

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.

	Tube	Theoretical Cell Mimic#	Actual Cell Mimic # Acquired		% Cell Mimics of Total CD45+	
			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 $\geq 0.05\%$ 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%.

Tube	%CD34+ of CD45+		Ave. %34+ of CD45+		%CV	
	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

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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.