BD OneFlow™ Assays performance: Detection capability of LST, B-CLPD T1, ALOT, PCST, PCD

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Abstract

The BD OneFlow™ Assays are used to investigate the expression of markers known to be present in normal and abnormal cell populations found in bone marrow (BM), peripheral blood (PB), and lymphoid tissues. The BD OneFlow™ Assays consist of LST (Lymphoid Screening Tube), B-CLPD T1 (B-Cell Chronic Lymphoproliferative Diseases Tube 1), ALOT (Acute Leukemia Orientation Tube), PCST (Plasma Cell Screening Tube) and PCD (Plasma Cell Disorders) Tube. These single-use tubes contain fluorochrome-conjugated antibodies as an optimized air-dried panel.

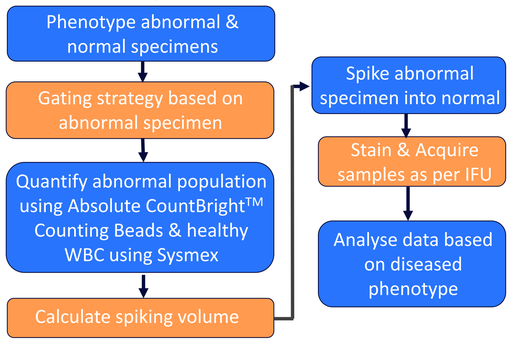
As qualitative assays, their detection capability refers to the ability to distinguish abnormal cells from normal cells. We evaluated Limit of Blank (LoB) and Limit of Detection (LoD) of these five BD OneFlow™ Assays. The study was designed with reference from the International Clinical Cytometry Society (ICCS) and International Council for Standardization in Hematology (ICSH) Practice Guidelines. We spiked diseased specimen into normal (BM/PB) at three known LoD ranges of abnormal cells: 0.02%, 0.05% and 0.1% of All Events and stained using BD OneFlow™ Reagents. Normal unspiked specimens were used as a control and for LoB assessment. Acquisitions were carried out using BD FACSLyric™ Flow Cytometers and BD FACSuite™ Software. Analysis was based on phenotype of the abnormal specimen. Phenotypical differences between the normal and abnormal specimens were used to distinguish the normal and abnormal populations within a spiked sample. LoB was determined based on the maximum value of either the maximum value observed or the 95th percentile of the observed background events in normal unspiked specimen. Trueness and standard deviation of the bias were calculated along with 95% lower and upper bound values to determine LoD. The results suggest that LoB of the five BD OneFlow™ Assays i.e., LST, B-CLPD T1, ALOT, PCST and PCD is 0.03% of All Events and LoD is 0.1% of All Events.

Methods

Study Design

BD OneFlow™ Reagents	LST/B-CLPD T1/ALOT/PCD/PCST	
Test System	12-Color BD FACSLyric™ Flow Cytometer x 4 instruments	
Concentration Range	0.0% (Unspiked normal sample i.e., control), 0.02%, 0.05% and 0.1% of All Events	
Sample Size	1 abnormal (diseased) specimen and 2 normal (healthy donor) specimens	
Replicates	Per Concentration	10
	Reagent Lots	2
Total Acquisitions	80 (10 replicates per concentration per reagent lot X 2 reagent lots X 4 concentration range)	

Workflow to evaluate detection capability of BD OneFlow™ Assays



Analysis Plan

The cytometric analysis strategy utilized a multiparameter approach as dictated by the phenotype of the abnormal specimen. Discrimination between the normal and abnormal populations within a spiked sample was dependent on the phenotypical differences between the two populations (normal and abnormal) and the markers available within the given tube.

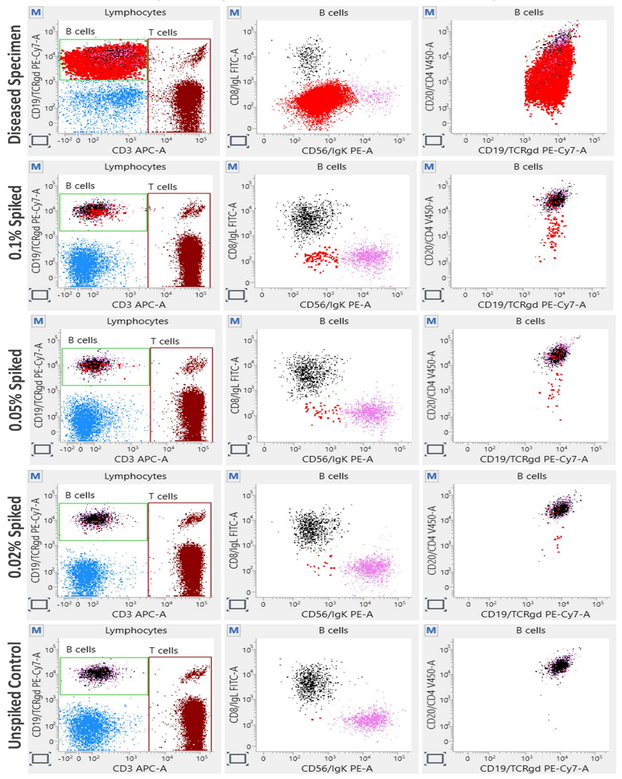
Statistical Analysis

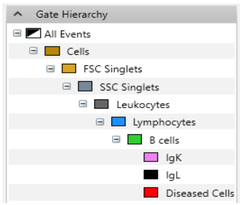
LoB: The endpoint is the percentage of abnormal cell population in the unspiked control samples. There are two metrics: the maximum value observed and the 95th percentile. The maximum was reported as the LoB.

