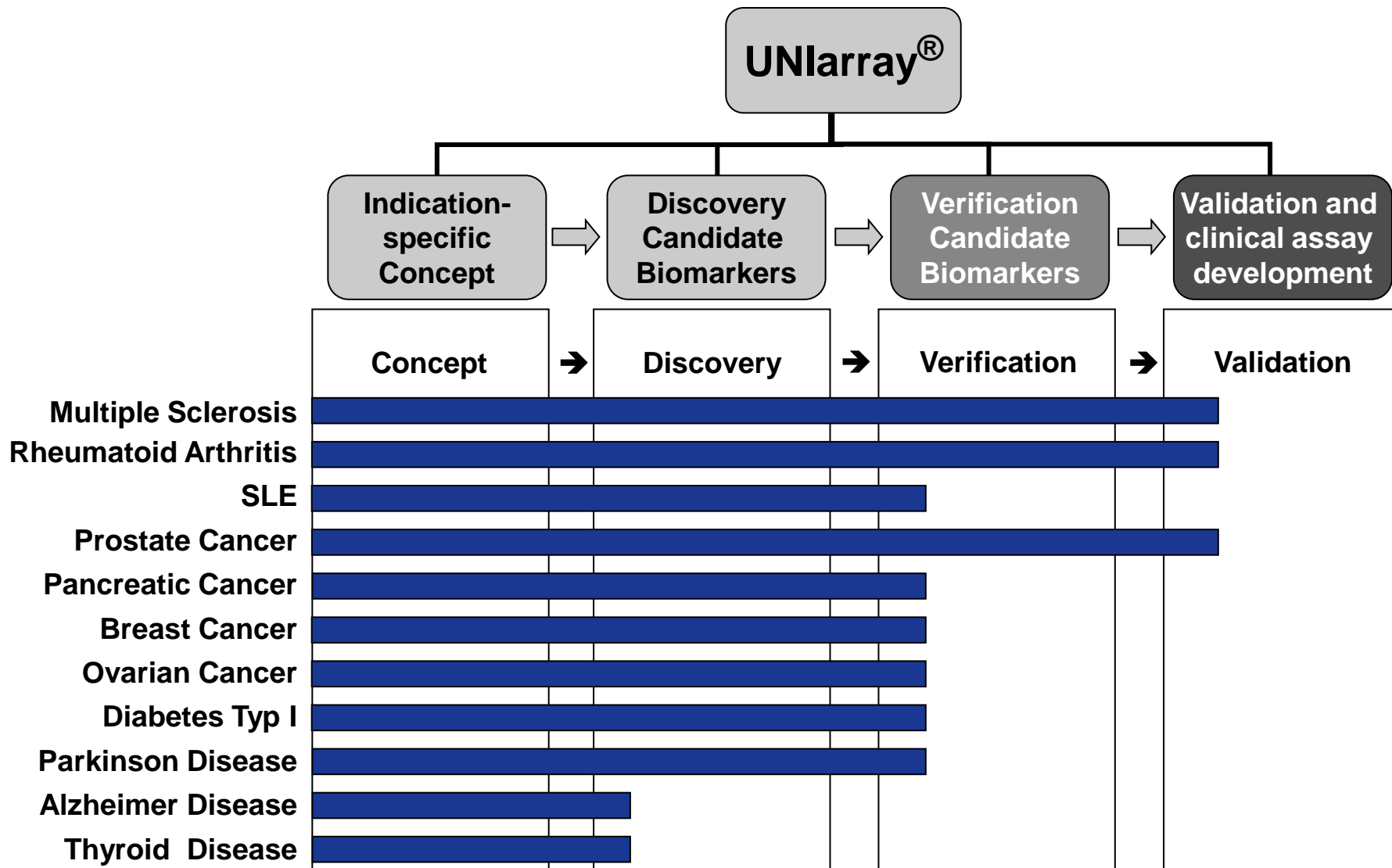
Expression libraries from human organs:

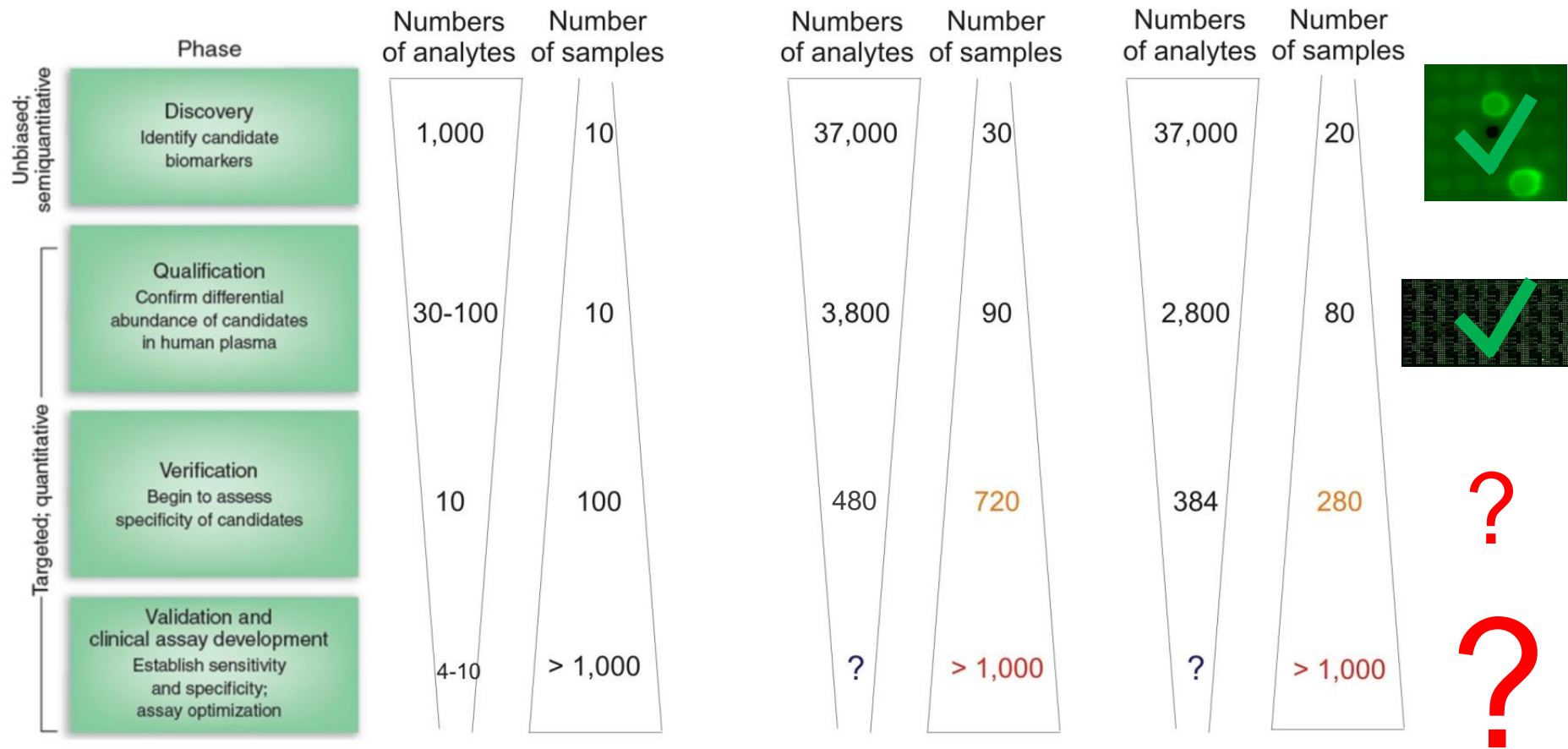
- High complexity (>155,000 expression clones)
- High diversity (>10,000 unique human proteins)
- High-throughput production of recombinant proteins
- Recombinant proteins fused to His-tag
- Affinity protein purification via His-Tag in a high-throughput manner



