Residual leukocyte count in canine purified platelet samples


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Abstract

Assessment of residual leukocytes in blood component samples is crucial for the determination of quality. Standard hematology analyzers are typically not suitable for these samples due to their lower detection limit. The objective of this study was to describe the use of a bead-based flow cytometric method (LeucoCOUNT, BD Biosciences, San Jose, CA) for counting leukocytes remaining in purified canine platelet samples. Leukocytes were also manually counted utilizing the hemocytometer method. Platelets were isolated from whole blood through density gradient centrifugation and leukocyte depletion with CD45-labeled beads. For manual counting, samples were loaded in the chamber, with or without dilution, depending on platelet concentration as to allow adequate visualization of the leukocytes. The LeucoCOUNT method was performed according to manufacturer’s instructions. Briefly, samples and the detergent/propidium iodide reagent were added to the bead-containing tubes. A FACScalibur flow cytometer was used to analyze the samples to a total of 30,000 bead events. From a total of 52 samples, no leukocytes were found by the hemocytometer method in 71% (37) samples. The LeucoCOUNT method counted leukocytes in all but 4 samples (7.7%) that had fewer cells than the detection limit of the test (for these samples, no cells were found on the hemocytometer method as well). In samples quantified by both methods (15), the LeucoCOUNT numbers were, on average, 1.8 times higher than the manual count. These results demonstrate that the LeucoCOUNT has a higher sensitivity than the manual hemocytometer count for enumerating canine leukocytes and that it is suitable for use in samples with low leukocyte numbers.

Introduction

Techniques for the characterization of platelet biology, such as proteomics and transcriptomics, and functional assays require samples with very low levels of leukocyte contamination, since they contain 12,500 times more mRNA and 65 times more protein than platelets (1). Therefore, it is crucial that the leukocyte number of these samples is accurately assessed.

Standard hematology analyzers are typically not suitable for these samples due to their lower detection limit (2), which is inappropriate for samples with very small concentrations of leukocytes, and will result in inaccurate results. They also usually need a large sample considering the small final volume of neat purified highly concentrated platelet samples (e.g. 130μl by Cell Dyn 3700).

In human transfusion medicine, both manual counting and flow cytometric methods are used for quality control of blood components samples (3).

The objective of this study was to describe the use of a bead-based flow cytometric method (LeucoCOUNT, BD Biosciences, San Jose, CA) for counting leukocytes remaining in purified canine platelet samples.

Materials and Methods

Canine purified blood platelets, diluted samples

Leukocytes counted in hemocytometer

Leukocytes counted by LeucoCOUNT system, using a FACScalibur (BD Biosciences)

Results

Neither methods found leukocytes (4)

Leukocyte number determined by both methods (15)

Figure 1. Number of samples with leukocytes assessed by either LeucoCOUNT, hemocytometer, or both methods. Total of 52 samples.

Less (8)

More (4)

Similar(4)

Figure 2. Leukocytes concentration found by the LeucoCOUNT method compared with hemocytometer results.

In these 15 samples where leukocytes were quantified by both methods, LeucoCOUNT numbers were in average 1.8 times higher than the hemocytometer.

Discussion and Conclusion

The manual method failed to find leukocytes in the majority of the samples, due to its lower detection limit compared to the flow cytometric method. The correlation between the two methods was low in the 15 samples that were quantified by both, probably due to the inherent error-variability of the manual method (4).

The results demonstrate that the LeucoCOUNT has a higher sensitivity than the manual hemocytometer count for enumerating canine leukocytes and that it is suitable for use in samples with low leukocyte numbers.

References


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