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Application Note

Use of Slingshot CTx TruCytes™ CD34 for assay sensitivity testing

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CD34 Assay: Sensitivity Testing

Introduction

The estimation of assay limits (of blank, detection, and quantification) is commonly performed during the validation of immunochemistry tests. The most important prerequisite to performing this is a standard material of known analyte quantity. This would be spiked in at various levels into the tested matrix and the readout compared. For flow cytometry based tests, this is traditionally not possible because standard cells expressing the epitope of interest at a fixed level do not exist.

Slingshot Biosciences has a range of hydrogels having lymphocyte scatter properties and the ability to be coated with certain antigens. Recently, we have obtained a vial of TruCytes™ bearing CD45 and CD34 (NPI-00049A). We specifically used the 'High' version where 90% of the cell mimics in the vial are coated with both CD45 and CD34, with a total of ~250,000 cell mimics per vial (i.e. ~225,000 CD34+ cell mimics per vial). While not a complete validation, herein we experimented to see if these cell mimics could possibly assist in the estimation of sensitivity on a flow based clinical test.

Method

The lyophilized vial kept at -20°C was spun down to pellet all cell mimics before reconstituting with 500 µL of PBS with 1% BSA. The cell mimics were mixed by pipetting and then directly pipetted from the vial into whole blood or diluted with PBS with 1% BSA before adding to whole blood at different volumes.

Whole blood was obtained from two healthy adult donors on the same day as testing. All assays were performed within 8 hours of cell mimic reconstitution to avoid degradation.

Blood and cell mimic mixtures were lysed with ammonium chloride after being stained with CD45 FITC and CD34 PE. Washed samples were then incubated with 7AAD for 10 minutes before being topped up using PBS with 4% FBS, and ran on a BD Bioscience FACS Lyric. Resulting data was analyzed on FCS Express v6.

Results

We first examined the system linearity by spiking in decreasing volumes of cell mimics into a similar volume of whole blood. This was performed in two sets of 'high' and 'low' cell mimic numbers. The data below compares the theoretical number of cell mimics that should be collected against the actual number of cell mimics gated. Values normalized for volume acquired on the cytometer and naturally occuring CD34+ cells from the donor themselves which was estimated from a tube ran without any spiked in cell mimics. The protocol includes multiple wash and spin steps and so we expect some loss in the experimental cell mimic counts.

Highly linear values were found throughout the test range, down to approximately 150 spiked in cell mimics per test sample (0.2% of lymphocytes) with R2 values exceeding 0.99. Tubes J and K showed persistently higher experimental values compared to theoretical values, suggesting background noise overcoming the signal or possibly from variability in the number of naturally occurring CD34+ cells. If repeated on a sample with depleted CD34 cells, we predict that these values could be more accurate.

Table 1 Layout of blood spiked with varying number of cell mimics. Experiment done in two parts for high and low cell mimic counts. The number of theoretical cell mimics spiked and actual number gated displayed showing as expected slightly lower values experimentally. The percentage cell mimics of total CD45+ events also displayed. n.d. no data, where a technical error forced that datapoint to be removed.

			Actual Cell Mimic # Acquired		% Cell Mimics of Total CD45+	
	Tube	Theoretical Cell Mimic#	Donor 1	Donor 2	Donor 1	Donor 2
High set	A	18000	14846	13870	9.03	14.37
	B	9000	7722	7464	4.86	7.77
	C	4500	3789	3874	2.45	4.19
	D	2250	1870	1834	1.27	2.27
	E	1125	992	871	0.68	1.13
Low set	F	1218	873	865	0.73	1.03
	G	609	485	490	0.37	0.62
	H	305	n.d.	272	n.d	0.35
	I	152	151	161	0.12	0.20
	J	76	84	90	0.08	0.12
	K	38	68	69	0.07	0.09

