CytoFLEX Instrument Evaluation Using Biological Specimens

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ABSTRACT

Evaluation of the CytoFLEX flow cytometer, by independent research flow cytometry laboratories and Beckman Coulter senior research and development scientists, was performed using flow cytometers equipped with 12 parameters and 9 fluorescent detectors. Instruments were equipped with the enhanced Side Scatter channel (VSSC) option. Evaluation of biological specimens included fresh human whole blood, human leukocyte control cells, and smaller cells and particles, such as RBCs, platelets, microbes, liposomes, and vesicles. A 9-color data set was collected, using a prototype human leukocyte control cells, developed by BCI. Analysis of the human control leukocyte cells clearly demonstrates expected populations of (T cells, NKT cells, NK cells, and B cells). To explore the instrument capabilities to resolve small particles, analysis of red blood cell micro particles was performed. A three month old human blood sample, stored under standard blood banking conditions, was stained with Annexin V. Forward scatter extinction and side scatter revealed red blood cell micro particles, transition events and intact red blood cells. A homogeneous population of *Listeria innocua*, a rod shaped bacteria with 0.5 μm in width and 1–1.5 μm in length was stained with Propidium Iodide and Syto9. The specimen was analyzed for live cell versus dead cell populations. In another experiment, micro particles were examined using vesicles isolated from rat plasma. Vesicles were stained with AnnV Dy488 and CD42d. Resolution of vesicles from instrument noise was achieved through the VSSC (Violet laser side scatter signal) and fluorescence. An increase in scatter signal sensitivity was achieved using the VSSC collection channel, as well as an increase in background noise. Data and figures presented in this poster are a collaborative effort, through the efforts of scientists representing the following laboratories: Purdue University Cytometry Laboratory (PUCL), La Jolla Bioengineering Institute, University of Pittsburgh, and Beckman Coulter, Miami Site. The flow cytometry platforms used for the evaluations are for research use only.

MATERIAL AND METHODS

Beckman Coulter in-house human leukocyte control cells were stained with the following combination. The control cells were stained for 15 minutes in the dark, then washed, centrifuged, and resuspended prior to data collection. Human blood staining followed the use of the VersaLyse procedure to remove red blood cells.

Three month old blood, stored under standard blood banking conditions was used. Events were thresholded on Gly-A+ to eliminate non-RBC debris and WBC.

Listeria innocua prepared from overnight culture.

Vesicles were prepared from rat plasma. 2 spin cycles at 2500g was performed.

Multicolor flow cytometry was performed on a CytoFLEX flow cytometer (Beckman Coulter). The flow cytometry analysis was performed with CytExpert software (Beckman Coulter), Kaluza Software (Beckman Coulter) and VenturiONE software, Applied Cytometry.
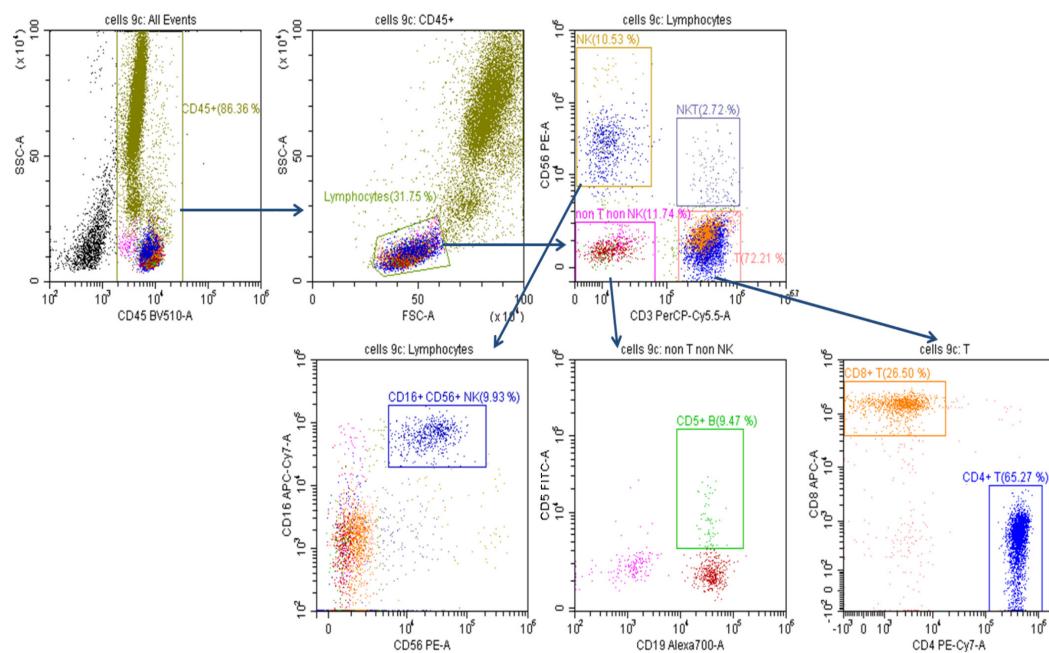
Antibody Conjugates.