LoD: The endpoints are- 1) the percentage of abnormal cell population in the candidate concentrations. 2) the number of spiked samples with abnormal phenotype readings. The two metrics are- 1) the standard deviation of the bias between the target percent abnormal and the measured percent abnormal. The spiking was done consistently if the standard deviation, or SD, of the bias is less than the precision limit. 2) Trueness, which indicates that samples with a low percentage of abnormal cells can be used to detect disease. A concentration can be the LoD if it, and all the higher concentrations, meet the trueness criteria.

Results

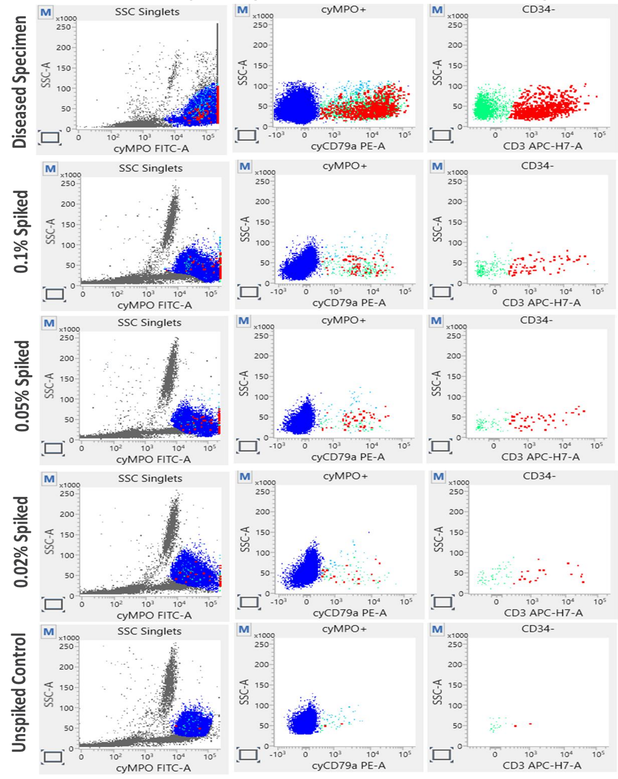
Detection Capability of BD OneFlow™ LST Assay

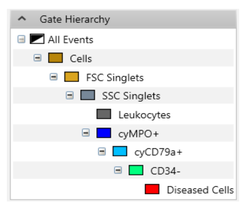




The phenotype of the diseased specimen using BD OneFlow™ LST was CD45 heterogenous, CD19$^+$, CD20 heterogenous, sIgL$^-$ sIgK$^-$, CD5$^+$, CD3$^-$, CD38$^-$, and CD56$^-$. With BD OneFlow™ B-CLPD T1, the specimen was CD45 heterogenous, CD19$^+$, CD20 heterogenous, CD43$^+$, CD200$^+$ and CD79b$^-$.

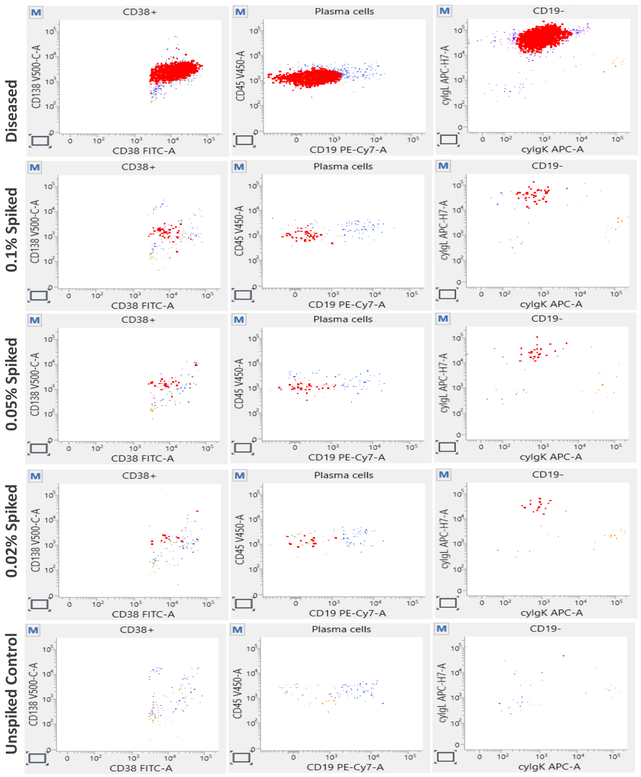
Detection Capability of BD OneFlow™ ALOT Assay

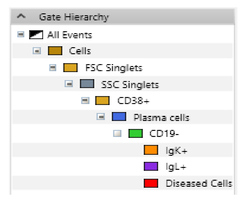




The phenotype of the diseased specimen using BD OneFlow™ ALOT was cyMPO$^+$, cyCD79a$^+$, CD3$^+$, CD34$^-$, CD45 heterogenous.