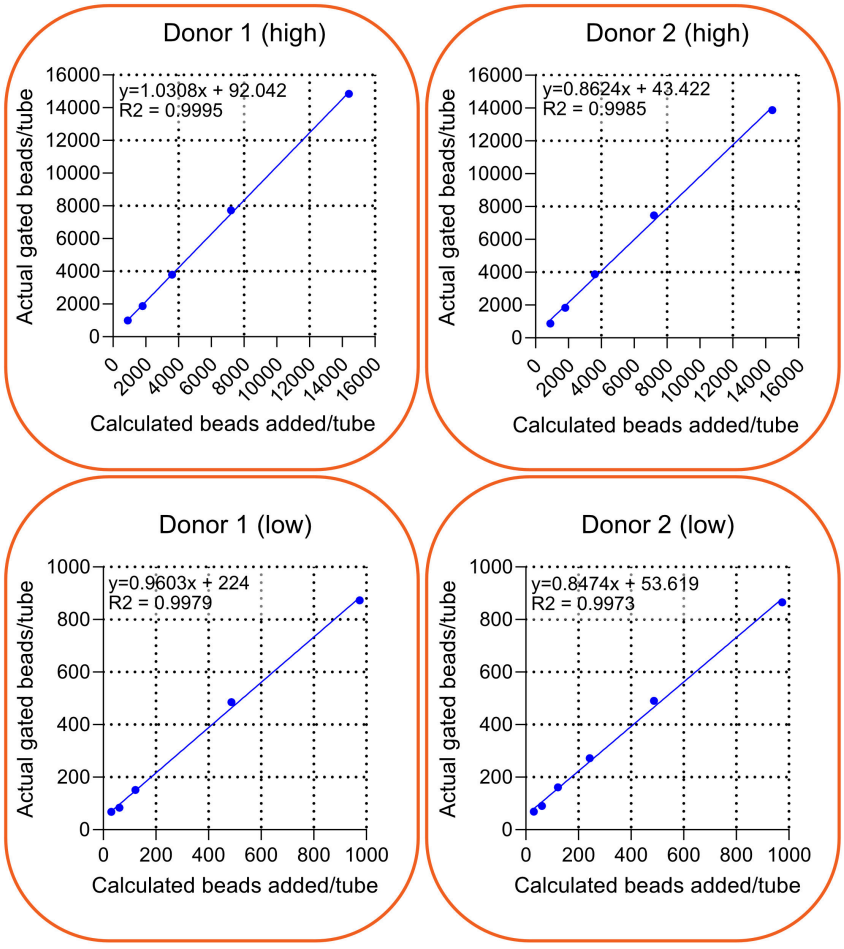

Figure 1 Graph plotting data displayed in Table 1. Very high R2 values found on both samples, and regardless of spiked cell mimic concentration.

Tubes F-K were repeated a total of three times and the percentage of CD34+ cell mimics of total CD45+ events extracted. The %CV from the three repeats are calculated and presented in the table below. These values have been corrected for volume analyzed on the cytometer and the natural CD34+ cells found per donor. Traditionally, a limit of CV <30% is taken for determining the limits of quantification. From the table below, while Donor 2 shows a small %CV down to the last dilution, Donor 1 has a CV>30% after dilution J (in red). For both these samples, the lower limit of quantification (LLOQ) can be deduced to be close to 0.05% (in green). These results indicate that only levels ≥0.05% CD34+ of total CD45+ events are reportable for this assay. Percentages found lower than 0.05% cannot be reported confidently due to possibly large %CVs.

Table 2. Estimating the LLOQ of the assay based on the CV>30% cutoff rule. In green are the limits where CV<30% are noted with both hovering around 0.05%.

	%CD34+ of CD45+		Ave. %34+ of CD45+		%CV	
Tube	**Donor 1**	**Donor 2**	**Donor 1**	**Donor 2**	**Donor 1**	**Donor 2**
F1	0.708	1.055	0.71	0.99	2.70	10.70
F2	0.724	0.865				
F3	0.686	1.039				
G1	0.346	0.567	0.34	0.57	4.20	0.86
G2	0.325	0.573				
G3	0.353	0.577				
H1	n.d.	0.316	n.d	0.30	n.d	10.64
H2	n.d.	0.327				
H3	n.d.	0.266				
I1	0.084	0.164	0.09	0.15	6.85	9.18
I2	0.093	0.162				
I3	0.095	0.138				
J1	0.051	0.069	0.05	0.07	13.05	11.02
J2	0.041	0.074				
J3	0.052	0.059				
K1	0.023	0.046	0.03	0.04	65.72	13.90
K2	0.058	0.035				
K3	0.018	0.040				

Conclusion

In this short report, we have evaluated the use of Slingshot CD34+ Trucytes as a possible tool for estimating limits for flow cytometry based tests. Based on the experimental results, we can expect robust linearity of results down to 0.2% of total gated CD45+ events with a lower limit of quantification set at 0.05% CD34+ of total CD45+ events.

Acknowledgments

CD34+ cell mimics were a kind gift from Slingshot Biosciences.

The Diagnostic Immunology Laboratory (DIL) is a CLIA certified and CAP accredited clinical laboratory at Cincinnati Children's Hospital Medical Center. Our comprehensive test menu has been developed to assist in the diagnosis and management of rare inborn errors of immunity (IEI), pediatric oncology, platelet and red blood cell disorders.