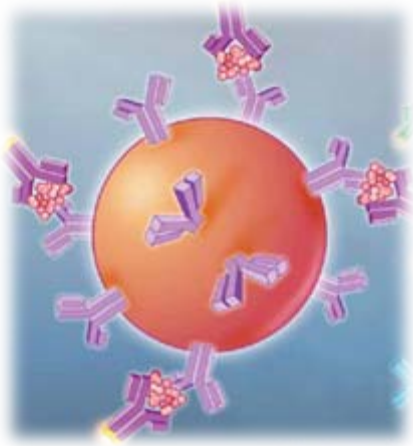
BD™ CBA on the BD Accuri™ C6:

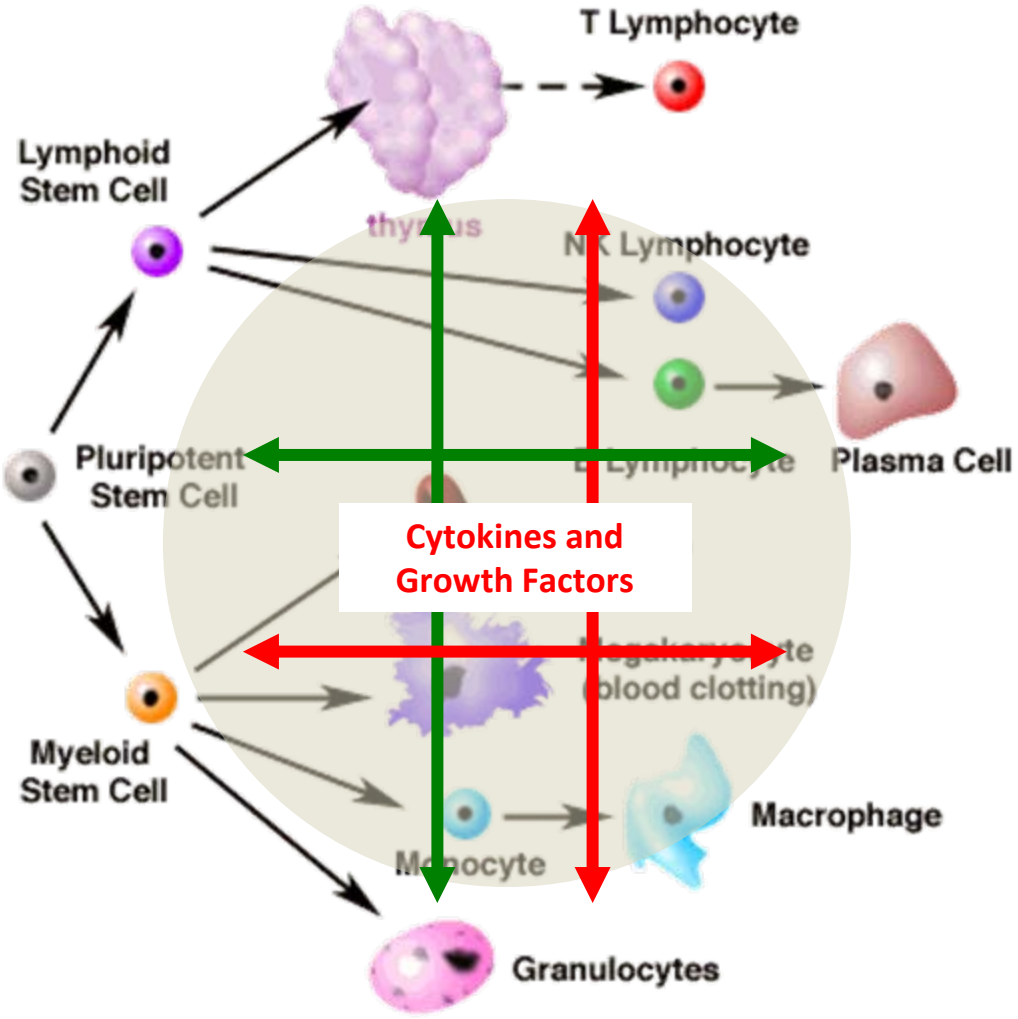
Bringing Multiplexed Cytokine Detection to the Benchtop



Maria Dinkelmann, PhD
Senior Marketing Applications Specialist
BD Biosciences, Ann Arbor, MI

BD

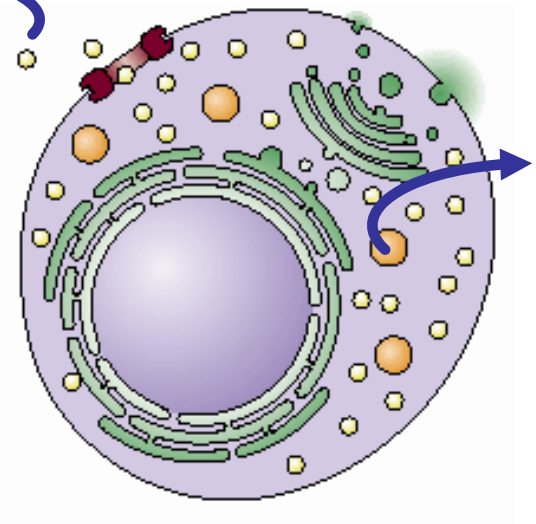
Cellular Communication



Techniques for Measurement of Cytokines

Soluble Proteins

- ELISA
- ELISPOT
- BD CBA



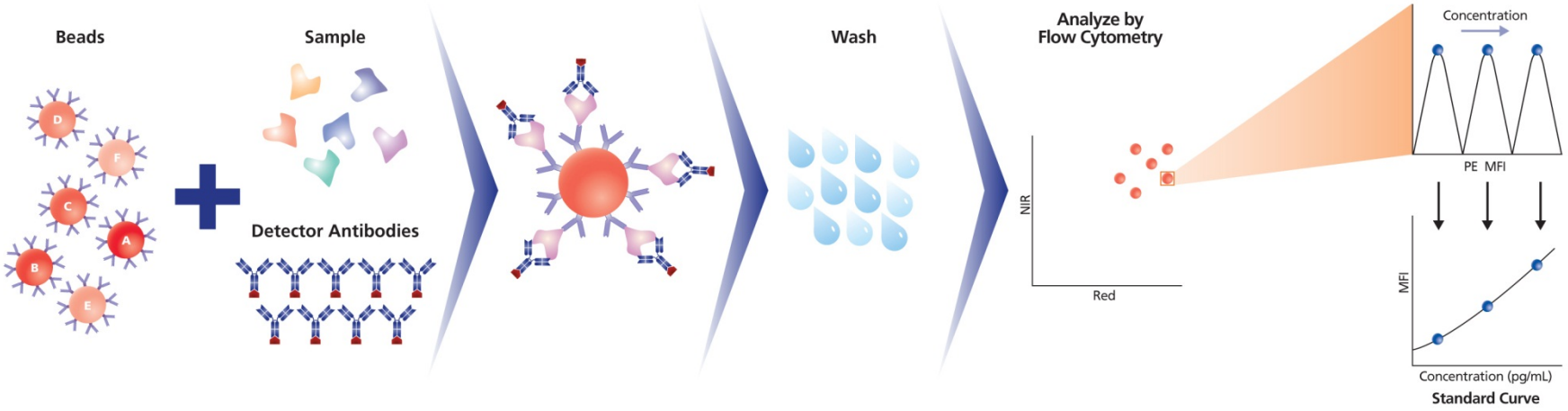
Intracellular Proteins

- Flow Cytometry
- Western Blot
- Immunohistochemistry

BD

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**

BD

The BD CBA Workflow

BD CBA Kits
BD CBA Flex Sets

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

FCAP Array v3.0.1 software
(Microsoft® Windows)

BD

CBA Kit 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 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

BD

The BD CBA Kit Workflow

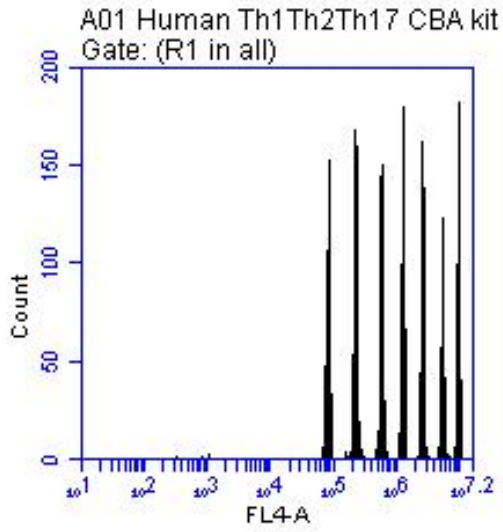
BD CBA Kits

BD CBA Kits

Stain

- 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

BD CBA Kits

Stain

- Reconstitute lyophilyzed 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

	Concentration (pg/mL)	Dilution
1	0	N/A
2	20	1:256
3	40	1:128
4	80	1:64
5	156	1:32
6	312.5	1:16
7	625	1:8
8	1250	1:4
9	2500	1:2
10	5000	neat
11	Sample A SUP	1:100
12	Sample A SUP	1:10
13	Sample A SUP	neat
14	Sample B SUP	1:100
15	Sample B SUP	1:10
16	Sample B SUP	neat

