

Bio-Plex Cytokine Immunoassays and ELISA: Comparison of Two Methodologies in Testing Samples From Asthmatic and Healthy Children

Maria C Jenmalm,¹ Michele Malit,² Jim Torrence,² and Aiguo Zhang,²
¹ Department of Molecular and Clinical Medicine, Division of Paediatrics, Linköping University, Sweden, and ² Bio-Rad Laboratories, Inc., 2000 Alfred Nobel Drive, Hercules, CA 94547 USA

Introduction

Cytokines play a significant role in hematopoiesis, inflammation, and wound healing. These important immunoregulators mediate interactions between various cells, and their dysregulated production may contribute to disease pathogenesis. Elevated levels of cytokines may indicate inflammation or disease progression. Although cytokine functions are complex, cytokine profiles are highly relevant parameters of an immune response. Different cytokines possess biologically overlapping functions, and they have the ability to regulate production of other cytokines. Therefore, analysis of the function of the complete set of cytokines expressed within microenvironments (e.g., a site of inflammation) is often of more value than analysis of a single isolated cytokine (O'Garra and Murphy 1994).

A number of methodologies can be used to measure cytokines. The most common one is the enzyme-linked immunosorbent assay (ELISA), which allows measurement of different cytokines with high sensitivity and specificity. This technology, however, is restricted to analyzing one cytokine target at a time, which limits its usefulness when simultaneous measurement of multiple cytokine targets is desired. The new suspension bead array technology employed in the Bio-Plex system (Luminex xMAP technology) allows multiple cytokine targets to be measured from a single sample in a microplate well (Vignali 2000).

The goal of our study was to compare the Bio-Plex cytokine assays with cytokine ELISA developed in-house for testing cell culture samples from asthmatic and healthy children.

Methods

Cell Isolation and Cultures

Human peripheral blood mononuclear cells (PBMC) were isolated from fresh blood donated by atopic asthmatic children and non-atopic healthy children between the ages of 13 and

15 (Böttcher et al. in press). The cells (1×10^6 /ml) were cultured in AIM-V serum-free medium (Life Technologies AB, Sweden) with 20 mM mercaptoethanol (Sigma-Aldrich, Stockholm, Sweden). The cells were stimulated with timothy grass, cat, and dog allergen extracts from ALK (Horsholm, Denmark) at 10,000 standardized units/ml each. The cells were cultured at 37°C with 5% CO₂ for 144 hr. The supernatants were collected after centrifugation and stored at -70°C. Samples were tested simultaneously for cytokines IL-5, IL-10, IL-13, and IFN- γ using both a Bio-Plex human cytokine 4-plex assay and an ELISA made from commercially available antibodies.

Multiplex Assay

A Bio-Plex human cytokine assay for simultaneous quantitation of IL-5, IL-10, IL-13, and IFN- γ was run according to the recommended procedure. In brief, the premixed standards were reconstituted in 0.5 ml of culture medium, generating a stock concentration of 50,000 pg/ml for each cytokine. The standard stock was serially diluted in the same culture medium to generate 8 points for the standard curve. The assay was performed in a 96-well filtration plate supplied with the assay kit. Premixed beads (50 μ l) coated with target capture antibodies were transferred to each well of the filter plate (5,000 beads per well per cytokine) and washed twice with Bio-Plex wash buffer. Premixed standards or samples (50 μ l) were added to each well containing washed beads. The samples were used directly without further dilution. The plate was shaken for 30 sec and then incubated at room temperature for 30 min with low-speed shaking (300 rpm). After incubation and washing, premixed detection antibodies (50 μ l, final concentration of 2 μ g/ml) were added to each well. The incubation was terminated after shaking for 10 min at room temperature. After 3 washes, the beads were resuspended in 125 μ l of Bio-Plex assay buffer. Beads were read on the Bio-Plex suspension array system, and the data were analyzed using Bio-Plex Manager™ software (v 3.0) with 5PL curve fitting.

ELISA

ELISAs were performed as described in Jenmalm et al. (2001). Human cytokines IL-10 and IL-13 were analyzed using PeliPair reagent set antibodies (CLB, Amsterdam, the Netherlands). IL-5 was analyzed using an antibody pair from Pharmingen (San Diego, CA): capture antibody TRFK5 and detection antibody JES1-5. IFN- γ was measured using an antibody pair from R&D Systems (Abingdon, UK): capture antibody 2571.811 and a polyclonal detection antibody (goat anti-human). The detection antibodies were biotinylated and streptavidin-horseradish peroxidase was the enzyme label reagent.

