Application Note

Good Correlation Between Measured Cytokine Levels Using Multiplexed Bead-based Detection and ELISA Platforms

Introduction

Cytokines are a diverse group of soluble proteins and peptides that regulate cell and tissue functions, such as immune cell responses, under both normal conditions and disease states such as sepsis, autoimmune disease, inflammation and cancer. These proteins also directly mediate interactions between cells and also regulate extracellular processes.

Because multiple cytokines frequently function in concert, and because their effects are highly sensitive to their precise cellular concentrations, identifying specific cytokines involved in any inflammatory or immune response may require screening for the presence of numerous cytokines. Efficient screening requires a detection platform, such as Luminex® xMAP® bead-based immunoassays, that requires minimal sample volume and has the potential for multiplexed detection. Following screening for large panels of cytokines, however, continued research often focuses on two or three cytokines identified as significant and requires highly validated, robust assays that enable consistent quantitation of these selected cytokines across many samples and across the lifetime of the research project. Enzyme-linked immunosorbent assays (ELISAs) are the gold standard assays for single-analyte quantitation.

Drawing biological conclusions from cytokine immunodetection, therefore, requires both a broad screening approach as well as single analyte-focused assays, using platforms that provide complementary, correlated data. Here we compare sample data from a bead-based immunoassay (MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel 1) with data from cytokine ELISAs, showing correlation in samples from normal subjects and sepsis patients. We also tested both assay platforms on lysates from peripheral blood mononuclear cells (PBMCs) treated with lipopolysaccharide (LPS) or concanavalin A (ConA) to induce elevation in cytokine levels.

Materials and Methods

Samples. Normal and septic human serum samples and human PBMCs were purchased from Bioreclamation (Bioreclamation Inc., Hicksville, NY.) Human PBMCs were removed from liquid nitrogen storage, thawed in a 37 °C waterbath, washed, and resuspended in complete medium (RPMI 1640 media containing 10% fetal bovine serum (FBS) and 1x penicillin/streptomycin). Then, the PBMCs were plated in complete medium, overnight, in a 37 °C cell culture incubator containing 5% CO_2. Following overnight culture, cells were stimulated with vehicle (control), 10 µg/mL LPS (w/v), or 5 µg/mL ConA (w/v) for 48 hours. After 48 hours, the medium from each culture well was clarified via centrifugation, collected, and utilized in subsequent immunoassays.

Immunoassay Protocols. Assays were conducted according to the immunoassay protocol for the MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel 1 (Cat. No. HCYTOMAG-60K) or the individual cytokine ELISA protocols.

The general procedure for the MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel is as follows:

1. Block the plate with 200 µL of wash buffer per well.
2. Add 25 µL standard or buffer, 25 µL matrix or sample, and 25 µL beads per well.
3. Incubate overnight with shaking at 4° C or room temperature for 2 hours.
4. Wash the assay plates twice with wash buffer.
5. Add 25 µL of detection antibodies per well.
6. Incubate at room temperature for 1 hour.
7. Add 25 µL of streptavidin-phycoerythrin (SA-PE) per well.
8. Incubate at room temperature for 30 minutes.
9. Wash the assay plates twice with wash buffer.
10. Resuspend the beads with 150 µL of sheath fluid.
11. Analyze the assay plates using Luminex® systems.

The MILLIPLEX® MAP Cytokine/Chemokine Magnetic Bead Panel assays were washed using the BioTek®ELx405RM Plate Washer (Cat. No. 40-020) according to suggested settings described in the kit protocol. This is a compact unit for automated washing of 96-well solid plates.

Table 1. Assay sensitivity is comparable between MILLIPLEX® MAP and ELISA detection platforms; MILLIPLEX® MAP assays, however, provide greater dynamic range by 1-2 orders of magnitude.

Human Cytokine	Method	Sensitivity (pg/mL)	Dynamic Range (pg/mL)	Sample Requirement (µL)
IL-2	ELISA	4.0	15.6-1000	50
IL-2	MILLIPLEX® MAP	1.0	3.2-10,000	25
IL-4	ELISA	0.6	3.2-200	50
IL-4	MILLIPLEX® MAP	4.5	3.2-10,000	25
IL-6	ELISA	1.6	7.8-500	50
IL-6	MILLIPLEX® MAP	0.9	3.2-10,000	25
IL-10	ELISA	2.0	3.9-250	50
IL-10	MILLIPLEX® MAP	8.6	3.2-10,000	25
TNFα	ELISA	3.5	15.6-1000	50
TNFα	MILLIPLEX® MAP	0.7	3.2-10,000	25

Multiplexed assay data were acquired and analyzed on the Luminex 200™ system, which consists of the Luminex 200™ instrument, Luminex XYP™ plate handling platform, and Luminex SD™ sheath fluid delivery system, xPONENT® software and personal computer (PC).

