ProbePlex: Simultaneous Immunophenotyping and Immunoassay

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Abstract

Cellular marker expression profiles (immunophenotyping by flow cytometry) and immunoassay technology for the quantitation of soluble (serum) markers have existed for decades. Recently, beads coated with antibody, or antigen, have been used with flow cytometry platforms to detect serum analytes. The ability to combine immunophenotyping and serum marker analysis in one tube (ProbePlex*) can provide a more complete picture of a patient´s medical status. It opens the door to a more comprehensive `snapshot´ of patient status or drug effects.

Anti-cytokine antibody-conjugated fluorescent capture beads with distinct fluorescent intensities were added to 100 mL of anticoagulated whole blood along with PE-conjugated anti-cytokine detector reagent and various fluorochrome-conjugated anti-CD markers. The sample was allowed to incubate for 1 hour with gentle mixing, protected from light. At the end of the incubation the blood was lysed using the IMMUNOPREP™ Reagent System and analyzed on a Beckman Coulter FC 500 Flow Cytometer

There was no significant difference in cytokine values when capture beads/detector reagents were incubated in plasma or in whole blood, with or without fluorochrome anti-CD markers. Neither CD expression, as measured by mean fluorescent intensity, nor cellular scatter parameters were effected by the capture beads thus providing reliable information on white blood cell percentages, cell surface expression and serum cytokine levels in a single tube.

ProbePlex is structured to take advantage of those instances when both cellular and soluble markers are needed to provide a more complete picture of patient status. It enables multiple simultaneous assays in a single tube utilizing a maximum of 100 mL blood, important for both pediatric and geriatric populations. Some potential applications are in the areas of sepsis/inflammation, autoimmune disease, cardiovascular risk and viral vs. bacterial infection.

* ImmunoPlex, patent pending

Introduction

- Both cell surface marker analyses by flow cytometric methods and Immunoassay technology for the quantitation of specific serum markers termed here 'analytes' (proteins, viruses, hormones, etc.), have existed for decades.
- Recently, beads coated with antibody, or antigen have been utilized with serum or plasma to detect these serum analytes using a flow cytometer platform
  - This type of bead technology is well known with patents going back to the 1980's.
  - Numerous papers and some kits are now available for these 'multi-plexed' and multi-analyte systems.
  - These assays generally employ a wash step to remove the bound analyte from the free analyte and are performed in a serum, plasma or media matrix devoid of cells.
- The ability to perform ProbePlex assays is accomplished by performing the assays in such a manner as to inhibit the phagocytosis of the particles by myeloid cells.
- Soluble and cellular expression patterns may be useful in the assessment of cardiovascular risk, cancer, inflammation/sepsis, or autoimmune disease as well as studies involving cell lines and their products affected by targeted drugs
- ProbePlex reagents combine the strengths of mutiplex bead assays for soluble analytes with those of cellular immunophenotyping and traditional cellular analysis.

