Development of sample type specific surrogate matrices for dilution of blood samples to overcome matrix effects in multiplexing immunoassays

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ABSTRACT

Beside the advantage of bead-based multiplex immunoassays being a high-throughput screening tool, saving time and sample volume and generating confer data that are internally consistent by sample, additional requirements in this technology are getting more and more important: since in recent years multiplexing assays got widespread in the clinical research field, the need of performance specifications comparable to those of classical ELISA assays - which are still the “golden standard” of immunoassays - is growing.
Benchmark analysis of bead-based multiplex immunoassays from different vendors and different platforms all showed up the same limitations for these assays in regards of spike- and dilution-recovery performance in blood samples. Due to the complexities of the matrix, both serum and plasma show to inhibit the readout of many cytokines (low spike recovery) with some variations between different donors and also with differences between serum and plasma. Some of these data also conclude that dilution of samples should not be assumed to be linear.
eBioscience/Affymetrix took the advantage of its long year expertise in high-validated immunoassay development to develop matrix type specific sample diluents for serum and plasma to overcome described matrix- and multiplexing-effects and to fulfill high performance specifications comparable to those of traditional ELISA.
The newly developed surrogate matrices for dilution of serum or plasma samples give spike- and dilution-recovery results in the range of 70-130% and help to fulfill these specifications over the broadest individual assay range, with lowest variation between individual donors and the broadest portfolio on the market.

Materials and Methods

A 42 plex multiplex immunoassay (ProcartaPlex® Platinum, eBioscience Vienna) was developed by testing a variety of surrogate assay buffers to find the best performance in inhibiting and blocking matrix effects. These matrices contained different combinations and ratios of biological, synthetic, partly heat-denaturated compounds. Spike- and Dilution recovery results obtained from these Luminex® based assays were used to select the best performing surrogate matrices. ProcartaPlex Platinum assays were performed as described in the workflow and according to the user manual. Assay Performance regarding accuracy and linearity in different sample matrices was compared with Luminex immunoassays from 3 additional vendors.

ProcartaPlex Immunoassay Workflow

Figure 1

Figure 2

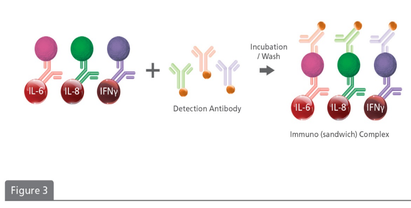


Figure 3

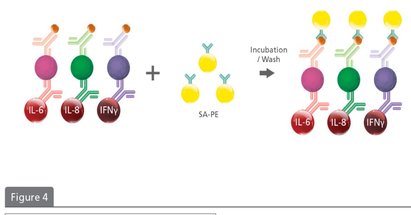
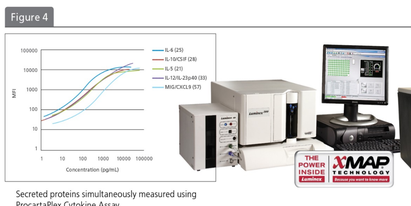