The general procedure for the Human Cytokine ELISAs is as follows:

1. Wash the plate 4 times with 300 µL of wash buffer per well.
2. Add 50 µL assay buffer to each well that will contain either standard or samples. (For IL-8 and IL-4 kits, add 50 µL of matrix to the standard curve wells instead of assay buffer.)
3. Add 50 µL of each standard or sample to the appropriate well.
4. Incubate 2 hours with shaking at or room temperature.
5. Wash the assay plates four times with wash buffer.
6. Add 100 µL of detection antibodies per well.
7. Incubate at room temperature for 1 hour with shaking.
8. Wash the assay plates five times with wash buffer.
9. Add 100 µL of substrate solution.
10. Incubate at room temperature in the dark for 15-30 minutes.
11. Add 100 µL of stop solution.
12. Analyze plates on the SPECTRAmax® 340PC[38] Absorbance Microplate Reader or similar plate reader at 450 and 570 nm.

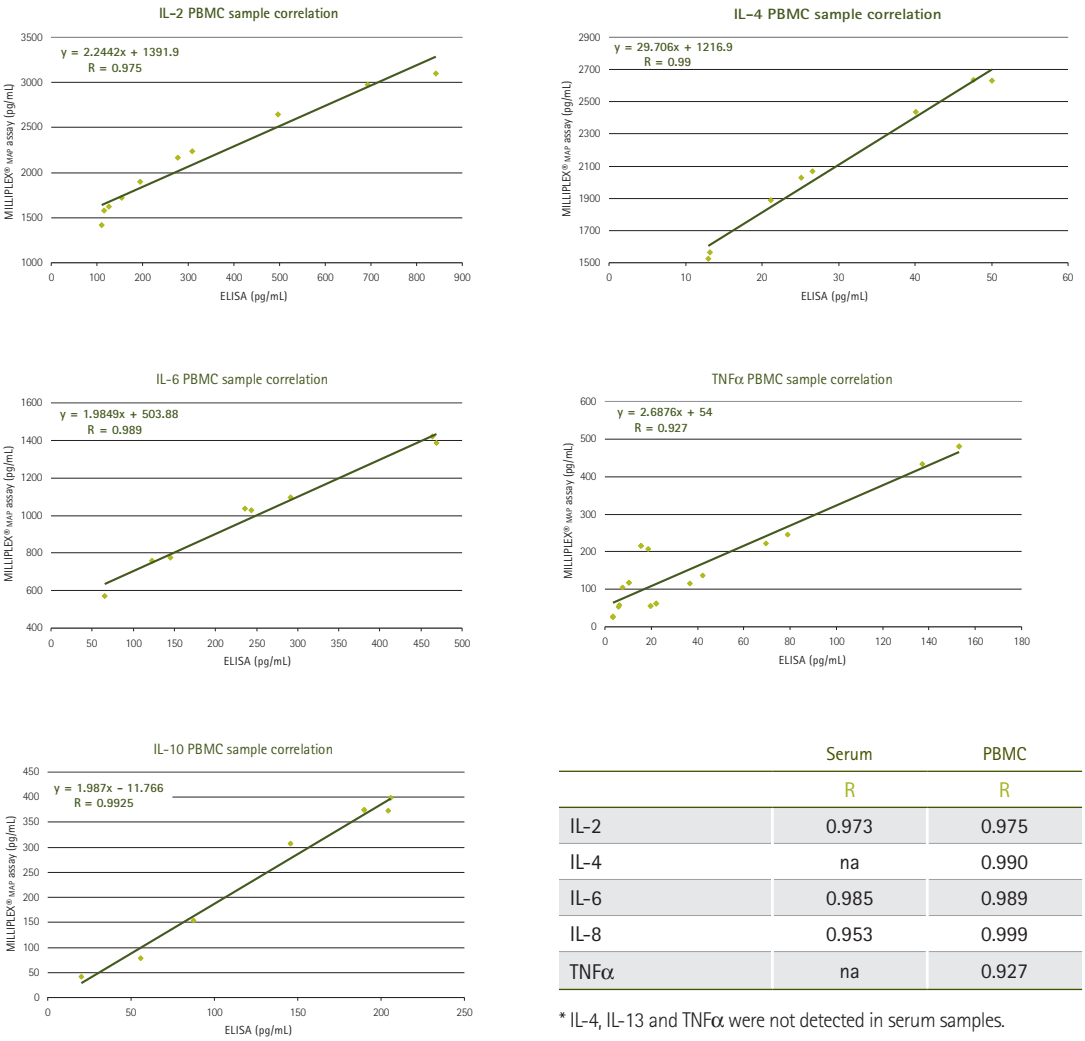
Results

Using the single bead assays for MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel and each individual Human Cytokine ELISA, we ran assays for each of the following cytokines: IL-2, IL-4, IL-6, IL-10 and TNFα. Specifically, normal/septic human sera and human PBMC cell culture supernatant samples were analyzed in each immunoassay. Septic sera often contains elevated concentrations of cytokines, compared to normal sera, and is useful for comparing biomarker detection platforms.

In addition, challenge of PBMCs with LPS and/or Con A induces expression/secretion of various cytokines, and therefore, valuable in immunoassay comparisons. Moreover, since the matrix environments of human sera and cell culture media are so different, using both types of samples provides a more complete picture of how the immunoassays for each analyte perform in distinct biological samples.

We analyzed the same set of serum and PBMC samples using the single bead MILLIPLEX® MAP assay and the ELISA for each individual cytokine. The correlation coefficients demonstrated highly positive correlation between the assay platforms for each individual analyte (Figure 2). Specifically, PBMC samples provided a mean R value of 0.959 when comparing the ELISAs with the bead assays (Fig. 2, Table 2). The range of correlation coefficients for PBMC samples, 0.927-0.999, is also fairly consistent. Moreover, for serum samples we observed a mean R value of 0.948, and a range of correlation coefficients of 0.953-0.985. The high level of positive correlation observed in both PBMC and serum samples demonstrates that researchers may reliably use either the MILLIPLEX® MAP or ELISA platform, and obtain similar data trends.

