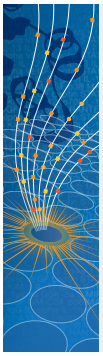

Comparison of Bio-Plex Pro Assay Performance Using the Bio-Plex 200 and Luminex xMAP INTELLIFLEX Systems

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Abstract

In this study, we compared the performance of the Bio-Plex Pro Human Cytokine Screening Panel, 48-Plex and the Bio-Plex Pro Rat Cytokine 23-Plex Assay using the Bio-Plex 200 System versus the Luminex xMAP INTELLIFLEX System. This application note demonstrates compatibility of Bio-Plex Assays with Luminex xMAP INTELLIFLEX Systems.

Introduction

Each new Bio-Plex Pro Multiplex Immunoassay is developed and designed for use on all research use only xMAP Systems available at the time of development. Bead region selection during development ensures compatibility with the bead number ranges present in systems such as the Luminex MAGPIX System (50 bead regions), Luminex 100/200 System (100 bead regions), Bio-Plex 200 System (100 bead regions), Bio-Plex 3D System (500 bead regions), and Luminex xMAP INTELLIFLEX Systems (500 bead regions). Assays are optimized to perform consistently across different systems when using each system's recommended photomultiplier tube (PMT) settings. By comparing the performance of two assays (the Bio-Plex Pro Human Cytokine Screening Panel, 48-Plex and the Bio-Plex Pro Rat Cytokine 23-Plex Assay) on two systems (the Bio-Plex 200 System and the Luminex xMAP INTELLIFLEX System) using a variety of PMT settings, we offer data supporting the recommended instrument settings that provide the most concordant data across systems.

Methods

The Bio-Plex Pro Human Cytokine Screening Panel, 48-Plex (Bio-Rad Laboratories, Inc., catalog #12007283) and Bio-Plex Pro Rat Cytokine 23-Plex Assay (Bio-Rad, #12005641) were tested on the Bio-Plex 200 System (Bio-Rad, #171000205) and the Luminex xMAP INTELLIFLEX System (Luminex Corporation, #INTELLIFLEX-RUO) using a 96-well plate. The standards, blanks, and samples were run in replicates of two (Figure 1). Samples consisted of seven human serum and six spiked plasma samples (total N=13). Enough reagents were prepared to acquire data from the same well multiple times to minimize variation introduced during assay preparation. Assays were prepared and tested as described in the Bio-Plex Pro Cytokine, Chemokine, and Growth Factors Assays Instruction Manual (#10000111560).

Data generated on the Bio-Plex 200 System for the Bio-Plex Pro Human Cytokine Screening Panel, 48-Plex were acquired using the low PMT setting. These data were compared to data acquired using the different PMT settings available with the Luminex xMAP INTELLIFLEX System. Data generated on the Bio-Plex 200 System for the Bio-Plex Pro Rat Cytokine 23-Plex Assay were acquired at the high PMT setting and were compared to data acquired using the different PMT settings available with the Luminex xMAP INTELLIFLEX System. Bio-Plex Manager 6 Software was used to analyze all data. At the time of this study, the software for the Luminex xMAP INTELLIFLEX System was limited to data acquisition only and had no analysis functionality.

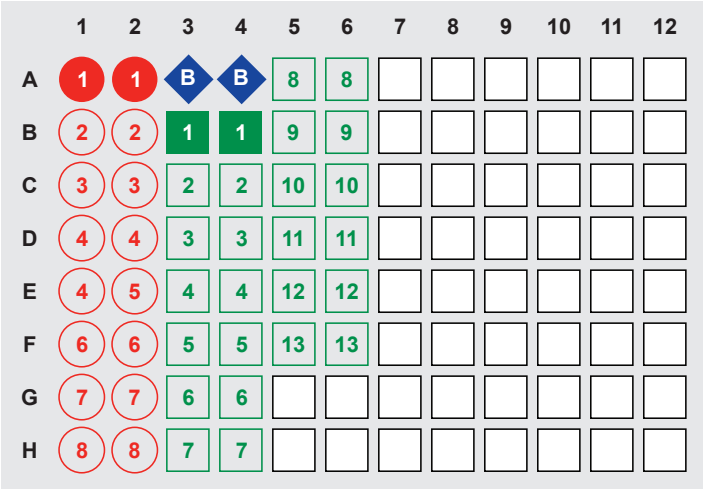

Fig. 1. Plate layout for data acquisition performed on the Bio-Plex 200 System and the Luminex xMAP INTELLIFLEX System. Blanks (■), standards (■), and samples (■) were run in duplicate.