Production of protein microarrays

- Content: up to 3000 proteins per microarray
- Contact printing onto nitrocellulose surface

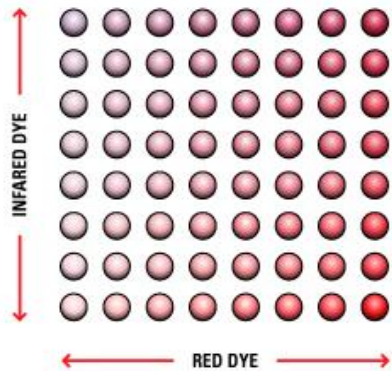
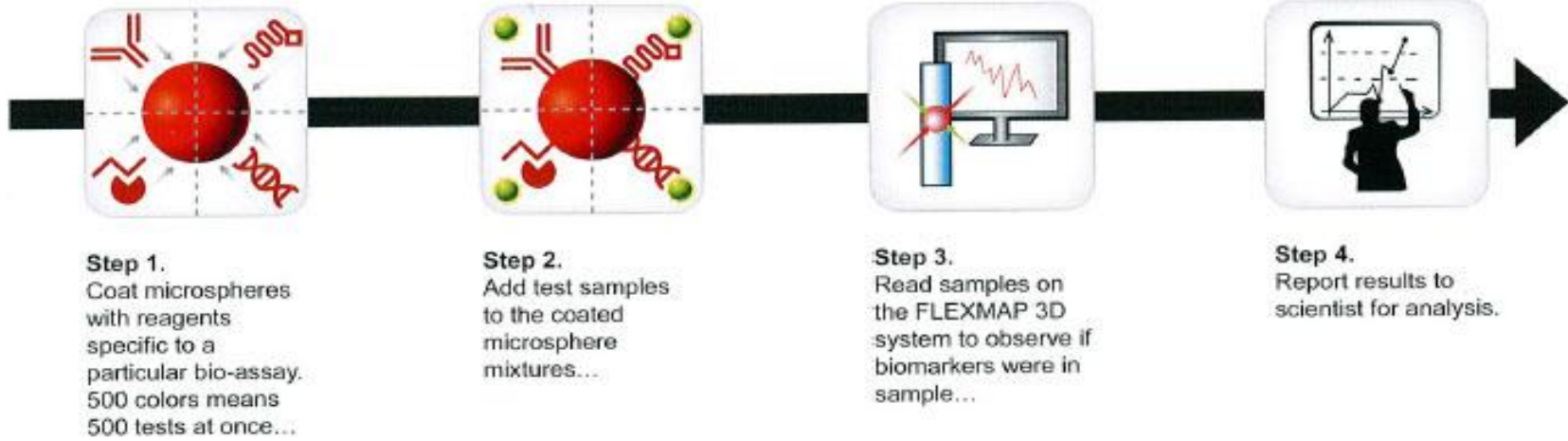




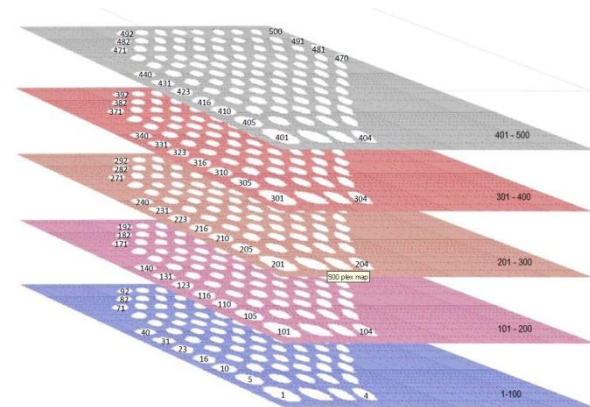
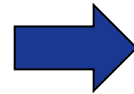
Rafai et al., 2006

Prostate Cancer

Multiple Sclerosis



Labeled mirospheres



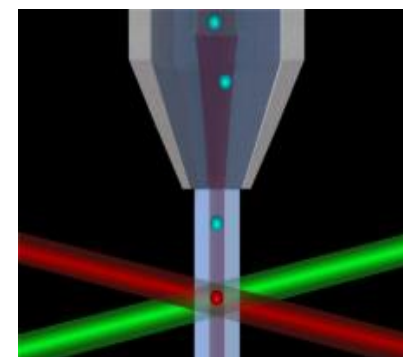
500 Bead Region Map

Features	Planar Array	Bead Based Array
Protein content	3000	50 / 100 / 500
Batch size	50	1000
Protein per batch	1 μ g	12 μ g
Sample volume	3 μ l	5 μ l
Samples per week	100	1000
Data points per measurment	2-4	> 35
Coefficient of variation	10-30 %	< 10 %
Dynamic Range	1-2 decades	3-4 decades