Figure 2.
PBMC sample correlation between the MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel and each individual cytokine ELISA.



	Serum	PBMC
	R	R
IL-2	0.973	0.975
IL-4	na	0.990
IL-6	0.985	0.989
IL-8	0.953	0.999
TNFα	na	0.927

Table 2.
R value and slope for ELISA (x) vs MILLIPLEX® MAP assay (y) in serum and PBMC samples.

* IL-4, IL-13 and TNFα were not detected in serum samples.

Conclusion

Analyzing mechanisms underlying inflammation, immunology and related processes can be facilitated by using Merck Millipore's complementary MILLIPLEX® bead-based immunoassays and ELISAs for classical cytokines. We have demonstrated that the bead-based assays and ELISAs show similar sensitivity for each cytokine analyte and that MILLIPLEX® MAP assays provide greater dynamic range of detection. Furthermore, side-by-side analysis of serum and PBMC samples using both the MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel and each individual human Cytokine ELISA showed that the correlation and sample trends between the two assay formats was generally similar. Together, our data demonstrate that users may obtain similar data trends using the MILLIPLEX® or ELISA platforms for cytokine quantitation.

Ordering Information

Description	Catalogue No.
MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel 1	HCYTOMAG-60K
Human IL-2 ELISA	EZHIL2
Human IL-4 ELISA	EZHIL4
Human IL-6 ELISA	EZHIL6
Human IL-10 ELISA	EZHIL10
Human TNFα ELISA	EZHTNFA

Related Products	
MILLIPLEX® MAP Mouse Cytokine/Chemokine Magnetic Bead Panel 1	MCYTOMAG-70K
MILLIPLEX® MAP Human Adipokine Panel 2	HADK2MAG-61K
MILLIPLEX® MAP Human Adipocyte	HADCYMAG-61K
MILLIPLEX® MAP Human Bone Panel	HBNMAG-51K
MILLIPLEX® MAP Human Circulating Cancer Biomarker	HCCBP1MAG-58K
MILLIPLEX® MAP Human Metabolic Hormone	HMHMAG-34K
MILLIPLEX® MAP Human Th17	HTH17MAG-14K
Mouse IL-2 ELISA	EZMIL2
Mouse IL-4 ELISA	EZMIL4
Mouse IL-6 ELISA	EZMIL6
Mouse IL-10 ELISA	EZMIL10
Mouse TNFα ELISA	EZMTNFA

* For pricing contact your local sales representative, or for more product specific information visit us online at: www.millipore.com/bmia

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