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Results

Precision

Intra-assay precision values, calculated as the mean percent coefficient of variation (%CV) of the mean fluorescence intensity (MFI) for each target in the Bio-Plex Pro Human Cytokine Screening Panel, 48-Plex, were similar for all targets when complex serum and spiked plasma samples were measured (N=13; Figure 2A) and were less than 10% for all eight points on the standard curve (N=8; Figure 2B).

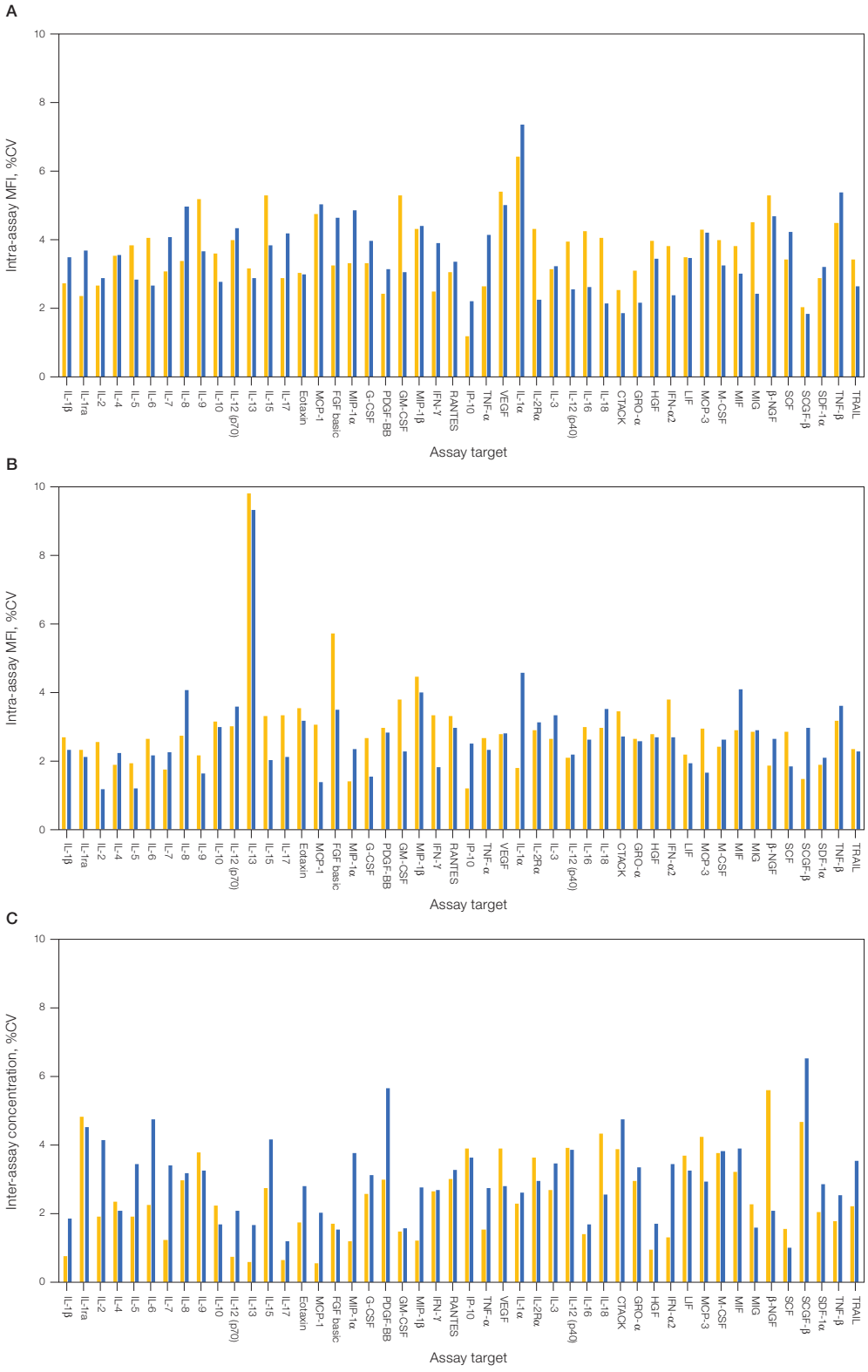

Fig. 2. Intra- and inter-assay precision values of the Bio-Plex Pro Human Cytokine Screening Panel, 48-Plex. Comparison of data obtained using the Bio-Plex 200 System (■) and a Luminex xMAP INTELLIFLEX System (■). Both systems were operated using low photomultiplier tube (PMT) settings. **A**, intra-assay precision for human serum and plasma sample measurements; **B**, intra-assay precision of the standard curve; **C**, inter-assay concentration %CV of the standard curve. MFI, mean fluorescence intensity; %CV, percent coefficient of variation.

Inter-assay precision calculations used the observed concentrations obtained from three assay plates prepared separately using the same batch of a Bio-Plex Pro Human Cytokine Screening Panel, 48-Plex. All three plates were run on the same day. The precision profile was found to be similar for all targets and the %CV were less than 10% for all eight points on the standard curve (N=8; Figure 2C). All data collected on the Bio-Plex 200 System used the low PMT setting, whereas data collected on the Luminex xMAP INTELLIFLEX System used a low PMT setting matched to the Bio-Plex 200 System or LX-200 System, indicated in the software as "LX200 LPMT." Data collected for IL-1ra by each system are presented in Table 1.

Table 1. Quantitative performance of the Bio-Plex Pro Human Cytokine Screening Panel, 48-Plex using the Bio-Plex 200 System and the Luminex xMAP INTELLIFLEX System. Data were collected using a Bio-Plex 200 System using the low PMT setting or a Luminex xMAP INTELLIFLEX System using the LX200 LPMT setting. Data shown are for IL-1ra and were analyzed using Bio-Plex Manager Software.

