Bio-Plex® suspension array system

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Multiplexing Compatibility of the Bio-Plex Pro™ Diabetes and Cytokine Assays: Human and Mouse Panels

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Introduction

Diabetes is a disease in which the body does not produce or properly use insulin. Type 1 diabetes has a largely genetic basis and results from an autoimmune attack on the insulin producing cells of the pancreas. Type 2 diabetes is impacted by both genetic and environmental factors, arising when the B cells fail to compensate for chronic elevated blood glucose, resulting in insulin insensitivity. In both cases, inflammatory molecules called cytokines play a role in the etiology, and are thus frequently measured in combination with disease-specific markers (metabolic markers) in diabetes research.

We have developed cytokine and diabetes assays on MagPlex microspheres which allow the option of multiplexing analytes of choice and enable easy automation of wash steps. However, the effectiveness of multiplex immunoassays depends on the analytical sensitivity and specificity of the antigen-antibody reaction for the analytes. To determine the compatibility of the Bio-Plex Pro diabetes assays with the cytokine assays of the same species, the assay performance based on the differences in the above-mentioned parameters was measured individually and in combination of the diabetes panel with group I and group II cytokine panels.

The results demonstrate the compatibility of 40 of the 50 available human cytokine assays with all 10 human diabetes assays. Likewise, the 32 available mouse cytokine assays are compatible with all 8 mouse diabetes assays. These analytes have the same sample dilution requirements (4-fold dilution). The diabetes assays are provided at a 20x stock concentration and the cytokine assays are provided at a 10x stock concentration. Table 1 shows the number of diabetes and cytokine assays that can be multiplexed to maintain a minimum 1x final concentration of assay reagents.

Materials and Methods

Preparation of the Protein Standards

The protein standards, capture beads, and detection antibodies from different panels were mixed as described in the Bio-Plex Pro Diabetes Assays instruction manual (bulletin 10010747, Rev B). A Bio-Plex Pro™ II wash station with magnetic plate carrier was used for wash steps.

Evaluation of Assay Sensitivity

The assay sensitivity or limit of detection (LOD) was calculated as the concentration of analyte for which the corresponding median fluorescent intensity (MFI) value is two standard deviations above the background signal. The impact on LOD when mixing assay panels was determined by comparing the difference in LOD between the individual and mixed panels.

Table 1. Number of mixable assays allowed when multiplexing between diabetes and cytokine panels.

Diabetes (20x stock)	Cytokine (10x stock)
0	10
2	9
4	8
6	7
8	6
10	5
12	4
14	3
16	2
18	1
20	0

Evaluation of Assay Specificity

The assay specificity when mixing the analytes from two assay panels was determined by calculating the cross-reactivity between mixed panels using the formula:

$$\text{Specific Cross-reactivity (\%)} = \frac{[\,(\text{Std MFI} - \text{Bkgd MFI})_{\text{Combined Panel}} - (\text{Std MFI} - \text{Bkgd MFI})_{\text{Individual Panel}}\,]}{(\text{Std MFI} - \text{Bkgd MFI})_{\text{Individual Panel}}}$$

The increase in background MFI due to target mixing was also evaluated. This was determined in the presence of multiplexed beads coupled to capture antibodies and in the presence of detection antibodies in standard diluent lacking the standard proteins (blank). A background MFI threshold of <10-fold increase in multiplex conditions was set.

Evaluation of Assay Accuracy

Assay accuracy is measured by standard recovery which is calculated as the percentage of the observed concentration of antigen standard in a serum-based matrix relative to its expected concentration. The standard curve recovery of three replicates was compared between individual and mixed panels.

Results and Discussion

Species-specific diabetes and cytokine assays, with unique bead regions, were assayed and then analyzed with respect to sensitivity, specificity, and accuracy. The strongest indicator that cross-panel mixing of analytes did not interfere was a lack of significant changes in assay sensitivity. The comparison of assay performance parameters of individual and combined panels is summarized in Tables 2-5.

