BD OneFlow™ ALOT performance: Interfering substances study

Jay Venkatachari, Milan Aggarwal, Sreenithya Ravindran, Nami Fuseya, Michael Herrler, Yuming Tang, Sheetal Bodhankar, Elena Afonina.

BD Biosciences, 2350 Qume Drive, San Jose, CA

Abstract

The BD OneFlow™ Acute Leukemia Orientation Tube (ALOT) is used to investigate markers present in normal and abnormal populations of hematopoietic cells present in bone marrow (BM) and peripheral blood (PB). Substances commonly found within BM and PB may interfere with assays and consequently cause significant difference in results.

We evaluated the performance of the BD OneFlow™ ALOT in the presence of substances potentially found within PB or BM that may interfere with immunofluorescence staining. The study design was based on the CLSI guidelines (EP07-A3). We used healthy BM spiked with endogenous and exogenous substances. Endogenous substances included hemoglobin, albumin, bilirubin (conjugated and unconjugated), triglycerides and erythrocytes. The exogenous compounds were acetaminophen, acetylsalicylic acid, ibuprofen, oseltamivir, ondansetron, dexamethasone, prednisolone, albuterol, guaifenesin, promethazine, cefotaxime, meropenem, and vancomycin. Test concentrations for exogenous substances were at 3X the highest concentration reported following a drug therapeutic dose and test concentrations for endogenous substances were at the highest expected concentration. For each interferent sample type, solvent controls were run in parallel. Spiked interference samples were compared to its solvent control for qualitative and quantitative evaluation by paired-difference analysis. Results denote that there was 100% agreement of qualitative assessment for all populations present when comparing between test and reference. The mean bias between test and control for comparing populations ranged between 0.9 to -0.82, suggesting that the presence of interferent substances evaluated in this study did not significantly affect performance of the BD OneFlow™ ALOT.

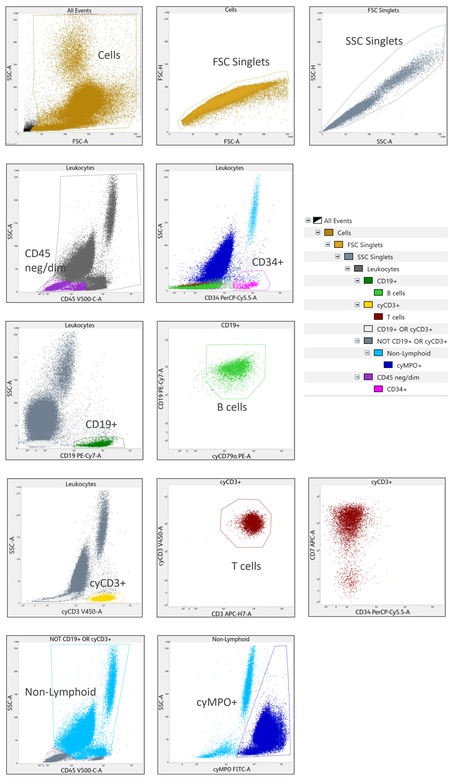
Introduction:

The BD OneFlow™ Acute Leukemia Orientation Tube (ALOT) is intended for flow cytometric immunophenotyping of aberrant immature populations of hematopoietic cells (lymphoid and nonlymphoid lineage) in bone marrow (BM) and peripheral blood (PB). This air dried-down 8-color immunophenotyping panel aids in the diagnosis of acute lymphoblastic leukemia and non-lymphoid acute leukemia. ALOT includes two tubes, one containing three cytoplasmic markers (C tube) and the other containing five surface markers (S tube). Substances commonly found within BM and PB may interfere with biochemical and fluorescence assays and consequently cause significant difference in the assay test results and interpretation.

BD OneFlow™ ALOT reagent composition

Antibody	MPO	CD79A	CD34	CD19	CD7	CD3	CD3	CD45
Fluorochrome	FITC	PE	PerCP-Cy 5.5	PE-Cy7	APC	APC-H7	BD Horizon™ V450	BD Horizon™ V500-C
Tube	C	C	S	S	S	S	C	S

Representative expression pattern of markers in normal bone marrow specimen stained with BD OneFlow™ ALOT single-test reagent acquired on the BD FACSLyric™ Flow Cytometer with BD FACSuite ™ Clinical Application v1.4



Methods

The study design for evaluation of interfering substances to the BD OneFlow™ ALOT was based on the guidelines from CLSI EP07 A3: Interference Testing in Clinical Chemistry, 3rd Edition and CLSI EP37: Supplemental Tables for Interference Testing in Clinical Chemistry, 1st Edition.