BD

The BD CBA Kit Workflow

BD CBA Kits

BD Accuri C6

The BD Accuri C6 Flow Cytometer System

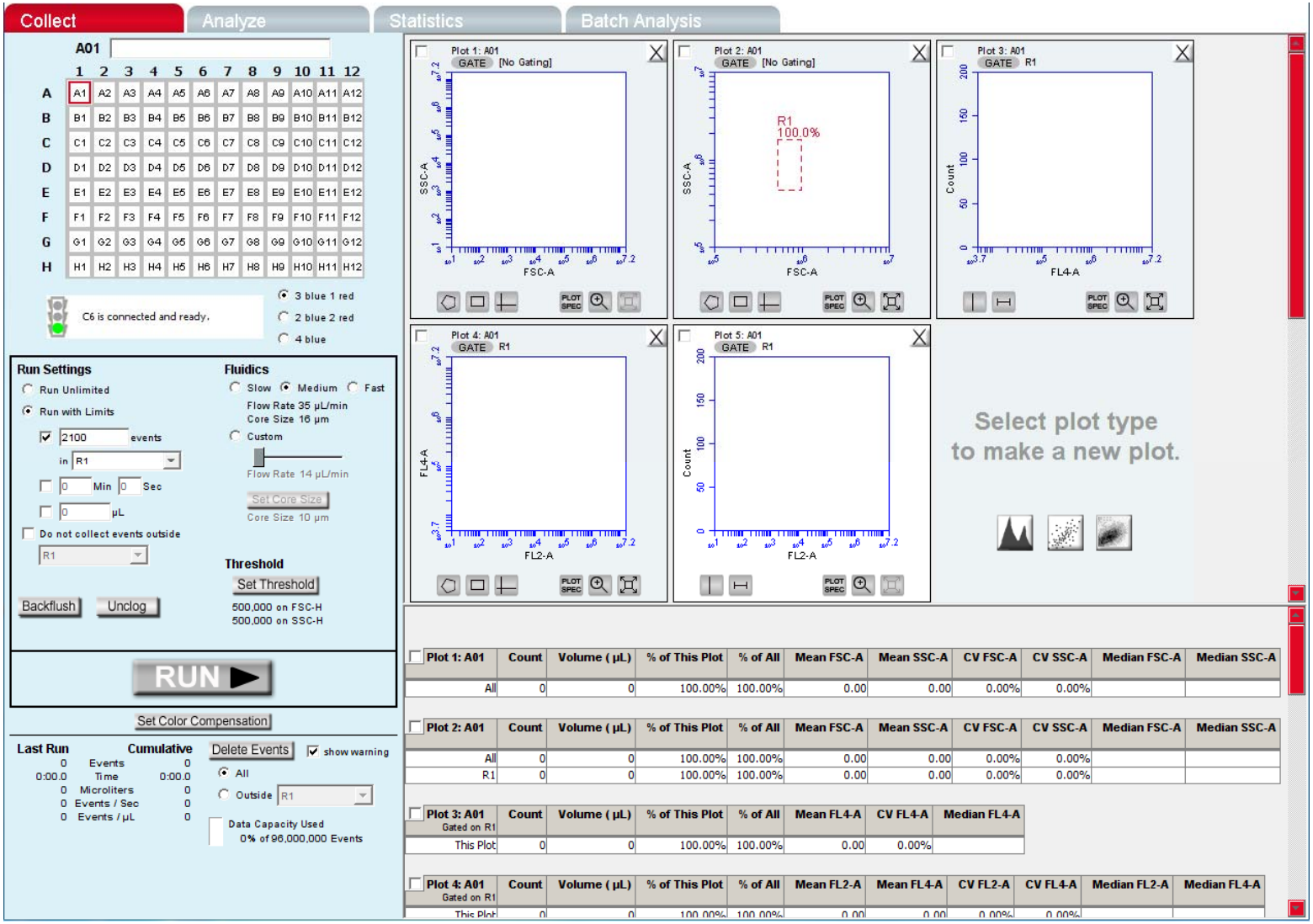
Acquire

- An affordable, full-featured, easy-to-use flow cytometer
- Equipped with two lasers and six detectors

BD

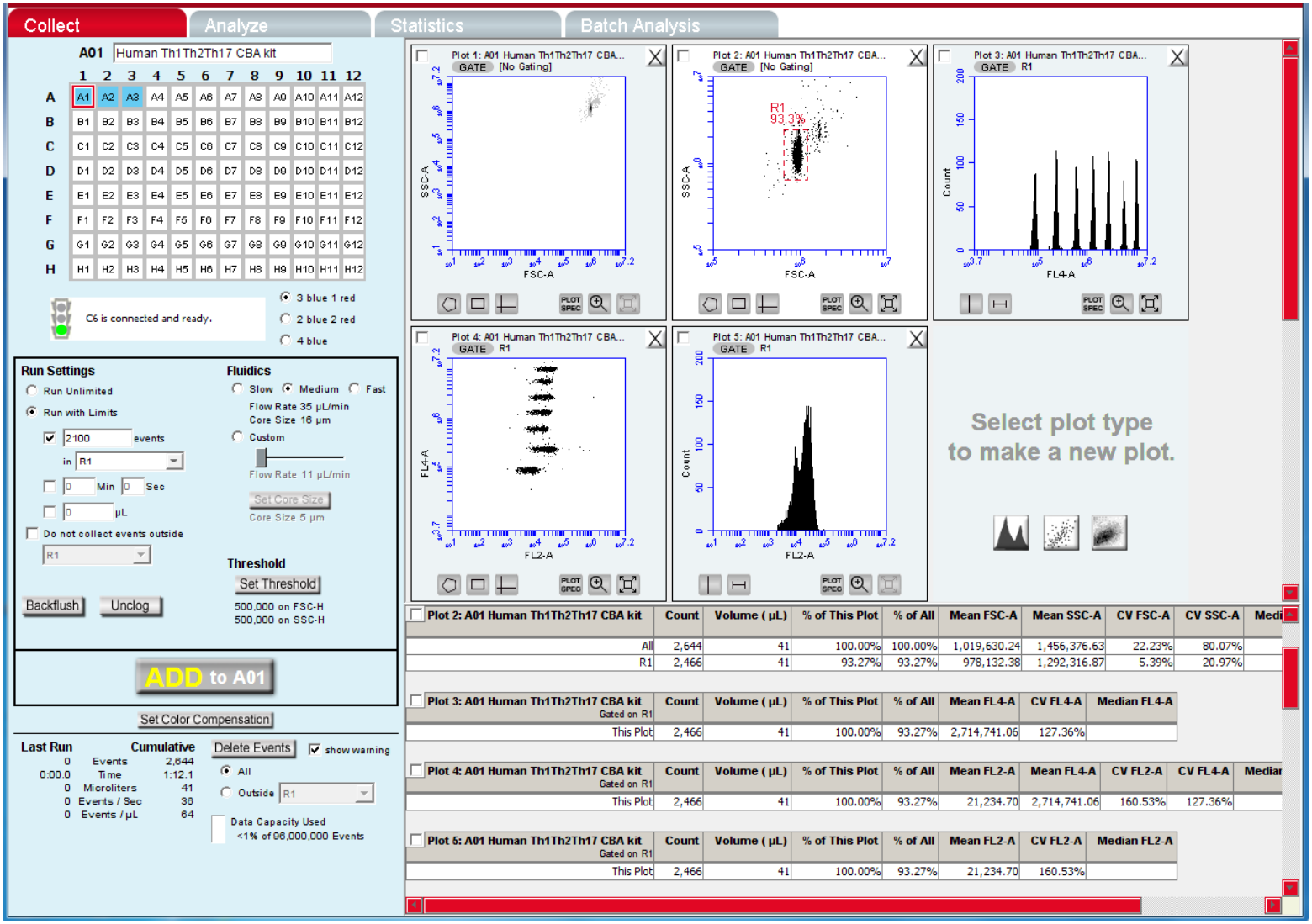
BD Accuri CBA Kit Template

Acquire



Human Th1/Th2/Th17 Cytokine Kit

Acquire



BD

The BD CBA Kit Workflow

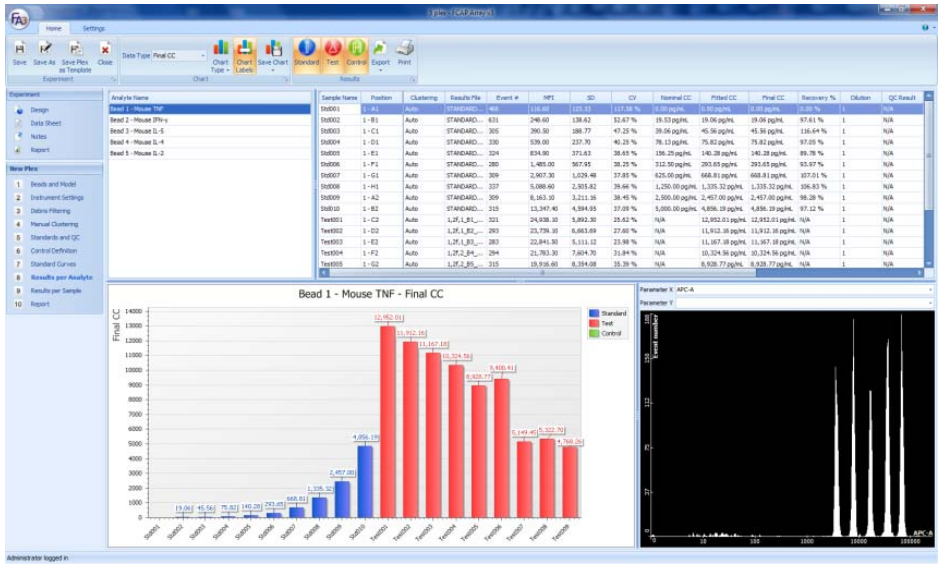
BD CBA Kits

BD Accuri C6

FCAP Array v3.0.1 (Microsoft® Windows)

FCAP Array Analysis Software

Analyze

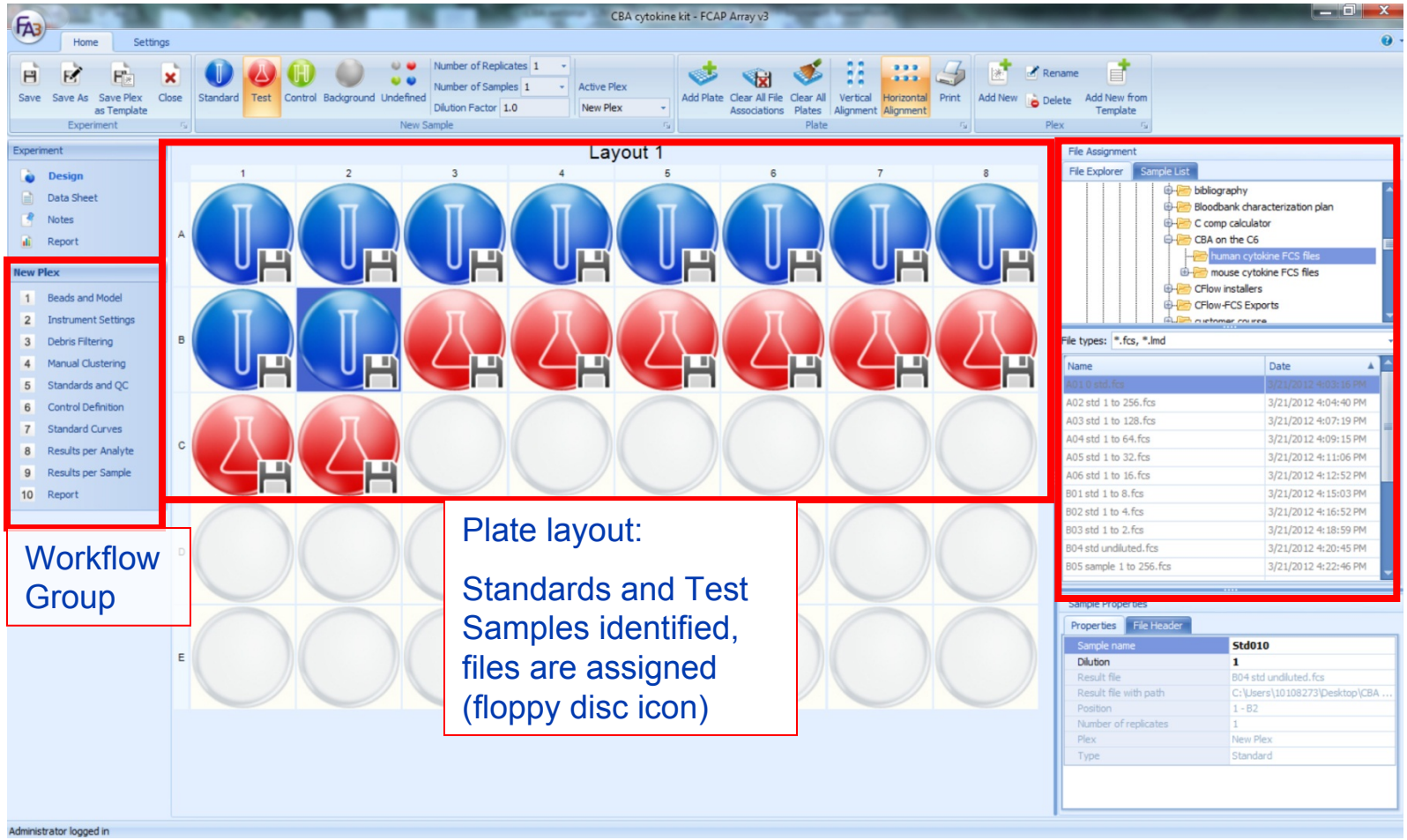


- Version 3.0.1 compatible with BD Accuri FCS files
- Compatible with FCS 2.0 or 3.0 files from any BD flow cytometer
- Results in graphical and tabular format
- Ability to save plex templates for routine panels
- Automatic and manual gating options