Planar Microarray



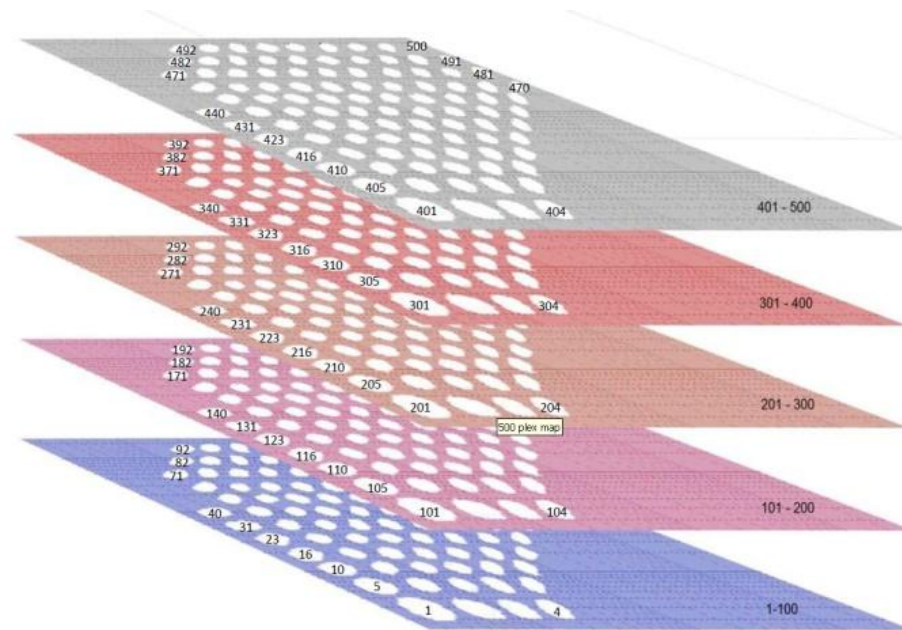
Bead Based Array





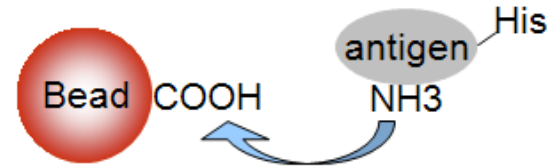
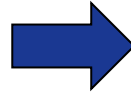
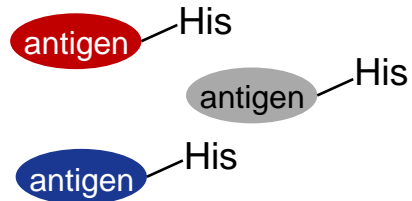
FlexMap3D

500 Bead Region Map

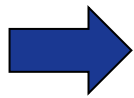
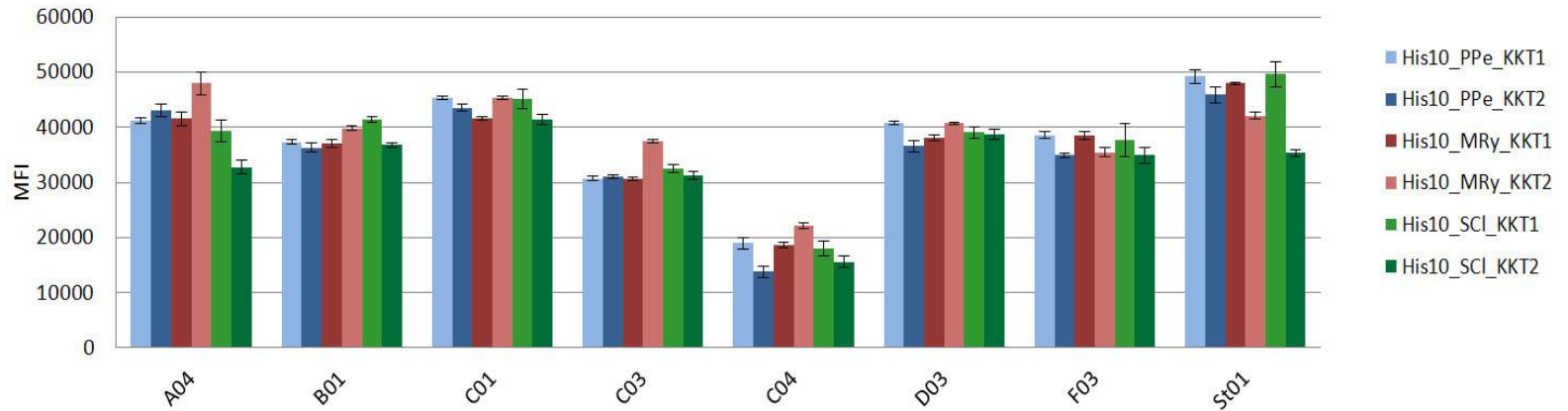


