

Cytokine profiling by multiplex immunoassay as an effective approach in monitoring inflammatory and immune responses

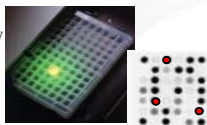
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Abstract

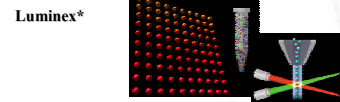
Cytokines are a family of secreted polypeptides that play a pivotal role in regulating immune responses, inflammation, development and tissue repair. Because cytokines function in a complex regulatory network where one cytokine influences production of many other cytokines and vice versa, there is a need for profiling an array of cytokines in a given pathological condition. For example, secretion of IL-1, IL-6, IL-8, IL-12 and TNF- α from macrophages is increased in response to bacterial infection. Current technology development of multiplex immunoassays for simultaneous measurement of multiple analytes in a single assay has greatly improved the throughput and cost effectiveness of cytokine profiling. While many different technologies are available for multiplex measurement, there are two major categories of the platforms. One platform, utilizing spot-based microarrays with antibodies immobilized on the solid support, is analyzed by imaging. The other is bead-based ("liquid") arrays, with antibodies conjugated on the beads, and is analyzed by flow cytometry. Most systems based on spots or beads can achieve sensitive cytokine assays with less than 10 pg/ml sensitivity and a dynamic range upper limit of 5000-10,000 pg/ml. The pros and cons of these two major technologies and the application of multiplex immunoassay for pre-clinical and clinical research, as well as its potential in diagnostics, will be discussed.

Note: For research use only. Not for use in diagnostic procedures. The example of spot-based multiplex assay system:

A²™ MicroArray System



The example of bead-based multiplex assay system:



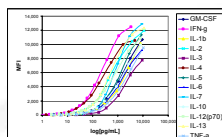
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Comparison of Spot and Bead Multiplex Technologies

	Spot Arrays	Bead Arrays
Basic Format	Printed spots with X, Y coordinates on a solid surface, e.g. glass slides or microtiter plate wells.	Color or size differentiation and identification using small particles (beads).
Flexibility	Arrays are typically manufactured as fixed arrays.	Panels can be mixed and matched according to experiments.
Reaction kinetics	Slower, usually requires 1-4h incubation.	Usually 10-30 min reaction time.
Detection Time	Fast CCD camera read time, typically less than 5-10 min per 96-well plate.	Slow. Beads must be counted in a flow cytometer; typically 30-60 min per 96-well plate equipped with a microtiter plate sample loader.
Multiplex factor	Dozens to hundreds	100 or less
Assay throughput	Microtiter plate type of arrays such as A ² plate can have automatic high throughput assay processes similar to that of ELISA.	Lower throughput, with up to a few hundred samples at a time.

A² MicroArray System

Validated assays: Human Cytokine panel



A² Plate Reader



- Sensitive fluorescent CCD detection
- Small footprint and automation friendly
- Fast 5 min reading and processing
- 5 min warm up time
- Controller with user friendly software

Integrated software solution Data reduction capability.

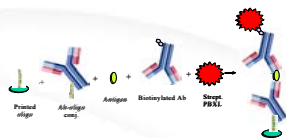


A² Universal Plate

- Polypropylene 96 well format
- Can be handled by automated liquid handling system (SBS Compliant)
- Preprinted with 14 different oligonucleotide sequences to act as zipcodes
- Enables proprietary self-assembling quantitative microarray for multiplex assays.

Cytokine Assays

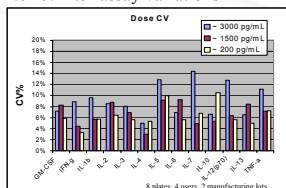
Principle



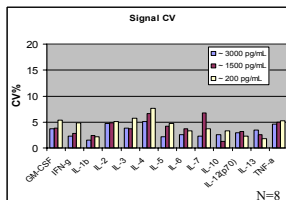
A² Plate immunoassay protocol

- Add antibody-oligo conjugates
- Incubate and wash
- Add samples
- Incubate and wash
- Add detection antibodies
- Incubate and wash
- Add streptavidin-PBXL1
- Incubate and wash
- Read on A² reader