BD

Design View

Analyze



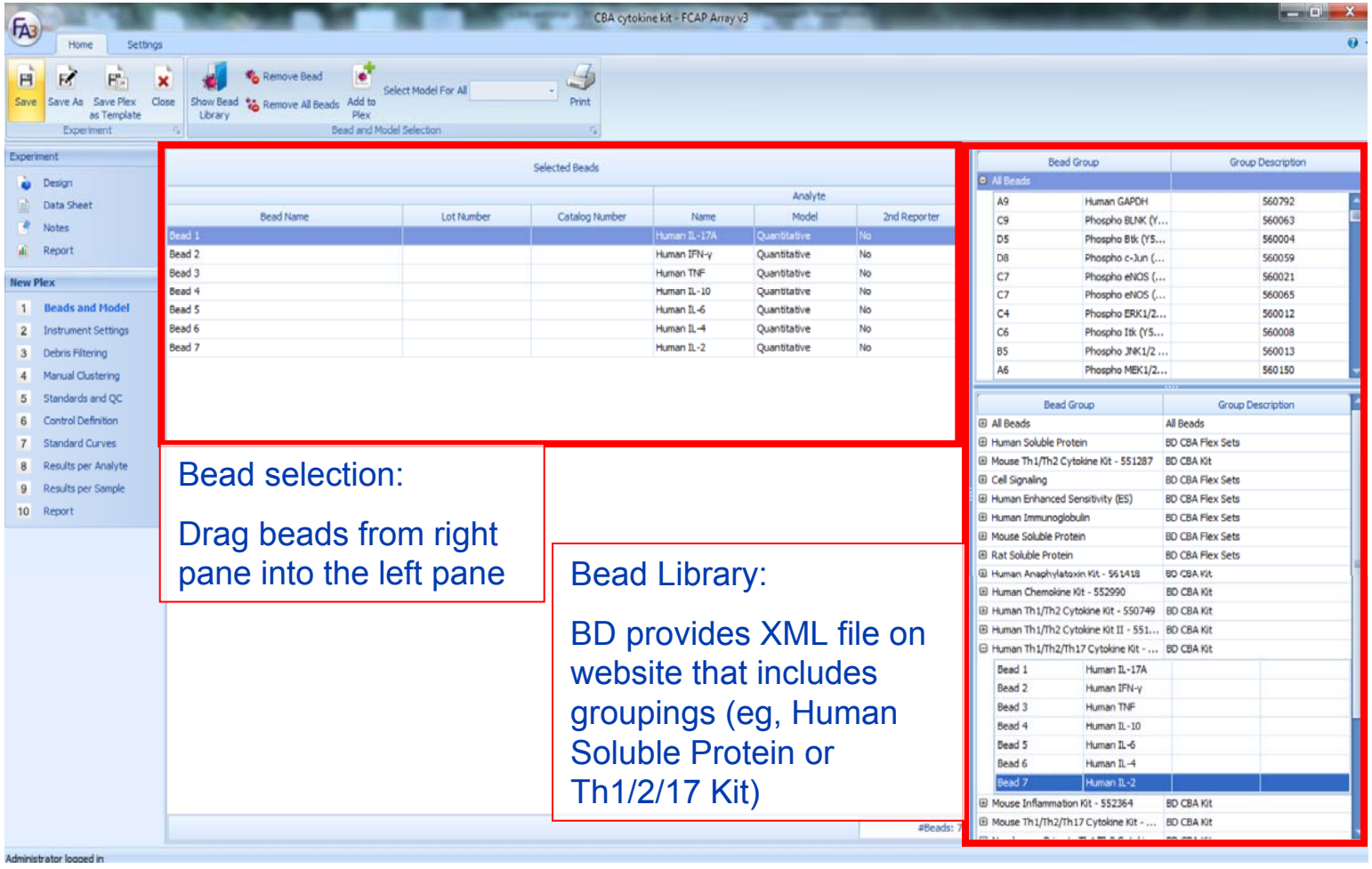
Workflow Group

Plate layout:

Standards and Test Samples identified, files are assigned (floppy disc icon)

Beads and Model

Analyze



Bead selection:

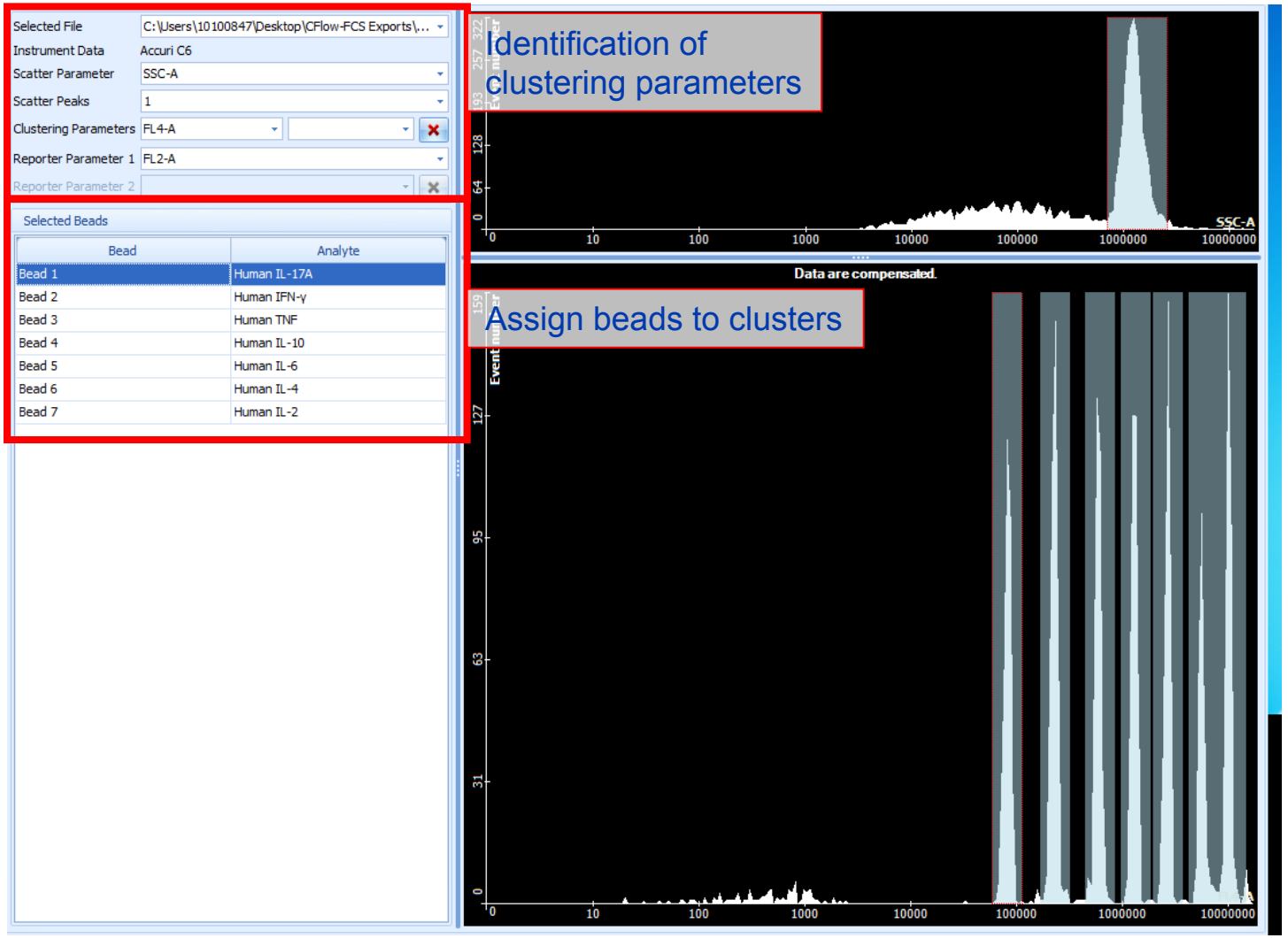
Drag beads from right pane into the left pane

Bead Library:

BD provides XML file on website that includes groupings (eg, Human Soluble Protein or Th1/2/17 Kit)