All conjugates used in 9-color stainings are from Beckman Coulter.

Target	Clone	Fluorochrome
CD3	UCHT1	PC5.5
CD3	UCHT1	Pacific Blue
CD4	13B8.2	PC7
CD4	13B8.2	APC A750
CD5	BL1a	FITC
CD8	B9.11	APC
CD8	B9.11	APC A700
CD16	3G8	APC A750
CD19	J3-119	APC A700
CD20	B9E9	Pacific Blue
CD25	B1.49.9	PC7
CD38	LS198-4-3	APC
CD45	J.33	Krome Orange
CD45RA	2H4LDH11LDB9	FITC
CD56	N901	PE

RESULTS

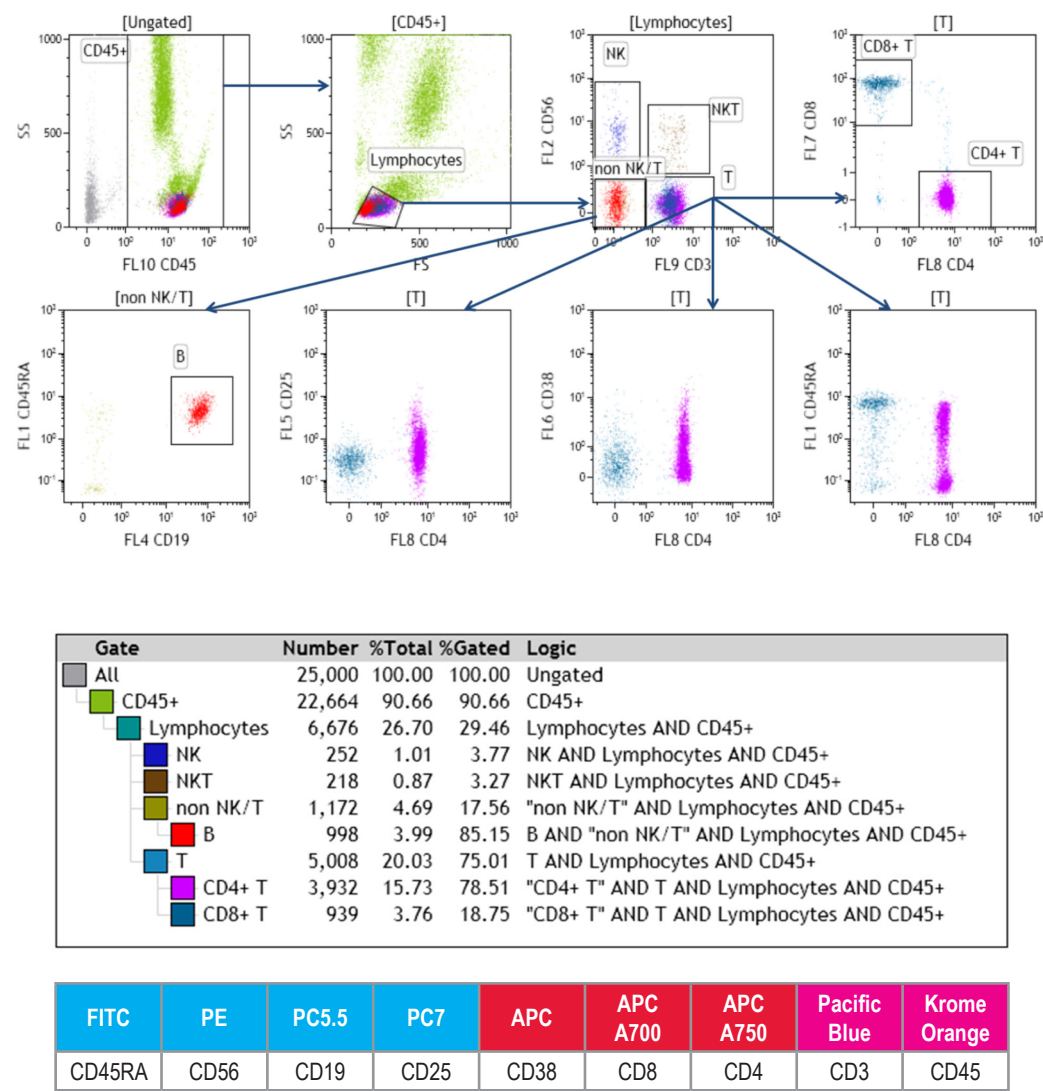**Figure 1.** 9-color Human Control Leukocytes Immunophenotyping Staining

