Application Note

MAGPIX® and Luminex 200™ Instruments Provide Equivalent Multiplex Detection of Inflammation Pathway Analytes

Abstract

Multiplex detection is a highly efficient tool for measuring the levels of multiple analytes in a single sample. MILLIPLEX® MAP assay panels from EMD Millipore provide a complete solution for multiplex detection of analytes within particular research areas using the bead-based Luminex® xMAP® technology. Previously, the only option for analyzing MILLIPLEX® MAP assays required the Luminex 200™ or FLEXMAP 3D® analyzers. With the launch of the MAGPIX® system, MILLIPLEX® MAP magnetic bead assays can be analyzed without the use of lasers or hydrodynamic focusing. The MAGPIX® instrument is a compact, cost-effective multiplexing system based on charged-coupled device (CCD) imaging technology. The MAGPIX® instrument was specifically designed to yield comparable results to the Luminex 200™ instrument. To demonstrate similarity between the two systems, we ran parallel assays using the MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel. Standard curve median fluorescence intensity (MFI) and sample range data are similar between the two instrument systems. Furthermore, sample concentration data between the MAGPIX® and Luminex 200™ systems demonstrate correlation coefficients ≥ 0.97.

Introduction

The immune system involves an organized, complex network of biological structures and processes that protect against disease. Cytokine and chemokine proteins of the inflammation pathway mediate interactions between cells and regulate target immune cell responses. These responses often result in an inflammatory state that attempts to eradicate foreign antigens and ultimately commence healing processes. Consequently, the inflammatory process may play a key protective role in disease.

Uncontrolled inflammation, however, can lead to sepsis, also known as SIRS (systemic inflammatory response syndrome). Sepsis results from infection, whether bacterial, viral, fungal or parasitic. This hyper-reaction to infection interrupts homeostasis through an uncontrolled inflammatory response, including glucocorticoids and catecholamines, mediators of the humoral immune response, and pro-inflammatory cytokines such as IL-1α, IL-6 and TNFα, as well as increased apoptosis of lymphoid organs, leading to immune suppression. Severe sepsis can cause septic shock, multiple organ dysfunction syndrome (MODS) and death.

MILLIPLEX® MAP assays utilizing Luminex® xMAP® technology enable simultaneous investigation of the modulation and expression of many cytokines involved in sepsis and many other inflammatory disease states. By enabling the measurement of relative cytokine levels within the same sample, the assays provide a unique opportunity for greater understanding of cytokine/chemokine regulation and inflammatory disease.

EMD Millipore is a division of Merck KGaA, Darmstadt, Germany

The 39-plex MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel is tailored for simultaneous quantification of multiple cytokines/chemokines in human serum/plasma and 42-plex for cell culture supernatant samples. Because many of these cytokines are elevated under conditions of sepsis, we analyzed normal and septic human serum samples using the 39-plex panel. By simultaneously acquiring data from the assay on both the MAGPIX® and Luminex 200™ systems, we were also able to compare the performance of these two instruments.

Materials and Methods

Luminex 200™ System. This platform includes a Luminex 200™ instrument, Luminex XYP™ plate handling platform, and Luminex SD™ sheath fluid delivery system, xPONENT® software and personal computer (PC).

MAGPIX® System. This compact system includes a PC and MAGPIX® instrumentation utilizing innovative CCD detection technology.

Samples. Normal and septic human serum samples were purchased from Bioreclamation (Bioreclamation Inc., Hicksville, NY).

Immunoassay Protocol. Assays were conducted in 96-well plates according to the immunoassay protocol for the MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel (Cat. No. HCYTOMAG-60K).

The general procedure is as follows:

1. Wash 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.
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.

Results

Using the 39-plex MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel assays, we measured cytokine/chemokine levels in serum samples collected from normal subjects or sepsis patients. In this application note, data for IFNγ, IL-6, IL-10, IL-12 (p40), and GM-CSF are shown and assumed to be representative of the entire panel. Standard curve MFI generated from the two Luminex® analyzers were similar for all five representative analytes (Figure 1).