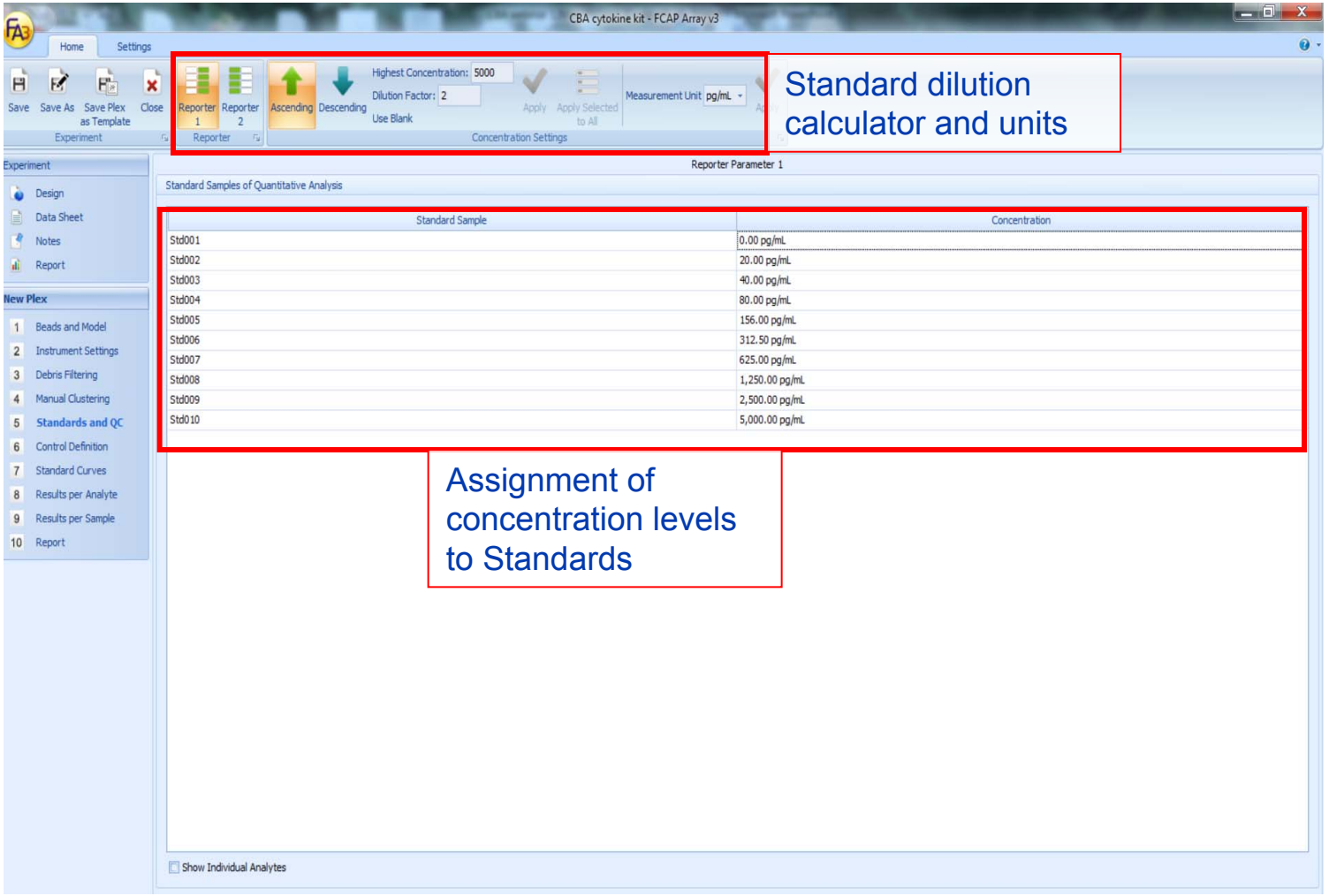
Instrument Settings: Cytokine Kit

Analyze



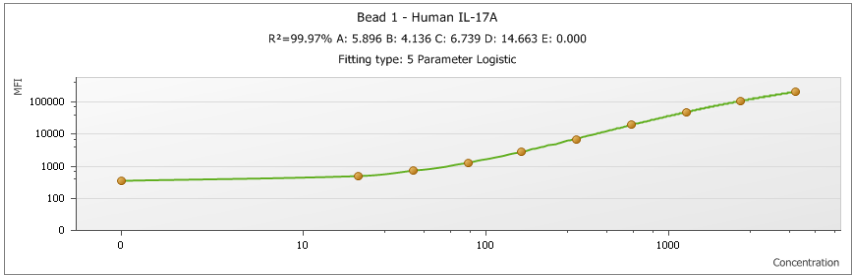
Standards and QC

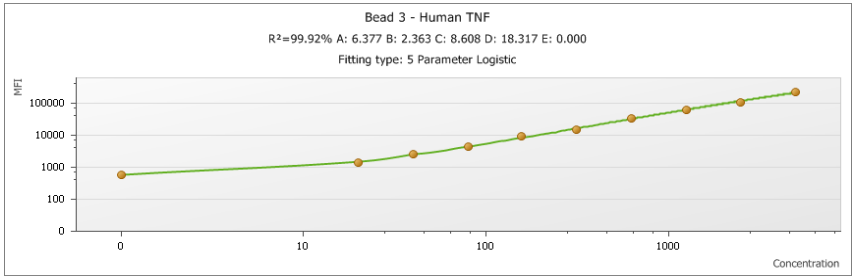
Analyze

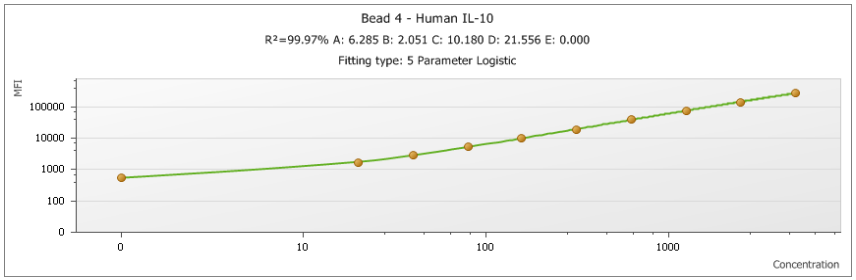


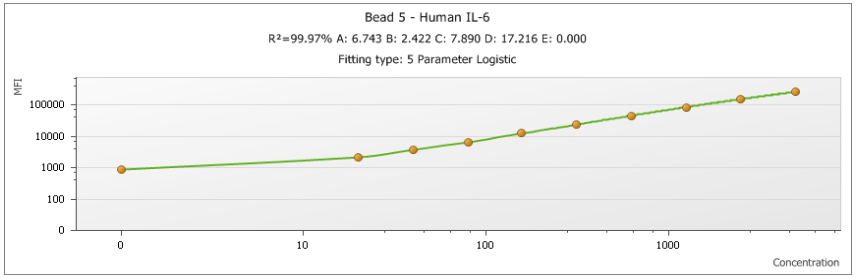
Standard Curves

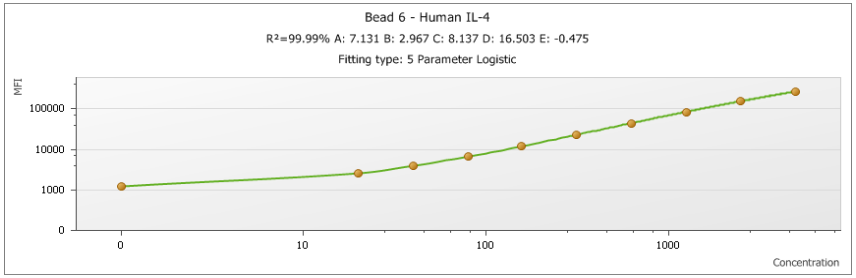
Analyze

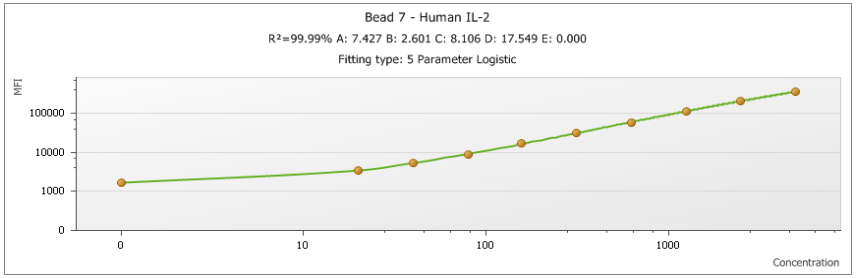












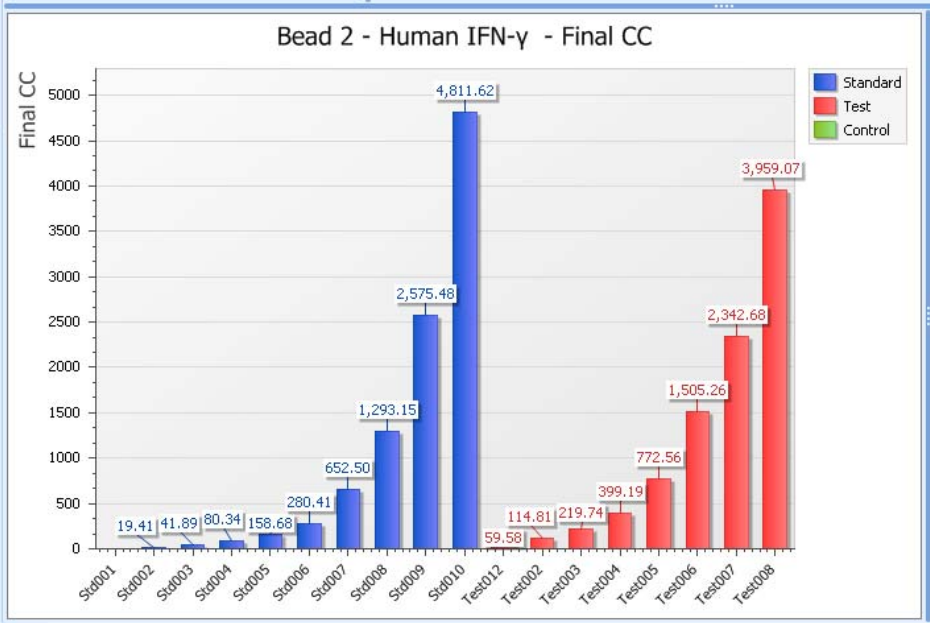
BD

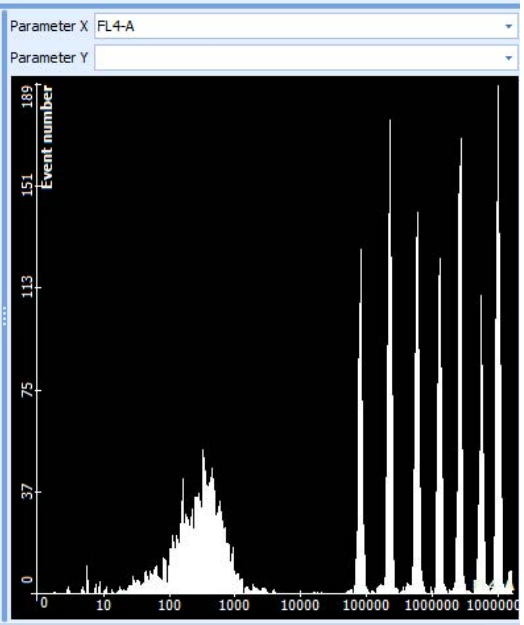
Results per Analyte

Analyze

Analyte Name
Bead 1 - Human IL-17A
Bead 2 - Human IFN-γ
Bead 3 - Human TNF
Bead 4 - Human IL-10
Bead 5 - Human IL-6
Bead 6 - Human IL-4
Bead 7 - Human IL-2