Population	Events	% Total	% Parent
All Events	20015	100.00%	100.00%
CD45+	17284	86.36%	86.36%
Lymphocytes	5487	27.41%	31.75%
T	3962	19.80%	72.21%
CD4+ T	2586	12.92%	65.27%
CD8+ T	1050	5.25%	26.50%
NKT	149	0.74%	2.72%
NK	578	2.89%	10.53%
non T non NK	644	3.22%	11.74%
B	474	2.37%	73.60%
CD5+ B	61	0.30%	9.47%
CD16+ CD56+ NK	545	2.72%	9.93%

Tube Name: cells 9c
Sample ID:

FITC	PE	PC5.5	PC7	APC	APC A700	APC A750	Pacific Blue	Krome Orange*
CD5	CD56	CD3	CD4	CD8	CD19	CD16	CD20	CD45

* BV510 designation is equivalent BP filter used for KrO conjugates

Figure 2. 9-color Human Whole Blood Immunophenotyping Staining

Gate	Number	%Total	%Gated	Logic
■ All	25,000	100.00	100.00	Ungated
■ CD45+	22,664	90.66	90.66	CD45+
■ Lymphocytes	6,676	26.70	29.46	Lymphocytes AND CD45+
■ NK	252	1.01	3.77	NK AND Lymphocytes AND CD45+
■ NKT	218	0.87	3.27	NKT AND Lymphocytes AND CD45+
■ non NK/T	1,172	4.69	17.56	"non NK/T" AND Lymphocytes AND CD45+
■ B	998	3.99	85.15	B AND "non NK/T" AND Lymphocytes AND CD45+
■ T	5,008	20.03	75.01	T AND Lymphocytes AND CD45+
■ CD4+ T	3,932	15.73	78.51	"CD4+ T" AND T AND Lymphocytes AND CD45+
■ CD8+ T	939	3.76	18.75	"CD8+ T" AND T AND Lymphocytes AND CD45+

FITC	PE	PC5.5	PC7	APC	APC A700	APC A750	Pacific Blue	Krome Orange
CD45RA	CD56	CD19	CD25	CD38	CD8	CD4	CD3	CD45

Figure 3.