Assay Sensitivity

A marked increase in the sensitivity of an assay when multiplexed with other assays would indicate nonspecific binding and could negatively impact assay performance. LOD for all targets, as shown in Tables 2–5 for individual and mixed panels of the same species, remained within normal assay-to-assay variation. Therefore, multiplexing the human or mouse diabetes and cytokine analytes has no negative impact on assay sensitivity.

Assay Specificity

Upon mixing of diabetes and cytokine assays, a few analytes displayed increased background MFI (resistin and PAI-1 for human assays and insulin, PAI-1, resistin, and GLP-1 for mouse assays). However, the background MFI was low in the separate panels and the ratio between mixed and individual panels was always less than 4-fold, and much lower than the 10-fold threshold. This small background increase, and the fact that the LOD and sample concentration measurements were comparable in mixed and individual panels, indicated that the sensitivity and specificity of the assays were unaffected by multiplexing across the two panels.

Assay Accuracy

The recovery values of a highly accurate assay should approach 100% at all dilution points across the assay range. To ensure that the diabetes and cytokine assays were accurate when combined in a multiplex, we examined whether the recovery values still approached 100% in multiplexed conditions. There were no significant differences in the standard curve recoveries of individual and multiplexed panels. This result indicates that the assay accuracy is unaffected across the assay ranges for all diabetes and cytokine targets when the two panels were multiplexed.

Table 2. LODs of human diabetes and human group I cytokine assays.

Panel	Analyte	Bead Region	LOD, pg/ml	
			Separate Panel	37-Plex
Human Diabetes	C-Peptide	72	14.9	14.7
	Ghrelin	26	1.5	3.0
	GIP	14	1.6	1.0
	GLP-1	27	9.7	7.1
	Glucagon	15	16.4	2.1
	Insulin	12	0.6	0.6
	Leptin	78	3.2	2.3
	PAI-1	61	0.4	6.1
	Resistin	65	1.2	0.8
	Visfatin	22	55.7	47.4
Human Cytokine Group I	IL-1β	39	0.1	0.0
	IL-1Rα	25	0.4	0.7
	IL-2	38	2.2	0.5
	IL-4	52	0.1	0.0
	IL-5	33	0.1	0.1
	IL-6	19	0.0	0.2
	IL-7	74	0.0	0.1
	IL-8	54	0.1	0.6
	IL-9	77	0.5	0.2
	IL-10	56	0.1	0.1
	IL-12 (p70)	75	0.1	0.9
	IL-13	51	0.1	0.3
	IL-15	73	0.1	0.3
	IL-17	76	0.4	0.1
	Eotaxin	43	1.7	2.3
	FGF basic	44	4.1	1.4
	G-CSF	57	0.4	0.6
	GM-CSF	34	5.4	0.8
	IFNγ	21	0.3	1.6
	IP-10	48	1.6	0.9
	MCP-1	53	0.2	0.1
	MIP-1α	55	0.4	0.3
	MIP-1β	18	0.1	0.1
	PDGF BB	47	1.4	2.1
	RANTES	37	0.4	1.0
	TNF-α	36	2.9	2.3
	VEGF	45	0.1	0.1

Table 3. LODs of human diabetes and human group II cytokine assays.

Panel	Analyte	Bead Region	LOD, pg/ml	
			Separate Panel	23-Plex
Human Diabetes	C-Peptide	72	14.9	21.2
	Ghrelin	26	1.5	0.3
	GIP	14	1.6	0.7
	GLP-1	27	9.7	3.6
	Glucagon	15	16.4	11.3
	Insulin	12	0.6	1.9
	Leptin	78	3.2	4.3
	PAI-1	61	0.4	1.1
	Resistin	65	1.2	1.3
	Visfatin	22	55.7	40.9
Human Cytokine Group II	HGF	62	1.9	0.1
	IFNα2	20	1.1	1.3
	IL-1α	63	0.1	0.1
	IL-2Rα	13	2.9	0.2
	IL-3	64	0.4	0.3
	IL-12 (p40)	28	4.4	3.5
	IL-18	42	0.1	0.1
	LIF	29	1.4	0.3
	M-CSF	67	0.7	0.1
	MIF	35	1.5	0.5
	β-NGF	48	0.0	0.0
	TNFβ	30	0.1	0.1
	TRAIL	66	1.2	0.1

Table 4. LODs of mouse diabetes and mouse group I cytokine assays.