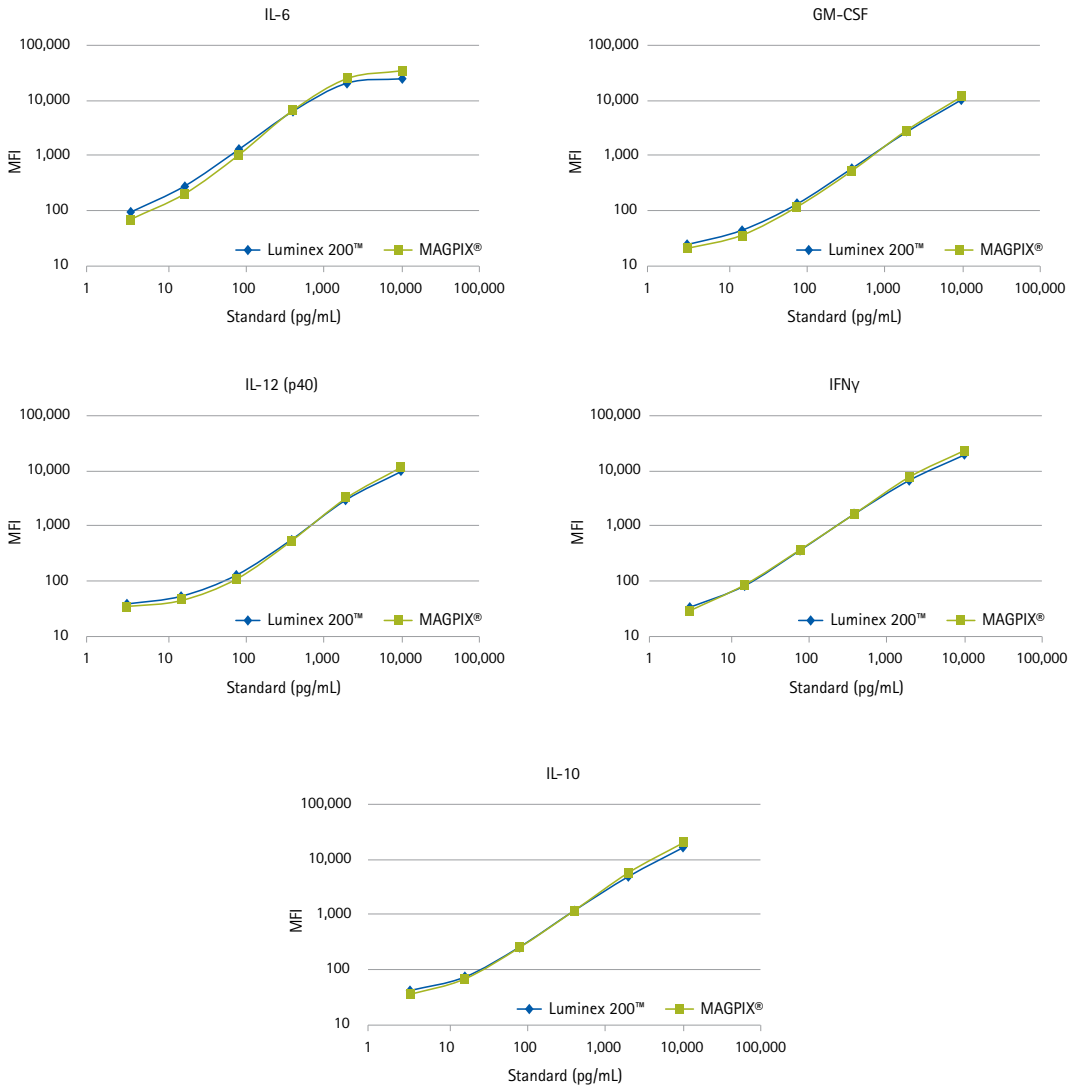

Figure 1.

Standard curve data from 5 analytes as representative of the 39-plex Human Cytokine/Chemokine Magnetic Bead Panel show nearly identical curves, regardless of the detection instrument used (Luminex 200™ or MAGPIX® system).

Sample concentration ranges generated for both normal and septic serum samples were comparable between the Luminex 200™ and MAGPIX® systems (Figure 2). All five cytokines were elevated in septic serum samples, and IL-6, GM-CSF and IL-10 showed significant elevation.

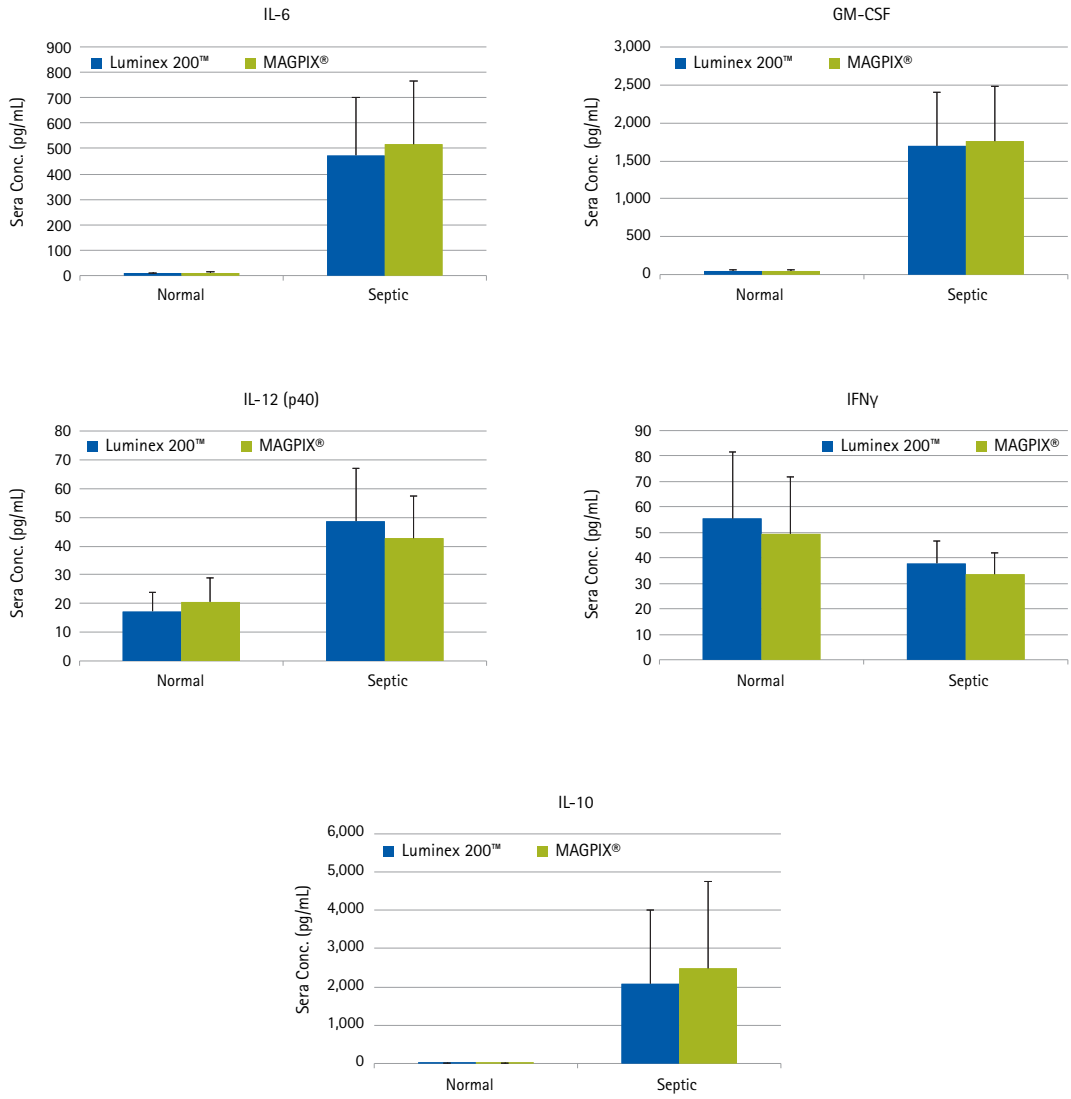

Figure 2:

IL-6, GM-CSF and IL-10 levels were significantly elevated in samples from patients with sepsis. Measured serum concentrations for 5 analytes were equivalent, regardless of the detection instrument used (Luminex 200™ or MAGPIX® system).

In addition, the correlation graphs comparing the two Luminex® systems for the five cytokines demonstrated highly positive correlation. Specifically, we observed an average slope of 0.997 and an average correlation coefficient of 0.991 (Figure 3). These sample correlation data demonstrate that comparable sample measurements can be obtained on Luminex 200™ and MAGPIX® systems.

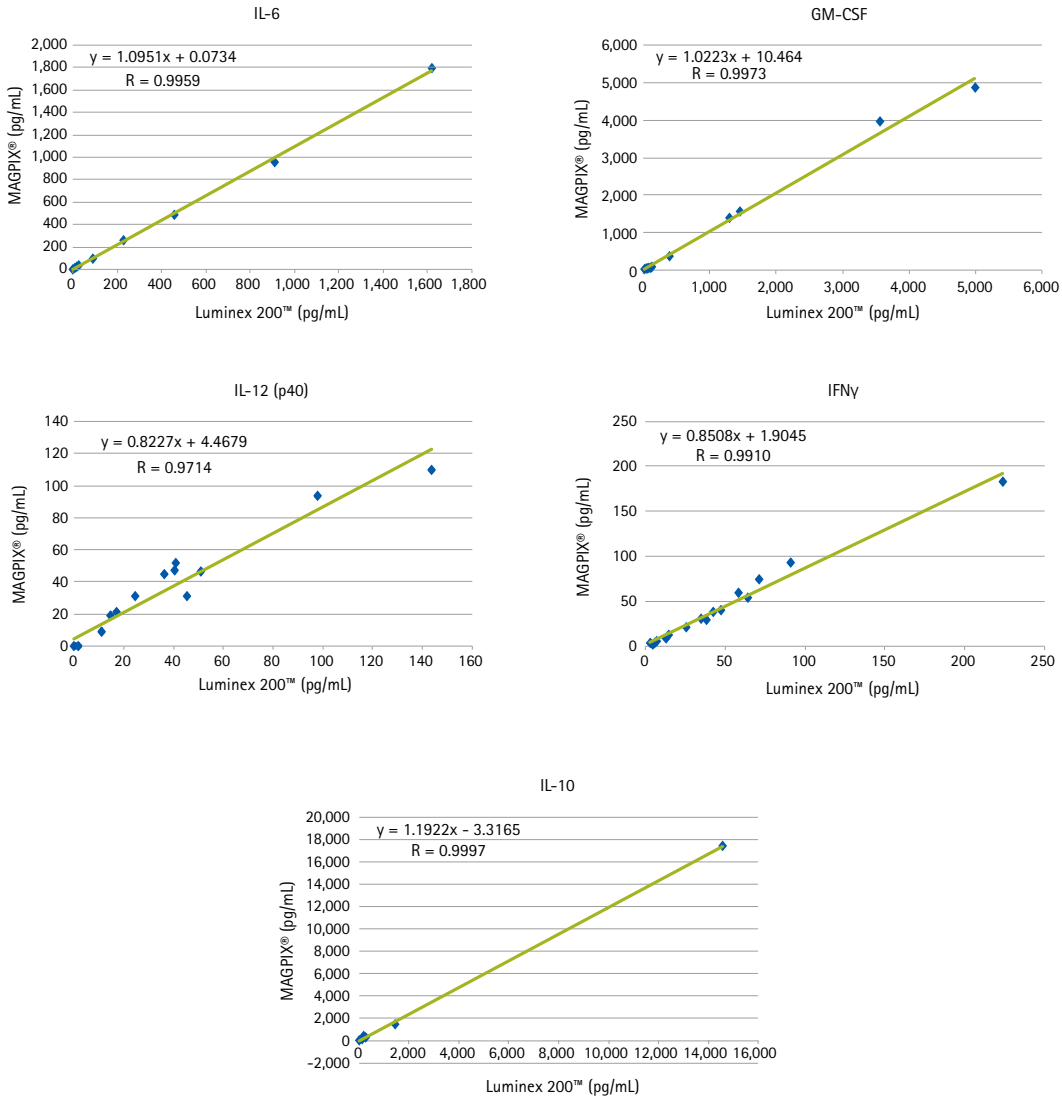

Figure 3:

Levels of 5 cytokines as measured using the MAGPIX® instrument plotted versus Luminex 200™ measurements in order to determine slope and correlation coefficients of linear regression fit lines.

Conclusion

Because inflammation responses, including sepsis, involve coordinated changes in the levels of multiple analytes, and because samples from affected subjects are frequently limiting, it is crucial to measure multiple analytes simultaneously when assessing the progression of inflammation responses. By enabling the measurement of up to 39 cytokines/chemokines from each sample, the MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic Bead Panel was particularly useful to extract maximum information from small sample volumes. The adoption of these multiplexed detection assays can be greatly facilitated by the use of smaller-footprint, flexible instruments such as the MAGPIX® analyzer, and we have shown comparable performance between the MAGPIX® instrument and a traditional, laser-equipped analyzer.

The MAGPIX® analyzer utilizes innovative CCD technology, and was designed to yield similar measurements compared to the Luminex 200™ system. Through evaluation of standard curve MFI, sample range, and sample correlation using the same assay panel and the same samples, we demonstrate comparable measurements between the two Luminex® systems. Thus, although the two Luminex® systems utilize different technologies for identification of bead regions and detection of the reporter molecule, SA-PE, investigators may use either instrument for reliable quantification of MILLIPLEX® MAP immunoassays, facilitating multiplexed analysis of cytokines for inflammation research.

System Specifications

Instrument	MAGPIX®	Luminex 100™ / 200™	FLEXMAP 3D®
Software	xPONENT® 4.2	xPONENT® 3.1	xPONENT® 4.0
Optic	LED / CCD Camera	Lasers / APDs / PMTs	Lasers / APDs / PMTs
Hardware	Fluorescent Imager	Flow Cytometry-based	Flow Cytometry-based
Bead Compatibility	Magnetic	Magnetic and non-Magnetic	Magnetic and non-Magnetic
Multiplex Capacity	50	100 (80 for Magnetic)	500
Read Time	~60 mins / 96-well	~40 mins / 96-well	~20 mins / 96-well
Applications	Protein / Nucleic Acid	Protein / Nucleic Acid	Protein / Nucleic Acid
Dynamic Range	3.5 logs	3.5 logs	4.5 logs
Microtiter Plate	96-well	96-well	96-well and 384-well
Footprint including PC (linear bench space)	64.8 cm (24 in.)	80.0 cm (32 in.)	64.8 cm (24 in.)
Weight (Analyzer)	17.5 kg (38.5 lbs)	49 kg (113 lbs)	77.1 kg (170 lbs)

Ordering Information

Description	Catalogue No.
MILLIPLEX® MAP Panels	
Human Cytokine/Chemokine Panel 1	HCYTOMAG-60K
Human Cytokine/Chemokine Panel 2	HCYP2MAG-62K
Human Cytokine/Chemokine Panel 3	HCYP3MAG-63K
Human Th17 Panel	HTH17MAG-14K
Related Products	
MAGPIX® System with xPONENT 4.2	40-072*
MAGPIX® xPONENT® 4.2 System with MILLIPLEX® Analyst Single Seat	40-073*
Luminex 200™ 3.1 xPONENT® System	40-012
Luminex 200™ 3.1 xPONENT® System with MILLIPLEX® Analyst	40-013*
FLEXMAP 3D®	40-014*
FLEXMAP 3D® with MILLIPLEX® Analyst	40-022*
BioTek® Magnetic 96-well Plate Washer, 110 V Complete	40-052*
BioTek® Magnetic/Vacuum Filtration 96-well Plate Washer, 110 V	40-057*
BioTek® Magnetic 96-well Strip Washer, 110 V Complete	40-062*
Hand-held Magnetic Separation Block for 96-well Plate	40-285

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

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