Evaluation of Red Blood Cell Microparticles on the CytoFLEX

APPLICATION NOTE

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IN THIS PAPER YOU WILL

- Learn how to prepare microparticles from red blood cells
- Learn how to stain microparticles for analysis by flow cytometry
- Identify RBC microparticles using the CytoFLEX flow cytometer

Introduction

With the understanding that micro- and nanoparticles are mediating many important biological responses, it is critical to be able to analyze these particles. To demonstrate the capabilities of the CytoFLEX to resolve small particles, analysis of Red Blood Cell (RBC) microparticles from human peripheral blood was performed. Three month old human blood samples, stored under standard blood banking conditions, were stained with Annexin V (AnnV). Forward scatter and side scatter revealed RBC microparticles, transition events, as well as, intact red blood cells.

The flow cytometry platforms used for these evaluations are for research use only.

Materials and Methods

1. Randomly selected packed RBC units were obtained from the Central Blood Bank, Pittsburgh, PA
2. Units were nonleukoreduced and preserved in ADSOL solution (standard practice at University of Pittsburgh Medical Center)
3. Quantification of microparticles was performed for each unit of packed RBC
4. 2 µL PE conjugated Glycophorin A was added to 5 µL RBC
5. Incubate 30 minutes at room temperature (RT) in the dark
6. Add 2 µL FITC-Annexin V followed by 500 µL of Annexin V binding buffer, per kit instructions
7. Incubate 30 minutes in dark at RT
8. Analyze by flow cytometry – acquire at least 100,000 events per sample at event rates not exceeding 10,000 eps
9. Use SSC and FSC both set to log scale
10. Microparticles were quantified as a percent of glycophorin A positive events

Full description of methods can be found in Reference 1.

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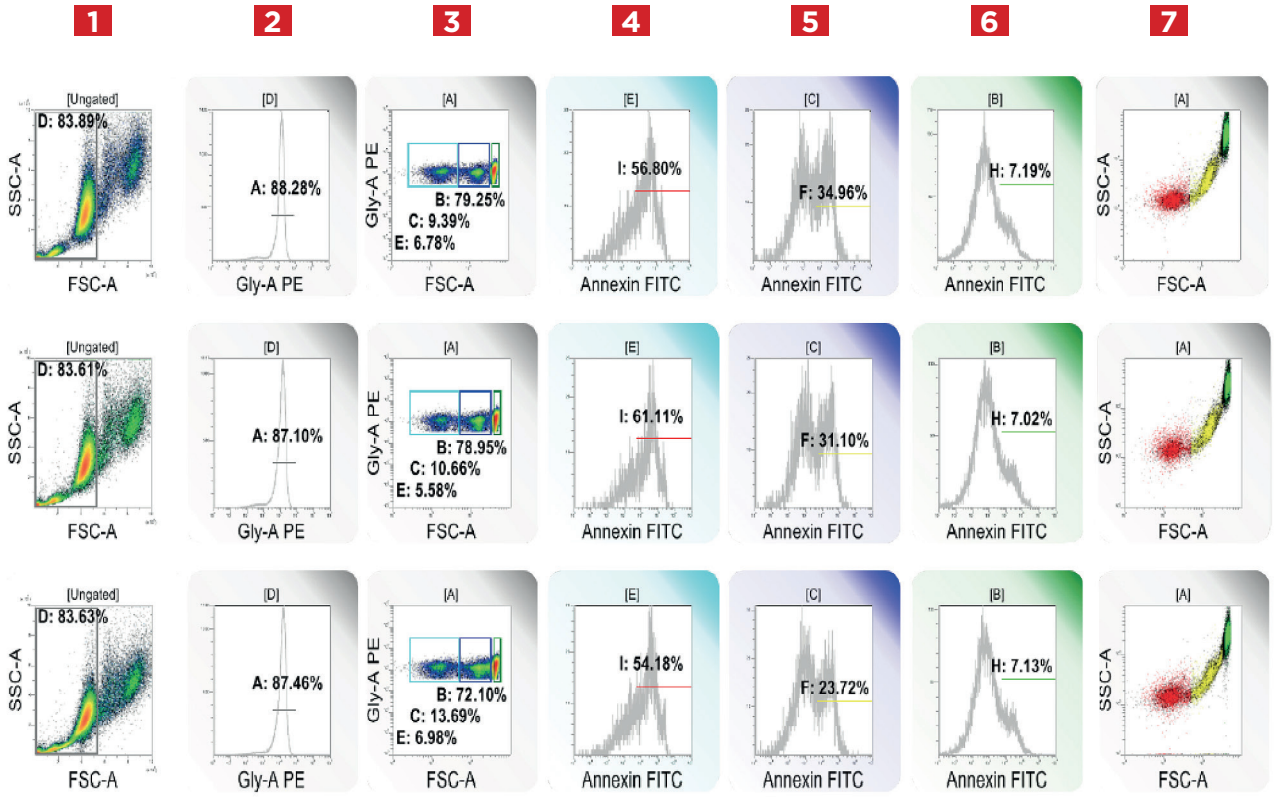
Results