3-20 Antigens

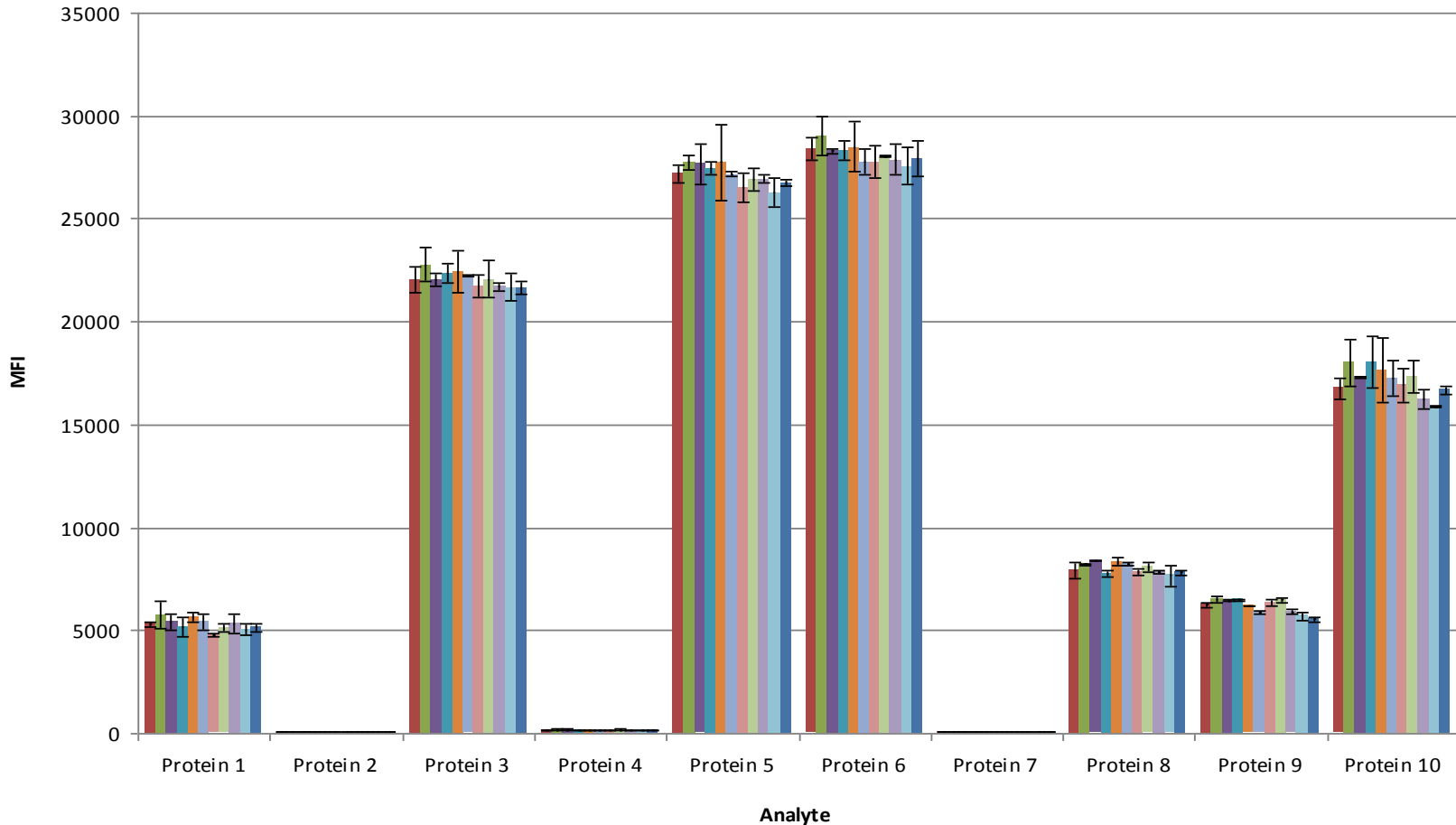
Coupling



Coupling Control Assay



Reproducibility of 10 Replicates



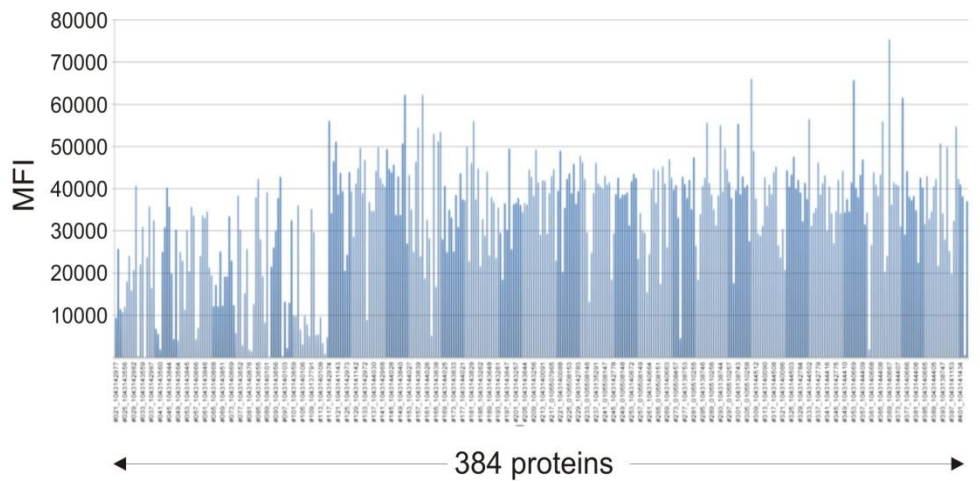
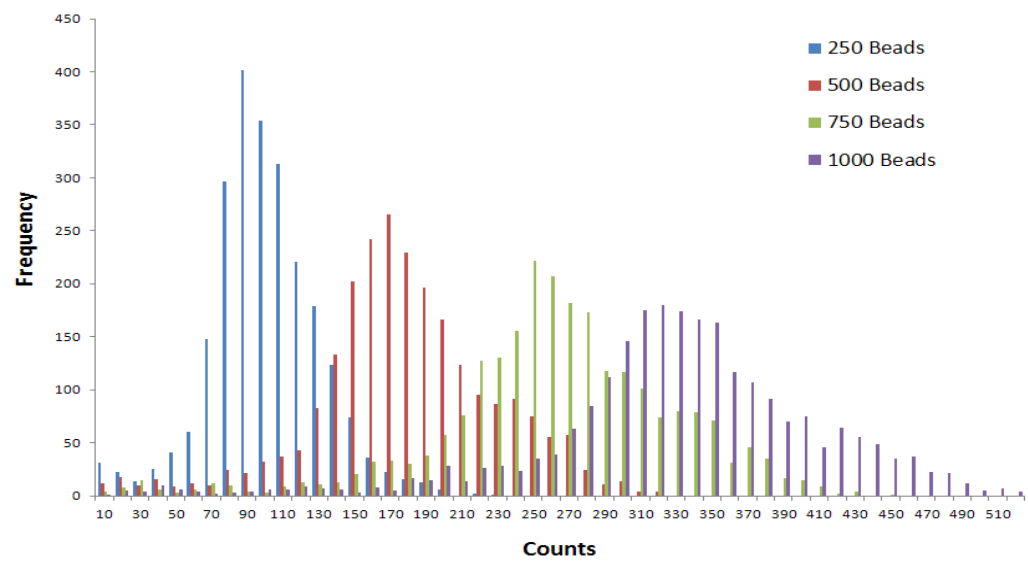
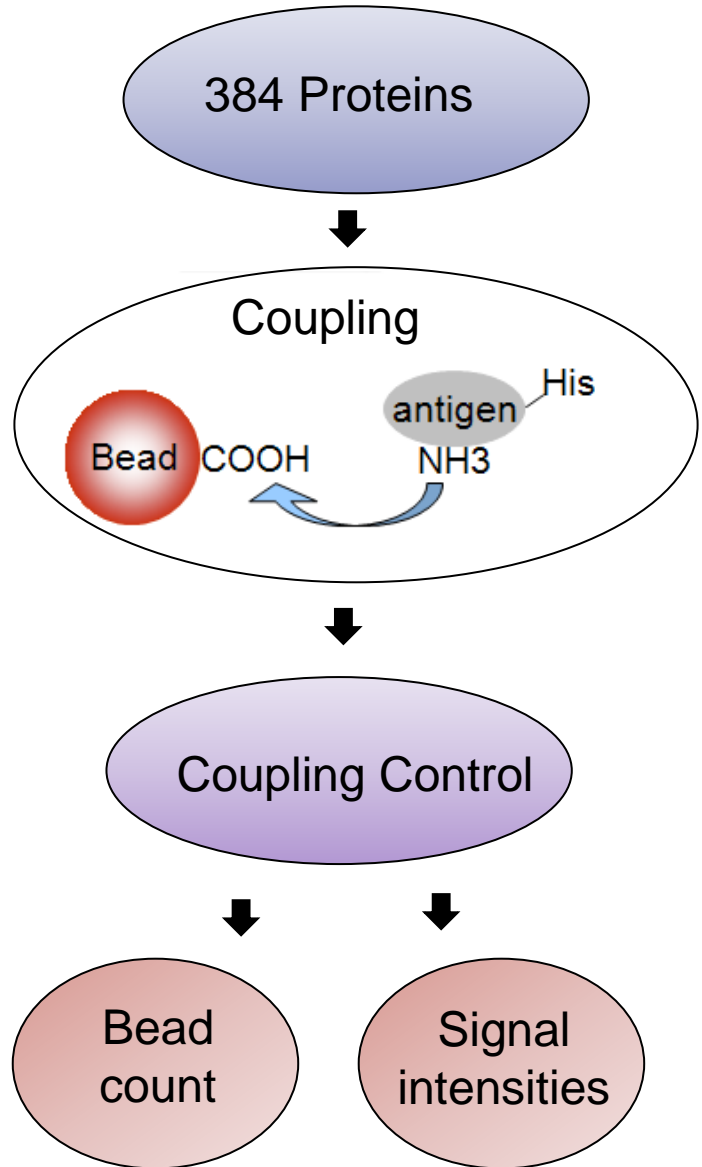
- Mean CV (Intra-Plate) : 4,2 %

