

Heather Darby Harding¹, Cari McDonald¹, and Kerry G. Oliver¹

Radix BioSolutions, Ltd, 111 W. Cooperative Way Suite 120, Georgetown, TX 78626

contact: heather@radixbiosolutions.com

fax: 512.868.9040

voice: 512.869.8000

ABSTRACT

The growing number of available xMAP® biomarker immunoassay kits presents both opportunity and challenge to today's scientist. With the benefit of a wide selection of biomarker immunoassays from a number of vendors comes the challenge of finding all biomarkers of interest available in one panel from one vendor. Further, there are still significant numbers of biomarkers of interest without commercial immunoassay kits available. The division of sample over several assay kits can come at significant cost and possibly still without providing a complete picture should a particular biomarker assay be unavailable.

Faced with this dilemma, we integrated immunoassay kits from two commercial vendors with three novel biomarker immunoassays to build a custom 6-plex biomarker panel. Using commercially-available recombinant standards, we extended the dynamic range beyond that of the commercial kits' standard curves. Integration of the AssayCheX™ process control panel instilled confidence in data quality. This level of customization allowed selection of available commercial assays adhering to stringent selection criteria, preservation of sample and resources, and a final product requiring no compromise. Though it's vital to examine each situation individually, the implications for custom integration of commercially available kits and novel assays are exciting.

INTRODUCTION

The Luminex® website lists more than 750 available xMAP-based biomarker immunoassays for more than 250 analytes from Luminex partners. Many partners have more unreleased immunoassays at various stages of development, and there is no way of knowing the number of homebrew assays currently in use in the field. In short, xMAP has firmly entrenched itself in the ever-growing biomarker field. However, the use of xMAP technology by a number of independent Luminex partners to build immunoassays for popular biomarkers has led to redundancy and significant gaps in availability. For example, seven Luminex partners offer an IL-8 immunoassay, yet none currently offer an assay for likely prostate cancer biomarker PDEF.

In this study three immunoassays (IL-8, IL-10, and MCP-1) were available from Luminex partners. Therefore we were able to select to best match stringent assay requirements without duplicating effort of development. However, there were no commercially available assays for 3 other analytes (MIG, MIP-1 δ , and Eotaxin-2) at the time of this work. Novel assays were developed for these three assays and integrated with selected commercial kits with no or negligible cross-reactivity. The final assay was customized to a more convenient incubation schedule and extended standard curves were established for all 6 analytes using commercially-available recombinant standard. Using modifications to buffer systems, incubation times, recombinant standards, and reagent concentrations a 10-plex assay was developed and its characteristics defined. The 10-plex assay integrated three novel assays, three commercial assays from two different Luminex partners, and four AssayCheX process control microspheres. The assay effectively integrates novel assays and assays from different Luminex partners to create a completely customized final product.

MATERIALS & METHODS

R&D Systems®: IL-8 (LUH208) & IL-10 (LUH217) Fluorokine® MAP Human Kits and Fluorokine MAP Human MultiAnalyte Profiling Base Kit A (LUH000); all recombinant standards; antibodies for MIG, MIP-1 δ , and Eotaxin-2

Invitrogen™: MCP-1 Human Singleplex Bead Kit (LHC1011)

Coupling of Antibodies to Microspheres: Capture antibodies were covalently attached to carboxylated polystyrene microspheres according to standard protocol established at Radix BioSolutions. The coupling was done in MES buffer pH 6.0 and coupled microspheres were stored in the dark at 2-8°C in storage buffer.

Assay Parameters: Standards and samples for all 6 analytes are diluted 1:10 in assay buffer to preserve valuable human serum samples. The assay includes a 10-plex (6 analytes and 4 AssayCheX process control panel microspheres) overnight (16-20h) capture reaction of 5000 beads per region per well at 2-8°C. Overnight incubation was selected for this assay to best coordinate technician time in intended implementation. After washing with assay buffer, the detection step with 6-plex biotinylated antibodies occurs for 1 hour at room temperature on an orbital shaker. Following further washing, a final 30 minute incubation with fluorescent reporter takes place. Data is acquired on an LX100 and analyzed with Masterplex® QT v4.0 with the AssayCheX plugin.

