EVALUATING A SYNTHETIC MAST CELL PARTICLE

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Background

Clinical flow cytometry laboratories require quality control materials for reagent qualification and assay performance. Typically, assay-specific materials include commercial cryopreserved blood, cell lines, and laboratory derived materials such as patient samples. However, finding suitable controls for populations with uncommonly expressed antigens, such as hairy cell leukemia, or for rare populations, such as mast cells, can be difficult. Standardized materials for these applications would be of substantial benefit to the flow cytometry community. To that end, we evaluated synthetic particles, developed together with, and manufactured by, Slingshot Biosciences, designed to phenotypically mimic abnormal mast cells. These particles have the same the forward/side scatter properties of mast cells and are presented with varying levels of CD45, CD33, CD117, CD2, CD25 and CD30 antigens consistent with mast cell disease. We evaluated several performance characteristics using ARUP's high sensitivity clinical mast cell assay.

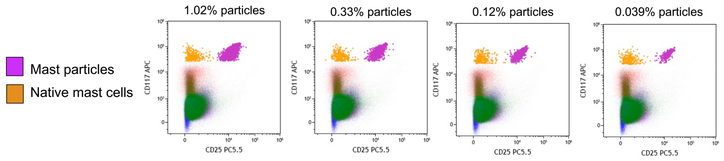
ARUP Clinical Mast Cell Assay

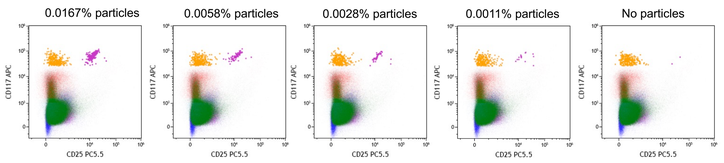
FL2	FL3	FL4	FL5	FL6	FL7	FL8	FL9	FL10
CD123 PE	CD34 PE-CF594	CD25 PC5.5	CD33 PC7	CD117 APC	CD30 APC A700	CD2 APC/Fire A750	DAPI	CD45 KrO

1.7 million events are acquired on a Navios flow cytometer using this panel for a clinically validated LOD of 0.003%

Limit of Detection

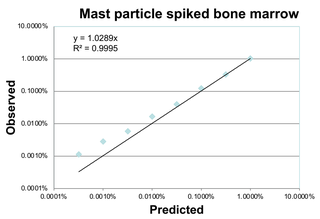
Mast cell particles were spiked into normal bone marrow at a starting concentration of 1.0%, and serially diluted by half-log dilutions into normal bone marrow to a final concentration of approximately 0.0003%. Cells were stained with ARUP's mast cell panel. 1.7 million events were acquired for each dilution on a Navios cytometer and analyzed using Kaluza software.





Mast particle spiked bone marrow

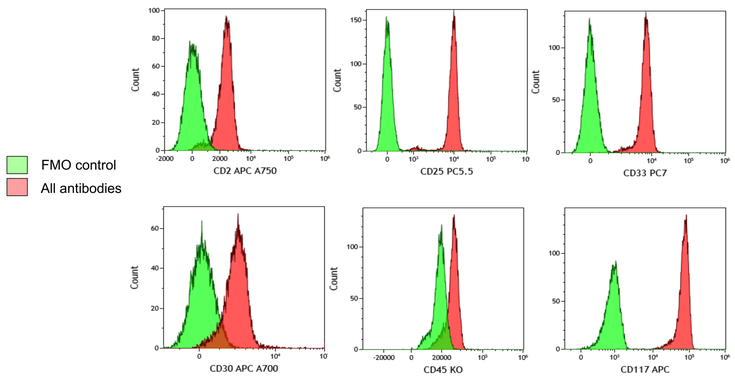
% Particles Predicted	# of particle events	% Particles Observed
1.0000%	15553	1.0243%
0.3162%	5034	0.3325%
0.1000%	1863	0.1232%
0.0316%	599	0.0394%
0.0100%	251	0.0166%
0.0032%	88	0.0058%
0.0010%	43	0.0028%
0.0003%	17	0.0011%
None	2	0.0001%



Mast cell particles could clearly be delineated from normal bone marrow down to a concentration of ≤0.0028%.