Sample Name	Position	Clustering	Results File	Event #	MFI	SD	CV	Nominal CC	Fitted CC
Std001	1 - A1	Auto	A01 0 std.fcs	443	370.00	313.57	69.01 %	0.00 pg/mL	0.00 pg/mL
Std002	1 - A2	Auto	A02 std 1 t...	300	1,976.00	1,226.48	53.31 %	20.00 pg/mL	19.41 pg/mL
Std003	1 - A3	Auto	A03 std 1 t...	385	3,917.00	1,580.08	35.71 %	40.00 pg/mL	41.89 pg/mL
Std004	1 - A4	Auto	A04 std 1 t...	453	7,328.00	3,111.61	36.87 %	80.00 pg/mL	80.34 pg/mL
Std005	1 - A5	Auto	A05 std 1 t...	439	14,438.00	6,782.90	46.41 %	156.00 pg/mL	158.68 pg/mL
Std006	1 - A6	Auto	A06 std 1 t...	413	25,600.00	14,198.49	48.04 %	312.50 pg/mL	280.41 pg/mL
Std007	1 - A7	Auto	B01 std 1 t...	420	59,298.00	24,588.92	40.52 %	625.00 pg/mL	652.50 pg/mL
Std008	1 - A8	Auto	B02 std 1 t...	406	114,732.00	31,456.51	31.66 %	1,250.00 pg/mL	1,293.15 pg/mL
Std009	1 - B1	Auto	B03 std 1 t...	364	217,274.00	79,093.56	37.08 %	2,500.00 pg/mL	2,575.48 pg/mL
Std010	1 - B2	Auto	B04 std und...	316	377,569.00	93,030.56	29.99 %	5,000.00 pg/mL	4,811.62 pg/mL
Test012	1 - B3	Auto	B05 sample ...	440	5,476.00	1,453.69	30.93 %	N/A	59.58 pg/mL
Test002	1 - B4	Auto	B06 sample ...	406	10,440.00	2,284.50	22.81 %	N/A	114.81 pg/mL
Test003	1 - B5	Auto	C01 sample...	329	20,034.00	4,894.80	25.07 %	N/A	219.74 pg/mL
Test004	1 - B6	Auto	C02 sample...	340	36,461.00	11,728.11	36.00 %	N/A	399.19 pg/mL



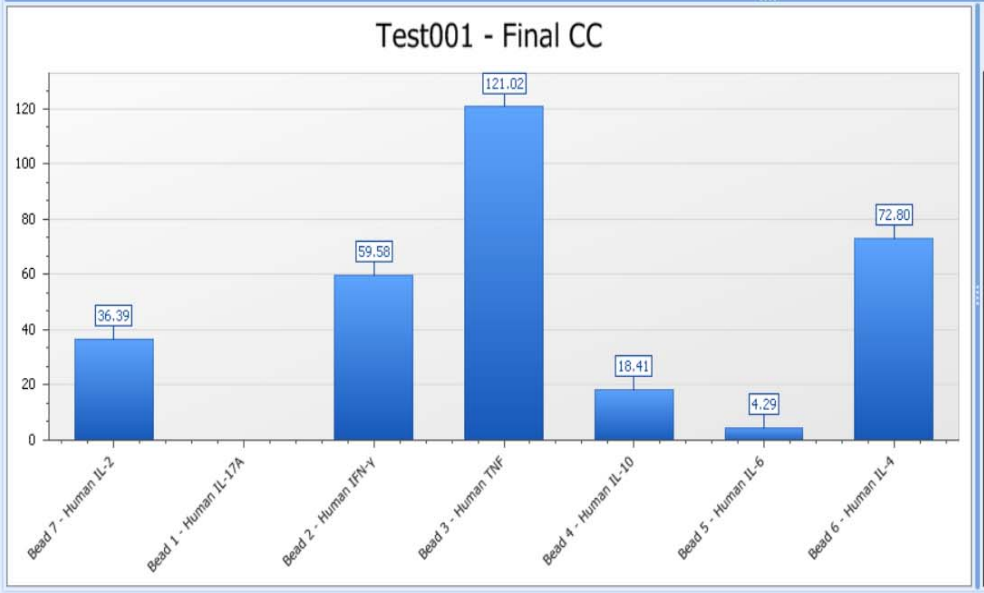


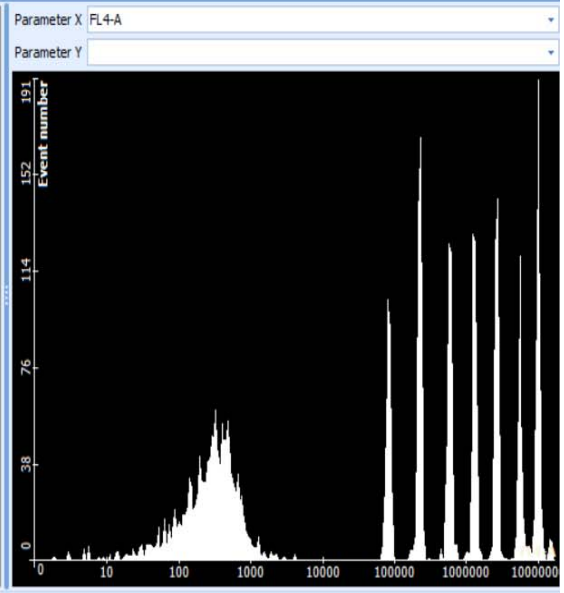
Results per Sample

Analyze

Sample Name	Clustering	Results File
Std006	Auto	A06 std 1 to 16.fcs
Std007	Auto	B01 std 1 to 8.fcs
Std008	Auto	B02 std 1 to 4.fcs
Std009	Auto	B03 std 1 to 2.fcs
Std010	Auto	B04 std undiluted...
Test001	Auto	B05 sample 1 to 2...
Test002	Auto	B06 sample 1 to 1...
Test003	Auto	C01 sample 1 to ...
Test004	Auto	C02 sample 1 to ...
Test005	Auto	C03 sample 1 to ...
Test006	Auto	C04 sample 1 to ...
Test007	Auto	C05 sample 1 to ...
Test008	Auto	C06 sample 1 to ...

Analyte Na...	Event #	MFI	SD	CV	Nominal CC	Fitted CC	Final CC	Recovery %	Dilution	QC Result	Qualitative...	Message
Bead 7 - Hu...	393	5,095.00	1,413.66	32.41 %	N/A	36.39 pg/mL	36.39 pg...	N/A	1	N/A	N/A	
Bead 1 - Hu...	307	314.00	338.77	77.38 %	N/A	0.00 pg/mL	0.00 pg/mL	N/A	1	N/A	N/A	
Bead 2 - Hu...	440	5,476.00	1,453.69	30.93 %	N/A	59.58 pg/mL	59.58 pg...	N/A	1	N/A	N/A	
Bead 3 - Hu...	339	6,506.00	2,372.16	33.05 %	N/A	121.02 pg...	121.02 p...	N/A	1	N/A	N/A	
Bead 4 - Hu...	369	1,657.00	569.32	31.90 %	N/A	18.41 pg/mL	18.41 pg...	N/A	1	N/A	N/A	
Bead 5 - Hu...	364	1,075.00	367.31	33.35 %	N/A	4.29 pg/mL	4.29 pg/mL	N/A	1	N/A	N/A	
Bead 6 - Hu...	242	6,195.00	1,905.14	30.95 %	N/A	72.80 pg/mL	72.80 pg...	N/A	1	N/A	N/A	

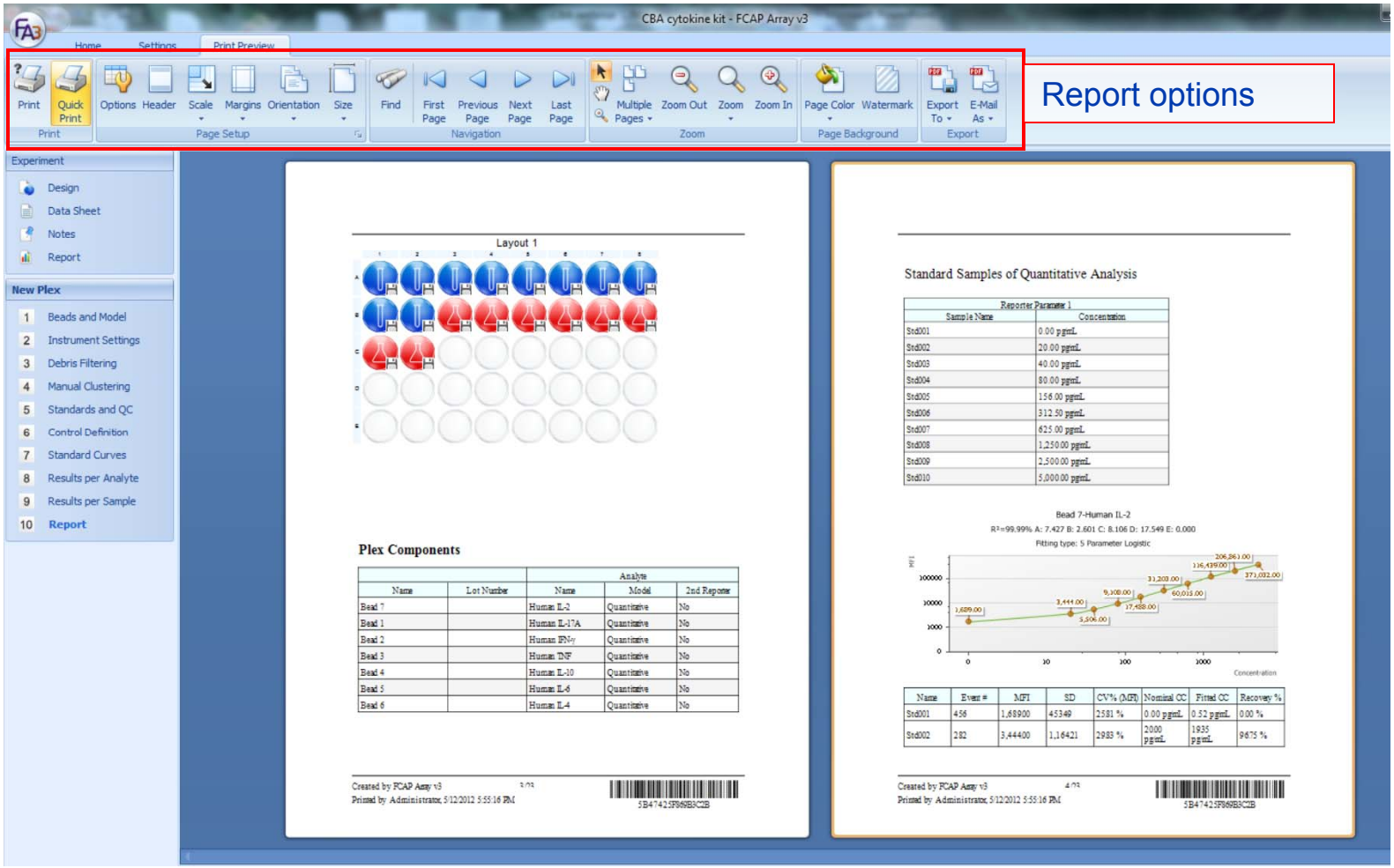




BD

Report

Analyze



The BD CBA Flex Set Workflow

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-2
  - IL-4
  - IL-5
  - IL-6
  - IL-10
  - IL-12p70
  - IL-17A
  - IFN-γ
  - TNF