The gating strategy is described shown in Figure 1 utilizing the glycophorin A staining as a method to discern RBC and derivative populations. Samples were prepared in replicate and run to calculate precision of instrument reading and provide statistical validity to the method and data.

Figure 1.



Legend from left to right colums:

1 RBC clusters are eliminated

2 Glycophorin dim events are eliminated

3 Glycophorin+ events are subsetted on the basis of forward light scatter into low, intermediate and high populations

4 5 6 Annexin V binding (surface phosphatidyl serine) is detected in low (E, red), intermediate (C, yellow), and high (B, green)

7 Pseudocolored events from the annexin V histograms backgated to a dotplot of FSC by SSC displays RBC microparticles (red), transitional events (yellow), and intact RBC (green).

Table 1

Replicates were performed and inter-replicate precision is shown by CV.

Sample	Total Clean events (A)	FS_Low	FS_Int	FS_Hi	Microparticles Annex P_FS_ Low	Transitional Annex P_ FS_Int	Intact FBCAnnex P_FS_Hi
W084514A	84010	11.2%	1.0%	86.4%	63.0%	18.9%	7.6%
W084514B	83365	11.7%	2.6%	83.7%	59.0%	19.6%	7.4%
W084514C	82680	11.0%	4.4%	81.8%	61.9%	17.5%	7.2%
Mean	83352	11.3%	2.7%	84.0%	61.3%	18.7%	7.4%
SD	665	0.4%	1.7%	2.3%	2.1%	1.1%	0.2%
CV	0.8%	3.2%	64.1%	2.7%	3.4%	5.7%	2.5%
W085315A	84586	9.7%	1.8%	86.2%	55.9%	15.6%	5.2%
W085315B	82371	9.2%	0.4%	88.5%	57.0%	31.0%	5.7%
W085315C	85298	8.6%	1.9%	87.1%	54.8%	15.1%	4.8%
Mean	84085	9.2%	1.4%	87.3%	55.9%	20.6%	5.2%
SD	1526	0.5%	0.9%	1.1%	1.1%	9.0%	0.4%
CV	1.8%	5.8%	62.6%	1.3%	2.0%	43.8%	8.4%
W086114A	79488	6.8%	9.4%	79.3%	56.8%	35.0%	7.2%
W086114B	73871	5.6%	10.7%	79.0%	61.1%	31.1%	7.0%
W086114C	79430	7.0%	13.7%	72.1%	54.2%	23.7%	7.1%
Mean	77596	6.4%	11.2%	76.8%	57.4%	29.9%	7.1%
SD	3226	0.8%	2.2%	4.0%	3.5%	5.7%	0.1%
CV	4.2%	11.7%	19.6%	5.3%	6.1%	19.1%	1.2%

Conclusions

Microparticles from RBC can be detected along with intermediate products and intact RBCs using the CytoFLEX flow cytometer. Additionally, inter-sample replicates showed a high degree of precision as calculated using CV.

Notes

The results demonstrated in this application sheet represent those generated on the Beckman Coulter CytoFLEX Flow Cytometer. As differences exist in the performance between analyzers, the author cannot guarantee a similar appearance with the use of other flow Cytometers.

Reagent Details

Reagent	Supplier	Order Details
Annexin V – FITC kit	BD-Pharmingen	556747
Glycophorin A – PE	BD-Pharmingen	340947

Reference

1. Donadee C, Raat NJH, Kanias T, Tejero J, Lee JS, Kelley EE, Zhao X, Liu C, Reynolds H, Azarov I, Frizzell S, Meyer EM, Donnenberg AD, QU L, Triulzi D, Kim-Shapiro DB, Gladwin MT. Circulatoin. 2011;124:465-476. Nitric Oxide Scavenging by Red Blood Cell Microparticles and Cell-Free Hemoglobin as a Mechanism for the Red Blood Cell Storage Lesion.

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