Study Design Summary

Study Parameters	Details
Instrument	12-color BD FACSLyric™ Flow Cytometer with loader
Software	BD FACSuite™ Clinical Application v1.4
Sample Conditions	**Test:** Specimen spiked with interferent **References:** Specimen spiked with PBS (to set baseline) or solvent in equal volume as interferent
Number of Interfering Substances	19 Total: 13 Exogenous, 6 Endogenous
Sample type	Healthy BM
Replicates	20

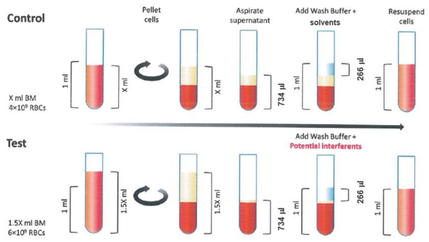
This study used healthy BM spiked with endogenous and exogenous substances. Endogenous substances were tested together, and the exogenous substances were tested in two groups, based on their solubility in DMSO or PBS. The groups are: (i) Endogenous substances (ii) Exogenous substances that are soluble in DMSO (iii) Exogenous substances that are soluble in PBS.

Test concentrations of Interferents

Endogenous Interferent	Highest Expected Conc (mg/mL)
Erythrocytes	$6X10^6$ cells/mL
Hemoglobin or hemolysate	10
Albumin or Total Protein	60
Bilirubin, conjugated	0.4
Bilirubin, unconjugated	0.4
Triglycerides	15
Exogenous Interferent (soluble in DMSO)	**Conc (mg/mL)**
Acetaminophen	0.156
Acetylsalicylic acid (Aspirin)	0.03
Ibuprofen	0.219
Oseltamivir phosphate	0.000399
Ondansetron	0.000342
Dexamethasone	0.012
Prednisolone	0.0012
Albuterol	0.000045
Guaifenesin	0.0045
Exogenous Interferent (soluble in PBS)	**Conc (mg/mL)**
Promethazine	0.000297
Cefotaxime	0.528
Meropenem	0.339
Vancomycin	0.12

For each interferent sample type, solvent controls were run in parallel. Test concentrations for exogenous substances were at 3X the highest concentration reported following a drug therapeutic dose and test concentrations for endogenous substances were at the highest expected concentration. 20 replicate samples for each group were acquired on a BD FACSLyric™ Flow Cytometer running BD FACSuite™ Clinical Application version 1.4.

Stepwise procedure (from left to right) to prepare the Control (top) and the Test (bottom) conditions



Stock solutions were prepared fresh. To add the potential interferents to the bone marrow (BM) in a combined mixture, the cells of the drawn BM were pelleted first, after which the supernatant was aspirated and then replaced with the potential interferents stock mixture to obtain the final test concentration. (Example: When pelleting $6X10^9$ RBCs in BM to target a final volume of 1 mL, up to 300µL of plasma can be easily aspirated without disturbing the cells).

Interference substances-spiked test samples were compared to its solvent control for qualitative and quantitative evaluation.

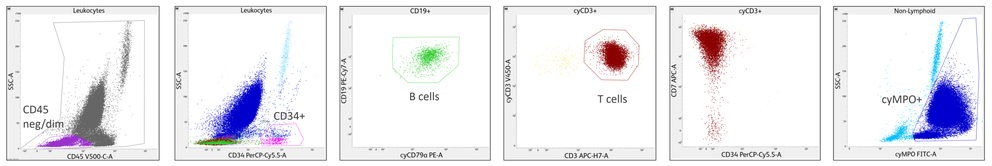
Quantitative: The average difference (sample bias) and the two-sided 95% confidence interval was calculated. Sample bias is defined as the test value minus the control value; the bias is expressed as a percentage because the test and control values are percentages.

Qualitative: The 95% lower bound for the percent agreement was calculated using the Clopper-Pearson exact method.