Bio-Plex 200 System						
Sample	**FI**	**SD**	**%CV**	**Concentration in Range, pg/ml**	**Expected Concentration, pg/ml**	**100(Observed/Expected)**
Blank	10	1.41	14.14	–	–	–
Standard 1	18,118.8	295.92	1.63	127,265.59	103,481	123
Standard 2	16,126.3	169.35	1.05	25,192.7	25,870.25	97
Standard 3	10,051.5	3.54	0.04	6,360.34	6,467.56	98
Standard 4	3,527.3	16.62	0.47	1,679.11	1,616.89	104
Standard 5	772	16.97	2.2	387.33	404.22	96
Standard 6	186.5	2.12	1.14	102.41	101.06	101
Standard 7	49.8	1.77	3.55	25.84	25.26	102
Standard 8	20	1.41	7.07	6.23	6.32	99
Sample 1	1,619	5.66	0.35	3,104.99	–	–
Sample 2	455.8	16.62	3.65	949.82	–	–
Sample 3	287.5	7.78	2.71	617.73	–	–
Sample 4	31	0	0	55.44	–	–
Sample 5	120	5.66	4.71	266.2	–	–
Sample 6	225.5	2.12	0.94	491.09	–	–
Sample 7	104	1.41	1.36	230.49	–	–
Sample 8	380.8	6.01	1.58	200.86	–	–
Sample 9	218.5	4.95	2.27	119.15	–	–
Sample 10	142.5	0.71	0.5	78.88	–	–
Sample 11	114.3	5.3	4.64	63.36	–	–
Sample 12	93.3	3.18	3.41	51.53	–	–
Sample 13	89.3	5.3	5.94	49.25	–	–
Luminex xMAP INTELLIFLEX System						
Sample	**FI**	**SD**	**%CV**	**Concentration in Range, pg/ml**	**Expected Concentration, pg/ml**	**100(Observed/Expected)**
Blank	11.1	0.54	4.86	–	–	–
Standard 1	19,097	451.07	2.36	105,868.17	103,481	102
Standard 2	17,404.1	326.19	1.87	26,513.49	25,870.25	102
Standard 3	10,815.3	295.75	2.73	6,321.89	6,467.56	98
Standard 4	3,599.4	63.76	1.77	1,668.72	1,616.89	103
Standard 5	730.8	7.81	1.07	393.55	404.22	97
Standard 6	156.4	3.67	2.35	100.5	101.06	99
Standard 7	42.1	0.56	1.33	26.1	25.26	103
Standard 8	18.1	0.4	2.2	5.65	6.32	89
Sample 1	1,627.3	43.14	2.65	3,187.87	–	–
Sample 2	421.3	7.82	1.86	973.13	–	–
Sample 3	262.8	4.99	1.9	642.05	–	–
Sample 4	29.7	0.52	1.76	64.86	–	–
Sample 5	104.1	1.86	1.78	274.05	–	–
Sample 6	209.3	7.49	3.58	523.9	–	–
Sample 7	93.1	3.1	3.33	245.99	–	–
Sample 8	381.3	0.43	0.11	222.91	–	–
Sample 9	214.7	13.73	6.4	134	–	–
Sample 10	133.4	7.22	5.41	86.71	–	–
Sample 11	106.2	4.88	4.59	69.88	–	–
Sample 12	88.1	6.84	7.77	58.22	–	–
Sample 13	88.3	7.79	8.81	58.39	–	–