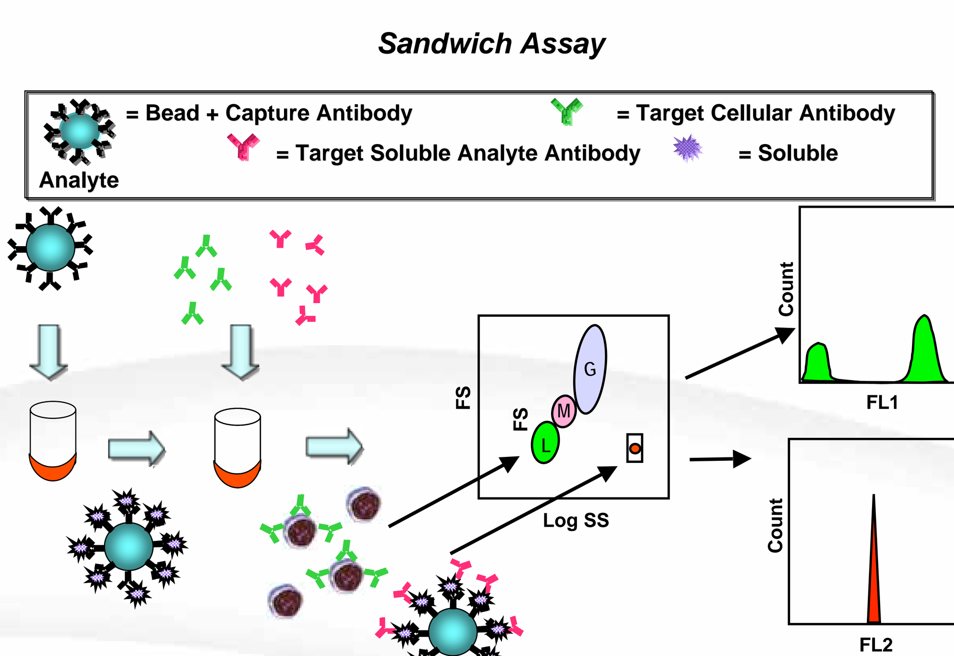
Materials & Methods

- 10 - 50 µL of antibody-conjugated fluorescent or non-fluorescent capture beads were added to 100 µL whole blood along with target cellular marker(s), CD45-FITC, alone, or CD45-PC5 and CD14-FITC (Beckman Coulter, Inc.).
- The sample was incubated for 30 to 150 minutes, at ≤ 25C, with continual mixing.
- The soluble target antibodies were then added and the sample was incubated for an additional 30 minutes prior to lysing with the ImmunoPrep™ Reagent System.
- Samples were analyzed on a Cytomics FC500 or Coulter® XL/MCL™ flow cytometer.

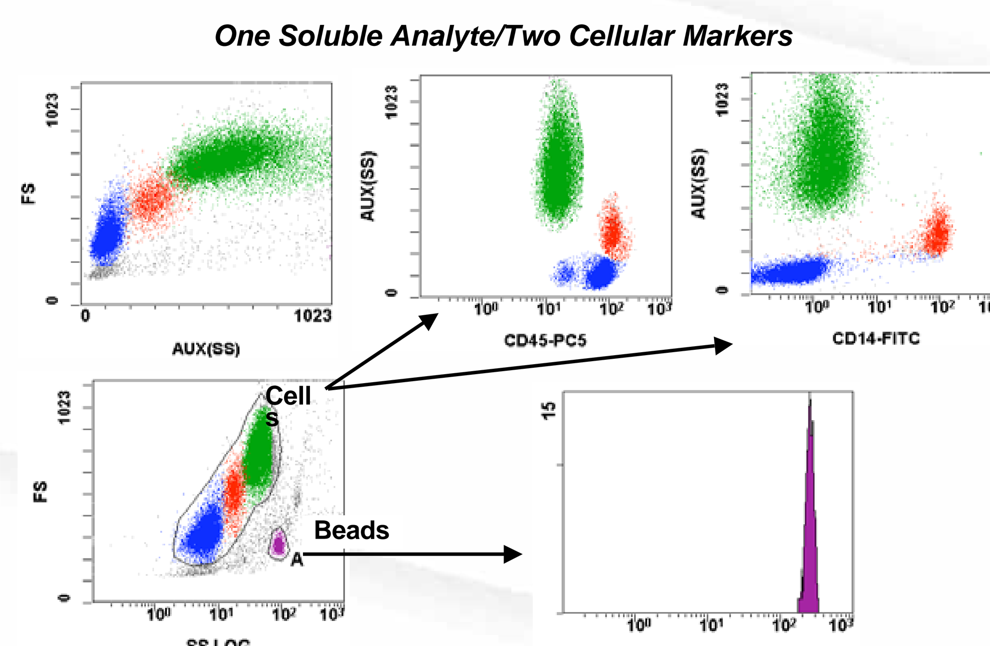
ProbePlex Concept

- All reactions occur in one tube.
- Tube may be lysed and /or washed depending upon sensitivity required.
- Assay can be 'sandwich' type, 'immune-complex' type or competitive inhibition. An example of a 'sandwich' type is shown
- Assay may use multiple analytes, multiple beads or both.

