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Determination of poultry heterophile functions by flow cytometry

Erdal Matur*[1], Mert Erek[1], