New Multiplex Assay Panels for Quantification of Cytokines, Interferons and Chemokines Involved in Innate and Adaptive Immune Responses

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Abstract

Pathogens, such as bacteria and viruses, trigger immune responses upon infection. Innate immune response is mediated by innate immune cells (e.g., macrophages, neutrophils, and dendritic cells) via the production of cytokines such as interferons (e.g., IFN-α, β, γ, λ), interleukins (e.g., IL-1, 6, 8, 10, 12, 18, 27, 33), TNF-α, and chemokines (e.g., MCP-1, IP-10, RANTES, MIP-1). Adaptive immune response is mediated by T cells and B cells. T helper cells secrete interleukins (IL-2, 4, 5, 9, 10, 13, 17, 21, 22), IFN-γ and TNF-α, and play pivotal roles in immune regulation. In addition, these cytokines and chemokines are also critically involved in inflammatory diseases. Therefore, expression profiling of these cytokines and chemokines is important in achieving in-depth understanding of the immune responses and various disease processes. We have developed multiplex assay panels, using fluorescence–encoded beads that are suitable for various flow cytometers. Each panel allows simultaneous quantification of 13 related analytes. Each antibody pair was carefully optimized for assay specificity, sensitivity, accuracy and reproducibility. These panels have been validated by detecting expected changes in biological samples. These panels are of high quality, low cost, and easy to use, providing an alternative multiplex solution to the biomedical and drug discovery community.

Materials and Methods

1. Instrument and Settings

Flow Cytometer	Laser to use	Reporter Channel	Reporter Channel Emission	Beads Classification Channel	Classification Channel Emission	Compensation needed?
BD FACS Calibur™ (single laser)	blue	FL2	575 nm	FL3	670 nm	Yes
BD FACS Calibur™ (dual laser)	blue & red	FL2	575 nm	FL4	660 nm	No
BD FACSCanto™, BD FACSCanto™ II	blue & red	PE	575 nm	APC	660 nm	No
BD LSR, BD LSRII, BD LSRFortessa™	blue & red	PE	575 nm	APC	660 nm	No
BD FACSAria™	blue & red	PE	575 nm	APC	660 nm	No
BD FACScan™ (single Laser)	blue	FL2	575 nm	FL3	670 nm	Yes

2. 96-well microtiter filter plates, V- or U-bottom plates, vacuum pump, filtration manifold and FACS tubes.
3. Capture antibody immobilized beads, biotinylated detection antibody cocktail, streptavidin-phycoerythrin conjugate and buffers.
4. Data analysis software and software dongle (free).
5. Biological Sample Preparation:

   Human PBMC from healthy donors were isolated using Ficoll-Paque (GE Healthcare) and seeded at 10^6 cells /mL into 48 -well plates with the appropriate stimulations as indicated.

