New Multiplex Panel for Quantifying Interferons and Cytokines Involved in Anti-Virus Responses

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Abstract

In response to pathogens, especially viruses, cells release interferons and other cytokines to fight the infections. Interferons are typically divided into three types: I (e.g., IFN-α, IFN-β), II (e.g., IFN-γ), and III (e.g., IFN-λ1, IFN-λ2). All interferons are important for fighting viral infections and for regulating the immune system. In addition, interferons are critically involved in cancer and autoimmune diseases such as psoriasis, systemic lupus erythematosus, and multiple sclerosis. Expression profiling of interferons and other related cytokines is critical in achieving a deeper understanding of the immune responses and various disease processes.

We have developed a multiplex Human Interferon Panel, using fluorescence–encoded beads that are suitable for use on various flow cytometers. This panel allows simultaneous quantification of 13 cytokines involved in anti-virus responses including interferons (α, β, γ, λ1 and λ2), interleukins (1β, 6, 8, 10, 12), TNF-α, IP-10, and GM-CSF. Each antibody pair was carefully selected for assay specificity, sensitivity, accuracy and reproducibility. The panel has been validated by detecting expected changes in biological samples. Further advantages include high sensitivity, small sample volume, flexible assay configurations, and time- and cost-effectiveness. The Interferon Panel can be used for serum, plasma, cell culture supernatant and other sample types, offering a useful tool for biomedical research and drug discovery.

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.

Assay Protocol

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

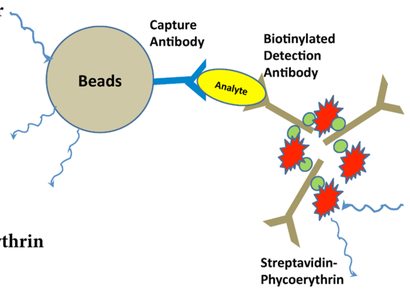
↓ Shaking for 1h, RT
No vacuum, no wash

25 µL Streptavidin-Phycoerythrin

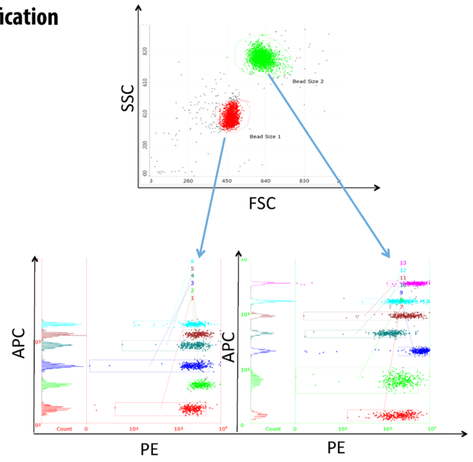
↓ Shaking for 30 min, RT
Vacuum and wash twice

Read on a flow cytometer

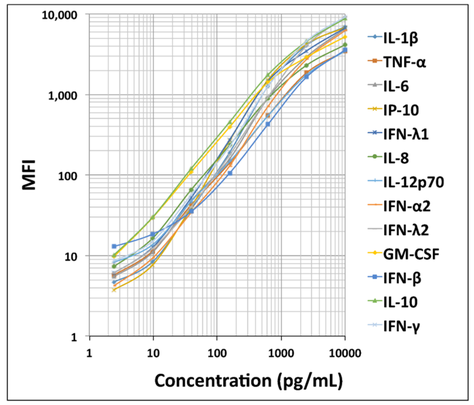
Principle of the Assay



Beads Classification



Representative Standard Curves



Assay Performance

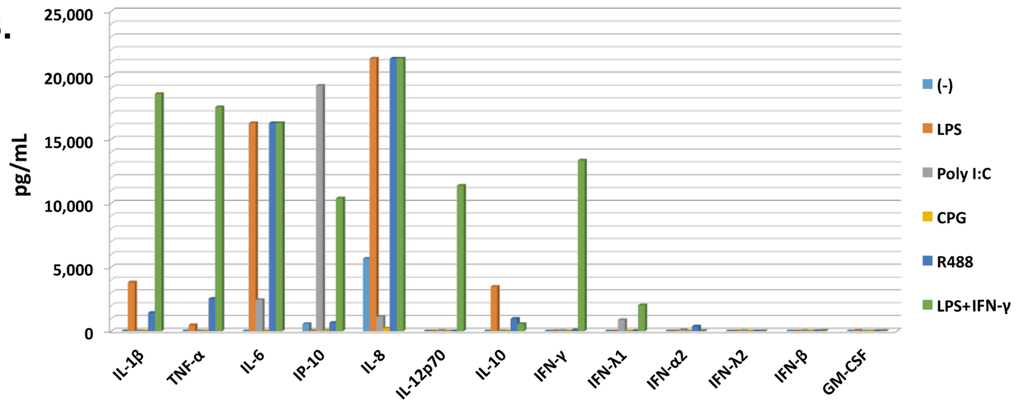
Analytes	Sensitivity in Medium (pg/mL)	Sensitivity in Serum (pg/mL)	Spike Recovery (Serum)	Dilution Linearity (Serum)
GM-CSF	1.5	1.8	99%	110%
IFN-α	1.0	1.8	130%	78%
IFN-β	0.7	2.0	85%	122%
IFN-λ1	2.0	3.0	130%	96%
IFN-λ2/3	1.2	2.0	50%	150%
IFN-γ	1.0	1.9	102%	120%
IL-1β	0.6	1.4	92%	110%
IL-6	0.8	1.5	110%	110%
IL-8	2.0	2.2	93%	125%
IL-10	1.0	1.2	98%	105%
IL-12p70	1.5	2.0	106%	108%
IP-10	1.6	1.6	97%	132%
TNF-α	1.5	1.8	98%	110%

Biological Validation

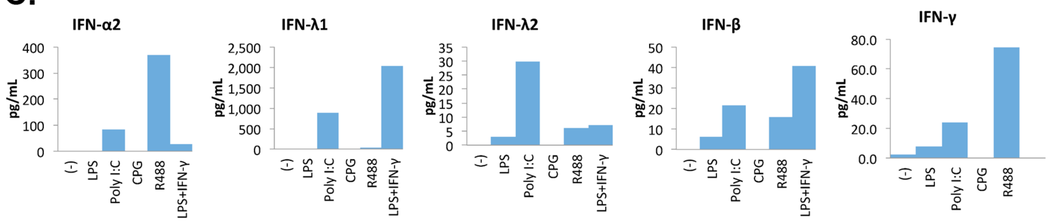
A.

Stimulation	TLRs	Potential Target Cells
Poly I:C (50 µg/mL)	TLR3	B cells, Macrophage, DC
LPS (1 µg/mL)	TLR4	Monocytes, DC, Macrophage, B cells
CPG –class B (5 µg/mL)	TLR9	B cells, pDCs, NK cells
R488 (2 µg/mL)	TLR7/8	B cells, Monocyte/Macrophages, DCs, Mast cells (TLR8)
IFN-γ + LPS (100 ng/mL + 1 µg/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 a bead-based multiplex assay for quantification of important interferons and cytokines involved in innate immune responses, especially those against viruses.
2. This assay panel offers high performance, low cost, is easy-to-use, with user-friendly software and no specialized instruments.
3. The utility of this multiplex assay was validated by using relevant biological samples, including serum and cell culture supernatants, offering a useful tool for biomedical research and drug discovery.

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