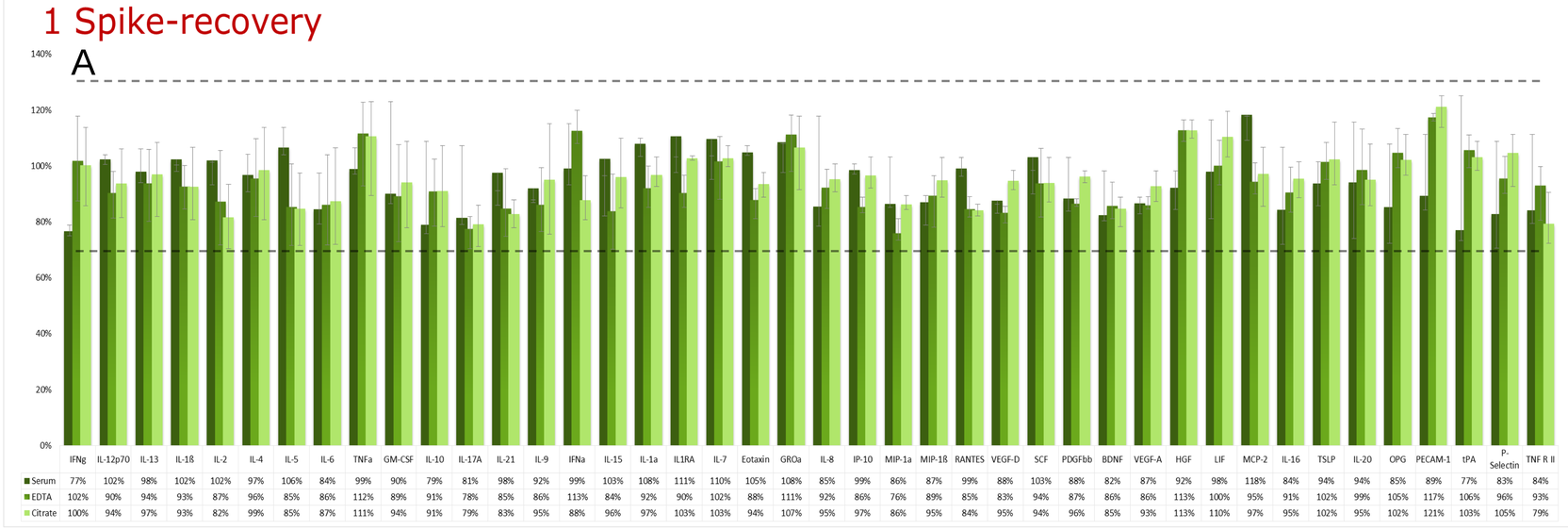
Figure 4



Secreted proteins simultaneously measured using ProcartaPlex Cytokine Assay.

Results

1 Spike-recovery



	IFNg	IL-12p70	IL-13	IL-1ß	IL-2	IL-4	IL-5	IL-6	TNFa	GM-CSF	IL-10	IL-17A	IL-21	IL-9	IFNa	IL-15	IL-1a	IL1RA	IL-7	Eotaxin	GROa	IL-8	IP-10	MIP-1a	MIP-1ß	RANTES	VEGF-D	SCF	PDGFbb	BDNF	VEGF-A	HGF	LIF	MCP-2	IL-16	TSLP	IL-20	OPG	PECAM-1	tPA	P-Selectin	TNF R II
Serum	77%	102%	98%	102%	102%	97%	106%	84%	99%	90%	79%	81%	98%	92%	99%	103%	108%	111%	110%	105%	108%	85%	99%	86%	87%	99%	88%	103%	88%	82%	87%	92%	98%	118%	84%	94%	94%	85%	89%	77%	83%	84%
EDTA	102%	90%	94%	93%	87%	96%	85%	86%	112%	89%	91%	78%	85%	86%	113%	84%	92%	90%	102%	88%	111%	92%	86%	76%	89%	85%	83%	94%	87%	86%	86%	113%	100%	95%	91%	102%	99%	105%	117%	106%	96%	93%
Citrate	100%	94%	97%	93%	82%	99%	85%	87%	111%	94%	91%	79%	83%	95%	88%	96%	97%	103%	103%	94%	107%	95%	97%	86%	95%	84%	95%	94%	96%	85%	93%	113%	110%	97%	95%	102%	95%	102%	121%	103%	105%	79%