Cell Staining Procedure

25 µL Matrix or Assay Buffer
25 µL Standard or samples
25 µL beads

↓ Shaking for 2h, RT
Vacuum and wash twice

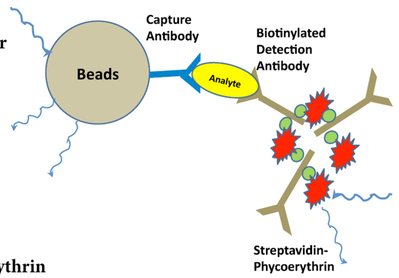
25 µL Detection Antibody

↓ Shaking for 1h, RT
No vacuum, no wash

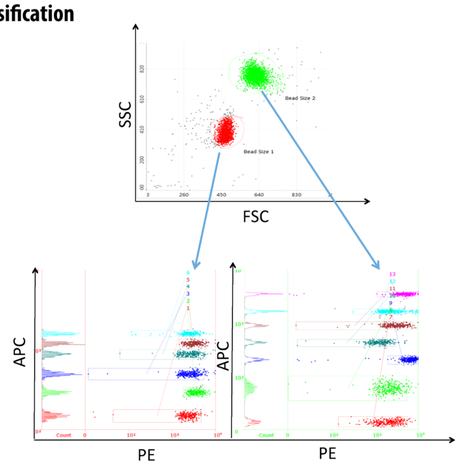
25 µL Streptavidin-Phycoerythrin

↓ Shaking for 30 min, RT
Vacuum and wash twice

Read on a flow cytometer



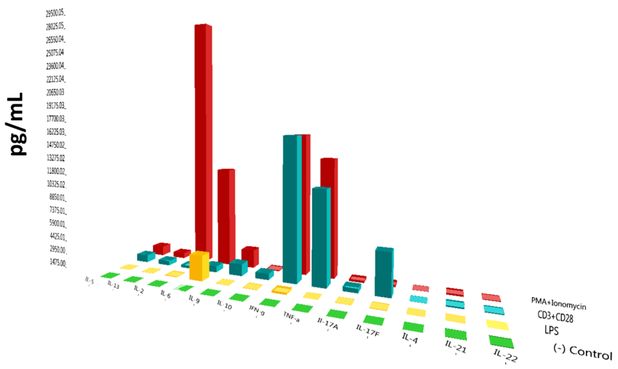
Beads Classification



Multiplex Assay Panels

Human Interferon Panel	Human Th Panel	Mouse Th Panel	Human Inflammation Panel	Mouse Inflammation Panel	Human Chemokine Panel	Mouse Chemokine Panel
GM-CSF	IL-2	IL-2	IL-1β	GM-CSF	CCL11	BLC
IFN-α	IL-4	IL-4	IFN-α	IFN-β	ENA-78	Eotaxin
IFN-β	IL-5	IL-5	IFN-γ	IFN-γ	Groα	IP-10
IFN-λ1	IL-6	IL-6	IL-6	IL-1α	IL-8	KC
IFN-λ2/3	IL-9	IL-9	IL-8	IL-1β	IP-10	LIX
IFN-γ	IL-10	IL-10	IL-10	IL-6	I-TAC	MCP-1
IL-1β	IL-13	IL-13	IL-12p70	IL-10	MCP-1	MDC
IL-6	IL-17A	IL-17A	IL-17A	IL-12p70	MIG	MIG
IL-8	IL-17F	IL-17F	IL-18	IL-17A	MIP-1α	MIP-1α
IL-10	IL-21	IL-21	IL-23	IL-27	MIP-1β	MIP-1β
IL-12p70	IL-22	IL-22	IL-33	IL-23	MIP-3α	MIP-3α
IP-10	IFN-γ	IFN-γ	MCP-1	MCP-1	RANTES	RANTES
TNF-α	TNF-α	TNF-α	TNF-α	TNF-α	TARC	TARC

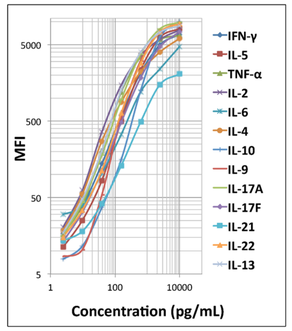
Biological Validation
(Representative Panel-Human Th Cytokine Panel)



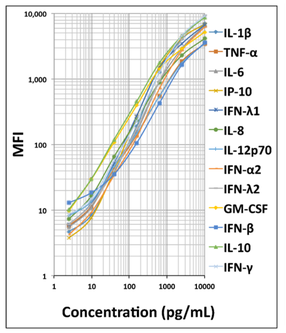
Human PBMCs were stimulated with LPS, CD3+ CD28 or PMA+ Ionomycin, supernatant collected after 24 hours and analyzed with the Human Th Cytokine panel. Shown above is a 3-D display of the data generated by the data analysis software.

Representative Standard Curves

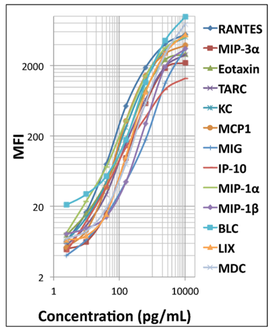
Mouse Th Cytokine Panel



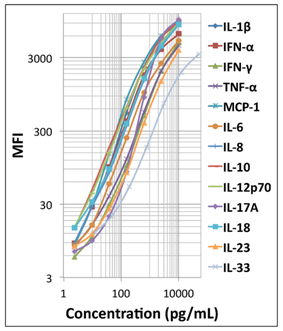
Human Interferon Panel



Mouse Chemokine Panel



Human Inflammation Panel

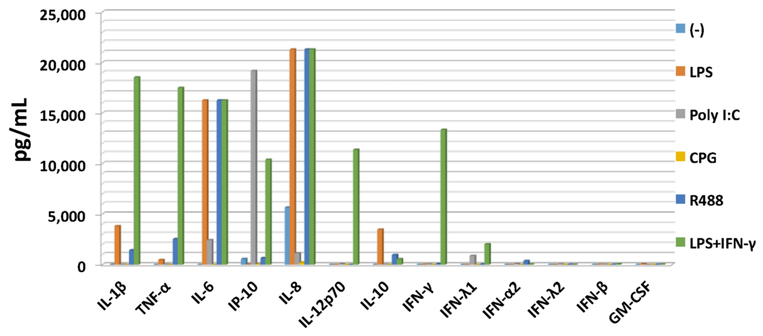


Biological Validation
(Representative Panel-Human Interferon Panel)

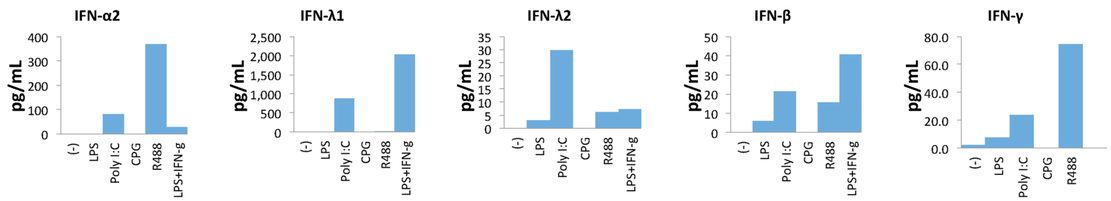
A.

Stimulation	TLRs	Potential Target Cells
Poly I:C (50 ug/mL)	TLR3	B cells, Macrophage, DC
LPS (1 ug/mL)	TLR4	Monocytes, DC, Macrophage, B cells
CPG –class B (5 ug/mL)	TLR9	B cells, pDCs, NK cells
R488 (2 ug/mL)	TLR7/8	B cells, Monocyte/ Macrophages, DCs, Mast cells (TLR8)
IFN-γ + LPS (100 ng/mL + 1 ug/mL)	TLR4	B cells, Monocyte/ Macrophages, DCs

B.



C.



Human PBMCs were stimulated under various conditions and supernatant collected after 24 hours and analyzed with the Human Interferon Panel. A. Stimulation conditions. B. Expression profile of all 13-analytes in the panel. C. Differential induction of interferons by various stimulations.

Conclusions

1. We have developed bead-based multiplex assays for quantification of important cytokines, interferons and chemokines involved in innate and adaptive immune responses.
2. These assay panels offer high performance, low cost, are easy to use, with user friendly software and no specialized instruments.
3. The utility of these multiplex assays were validated by using relevant biological samples. including serum, plasma, and cell culture supernatants, offering useful tools for biomedical research and drug discovery.