BD™ CBA on the BD Accuri™ C6: Bringing Multiplexed Cytokine Detection to the Benchtop

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Cellular Communication

Cytokines and Growth Factors
Techniques for Measurement of Cytokines

Soluble Proteins
- ELISA
- ELISPOT
- BD CBA

Intracellular Proteins
- Flow Cytometry
- Western Blot
- Immunohistochemistry
BD Cytometric Bead Array (CBA) Assay Overview

- Analytes are bound by specific capture antibodies conjugated to beads with distinct fluorescent properties.
- The reporter in the assay is a PE-labeled detection antibody.
- Analyte concentrations are estimated by comparison with a standard curve in FCAP Array™ software.
Bead-Based Immunoassay Overview

• Advantages
  – Analyze multiple cytokines simultaneously (≤30)
  – Reduced sample volume requirements
  – Reduced hands-on time with parallel analysis of samples
  – Wide dynamic range (fluorescence)
  – Requires fewer sample dilutions
  – High statistical relevance
    • 300 beads measured per cytokine → equivalent of 300 ELISA wells

• BD CBA is like doing multiple ELISAs at the same time by flow cytometry
The BD CBA Workflow

- **Stain**
  - BD CBA Kits
  - BD CBA Flex Sets

- **Acquire**
  - Flow cytometers:
    - BD Accuri™ C6
    - BD FACSVerse™
    - BD LSRFortessa™
    - BD™ LSR II
    - BD FACSCanto™ II
    - BD FACSArray™ III
    - BD FACSAria™
    - BD FACSCalibur™

- **Analyze**
  - FCAP Array v3.0.1 software (Microsoft® Windows)
Experimental samples
PBMCs were cultured for several days with plate-bound anti-CD3, soluble anti-CD28, IL-2, and IL-4. Cells were stimulated with PMA and ionomycin for several hours prior to collecting culture supernatants.

Staining
BD CBA Human Th1/Th2/Th17 Cytokine Kit
- IL-2
- IL-4
- IL-6
- IL-10
- TNF
- IFN-γ
- IL-17A

Acquisition
BD Accuri C6 standard configuration

Analysis
FCAP Array software v3.0.1 to measure cytokine concentrations
The BD CBA Kit Workflow

- Stain
- Acquire
- Analyze

BD CBA Kits
BD CBA Kits

- Preconfigured panels of 3 to 7 analytes
  - Cytokines
  - Inflammatory cytokines
  - Chemokines
  - Anaphylatoxins
  - Ig isotyping (mouse)
- 2-color assay
  - Red dye in beads
  - PE reporter

- Contents
  - Diluents and wash buffers
  - Setup beads
  - 3–7 vials of capture beads
  - 2 vials of standards
  - 1 bottle of detection reagent
BD CBA Kits

- Reconstitute lyophylized standards in assay diluent
- Prepare 10 serial dilutions of standards (typically 20–5,000 pg/mL)
- Mix capture beads in a single tube
- Dilute samples if necessary with assay diluent
- Combine capture beads, test sample (or standard), and detection reagent for each sample
- Incubate for 3 hours
- Wash 1X with wash buffer

<table>
<thead>
<tr>
<th>Concentration (pg/mL)</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
</tr>
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<td>3</td>
<td>40</td>
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<tr>
<td>4</td>
<td>80</td>
</tr>
<tr>
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<td>156</td>
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<td>6</td>
<td>312.5</td>
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<td>7</td>
<td>625</td>
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<tr>
<td>8</td>
<td>1250</td>
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<td>9</td>
<td>2500</td>
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<td>5000</td>
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<tr>
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<td>Sample A SUP</td>
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<td>Sample A SUP</td>
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<td>13</td>
<td>Sample A SUP</td>
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<tr>
<td>14</td>
<td>Sample B SUP</td>
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<tr>
<td>15</td>
<td>Sample B SUP</td>
</tr>
<tr>
<td>16</td>
<td>Sample B SUP</td>
</tr>
</tbody>
</table>
The BD CBA Kit Workflow

- **Stain**: BD CBA Kits
- **Acquire**: BD Accuri C6
- **Analyze**
The BD Accuri C6 Flow Cytometer System