Panel	Analyte	Bead Region	LOD, pg/ml	
			Separate Panel	31-Plex
Mouse Diabetes	Ghrelin	64	1.4	2.5
	GIP	46	6.4	1.2
	Glucagon	63	11.6	13.6
	Leptin	65	10.9	1.6
	PAI-1	48	0.8	0.6
	Resistin	30	23.8	1.6
	Insulin	66	11.5	66.3
	GLP-1	62	3.0	5.3
Mouse Cytokine Group I	IL-1α	53	0.6	2.3
	IL-1β	19	0.8	4.9
	IL-2	36	1.1	1.4
	IL-3	18	0.4	0.3
	IL-5	52	0.5	0.3
	IL-6	38	0.2	0.5
	IL-9	33	7.3	17.4
	IL-10	56	0.2	1.5
	IL-12 (p40)	76	0.2	0.5
	IL-13	37	8.1	2.8
	IL-17	72	0.2	0.6
	Eotaxin	74	20.3	117.4
	G-CSF	54	0.4	0.8
	GM-CSF	73	2.4	9.5
	IFNγ	34	2.5	2.1
	KC	57	0.5	0.5
	MCP-1	51	11.6	5.8
	MIP-1α	77	6.7	17.1
	MIP-1β	75	3.1	2.5
	RANTES	55	0.2	0.6
	TNF-α	21	2.4	5.2
	IL-4	39	0.7	0.9
	IL-12 (p70)	78	0.6	3.2

Table 5. LODs of mouse diabetes and mouse group II cytokine assays.

Panel	Analyte	Bead Region	LOD, pg/ml	
			Separate Panel	17-Plex
Mouse Diabetes	Ghrelin	64	0.3	0.2
	GIP	46	1.0	2.6
	Glucagon	63	6.8	13.6
	Leptin	65	0.8	4.1
	PAI-1	48	0.2	0.1
	Resistin	30	74.5	24.9
	Insulin	66	53.6	27.7
	GLP-1	62	2.1	2.2
Mouse Cytokine Group II	IL-15	42	4.2	1.6
	IL-18	20	10.2	3.0
	FGF-basic	25	2.4	1.1
	LIF	45	0.0	0.3
	M-CSF	26	0.2	0.0
	MIG	44	14.1	3.7
	PDGF-BB	35	6.0	1.7
	VEGF	47	1.2	0.7
	MIP-2	27	0.0	0.1

Conclusion

Based on the assay performance in terms of sensitivity, specificity, and accuracy, the human diabetes assays can be mixed with human cytokine assays as long as the bead regions are unique. However, 10 assays from human group II cytokine panel are not compatible with the human diabetes panel due to overlapping bead regions (MIG, SDF-1α, MCP-3, IL-16, Gro-α, SCF, CTACK, SCGF-β, ICAM, and VCAM). Therefore, those human assays cannot be multiplexed with the human diabetes assays.

The assay sensitivity, specificity, and accuracy were comparable for the mouse diabetes and cytokine assays, either in their separate panels or when multiplexed. Mouse diabetes assays do not share bead regions with any of the mouse cytokine assays. Therefore, all analytes from mouse diabetes and cytokine assay groups are mixable. Refer to the Bio-Plex Pro Assays Diabetes Panels instruction manual (bulletin number 10010747, Rev B) for details on standards, beads and detection antibody preparations, and panel mixing.

Information in this tech note was current as of the date of writing (2010) and not necessarily the date this version (rev A, 2010) was published.

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