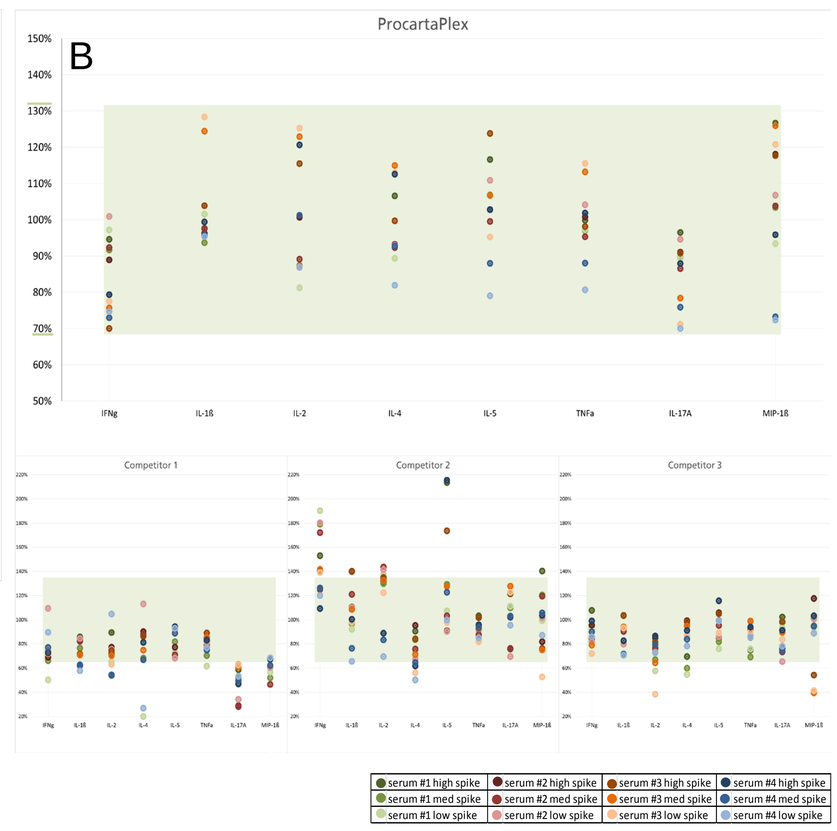


Figure 1: Spike-recovery was evaluated in a minimum of 5 individual donor samples per matrix by spiking recombinant proteins in 3 different known concentrations (high, medium and low) covering the dynamic range of the assays. (A) Mean recoveries for each matrix were calculated across the complete portfolio of available ProcartaPlex® Platinum analytes. Target specifications of 100% +/- 30% are fulfilled, also regarding min/max values. (B) Spike-recovery in ProcartaPlex Platinum assays of each individual serum sample and each individual spike concentration (shown for 8 key cytokines) was shown to be in the target range of 70-130%. Same experiments performed in the corresponding competitor assays resulted in a significant number of individual spiked serum samples out of target range for each competitor assay.

2 Dilution-recovery

	IFNg	IL-12p70	IL-13	IL-1ß	IL-2	IL-4	IL-5	IL-6	TNFa	GM-CSF	IL-10	IL-17A	IL-21	IL-9	IFNa	IL-15	IL-1a	IL1RA	IL-7	Eotaxin	GROa	IL-8	IP-10	MIP-1a	MIP-1ß	RANTES	VEGF-D	SCF	PDGFbb	BDNF	VEGF-A	HGF	LIF	MCP-2	IL-16	TSLP	IL-20	OPG	PECAM-1	tPA	P-Selectin	TNF R II
Serum	92%	96%	90%	95%	84%	90%	93%	95%	104%	110%	104%	103%	97%	106%	103%	101%	113%	108%	92%	82%	90%	94%	87%	95%	91%	90%	105%	98%	101%	95%	94%	97%	95%	99%	90%	99%	95%	92%	88%	91%	89%	94%
EDTA	91%	99%	90%	95%	92%	90%	91%	97%	81%	84%	89%	87%	83%	89%	92%	84%	94%	100%	93%	78%	84%	89%	96%	86%	82%	93%	86%	91%	101%	102%	92%	95%	94%	97%	79%	92%	88%	94%	82%	99%	99%	102%
Citrate	94%	99%	93%	100%	92%	96%	104%	94%	75%	84%	90%	88%	81%	87%	91%	80%	100%	97%	97%	90%	87%	90%	95%	96%	95%	99%	90%	92%	103%	106%	93%	100%	99%	94%	87%	88%	93%	105%	90%	101%	97%	97%

Figure 2: Dilution-recovery was evaluated in a minimum of 5 individual donor samples per matrix by performing 4-fold serial dilutions from 1:4 to 1:256. (A) Mean recoveries for each matrix were calculated across the complete portfolio of ProcartaPlex®. Platinum assays target specification of 100% +/- 30% are fulfilled, also regarding min/max values. (B) Dilution-recovery in ProcartaPlex Platinum assays of each individual serum sample (shown for 8 key cytokines) are calculated using observed vs. expected*100. Dilution linearity of 100% +/- 30% is given for each individual serum and plasma sample across all analytes. Same experiments were performed in corresponding competitor assays (data not shown) and gave following results: Competitor 1: 60-180%; Competitor 2: 60-180%; Competitor 3: 70-130%, however in this case the dynamic range was significantly narrower (factor 6).