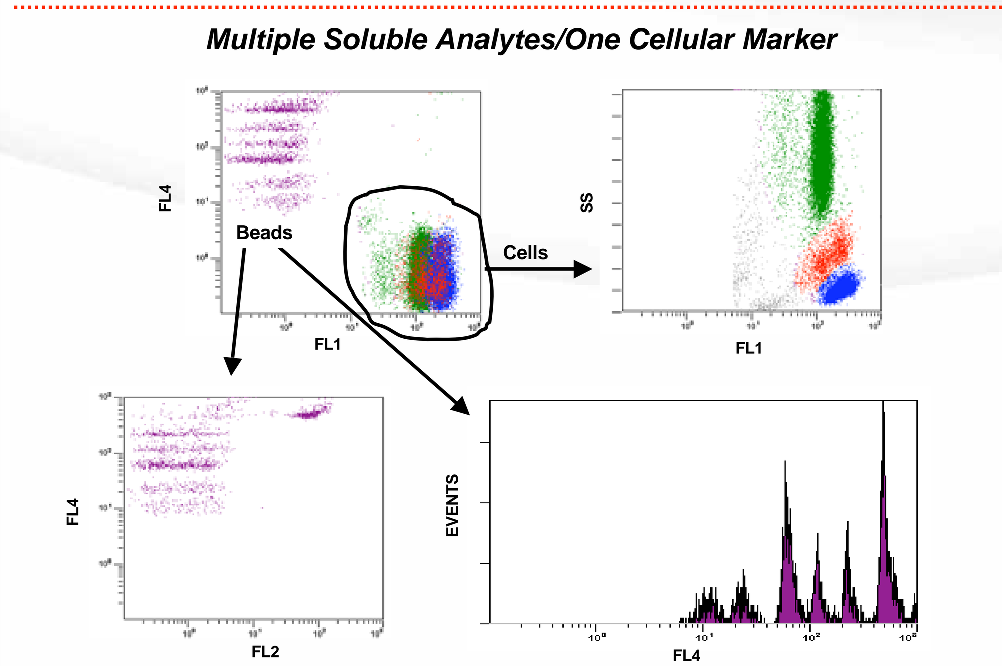
Sandwich Assay



One Soluble Analyte/Two Cellular Markers

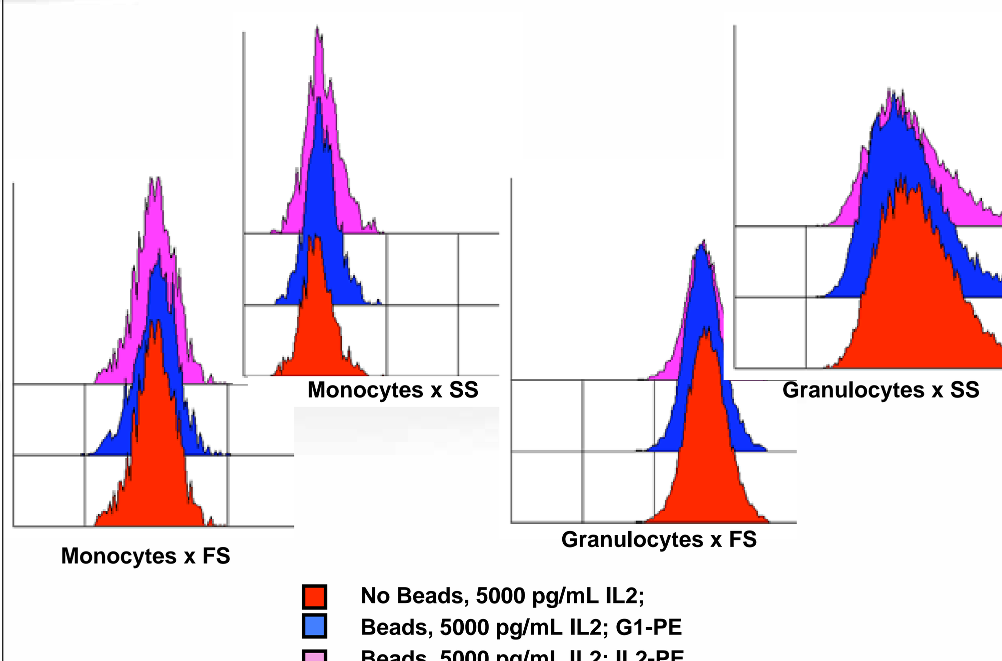


Multiple Soluble Analytes/One Cellular Marker



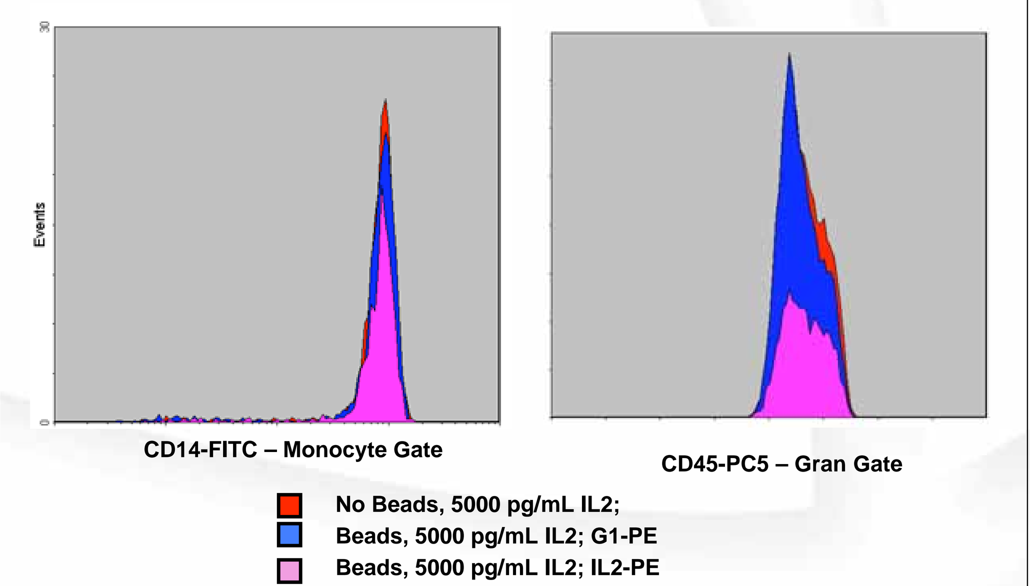
Effect of Beads on Light Scatter

- Samples were processed with and without beads to assess any impact on cellular light scatter patterns.
- Lymphocytes, monocytes and granulocytes were examined. Only monocytes and granulocytes, considered worst case scenario due to potential phagocytosis of particles, are shown.