FMO Controls

Mast particles were stained with ARUP's mast cell panel (above), or with the mast cell panel without CD2 APC A750, CD25 PC5.5, CD33 PC7, CD30 APC A700, CD45 KrO or CD117 APC. Overlay histograms are shown below with the full mast panel together with the panel missing the indicated marker. Particles appear to exhibit some binding of CD45 in the CD45 KrO FMO sample because the particles are designed with cell-like autofluorescence in violet channels.

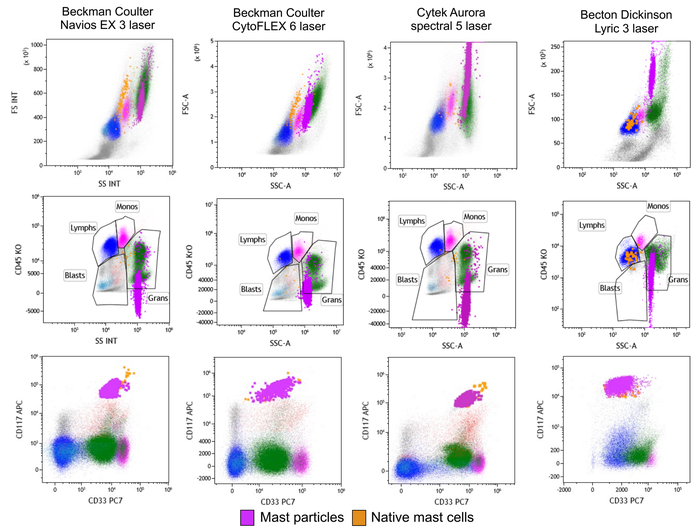


This data demonstrates specificity of each of the expressed markers.

Particle scatter properties on various cytometer platforms

Mast particles were spiked into normal bone marrow and stained with the mast cell panel. Samples were run on the cytometers shown below.

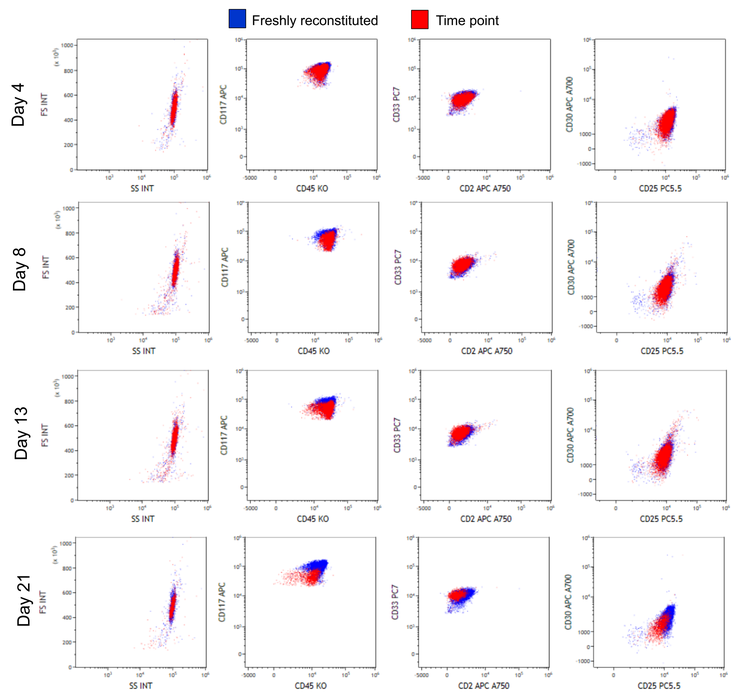
The Navios, CytoFLEX and Aurora were tested with the particles spiked into the same bone marrow sample. The Lyric was tested with particles spiked into a different sample and used CD34 FITC instead of CD34 PE-CF594 in the mast cell panel.



Although some differences in forward scatter were seen among the platforms, the particles performed similarly on all instruments tested.

Particle Stability

Mast particles were reconstituted from lyophilized vials as specified and stored at 4ºC. for the indicated number of days and compared to freshly reconstituted particles after staining with the mast panel. Overlay dot plots and MFI intensity changes are shown below of forward scatter, side scatter and each of the markers expressed by the particles.