%CV, percent coefficient of variation; FI, fluorescence intensity; SD, standard deviation.

Reported Concentration

Data collected from both platforms on low PMT settings were analyzed using the five-parameter logistic (5PL) curve fit equation with standard curve optimization in Bio-Plex Manager 6 Software. The resulting concentration calculations were compared for both the standard curve (N=8) and serum samples (N=13). The concentration difference from all the standard points and the samples was preserved at about one-fold (Figure 3). This confirms that the observed concentration calculated by the two different platforms is aligned.

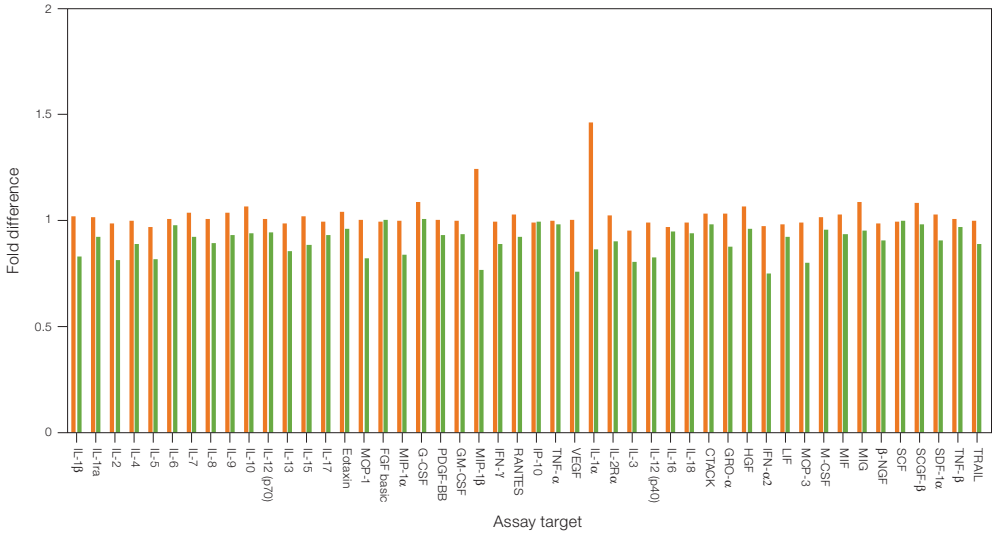

Fig. 3. Cross-platform comparison of concentrations obtained using the Bio-Plex Pro Human Cytokine Screening Panel, 48-Plex. Comparison of standard curve (■) and sample (■) concentrations obtained using the Bio-Plex 200 System and the Luminex xMAP INTELLIFLEX System, represented as fold difference between the systems (concentration obtained using the Bio-Plex 200 System divided by the concentration obtained using the Luminex xMAP INTELLIFLEX System).

PMT Setting Comparison

Each Bio-Plex or Luminex System has a recommended PMT setting. Because different PMT settings will elicit different fluorescence intensity responses, variations in these settings between platforms can affect the data collected. To ensure that these differences in response do not alter the shape of standard curves, ultimately affecting sample measurements, different PMT settings on the two instruments were compared.