- Acquisition

  BD Accuri C6

  Selectable Lasers: 2 Blue, 2 Red option with 780 BP in FL3

- Analysis

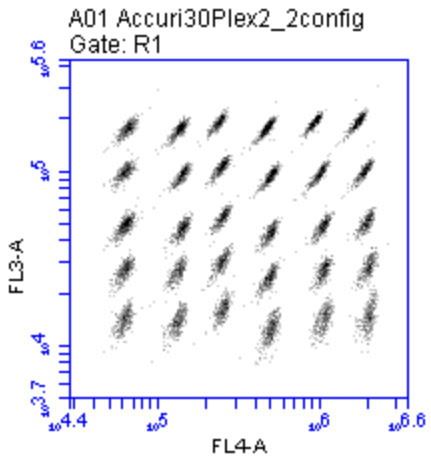
  FCAP Array software to measure cytokine concentrations

BD

BD CBA Flex Sets

Stain

- 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

BD CBA Flex Sets

Stain

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

Tube	Concentration (pg/mL)	Dilution
1	0	N/A
2	10	1:256
3	20	1:128
4	40	1:64
5	80	1:32
6	156	1:16
7	312.5	1:8
8	625	1:4
9	1250	1:2
10	2500	neat
11	test treatment A	1:10
12	test treatment A	neat
13	test treatment B	1:10
14	test treatment B	neat

BD

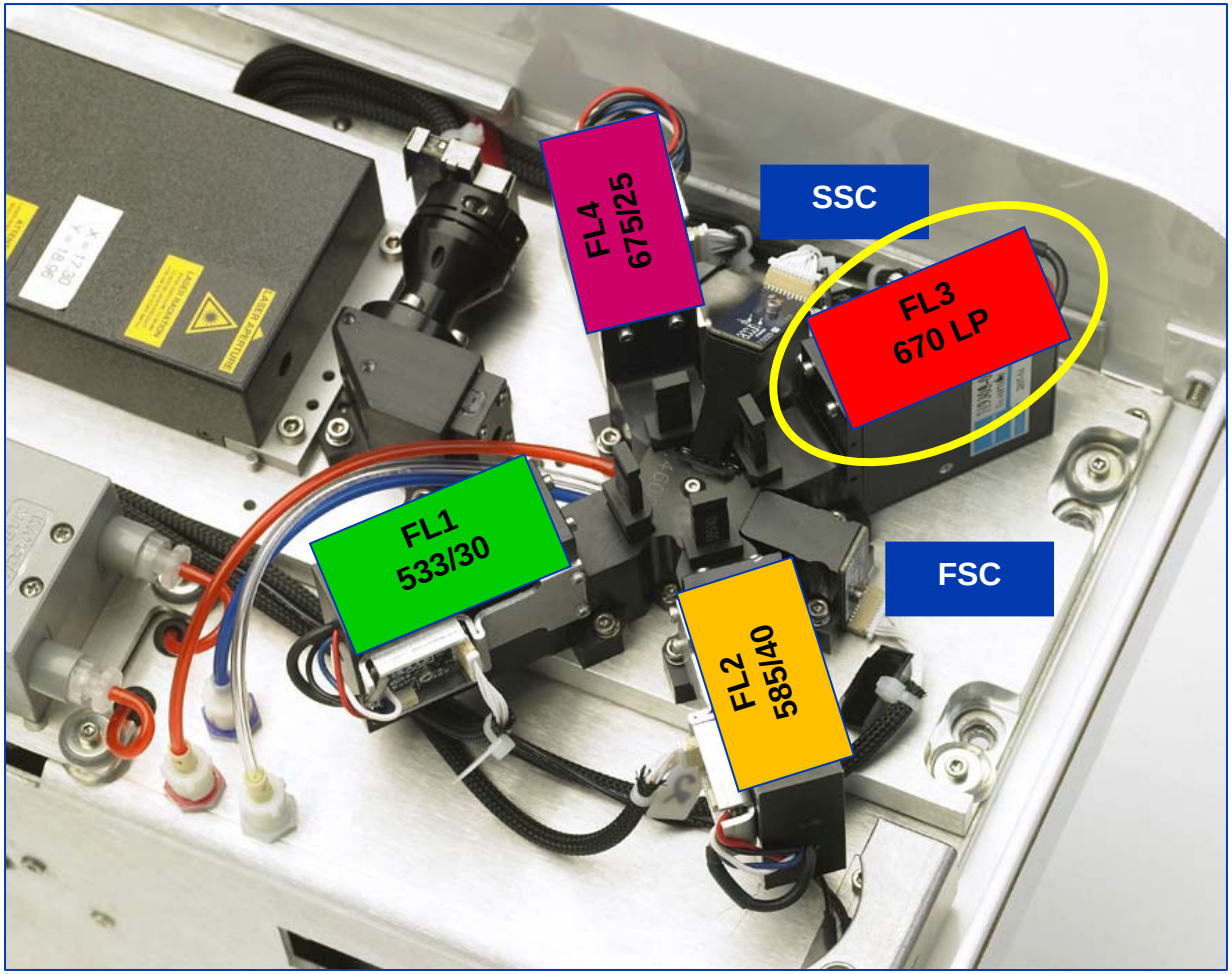
The BD CBA Flex Set Workflow

BD CBA Flex Sets

BD Accuri C6

Optical Configuration

Acquire



User changeable optical filters

510/15
540/20
565/20
610/20
780/60

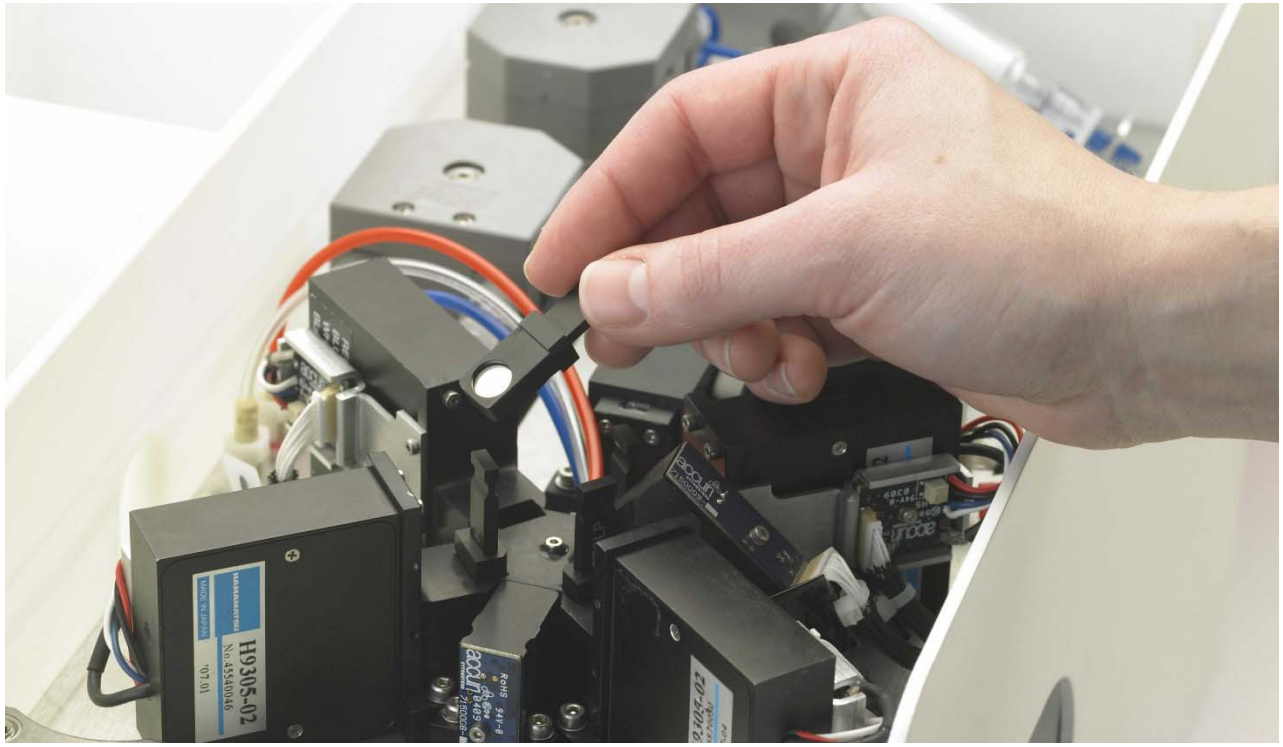
Selectable lasers

3 blue 1 red
2 blue 2 red
4 blue

BD

Selectable Lasers

Acquire



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

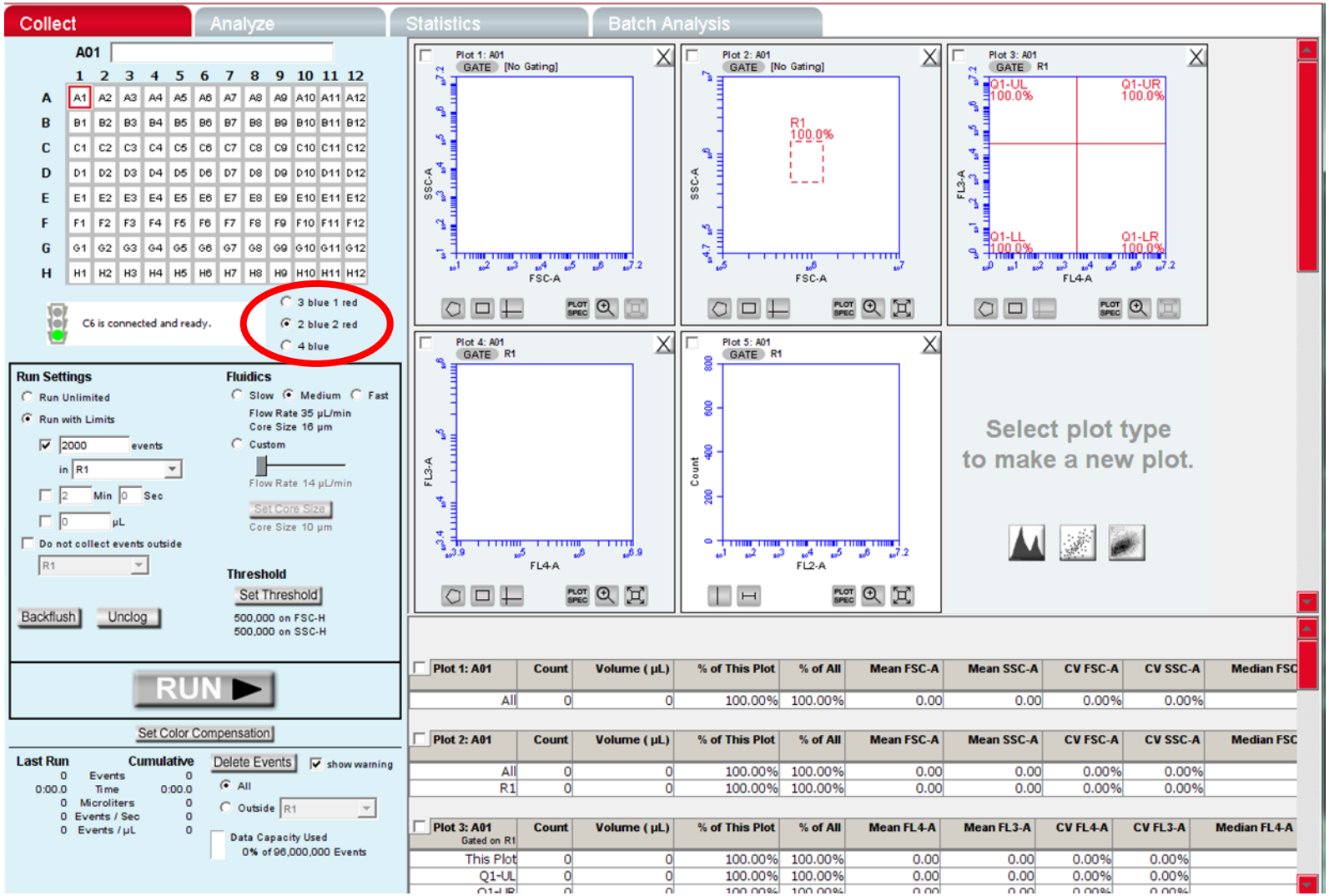
C6 is connected and ready.