- An affordable, full-featured, easy-to-use flow cytometer
- Equipped with two lasers and six detectors
BD Accuri CBA Kit Template

Select plot type to make a new plot.
Human Th1/Th2/Th17 Cytokine Kit

Acquire

BD
The BD CBA Kit Workflow

Stain
BD CBA Kits

Acquire
BD Accuri C6

Analyze
FCAP Array v3.0.1 (Microsoft® Windows)
FCAP Array Analysis Software

- Version 3.0.1 compatible with BD Accuri FCS files
- Compatible with FCS 2.0 or 3.0 files from any BD flow cytometer
- Results in graphical and tabular format
- Ability to save plex templates for routine panels
- Automatic and manual gating options
Design View

Plate layout:
Standards and Test Samples identified, files are assigned (floppy disc icon)
**Beads and Model**

**Analyze**

Bead selection:
Drag beads from right pane into the left pane

Bead Library:
BD provides XML file on website that includes groupings (eg, Human Soluble Protein or Th1/2/17 Kit)
Instrument Settings: Cytokine Kit

Identification of clustering parameters

Assign beads to clusters
Standards and QC

Standard dilution calculator and units

Assignment of concentration levels to Standards
Standard Curves
Results per Analyte

Bead 2 - Human IFN-γ - Final CC
Results per Sample
The BD CBA Flex Set Workflow

Stain → Acquire → Analyze

BD CBA Flex Sets
CBA Flex Set Workflow

- **Experimental samples**
  PBMCs were cultured for several days with plate-bound anti-CD3, soluble anti-CD28, IL-2, and IL-4. Cells were stimulated with PMA and ionomycin for several hours prior to harvesting. Alternatively, PBMCs were stimulated for several hours with IFN-γ. LPS was added to the culture overnight.

- **Staining**
  Master Buffer Kit = all buffers needed for assay
  Flex Set = capture beads, detection reagent, standard (2 curves)
  - IL-1β
  - IL-4
  - IL-6
  - IL-12p70
  - IFN-γ
  - IL-2
  - IL-5
  - IL-10
  - IL-17A
  - TNF

- **Acquisition**
  BD Accuri C6
  Selectable Lasers: 2 Blue, 2 Red option with 780 BP in FL3

- **Analysis**
  FCAP Array software to measure cytokine concentrations
BD CBA Flex Sets

• Build your own multiplex
  – Human soluble protein
  – Mouse or rat soluble protein
  – Enhanced sensitivity
    • (<1.0 pg/mL)
  – Cell signaling
  – Human Ig

• 3-color assay
  – Two red dyes in beads
  – PE reporter

• Master Buffer Kit
  – Includes buffers and setup reagents

• Flex Set
  – Capture beads
  – Detector reagents
  – Standard (x2)
BD CBA Flex Sets

- Combine flex set standards
- Combine flex set beads
- Combine flex set detection reagent
- Perform assay

<table>
<thead>
<tr>
<th>Tube</th>
<th>Concentration (pg/mL)</th>
<th>Dilution</th>
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</thead>
<tbody>
<tr>
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<td>N/A</td>
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<td>3</td>
<td>20</td>
<td>1:128</td>
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<td>4</td>
<td>40</td>
<td>1:64</td>
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<tr>
<td>5</td>
<td>80</td>
<td>1:32</td>
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<tr>
<td>6</td>
<td>156</td>
<td>1:16</td>
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<tr>
<td>7</td>
<td>312.5</td>
<td>1:8</td>
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<tr>
<td>8</td>
<td>625</td>
<td>1:4</td>
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<td>9</td>
<td>1250</td>
<td>1:2</td>
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<tr>
<td>10</td>
<td>2500</td>
<td>neat</td>
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<tr>
<td>11</td>
<td>test treatment A</td>
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<td>12</td>
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<td>13</td>
<td>test treatment B</td>
<td>1:10</td>
</tr>
<tr>
<td>14</td>
<td>test treatment B</td>
<td>neat</td>
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</tbody>
</table>
The BD CBA Flex Set Workflow