- 20 antigens coupled to different bead regions (20-plex)
- 20 serum samples measured in quadruplicates.

Table: CV in % for antigens and serum samples

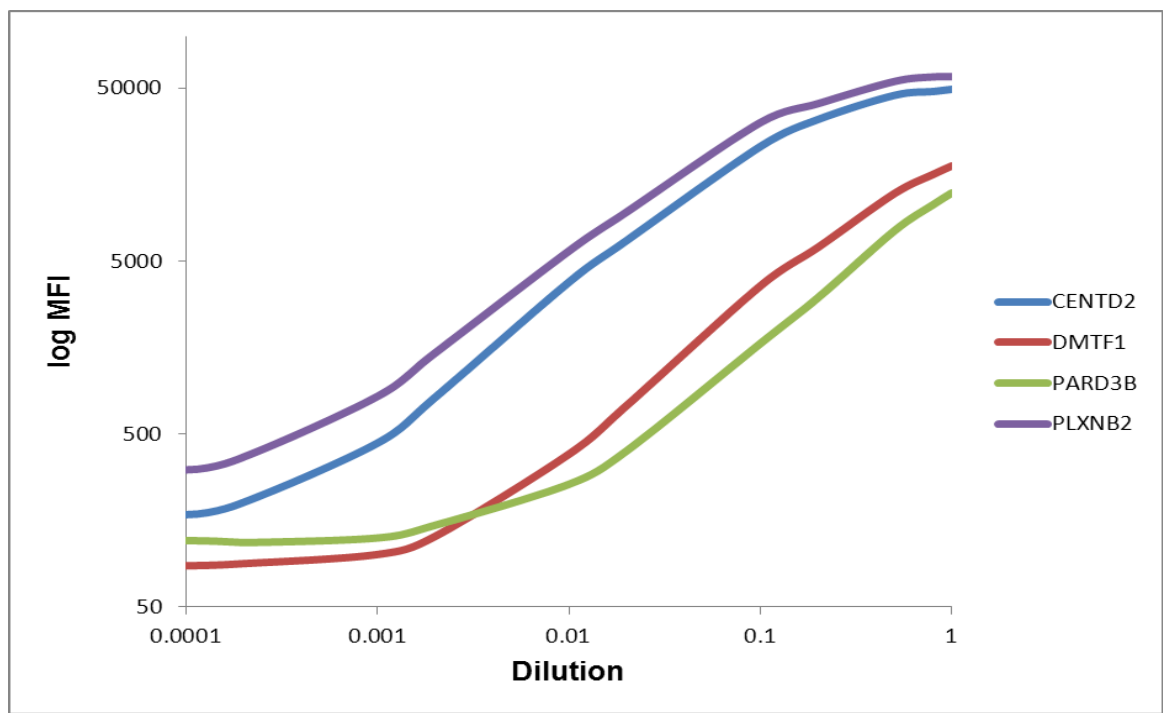
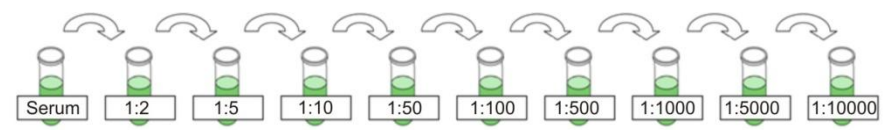
Sample	A01	A02	A03	A04	B01	B02	B03	C01	C03	C04	D03	E02	E03	F02	F03	G01	H01	H02	St01	St01 de	C01 de	Mean by sample
CA_154147 PG264-P01-2009-1351	6	7	11	6	9	8	3	29	5	7	12	13	5	3	4	3	4	13	4	6	12	8
CA_154224 PG264-P01-2009-1369	4	10	4	5	4	8	7	6	9	13	13	9	13	5	2	10	6	21	9	8	12	8
CA_159149 PG264-P01-2009-1360	2	6	2	6	1	10	4	2	10	3	2	2	13	3	5	3	3	9	1	1	13	5
CA_286957 PG264-P01-2009-1354	2	7	1	3	3	10	6	4	30	7	4	4	7	5	8	3	3	14	5	5	20	7
CA_287676 PG264-P01-2009-1361	1	5	0	1	1	1	2	2	9	1	2	3	13	4	4	3	4	11	1	0	20	4
CA_287778 PG264-P01-2009-1357	4	3	1	2	6	11	3	4	12	3	5	5	2	6	2	2	3	4	3	2	14	5
CA_310081 PG264-P01-2009-1358	7	19	11	9	11	12	11	7	23	9	18	12	11	13	10	19	17	15	10	10	18	13
CA_325086 PG264-P01-2009-1376	5	2	2	1	2	7	2	7	10	2	2	1	3	4	2	3	2	9	1	2	12	4
DB_226971 PG264-P01-2009-1414	2	7	3	14	8	7	3	13	11	11	7	17	10	6	14	6	8	12	4	4	16	9
DB_241028 PG264-P01-2009-1397	4	19	4	12	4	9	2	5	27	9	6	2	8	4	4	8	2	12	4	5	27	8
DB_246021 PG264-P01-2009-1409	6	4	1	4	4	2	1	3	16	2	1	3	2	3	11	3	3	5	1	2	31	5
DB_247546 PG264-P01-2009-1404	6	15	6	9	5	14	9	10	19	11	12	9	4	9	10	7	6	11	12	9	15	10
DB_252485 PG264-P01-2009-1403	2	2	1	10	3	2	2	4	9	3	5	1	0	6	1	2	2	3	1	2	11	3
DB_256445 PG264-P01-2009-1399	3	3	3	3	2	8	2	4	5	2	3	3	1	2	9	2	1	3	2	1	52	6
DB_287743 PG264-P01-2009-1413	2	3	2	5	1	2	3	3	4	4	4	1	5	4	6	3	2	9	2	2	8	4
DB_289601 PG264-P01-2009-1422	2	10	2	2	2	1	2	5	8	4	1	2	1	1	7	2	1	4	1	3	19	4
DB_299059 PG264-P01-2009-1421	2	6	2	4	0	19	3	2	8	3	5	2	2	3	5	3	2	9	4	2	9	4
DB_299097 PG264-P01-2009-1408	3	3	3	2	2	13	1	6	5	2	2	2	1	3	8	4	2	5	2	1	14	4
PG264-P01-2009-1366	3	1	2	8	1	8	2	5	27	3	2	1	4	1	4	3	3	10	3	2	5	5
PG264-P01-2009-1373	3	5	7	17	3	11	6	8	8	9	9	7	5	5	5	10	2	12	5	7	10	7
Mean by antigen	3	7	3	6	4	8	4	6	13	5	6	5	5	4	6	5	4	9	4	4	17	