From left to right: RBC clusters are eliminated; glycophorin dim events are eliminated; glycophorin+ events are subsetted on the basis of forward light extinction into low, intermediate and high populations; annexin binding (surface phosphatidyl serine) is detected in low (E, red), intermediate (C, yellow), and high (B, green) forward light extinction populations. Color eventing annexin binding events on forward light extinction and side scatter reveals RBC microparticles (red), transitional events (yellow), and intact rbc (green).
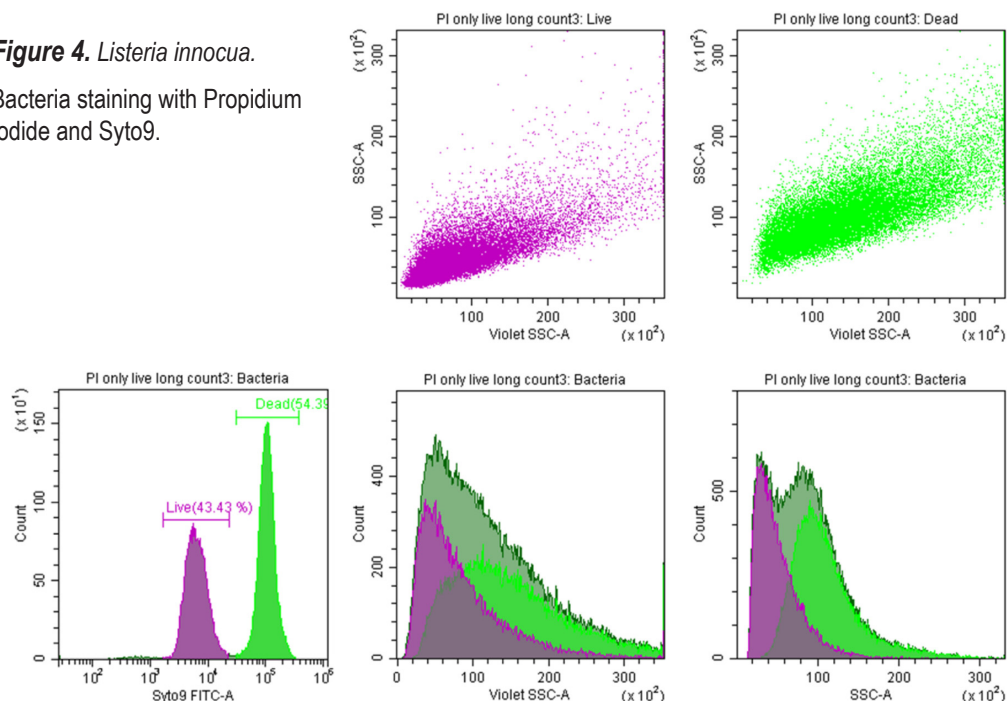
Analysis of RBC Microparticles

Sample W086114, triplicate determinations

Sample	Total Clean events (A)	FS_Low	FS_Int	FS_Hi	Microparticles Annex P_FS_Low	Transitional Annex P_FS_Int	Intact FBCAnnex P_FS_Hi
W084514A	84010	11.2%	1.0%	86.4%	63.0%	18.9%	7.6%
W084514B	83365	11.7%	2.6%	83.7%	59.0%	19.6%	7.4%
W084514C	82680	11.0%	4.4%	81.8%	61.9%	17.5%	7.2%
Mean	83352	11.3%	2.7%	84.0%	61.3%	18.7%	7.4%
SD	665	0.4%	1.7%	2.3%	2.1%	1.1%	0.2%
CV	0.8%	3.2%	64.1%	2.7%	3.4%	5.7%	2.5%
W085315A	84586	9.7%	1.8%	86.2%	55.9%	15.6%	5.2%
W085315B	82371	9.2%	0.4%	88.5%	57.0%	31.0%	5.7%
W085315C	85298	8.6%	1.9%	87.1%	54.8%	15.1%	4.8%
Mean	84085	9.2%	1.4%	87.3%	55.9%	20.6%	5.2%
SD	1526	0.5%	0.9%	1.1%	1.1%	9.0%	0.4%
CV	1.8%	5.8%	62.6%	1.3%	2.0%	43.8%	8.4%
W086114A	79488	6.8%	9.4%	79.3%	56.8%	35.0%	7.2%
W086114B	73871	5.6%	10.7%	79.0%	61.1%	31.1%	7.0%
W086114C	79430	7.0%	13.7%	72.1%	54.2%	23.7%	7.1%
Mean	77596	6.4%	11.2%	76.8%	57.4%	29.9%	7.1%
SD	3226	0.8%	2.2%	4.0%	3.5%	5.7%	0.1%
CV	4.2%	11.7%	19.6%	5.3%	6.1%	19.1%	1.2%