Stain  Acquire  Analyze

BD CBA Flex Sets  BD Accuri C6
Optical Configuration

User changeable optical filters
- 510/15
- 540/20
- 565/20
- 610/20
- 780/60

Selectable lasers
- 3 blue 1 red
- 2 blue 2 red
- 4 blue
Selectable Lasers

User changeable optical filters
- 510/15
- 540/20
- 565/20
- 610/20
- **780/60**

Selectable lasers
- 3 blue 1 red
- **2 blue 2 red**
- 4 blue

C6 is connected and ready.
BD Accuri CBA Flex Set Template
Set Color Compensation
The BD CBA Flex Set Workflow

- **Stain**: BD CBA Flex Sets
- **Acquire**: BD Accuri C6
- **Analyze**: FCAP Array v3.0.1 (Microsoft® Windows)
Instrument Settings: 10-plex Flex Set

Identification of clustering parameters

Assign beads to clusters
Results per Analyte

<table>
<thead>
<tr>
<th>Analyte Name</th>
<th>Sample Name</th>
<th>Position</th>
<th>Clustering</th>
<th>Results File</th>
<th>Event #</th>
<th>MFI</th>
<th>SD</th>
<th>CV</th>
<th>Nominal CC</th>
<th>Fitted CC</th>
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<tbody>
<tr>
<td>C4 - Human TNP</td>
<td>Std01</td>
<td>1 - A1</td>
<td>Manual</td>
<td>A01.fcs</td>
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<td>4,916.02</td>
<td>66,25</td>
<td>14.09%</td>
<td>20.00 pg/mL</td>
<td>15.71 pg/mL</td>
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<tr>
<td>A7 - Human IL-6</td>
<td>Std02</td>
<td>1 - A2</td>
<td>Manual</td>
<td>A02.fcs</td>
<td>192</td>
<td>4,207.00</td>
<td>45.29</td>
<td>15.07%</td>
<td>20.00 pg/mL</td>
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<td>A6 - Human IL-7</td>
<td>Std03</td>
<td>1 - A3</td>
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<td>A03.fcs</td>
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<td>A8 - Human IL-8</td>
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<td>1 - A4</td>
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<td>15.10%</td>
<td>80.00 pg/mL</td>
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<td>B9 - Human IL-11</td>
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<td>15.33%</td>
<td>625.00 pg/mL</td>
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<td>E5 - Human IL-18</td>
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<td>Manual</td>
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<td>5,000.00 pg/mL</td>
<td>50.00 pg/mL</td>
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<td>N/A</td>
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<td>C01.fcs</td>
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<td>15.37%</td>
<td>282.28 pg/mL</td>
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<tr>
<td>Test004</td>
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<td>Manual</td>
<td>C02.fcs</td>
<td>183</td>
<td>5,967.00</td>
<td>1,185.32</td>
<td>17.21%</td>
<td>130.63 pg/mL</td>
<td>13.00 pg/mL</td>
<td></td>
</tr>
</tbody>
</table>

**A4 - Human IL-2 - Final CC**

- Standard
- Test
- Control

**Parameter X: FL4-A**

**Parameter Y: FL3-A**

BD logo
Comparison of Cytokine Concentrations

![Cytokine Concentration Chart]

- **IL-1β**
- **IL-2**
- **IL-4**
- **IL-5**
- **IL-6**
- **IL-10**
- **IL-12p70**
- **IL-17**
- **IFN gamma**
- **TNF**

Legend:
- **PMA + Ionomycin**
- **LPS + IFN gamma**

Concentration (pg/mL)
Summary

BD offers a complete solution for soluble analyte measurement with BD CBA reagents, the BD Accuri C6 flow cytometer, and FCAP Array software.

Stain

• BD CBA assays provide a powerful technique for measuring soluble analytes such as cytokines.
• The BD product line includes pre-configured CBA kits, as well as configurable Flex Sets.

Acquire

• The BD Accuri C6 flow cytometer is an affordable, easy-to-use system for data acquisition.
• BD provides software templates that minimize instrument setup.

Analyze

• FCAP Array software provides simplified data analysis.
• FCAP Array v3.0.1 is compatible with BD Accuri FCS files.
Thank You!

- Trent Colville
- Stacey Roys
- Jacob Rabenstein