B

IFNg				
sample	dilution	Expected	Observed	dil-rec in %
serum #1	4		5229.3	
serum #1	16	1307.3	1219.8	93.3
serum #1	64	326.8	286.6	87.7
serum #1	256	81.7	64.3	78.7
plasma #1	4		5008.8	
plasma #1	16	1252.2	1180.1	94.2
plasma #1	64	313.0	267.5	85.5
plasma #1	256	78.3	62.1	79.4

IL-12p70				
sample	dilution	Expected	Observed	dil-rec in %
serum #1	4		2945.2	
serum #1	16	736.3	712.7	96.8
serum #1	64	184.1	169.9	92.3
serum #1	256	46.0	39.6	86.1
plasma #1	4		2536.2	
plasma #1	16	634.1	678.4	107.0
plasma #1	64	158.5	155.7	98.3
plasma #1	256	39.6	38.0	95.8

IL-1ß				
sample	dilution	Expected	Observed	dil-rec in %
serum #1	4		987.4	
serum #1	16	246.8	236.5	95.8
serum #1	64	61.7	52.3	84.8
serum #1	256	15.4	12.9	83.7
plasma #1	4		913.7	
plasma #1	16	228.4	224.5	98.3
plasma #1	64	57.1	50.8	89.0
plasma #1	256	14.3	12.3	85.9

IL-2				
sample	dilution	Expected	Observed	dil-rec in %
serum #1	4		2232.4	
serum #1	16	558.1	432.7	77.5
serum #1	64	139.5	99.2	71.1
serum #1	256	34.9	26.1	74.8
plasma #1	4		1798.3	
plasma #1	16	449.6	393.2	87.5
plasma #1	64	112.4	87.9	78.2
plasma #1	256	28.1	21.7	77.3

IL-4				
sample	dilution	Expected	Observed	dil-rec in %
serum #1	4		5137.7	
serum #1	16	1284.4	1255.9	97.8
serum #1	64	321.1	284.8	88.7
serum #1	256	80.3	63.4	79.0
plasma #1	4		4570.5	
plasma #1	16	1142.6	1178.9	103.2
plasma #1	64	285.7	271.3	95.0
plasma #1	256	71.4	60.9	85.3

IL-5				
sample	dilution	Expected	Observed	dil-rec in %
serum #1	4		2819.9	
serum #1	16	705.0	708.9	100.6
serum #1	64	176.2	175.3	99.5
serum #1	256	44.1	42.5	96.4
plasma #1	4		3106.7	
plasma #1	16	776.7	707.6	91.1
plasma #1	64	194.2	171.9	88.5
plasma #1	256	48.5	37.8	77.9

IL-6				
sample	dilution	Expected	Observed	dil-rec in %
serum #1	4		4880.3	
serum #1	16	1220.1	1045.2	85.7
serum #1	64	305.0	244.2	80.1
serum #1	256	76.3	66.5	87.2
plasma #1	4		4162.8	
plasma #1	16	1040.7	983.2	94.5
plasma #1	64	260.2	229.0	88.0
plasma #1	256	65.0	51.5	79.2

TNFa				
sample	dilution	Expected	Observed	dil-rec in %
serum #1	4		3458.4	
serum #1	16	864.6	793.8	91.8
serum #1	64	216.1	184.5	85.4
serum #1	256	54.0	42.8	79.2
plasma #1	4		3367.1	
plasma #1	16	841.8	775.9	92.2
plasma #1	64	210.4	175.4	83.4
plasma #1	256	52.6	42.4	80.6

Conclusion

Newly developed surrogate matrices for serum and plasma used in this 42 plex ProcartaPlex Platinum Multiplex Immunoassay guaranty performance characteristics comparable to highly validated ELISA assays. Our data demonstrate that spike- and dilution-recovery performance between 70-130% is achievable with bead based multiplex assays. Data also demonstrated that eBioscience provides the largest commercially available multiplex immunoassay panel that fulfills this performance criteria.