- ○ 3 blue 1 red
- ◉ 2 blue 2 red
- ○ 4 blue

BD

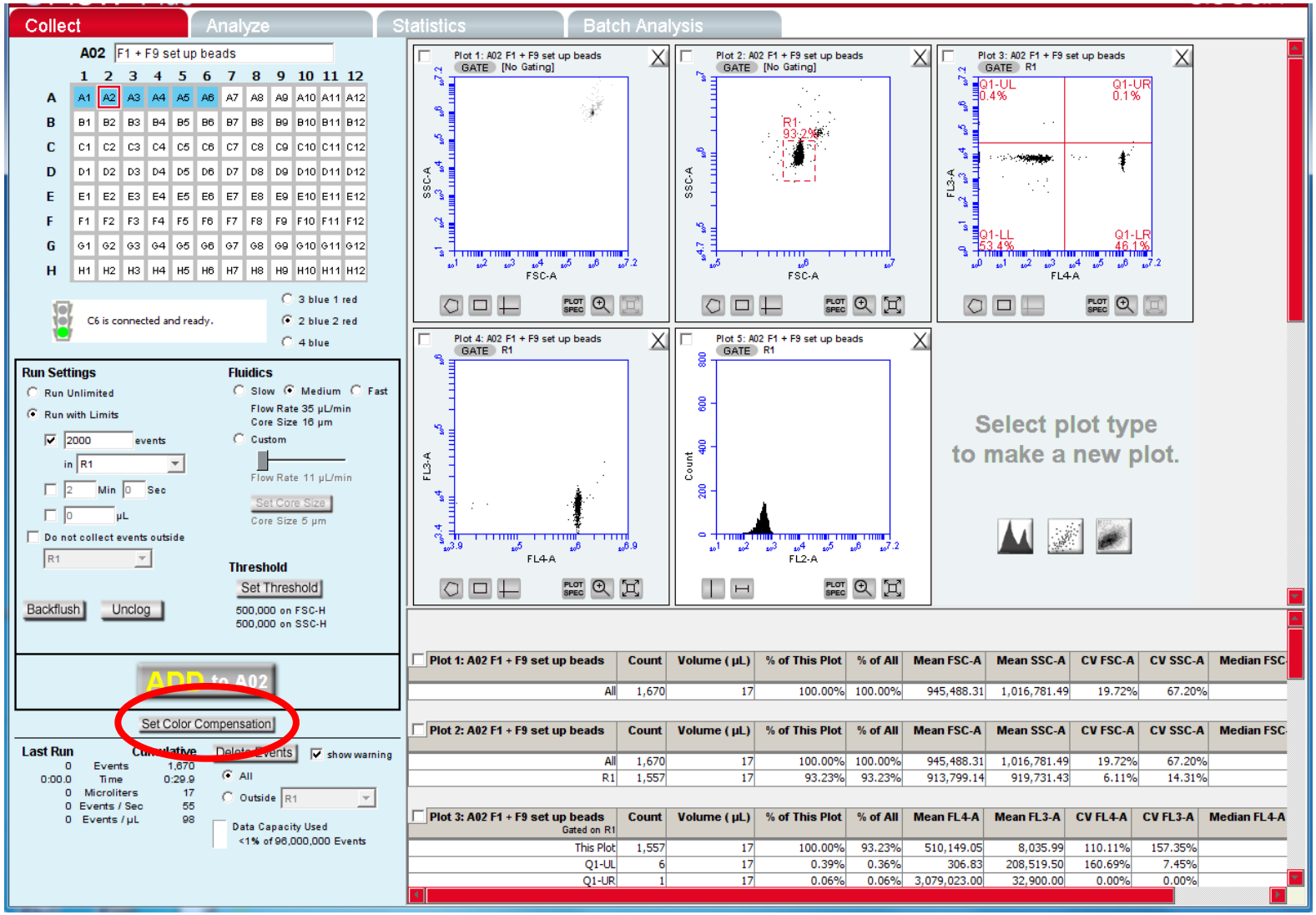
BD Accuri CBA Flex Set Template

Acquire



Set Color Compensation

Acquire



Plot 1: A02 F1 + F9 set up beads	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC
All	1,670	17	100.00%	100.00%	945,488.31	1,016,781.49	19.72%	67.20%	

Plot 2: A02 F1 + F9 set up beads	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC
All	1,670	17	100.00%	100.00%	945,488.31	1,016,781.49	19.72%	67.20%	
R1	1,557	17	93.23%	93.23%	913,799.14	919,731.43	6.11%	14.31%	

Plot 3: A02 F1 + F9 set up beads Gated on R1	Count	Volume (µL)	% of This Plot	% of All	Mean FL4-A	Mean FL3-A	CV FL4-A	CV FL3-A	Median FL4-A
This Plot	1,557	17	100.00%	93.23%	510,149.05	8,035.99	110.11%	157.35%	
Q1-UL	6	17	0.39%	0.36%	306.83	208,519.50	160.69%	7.45%	
Q1-UR	1	17	0.06%	0.06%	3,079,023.00	32,900.00	0.00%	0.00%	

The BD CBA Flex Set Workflow

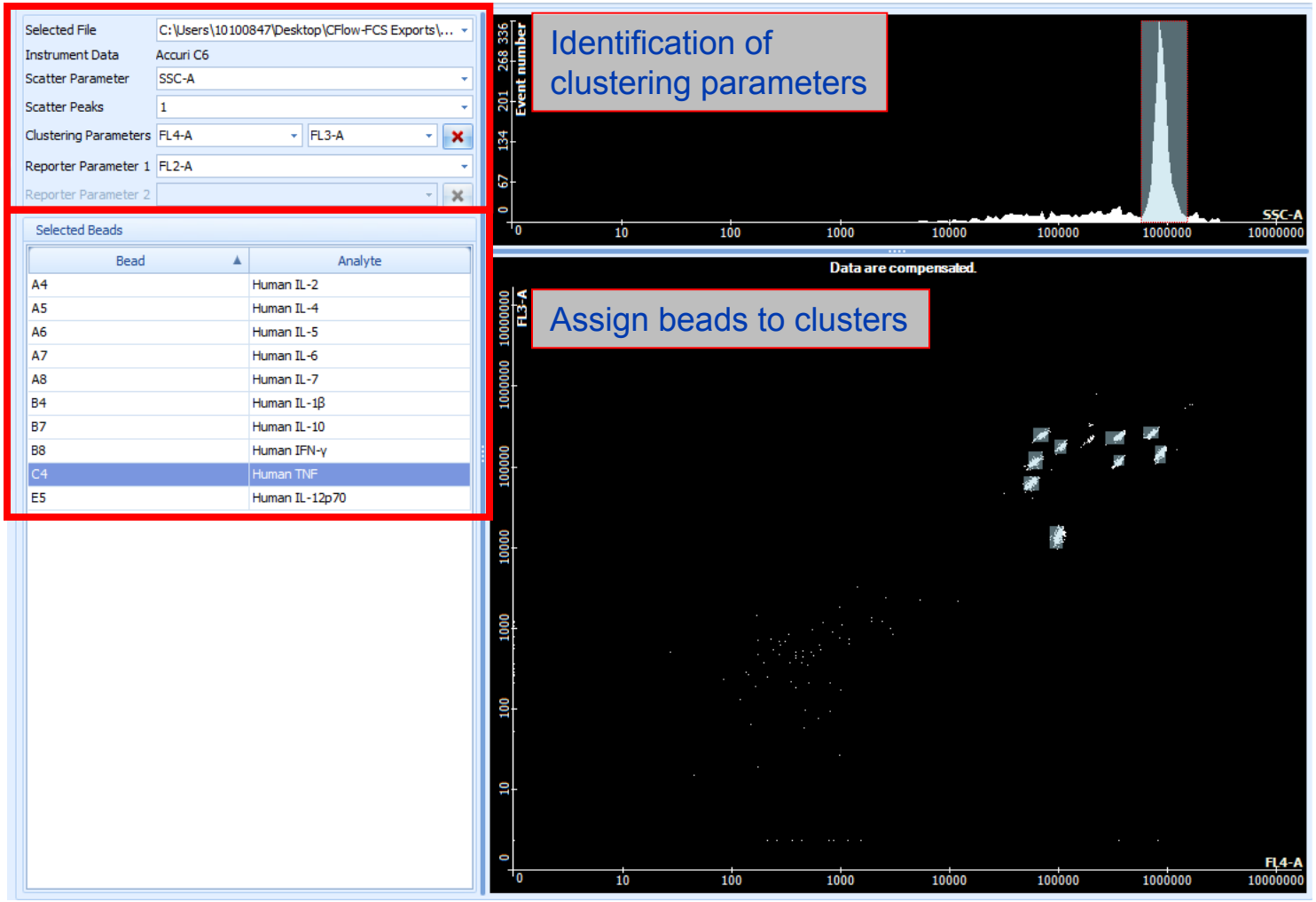
BD CBA Flex Sets

BD Accuri C6

FCAP Array v3.0.1 (Microsoft® Windows)