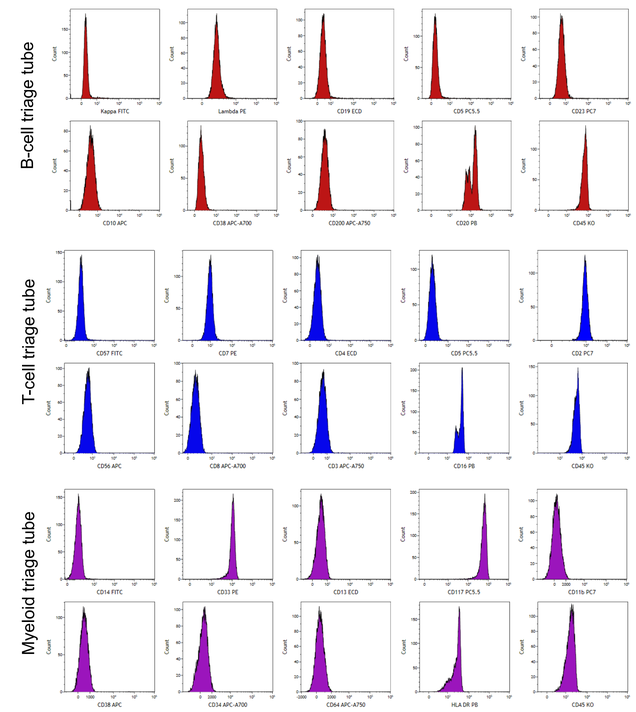
Change in MFI

	FS	SS	CD45	CD33	CD117	CD2	CD25	CD30
Day 4	0.0%	0.2%	-17.1%	-12.8%	-14.9%	-10.7%	-11.6%	-11.7%
Day 8	0.6%	0.8%	-10.1%	0.6%	-23.1%	-17.6%	-12.0%	-15.7%
Day 13	0.2%	0.2%	-8.7%	-0.2%	-29.5%	-22.2%	-10.5%	-15.6%
Day 21	0.4%	0.0%	-44.9%	-1.2%	-47.7%	-36.7%	-30.1%	-35.4%

Slight losses of marker intensities were seen at time points up to day 13, but more significant losses were observed by day 21. Forward scatter and side scatter were virtually unchanged at all time points tested.

Evaluation of Off-target Binding

Mast particles were stained with ARUP's 3 tube 10-color leukemia/lymphoma triage panel with B-cell, T-cell, and myeloid specific tubes. Particles appear to exhibit some binding with violet dyes because they are designed with cell-like autofluorescence in those channels.



Except for the markers they are designed to express, as well as the autofluorescence they exhibit in the violet channels, the particles exhibited no off-target activity on any of the other markers tested.

Precision

Mast particles were spiked into normal bone marrow in five replicate tubes and stained with the mast cell panel to measure between run precision. One replicate was run 5 times for within run precision. 250,000 events were collected, the mast particle percent of total FS/SS gated events was calculated for each of the replicates and runs, and the %CVs were calculated.

Between run precision

	# FS/SS total events	# particle events	% particles
Replicate 1	239562	2647	1.10%
Replicate 2	238333	2395	1.00%
Replicate 3	243562	2777	1.14%
Replicate 4	247689	2963	1.20%
Replicate 5	244568	2963	1.21%
		Average	1.13%
		Standard Deviation	0.08%
		% CV	**7.32%**

Within run precision

	# FS/SS total events	# particle events	% particles
Run 1	239562	2647	1.10%
Run 2	243144	2528	1.04%
Run 3	240698	2613	1.09%
Run 4	238112	2559	1.07%
Run 5	240156	2444	1.02%
		Average	1.06%
		Standard Deviation	0.04%
		% CV	**3.32%**

CVs for the percent of particles spiked into bone marrow for between run and within run precision were both <10%.

Conclusions

- The mast mimic particles were found to perform similarly on BC Navios and CytoFLEX, BD FACSLyric, and Cytek Aurora platforms.
- FMO controls demonstrated specificity of each of the coupled markers.
- Furthermore, when tested against our extensive library of antibodies, the particles exhibited no evidence of off target binding.
- Limit of detection was found to be ≤0.003% in a limiting dilution assay.
- Inter- and intra-assay precision were less than 10% CV.
- Finally, while Slingshot Biosciences recommends these particles be used within several days after reconstitution, we found they were stable to 13 days with minimal loss of fluorescence intensity.
- These particles represent an exciting new technology which fills a vital need in the clinical flow cytometry laboratory.

Conflict of Interest/Disclosures: Artificial mast cell particles were provided free of charge to ARUP by Slingshot Biosciences for testing