Using the Bio-Plex Pro Human Cytokine Screening Panel, 48-Plex, the measured concentration of IL-4 was quantified using the Bio-Plex 200 System's low PMT setting and the equivalent setting (LX200 LPMT) on the Luminex xMAP INTELLIFLEX System. IL-4 concentration was also measured on a Luminex xMAP INTELLIFLEX System using the 3D LPMT and High Sensitivity settings. The observed concentration was found to be similar when using the low PMT settings on both systems. Concentration values floated seamlessly at high PMT settings, and no significant benefit was found to using the High Sensitivity setting. Results are summarized in Figure 4.

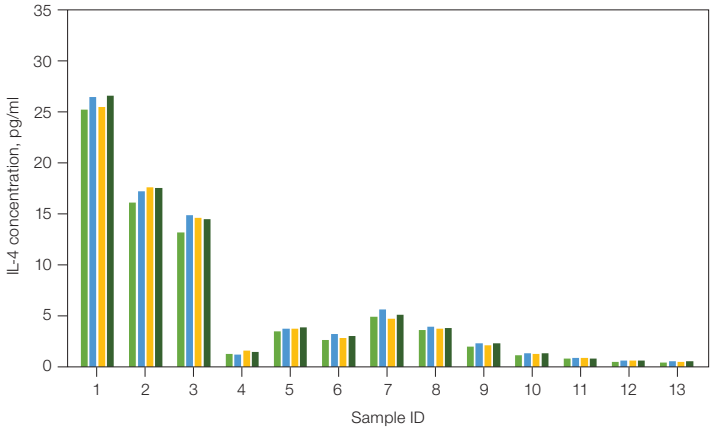

Fig. 4. Cross-platform comparison of human IL-4 concentrations using various PMT settings. IL-4 concentration quantified in samples using the Bio-Plex Pro Human Cytokine Screening Panel, 48-Plex. Samples were quantified using the Bio-Plex 200 System with the low PMT setting (■) and a Luminex xMAP INTELLIFLEX System with the LX200 LPMT (■), 3D LPMT (■), and High Sensitivity (■) PMT settings. PMT, photomultiplier tube.

To compare high PMT settings on the Bio-Plex 200 System to various PMT settings on the Luminex xMAP INTELLIFLEX System, the concentration of IL-1α was measured using the Bio-Plex Pro Rat Cytokine 23-Plex Assay (Figure 5).

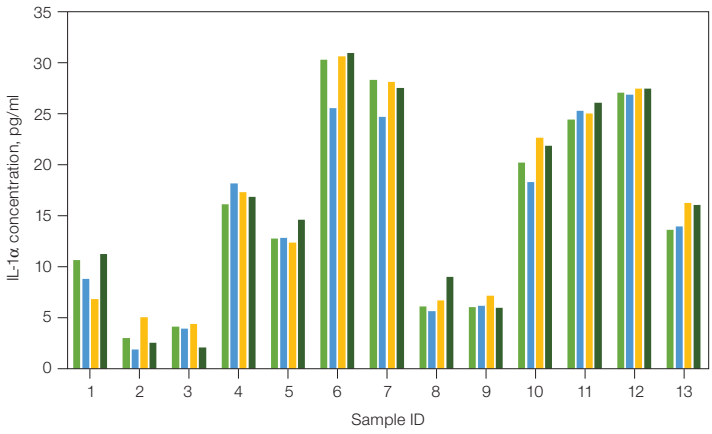

Fig. 5. Cross-platform comparison of IL-1α concentrations using high PMT settings. IL-1α concentration quantified in samples using the Bio-Plex Pro Rat Cytokine 23-Plex Assay. Samples were quantified using the Bio-Plex 200 System with the high PMT setting (■) and a Luminex xMAP INTELLIFLEX System with the LX200 HPMT (■), 3D HPMT (■), and High Sensitivity (■) PMT settings. PMT, photomultiplier tube.

Conclusion

The Bio-Plex Pro Assays show comparable performance when run on the Luminex xMAP INTELLIFLEX System as when run on the Bio-Plex 200 System in terms of precision for inter- and intra-assay reproducibility. The various instrument PMT settings on the Luminex xMAP INTELLIFLEX System can be set so that the output data will correlate to data run on the Bio-Plex 200 System.

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