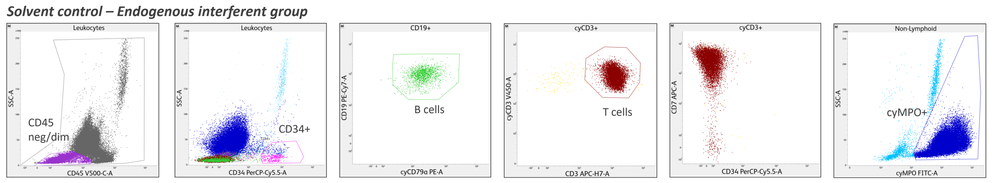
Results

Evaluation of Endogenous Interferent substances for BD OneFlow™ ALOT assay

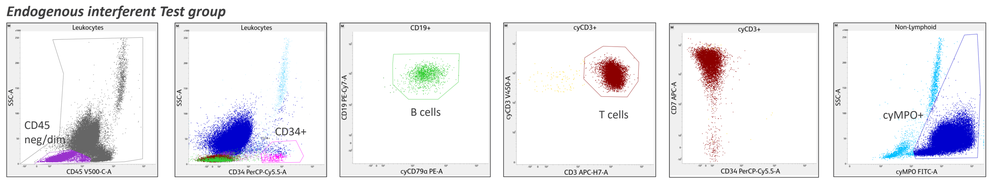
Untreated control



Solvent control – Endogenous interferent group



Endogenous interferent Test group



Representative expression pattern of markers in matching normal bone marrow specimen, solvent control group and Endogenous interferent test group. 20 replicate samples were stained with BD OneFlow™ ALOT and acquired on the BD FACSLyric™ Flow Cytometer with BD FACSuite ™ Clinical Application v1.4

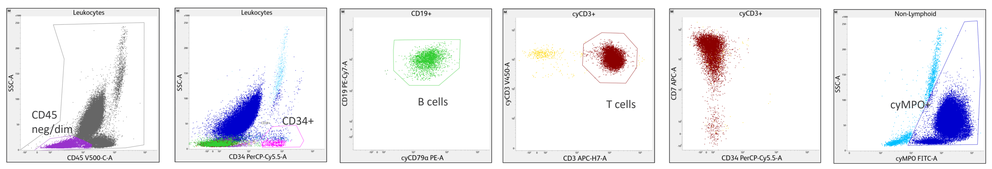
Summary of evaluation of Endogenous Interferent substances for BD OneFlow™ ALOT (Mean Bias).

Population	Mean Bias (95% CI)
Leukocytes % SSC singlets	**-0.13** (-0.16, -0.10)
cyCD3+ % of Leukocytes	**-0.52** (-0.65, -0.39)
T cells % of cyCD3	**-0.06** (-0.20, 0.08)
CD19+ % of Leukocytes	**-0.08** (-0.11, -0.04)
B cells % of CD19+	**-0.31** (-0.66, 0.05)
CD34 % of Leukocytes	**0.42** (0.30, 0.55)
cyMPO+ % of non-lymphoid	**0.90** (0.65, 1.15)

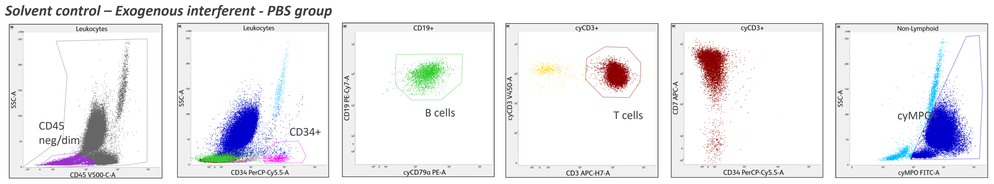
Hemoglobin, Albumin, conjugated Bilirubin are constituted in PBS, while NaOH helps to increase pH and get unconjugated Bilirubin in solution. Sucrose/NaCl are included along with Triglycerides in solution. Matching diluent solutions were included in the solvent controls (reference) group and evaluated in 20 replicates.

Evaluation of Exogenous Interferent substances dissolved in PBS for BD OneFlow™ ALOT assay