Results and Discussion

Correlation between the Bio-Plex assay and ELISA was evaluated by measuring 22 samples for four cytokine targets (IL-5, IL-10, IL-13, and IFN- γ ; see table). The concentrations of the majority of samples measured by ELISA compared well with concentrations obtained in the Bio-Plex assays (see figure). Correlation coefficients (r^2) ranged from 0.80 to 0.99 ($p < 0.001$ for all analyses).

The dynamic range of the ELISA used in this study was significantly narrower than that of the Bio-Plex assays. The ELISAs detected 1.6 to 600 pg/ml cytokines, while Bio-Plex assays detected 1.6 to 32,000 pg/ml.

Table. Correlation between Bio-Plex and ELISA results.

Cytokine	Correlation Coefficient	# of Assays
IL-5	0.99	22
IL-10	0.83	22
IL-13	0.96	22
IFN- γ	0.80	22

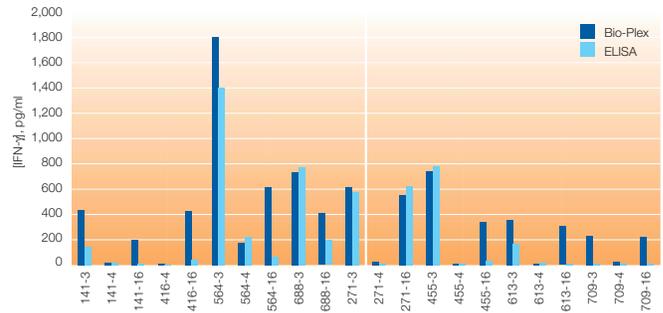


Figure. Comparison of Bio-Plex and ELISA assays for human IFN- γ .

Conclusions

In this study, we were able to demonstrate that testing PBMC samples from asthmatic and healthy children for IL-5, IL-10, IL-13, and IFN- γ by ELISA and Bio-Plex assays yielded similar results. Bio-Plex assays have broader dynamic range and allow for multiplex analysis of cytokines in a single sample. Therefore, Bio-Plex assays have an advantage over the current ELISA methodology when multiple samples have to be analyzed for several cytokines and when available sample volume is limited.

Acknowledgements

We thank Ulrika Bengtsson, Anne-Marie Fornander, Lena Lindell, Ing-Marie Sandberg, and Kristina Warstedt, Department of Molecular and Clinical Medicine, Division of Paediatrics, Linköping University, Sweden, for excellent technical assistance.

References

- Böttcher MF et al., Allergen-induced cytokine secretion in atopic and non-atopic asthmatic children, *Pediatr Allergy Immunol*, in press
- Jenmalm MC et al., Allergen-induced Th1 and Th2 cytokine secretion in relation to specific allergen sensitization and atopic symptoms in children, *Clin Exp Allergy* 31, 1528–1535 (2001)
- O'Garra A and Murphy K, Role of cytokines in determining T-lymphocyte function, *Curr Opin Immunol* 6, 458–466 (1994)
- Vignali DA, Multiplexed particle-based flow cytometric assays, *J Immunol Methods* 243, 243–255 (2000)

xMAP is a trademark of Luminex Corp. The Bio-Plex protein array system includes fluorescently labeled microspheres and instrumentation licensed to Bio-Rad Laboratories, Inc. by the Luminex Corporation.



**Bio-Rad
Laboratories, Inc.**

Life Science
Group

Web site www.bio-rad.com **USA** (800) 4BIORAD **Australia** 02 9914 2800 **Austria** (01)-877 89 01 **Belgium** 09-385 55 11 **Brazil** 55 21 2527 3454
Canada (905) 712-2771 **China** (86-21) 63052255 **Czech Republic** + 420 2 41 43 05 32 **Denmark** 44 52 10 00 **Finland** 09 804 22 00
France 01 47 95 69 65 **Germany** 089 318 84-177 **Hong Kong** 852-2789-3300 **Hungary** 36 1 455 8800 **India** (91-124)-6398112/113/114, 6450092/93
Israel 03 951 4127 **Italy** 39 02 216091 **Japan** 03-5811-6270 **Korea** 82-2-3473-4460 **Latin America** 305-894-5950 **Mexico** 55-52-00-05-20
The Netherlands 0318-540666 **New Zealand** 64 9 415 2280 **Norway** 23 38 41 30 **Poland** + 48 22 331 99 99 **Portugal** 351-21-472-7700
Russia 7 095 721 1404 **Singapore** 65-6415 3188 **South Africa** 00 27 11 4428508 **Spain** 34 91 590 5200 **Sweden** 08 555 12700
Switzerland 061 717-9555 **Taiwan** (8862) 2578-7189/2578-7241 **United Kingdom** 020 8328 2000