Bead Effect on CD Expression

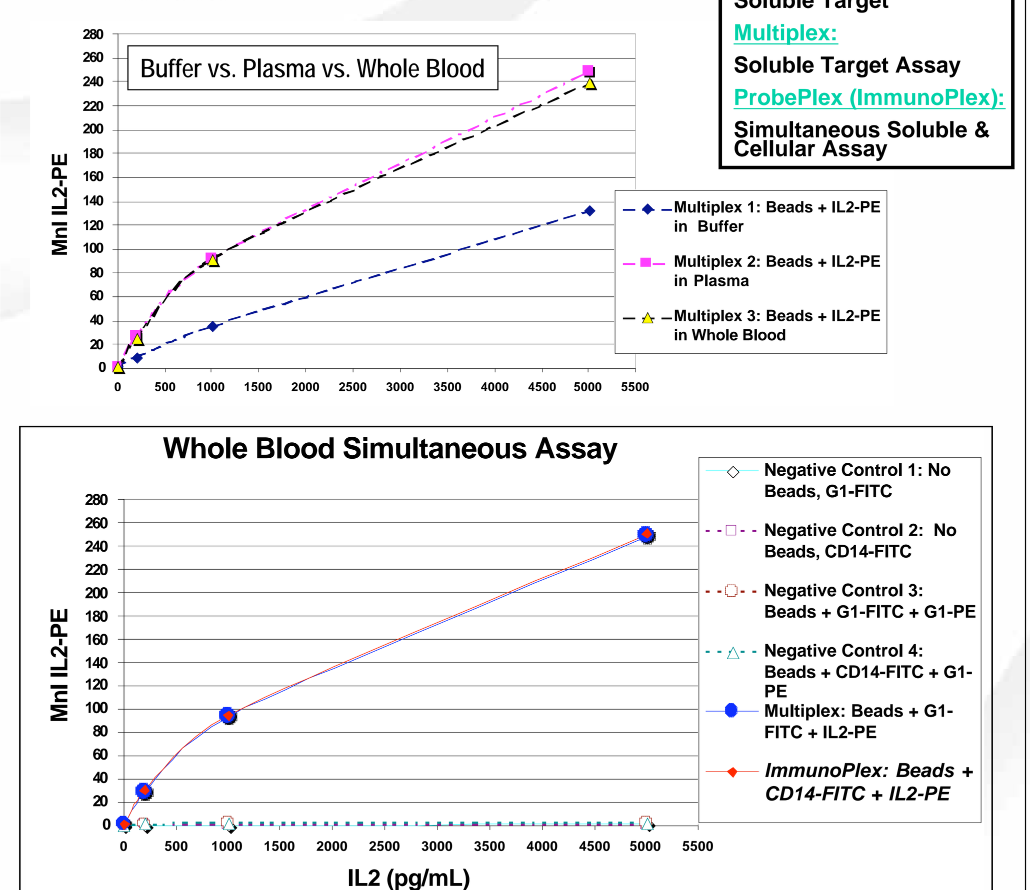
- Samples were processed with and without beads to assess any impact on mean fluorescent intensity (MFI)
- Lymphocytes, monocytes and granulocytes were examined. Only monocytes and granulocytes, considered worst case scenario due to potential phagocytosis of particles, are shown.
- As beads and detector antibody are added there are less events collected for the cellular markers but as can be seen in the histograms below there is no change in the MFI.



ProbePlex Assay

- IL2 was spiked into buffer, plasma and whole blood
- Assay was then performed with and without anti-IL2 beads, with and without soluble target antibody (IL2-PE), and with and without cellular target antibody (CD14-FITC).
- Isotypic controls were used as negative controls

G1-FITC:
Negative Control for Cellular Target
CD14-FITC:
Cellular Target
G1-PE:
Negative Control for Soluble Target
IL2-PE:
Soluble Target
Multiplex:
Soluble Target Assay
ProbePlex (ImmunoPlex):
Simultaneous Soluble & Cellular Assay



Conclusions/Discussion

- There was no difference in values obtained in plasma compared to whole blood confirming that phagocytosis of beads was inhibited.
- As expected, a distinctly different regression equation was seen when the sample media was buffer as compared to plasma or whole blood that is indicative of 'matrix' effects.
- The addition of beads and soluble target antibody did not adversely impact cellular scatter patterns or antigenic expression.
- ProbePlex allows for a more complete picture of a patient's medical status with regard to both cellular and soluble mediators, activators or inhibitors. It opens the door to a more comprehensive **'snapshot'** of patient status or drug effects.
- There would be expected benefits in throughput, decreased blood requirements, accuracy of clinical monitoring, and decreased overall cost to the patient.