Instrument Settings: 10-plex Flex Set

Analyze

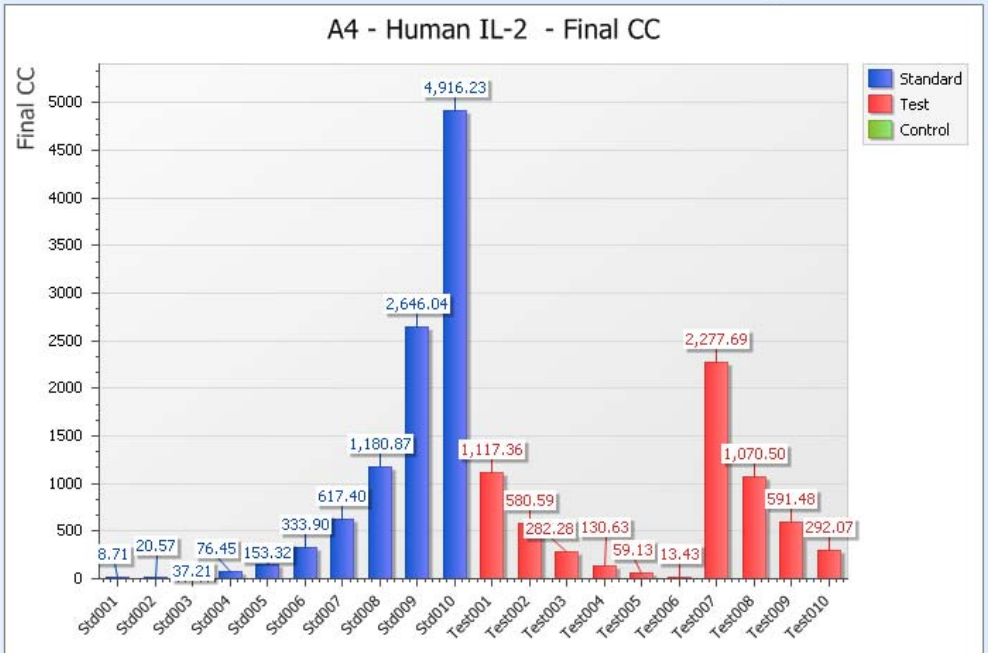


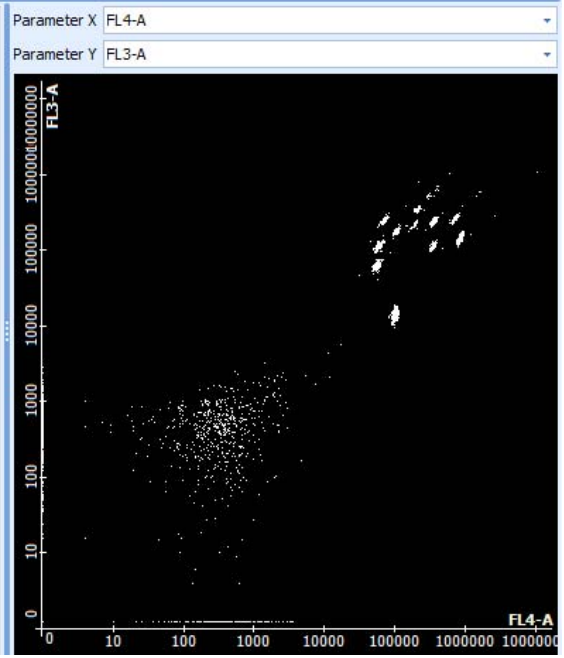
Results per Analyte

Analyze

Analyte Name
C4 - Human TNF
A7 - Human IL-6
A6 - Human IL-5
A5 - Human IL-4
A4 - Human IL-2
A8 - Human IL-7
B8 - Human IFN-γ
B7 - Human IL-10
B4 - Human IL-1β
E5 - Human IL-12p70

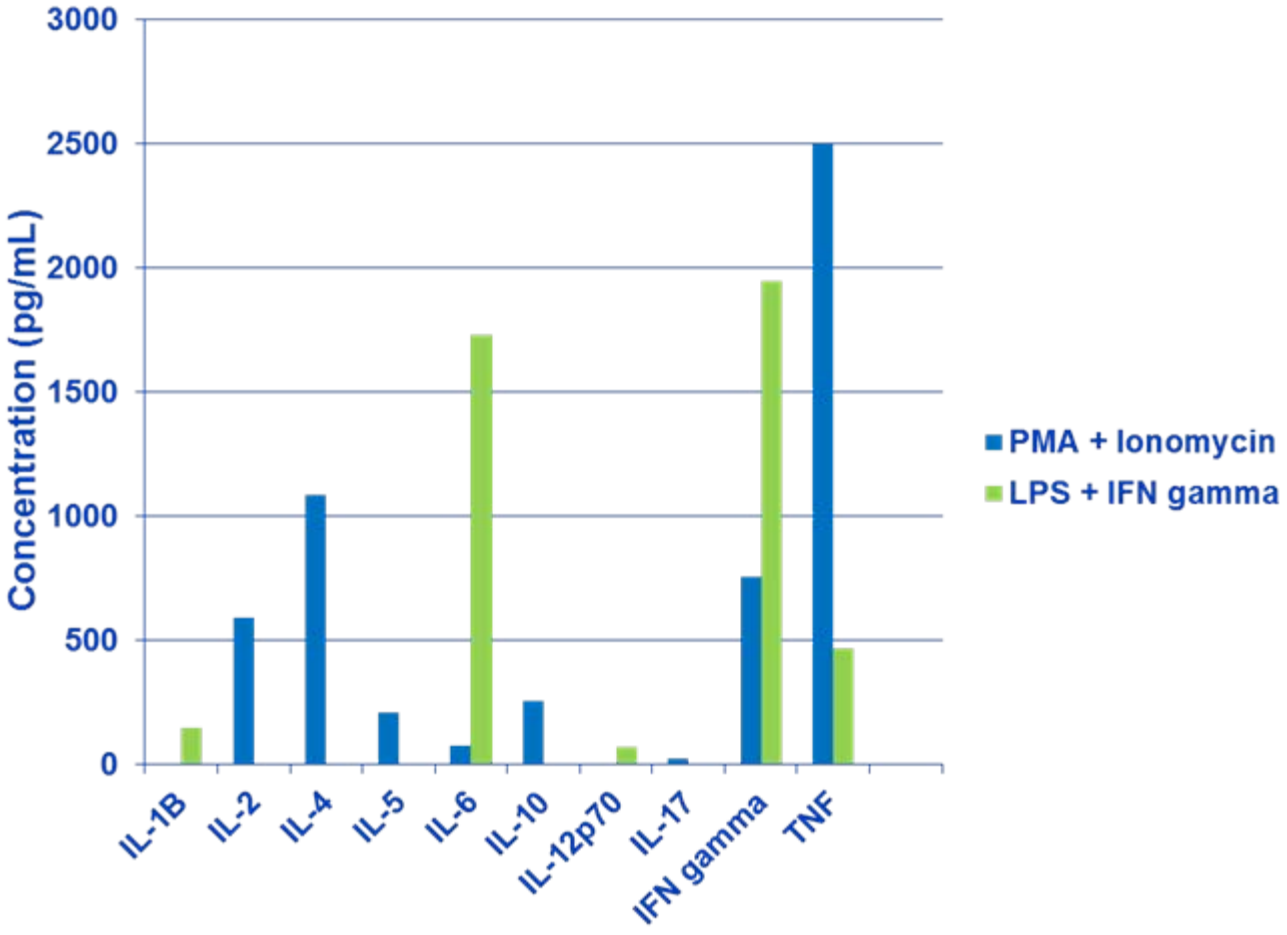
Sample Name	Position	Clustering	Results File	Event #	MFI	SD	CV	Nominal CC	Fitted CC
Std001	1 - A1	Manual	A01.fcs	161	3,027.00	489.26	14.69 %	0.00 pg/mL	8.71 pg/mL
Std002	1 - A2	Manual	A02.fcs	192	3,207.00	502.42	15.07 %	20.00 pg/mL	20.57 pg/mL
Std003	1 - A3	Manual	A03.fcs	203	3,542.00	681.25	17.26 %	40.00 pg/mL	37.21 pg/mL
Std004	1 - A4	Manual	A04.fcs	164	4,484.00	744.82	15.60 %	80.00 pg/mL	76.45 pg/mL
Std005	1 - A5	Manual	A05.fcs	155	6,632.00	956.28	15.49 %	156.00 pg/mL	153.32 pg/mL
Std006	1 - A6	Manual	A06.fcs	184	12,544.00	7,895.22	47.60 %	312.50 pg/mL	333.90 pg/mL
Std007	1 - A7	Manual	B01.fcs	208	23,017.00	6,653.17	45.44 %	625.00 pg/mL	617.40 pg/mL
Std008	1 - A8	Manual	B02.fcs	194	44,654.00	6,960.99	16.77 %	1,250.00 pg/mL	1,180.87 pg/mL
Std009	1 - B1	Manual	B03.fcs	174	94,621.00	21,812.75	22.80 %	2,500.00 pg/mL	2,646.04 pg/mL
Std010	1 - B2	Manual	B04.fcs	151	152,272.00	34,550.51	22.04 %	5,000.00 pg/mL	4,916.23 pg/mL
Test001	1 - B3	Manual	B05.fcs	169	42,244.00	7,602.03	18.78 %	N/A	1,117.36 pg/mL
Test002	1 - B4	Manual	B06.fcs	102	21,615.00	3,755.06	22.15 %	N/A	580.59 pg/mL
Test003	1 - B5	Manual	C01.fcs	195	10,764.00	1,805.81	15.37 %	N/A	282.28 pg/mL
Test004	1 - B6	Manual	C02.fcs	163	5,967.00	1,165.32	17.51 %	N/A	130.63 pg/mL





Comparison of Cytokine Concentrations

Analyze



BD

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.

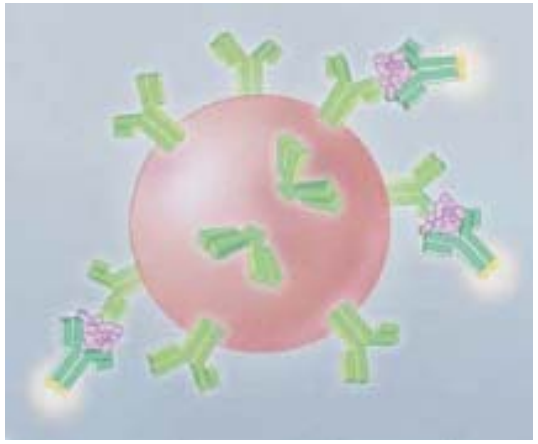
Analyze

- FCAP Array software provides simplified data analysis.
- FCAP Array v3.0.1 is compatible with BD Accuri FCS files.

BD

Thank You!

- Trent Colville
- Stacey Roys
- Jacob Rabenstein



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