- Mean CV of antigens: 6%
- Mean CV of samples: 6%
- FDA recommends CV < 15%



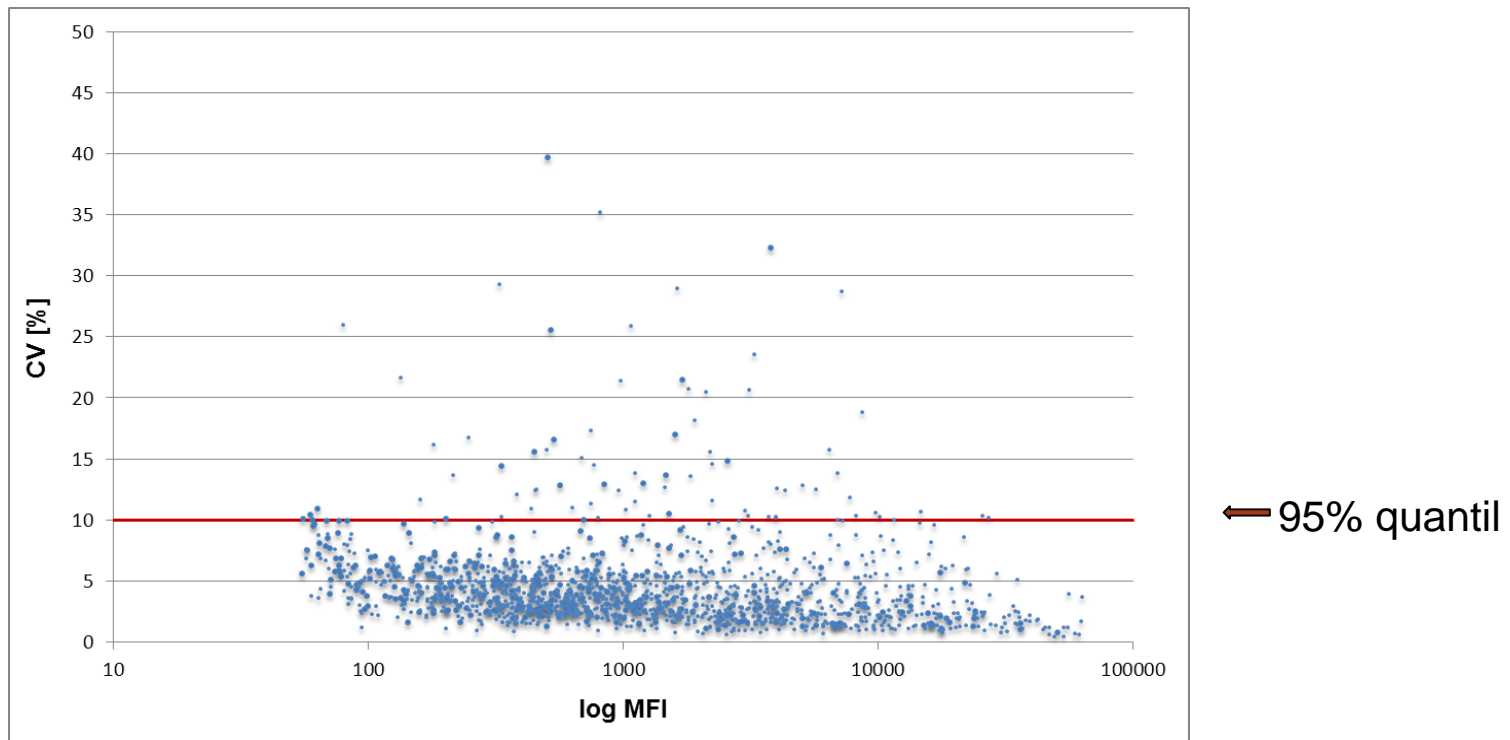
Dynamic Range of BBAs

- 400 antigens coupled to different bead regions (400-plex)
- Serial dilution of a serum sample



- Dynamic range: 3-4 decades
- Limit of detection: 100 - 500 MFI
- Limit of quantification: 500 MFI

- 400 antigens coupled to different bead regions (400-plex)
- 3 serum samples measured in 8 replicates