Detection Capability of BD OneFlow™ PCST Assay





The phenotype of the diseased specimen determined using BD OneFlow™ PCST was CD45 dim, CD38$^+$, CD138$^+$, CD19$^-$, Lambda monoclonal, CD56$^-$ and b2-microglobulin$^+$. With BD OneFlow™ PCD Tube, the phenotype of the specimen was determined as CD45 dim, CD38$^+$, CD138$^+$, CD19$^-$, CD117 heterogenous, CD27$^+$, CD28 dim and CD81$^-$.

Results

Summary Metrics from the Statistical Analysis of BD OneFlow™ LST, B-CLPD T1, ALOT, PCST and PCD Detection Capability Testing Data

Population	Targeted % Abnormal Population	Mean (N= 20)	95% CI for the Mean (Upper, Lower)	Bias SD (95% UB)	Bias SD Conclusion (Pass/ Fail)	Trueness (95% LB)	Trueness Conclusion
LST	0.000 (Unspiked control)	0.003	(0.002, 0.004)				
	0.020	0.020	(0.018, 0.021)	0.0036 (0.0067)	Pass	100.0 (88.1)	Pass
	0.050	0.049	(0.047, 0.051)	0.0046 (0.0086)	Pass	100.0 (88.1)	Pass
	0.100	0.084	(0.078, 0.089)	0.0114 (0.0214)	Pass	100.0 (88.1)	Pass
B-CLPD T1	0.000 (Unspiked control)	0.002	(0.001, 0.003)				
	0.020	0.019	(0.018, 0.021)	0.0035 (0.0066)	Pass	100.0 (88.1)	Pass
	0.050	0.044	(0.041, 0.047)	0.0065 (0.0122)	Pass	100.0 (88.1)	Pass
	0.100	0.074	(0.072, 0.077)	0.0051 (0.0096)	Pass	100.0 (88.1)	Pass
ALOT	0.000 (Unspiked control)	0.000	(0.000, 0.001)				
	0.020	0.017	(0.016, 0.018)	0.0023 (0.0043)	Pass	100.0 (88.1)	Pass
	0.050	0.044	(0.043, 0.046)	0.0032 (0.0059)	Pass	100.0 (88.1)	Pass
	0.100	0.092	(0.090, 0.094)	0.0038 (0.0071)	Pass	100.0 (88.1)	Pass
PCST	0.000 (Unspiked control)	0.004	(0.003, 0.004)				
	0.020	0.022	(0.020, 0.023)	0.0040 (0.0076)	Pass	100.0 (88.1)	Pass
	0.050	0.038	(0.033, 0.042)	0.0093 (0.0174)	Pass	100.0 (88.1)	Pass
	0.100	0.081	(0.075, 0.087)	0.0127 (0.0238)	Pass	100.0 (88.1)	Pass
PCD	0.000 (Unspiked control)	0.002	(0.000, 0.001)				
	0.020	0.019	(0.017, 0.021)	0.0040 (0.0075)	Pass	100.0 (88.1)	Pass
	0.050	0.045	(0.040, 0.051)	0.0120 (0.0225)	Pass	100.0 (88.1)	Pass
	0.100	0.073	(0.066, 0.081)	0.0163 (0.0306)	Pass	100.0 (88.1)	Pass

The 95% upper bound of the standard deviation of the biases at each concentration i.e., 0.1%, 0.05% & 0.02% of All Events was less than the precision limit of 5% indicating that the spiking was consistent for the tested diseased specimen. The 95% minimum is the minimum value for 95% of the data. All the three spiked concentrations met the trueness limit of 88%.

LoB Results

LoB Metrics and Observed Population

Population	Maximum Value	95th Percentile	Maximum Metric
LST	0.007	0.005	**0.007**
B-CLPD T1	0.005	0.006	**0.006**
ALOT	0.002	0.001	**0.002**
PCST	0.007	0.007	**0.007**
PCD	0.002	0.001	**0.002**

Conclusions

The five BD OneFlow™ Assays were evaluated to determine the highest apparent background observed in normal population and the maximum value of 0.007% was observed for B-CLPD T1 and PCST in the respective tested normal specimens. For each of the five BD OneFlow™ Assays, the trueness was observed up to the level of 0.02% spiking of the tested diseased specimen indicating that the BD OneFlow™ Assays could discriminate low percentage of abnormal cells from normal population.

The results suggest that LoB of the five BD OneFlow™ Assays i.e., LST, B-CLPD T1, ALOT, PCST and PCD is 0.03% of All Events and LoD is 0.1% of All Events.