Table 1. Samples WO85315A and WO886114 not displayed.
Note inter-replicate precision as shown by the CV.
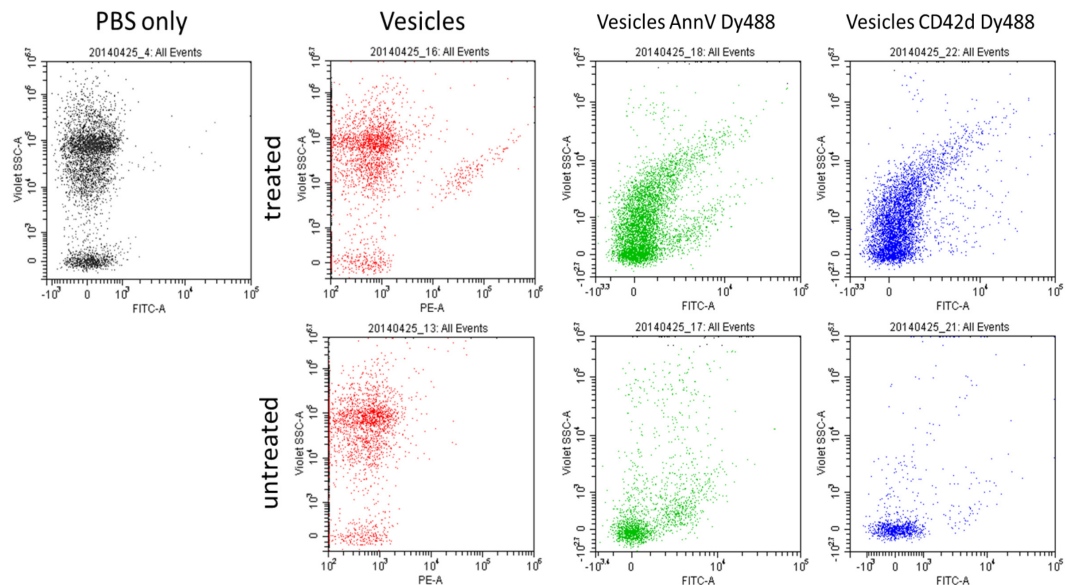
Figure 4. Listeria innocua.

Bacteria staining with Propidium Iodide and Syto9.

Live and Dead Bacteria have distinct Side Scatter (SSC) Profile

Figure 5. Vesicles prepared from Rat plasma.

Treated versus untreated, using both AnnexinV and CD42d Dy488.

SUMMARY

The CytoFLEX flow cytometer equipped with 12 parameters and 9 fluorescent detectors, including the enhanced Side Scatter channel (VSSC) option, was used to evaluate biological specimens at independent collaboration sites and Beckman Coulter, Miami. The biological specimens included human whole blood, human leukocyte control cells, and smaller cells and particles, such as RBCs, microbes, and vesicles. Fluorescent detection capability and scatter resolution capability, was typical across all specimen evaluations. Distinct and separate populations of cells and particles were visualized through fluorescent antibody tagging and fluorescent dyes, excited by distinct laser lines 405nm, 488nm and 638nm.