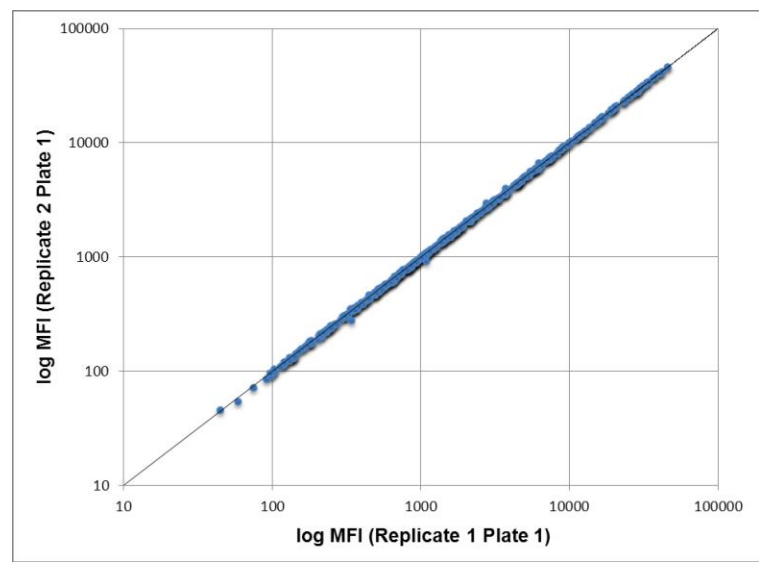


- 95% of all data points show a coefficient of variation $< 10\%$
- By increasing signal intensity the error of measurement is reduced

Antigen/Autoantibody Interaction Assays Using BBAs **PROTAGEN**[®]

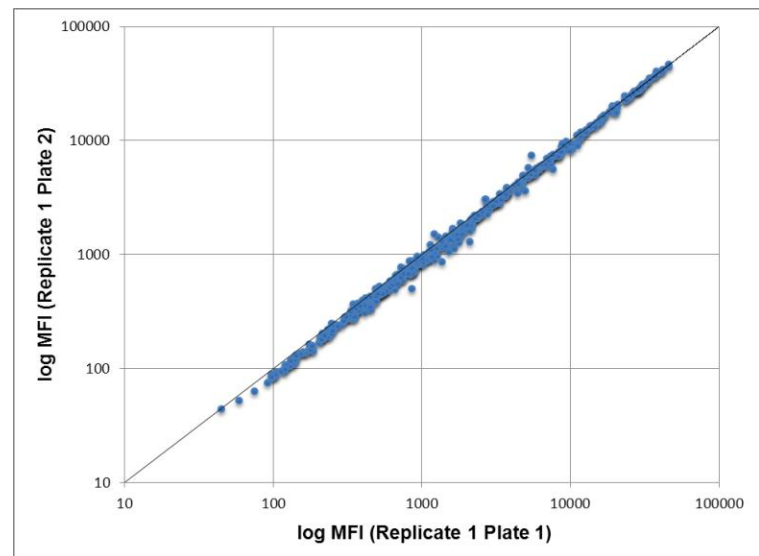
Intra-Plate:
Average CV Values of reference serum