RESULTS

Table 1. Custom assay characteristics

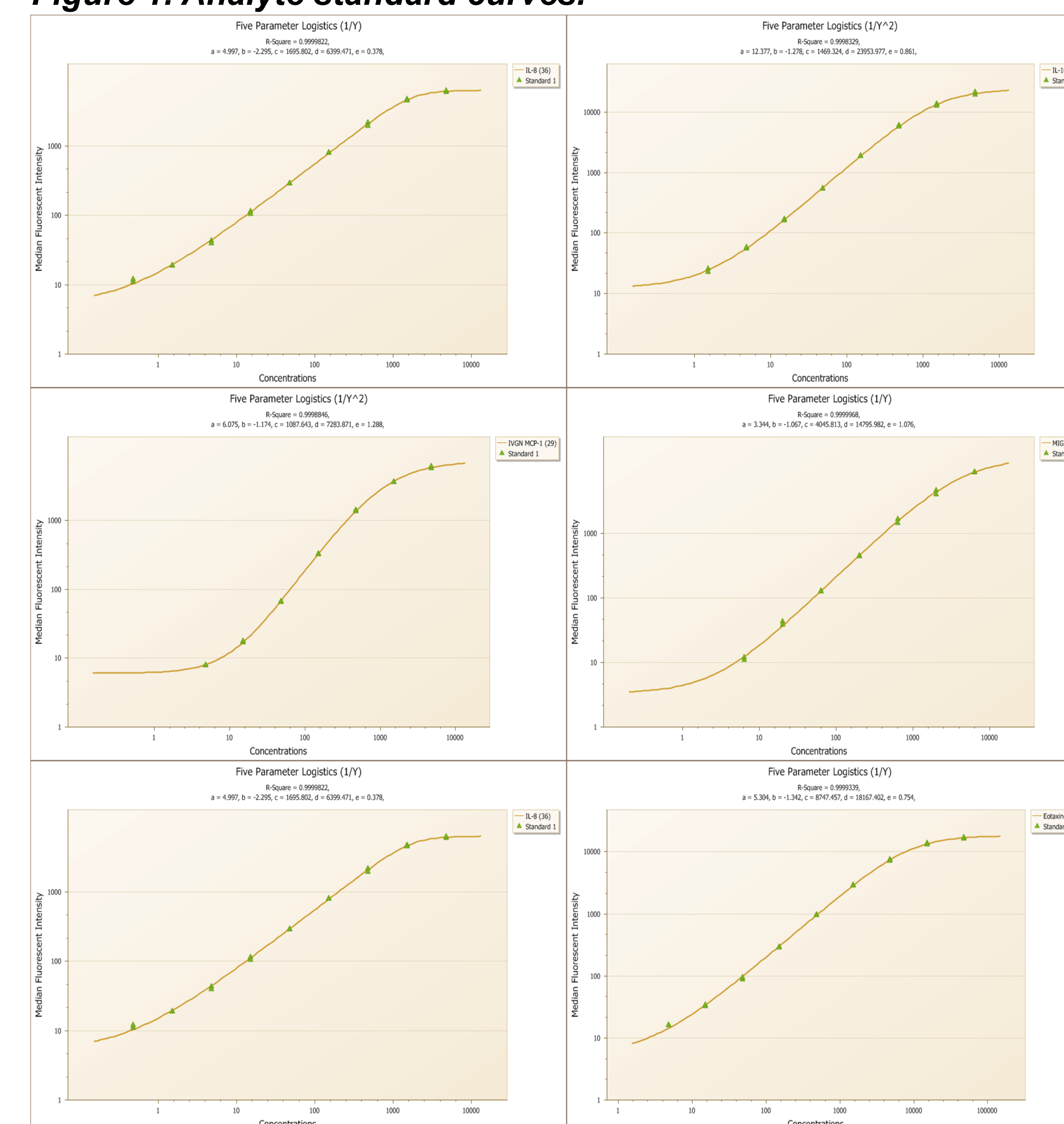
Cytokine	Assay Range, pg/ml	Avg Rec, %	LOD, pg/ml	Sample Intra-Assay Precision, %CV	Sample Inter-Assay Precision, %CV	Avg Human Basal, pg/ml
IL-8	0.48-4747	85-126	0.8	7.04	4.33	<30
IL-10	1.5-4747	80-142	3	6.77	5.06	<10
MCP-1	4.8-4747	83-114	6	4.55	9.5	400
MIG	6.4-6329	87-116	7.5	8.72	7.33	40
MIP-1 δ	25-25316	96-107	35	6	5.37	7000
Eotaxin-2	4.8-47468	78-132	6	5.87	5.56	200

Table 2. Commercial assays' characteristics.

Cytokine	Assay Range, pg/ml	LOD, pg/ml	Intra-Assay Precision, %CV	Inter-Assay Precision, %CV	Sample Matrix %Rec
IL-8	3-2500	0.39	4.6-7.8	11.6-18.7	91-129
IL-10	3-2320	0.13	5.2-6.4	7.3-10.1	90-122
MCP-1	22.6-16470	<10	5.8	5.8	80-119

Table 1 lists the characteristics of the assay. Accuracy and precision were examined over 3 days; sample spikes had a minimum n=10. Table 2 lists the published characteristics of the three commercially available assays. The custom assay's range exceeds that of the commercial assays with the exception of the MCP-1 assay, which gains sensitivity at the cost of dynamic range. Figure 1 provides examples of standard curves obtained in the 6-plex assay as analyzed with Masterplex QT v4.0. An 11-point standard curve with backgrounds is run to allow visualization of the complete fluorescence range of the assays, and extraneous points are designated as outliers in the software before analysis.

Figure 1. Analyte standard curves.



CONCLUSIONS

The customization of this assay allowed the extension of standard curves, the integration of kits from multiple vendors with novel assays, a change in assay parameters, the inclusion of AssayCheX microspheres, and the preservation of valuable serum sample through dilution. Use of Masterplex QT v4.0 with the AssayCheX plugin allowed the rapid confirmation of valid results before analysis. Incorporation of the 6 assays and the 4 AssayCheX microspheres resulted in no cross-reactivity and comparable behavior to the assays in their intended formats. The ability to integrate novel assays with commercial kits as well as commercial kits with one another opens a wide door of possibilities for custom final kit production. This customization makes the selection of assay kits limitless and avoids the duplication of cost and effort of developing already-available assays. In a scalable format we were able to demonstrate the ability to completely customize an assay to best suit our needs without redeveloping an assay already commercially available.

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