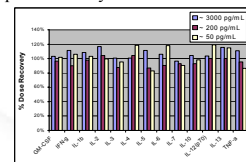
Plate-to-plate (n=8), user-to-user and lot-to-lot inter-assay variations



Intra-assay (well to well) variation



Spike recovery



Cross-reactivity: "-1" study

Reagent	Obs Conc	% Cross-reactivity
GM-CSF	8.1	0.13%
IFN-gamma	1.7	0.03%
IL-1b	8.2	0.16%
IL-2	8.7	0.13%
IL-3	7.3	0.15%
IL-4	5.4	0.11%
IL-5	7.1	0.14%
IL-6	7.5	0.15%
IL-7	20.1	0.40%
IL-10	8.2	0.12%
IL-12(p70)	5.3	0.11%
IL-13	8.8	0.13%
TNF-alpha	14.5	0.29%

- Antigen concentration: 5000 pg/ml of each analyte
- Assays consisted of 13 capture antibodies, all the other 12 recombinant antigens except the one examined ("-1") and 13 detection antibodies ("13-12-13" assay)
- Observed Concentration (Obs Conc) of a particular analyte was obtained from a standard curve of a "13-13-13" assay run on the same plate
- The % Cross-reactivity = observed concentration x 1000

Cross-reactivity: "+1" study

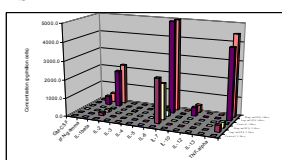
Assay	Obs Conc	% Cross-reactivity
IL-13	1.1	0.02%
IL-10	1.1	0.02%
IL-6	1.1	0.02%
IL-7	1.1	0.02%
IL-5	1.1	0.02%
IL-4	1.1	0.02%
IL-3	1.1	0.02%
IL-2	1.1	0.02%
IL-1b	1.1	0.02%
IFN-gamma	1.1	0.02%
GM-CSF	1.1	0.02%
TNF-alpha	1.1	0.02%

- Antigen concentration: 50,000 pg/ml
- Assays consisted of 13 capture antibodies, recombinant antigen of the analyte that was tested ("+1") and 13 detection antibodies ("13-13-13" assay)
- The % Cross-reactivity = observed concentration / 50,000

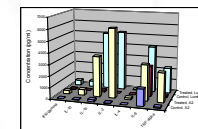
Assay sensitivity and dynamic range

Assay	BSL	LLSD	ULSD
IL-13	2.2	17	2500
IL-10	2.2	17	2500
IL-6	2.2	17	2500
IL-7	2.2	17	2500
IL-5	2.2	17	2500
IL-4	2.2	17	2500
IL-3	2.2	17	2500
IL-2	2.2	17	2500
IL-1b	2.2	17	2500
IFN-gamma	2.2	17	2500
GM-CSF	2.2	17	2500
TNF-alpha	2.2	17	2500

Cytokine production: PBMC treated with LPS



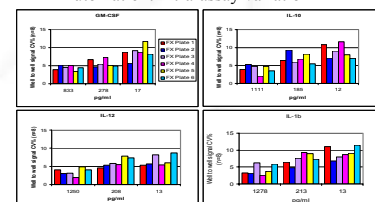
Cytokine production by PBMC: A² vs. Luminex assays



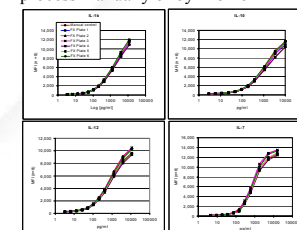
Automation of A² Assays

- Biomek® FX Laboratory Automation Workstation
 - Deck Bridge
 - Spun 8 Arm with disposable tips
 - 96 Head Pipetting Tool
 - 2 Orbital Shaking ALPs
 - Plate washer
- Process 6 plates of assays in a single run.

Automation: intra-assay variation



Comparison of assay signal intensity process manually or by Biomek FX



Summary

- A² MicroArray System
 - Flexible complete system for multiplexed immunoassays
 - Quantitative tool for biomarker validation
 - Easy to use and standardized format
 - Automation friendly.

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