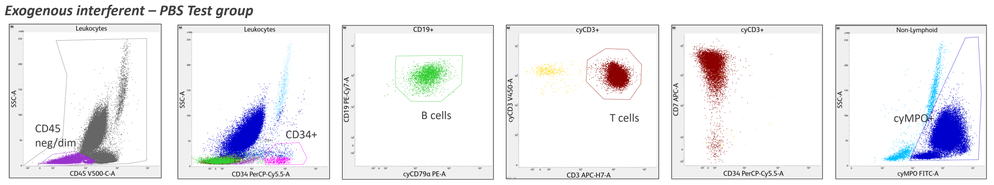
Untreated control



Solvent control – Exogenous interferent - PBS group

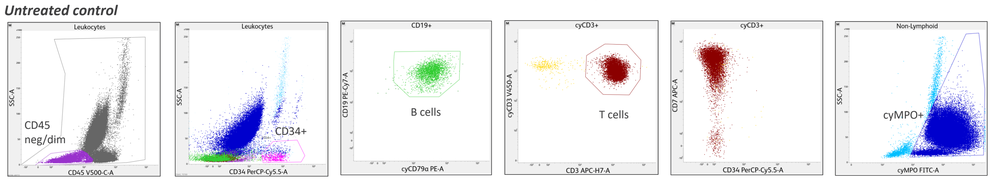


Exogenous interferent – PBS Test group

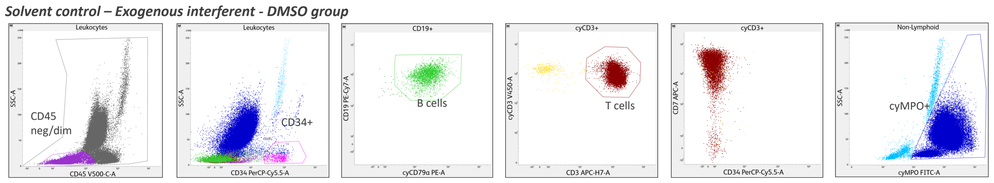


Evaluation of Exogenous Interferent substances dissolved in DMSO for BD OneFlow™ ALOT assay

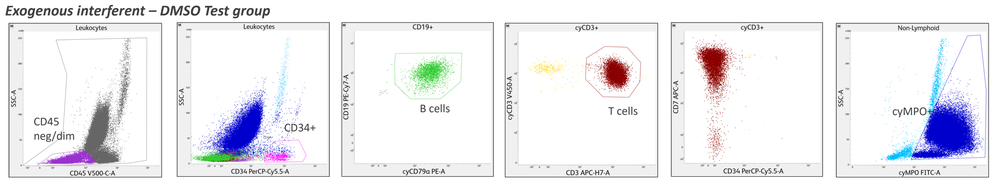
Untreated control



Solvent control – Exogenous interferent - DMSO group



Exogenous interferent – DMSO Test group



Representative expression pattern of markers in matching normal bone marrow specimen, solvent control group and Exogenous interferent dissolved in PBS or DMSO test group. 20 replicate samples were stained with BD OneFlow™ ALOT and acquired on the BD FACSLyric™ Flow Cytometer with BD FACSuite ™ Clinical Application v1.4

Summary of evaluation of Exogenous Interferent substances for BD OneFlow™ ALOT (Mean Bias).

Population	Exogenous Interferent substances - soluble in PBS Mean Bias (95% CI)	Exogenous Interferent substances - soluble in DMSO Mean Bias (95% CI)
Leukocytes % SSC singlets	**0.00** (0.00, 0.00)	**0.00** (0.00, 0.00)
cyCD3+ % of Leukocytes	**0.05** (-0.07, 0.17)	**0.09** (-0.05, 0.23)
T cells % of cyCD3	**-0.29** (-0.81, 0.23)	**-0.03** (-0.16, 0.10)
CD19+ % of Leukocytes	**0.03** (-0.02, 0.09)	**0.11** (0.03, 0.18)
B cells % of CD19+	**-0.82** (-1.12, -0.52)	**-0.61** (-0.84, -0.38)
CD34 % of Leukocytes	**-0.17** (-0.29, -0.05)	**-0.16** (-0.33, 0.01)
cyMPO+ % of non-lymphoid	**-0.04** (-0.08, 0.00)	**-0.16** (-0.34, 0.02)

Exogenous interferent substances were tested in two groups, one group that are soluble in PBS and the second group that are soluble in PBS. Each group had matching solvent control (reference) groups and had 20 replicates.

Conclusions

There was 100% agreement of qualitative assessment for all populations present when comparing between test and reference.

The mean bias between test and control for comparing populations ranged between 0.9 to -0.82.

- With endogenous interferents tested in the study, the maximum Mean bias of 0.9 was observed in percentage of cyMPO+ cells with the 95% CI of 0.65 and 1.15, while it was observed that cyCD3+ cells was reduced with a Mean bias of -0.52.
- With exogenous interferents tested in the study, the maximum Mean bias of -0.82 was observed in percentage of B cells with the 95% CI of -1.12 and -0.52.

These results suggest that the presence of interferent substances evaluated in this study did not significantly affect performance of the BD OneFlow™ ALOT.