Plate	Mean CV
# 1	5 %
# 2	2 %
# 3	2 %
# 4	2 %
# 5	3 %
# 6	2 %
Mean	3 %

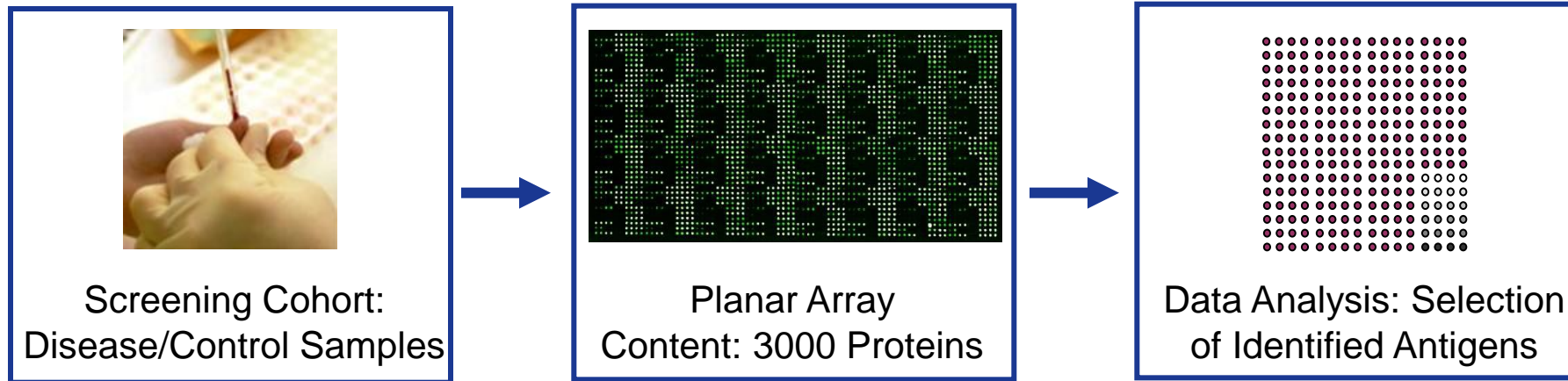


Inter-Plate:
Average CV Values of reference serum

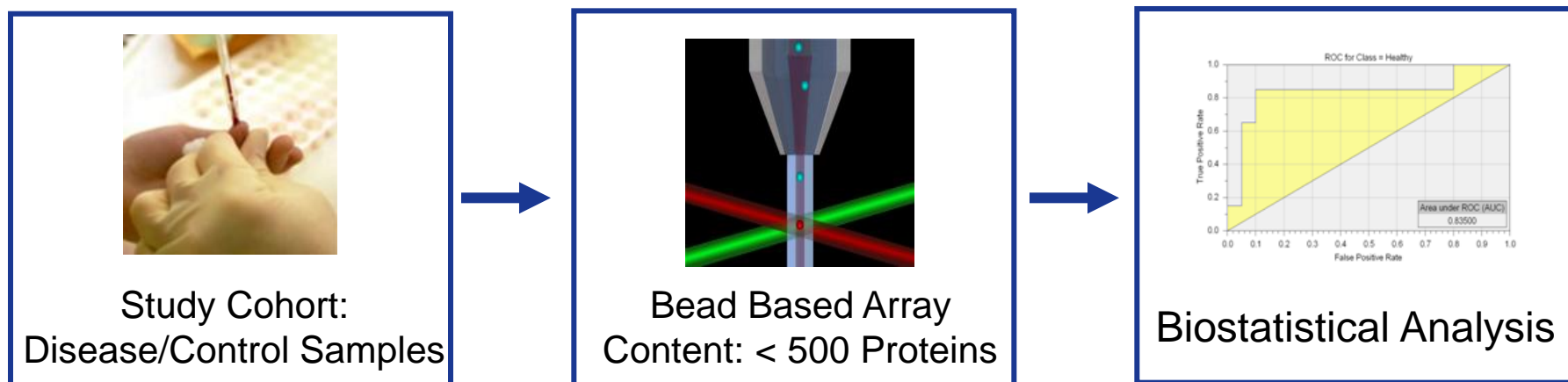
Plate	Mean CV
# 1 -6	10 %



- Screening of antigen/autoantibody interactions



- Validation of biomarker panels



Diagnostic Protein Panel

November 2010

Antigen No.	Microarray Serum 1	Luminex Serum 1	Microarray Serum 2	Luminex Serum 2	Microarray Serum 3	Luminex Serum 3	Microarray Serum 4	Luminex Serum 4
1	+	++	-	-	-	++	-	-
2	+	++	-	-	-	-	-	-
3	-	-	-	+	-	-	-	++
4	-	-	-	-	-	-	-	-
5	-	-	++	++	++	++	-	+
6	-	+	++	++	-	-	++	++
7	-	+	++	++	++	++	-	++
8	-	-	-	-	-	-	++	++
9	-	+	-	-	-	-	-	-
10	-	-	-	-	-	-	++	-
11	-	+	+	-	-	-	-	-
12	-	+	+	-	-	-	+	++
13	-	-	-	-	-	-	-	-
14	+	+	-	-	-	-	-	+
15	-	-	-	-	-	-	++	++
16	-	-	-	-	-	-	-	-
17	-	+	-	-	-	+	++	++
18	-	-	-	-	-	-	-	+
Overlap (%)	72		89		89		67	

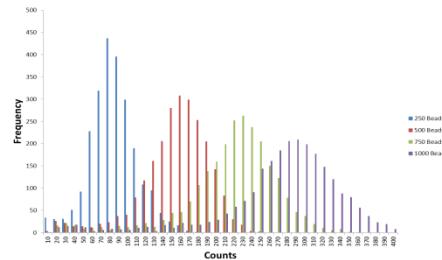
Proof of concept
Cooperation: NMI

February 2011



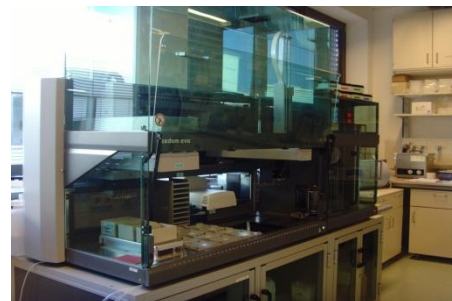
FlexMAP3D
500 Bead-Set
Set up of Assays

May 2011

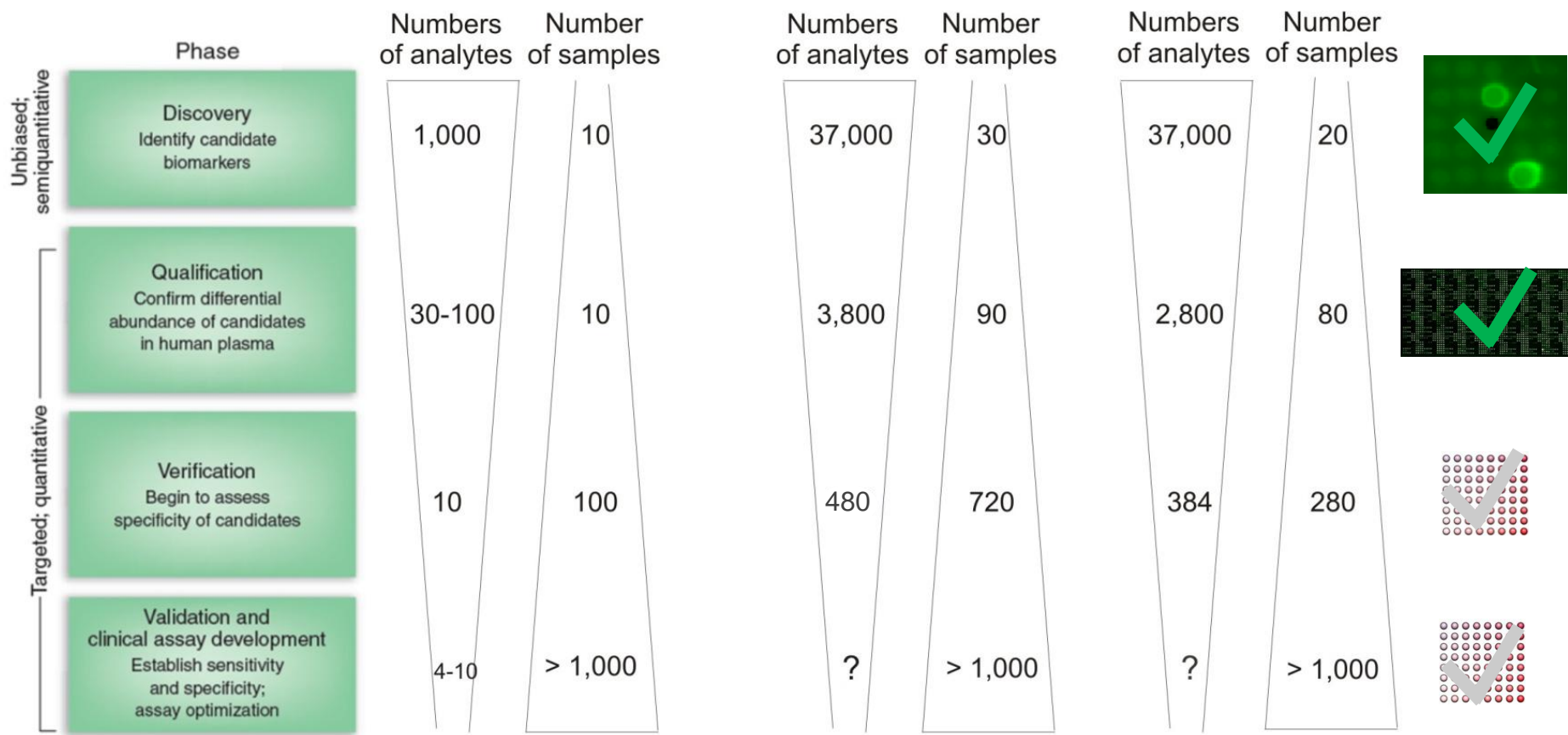


Training of staff
Quality control
Data analysis tools

August 2011



Automation of liquid
handling process



Rafai et al., 2006

Prostate Cancer

Multiple Sclerosis

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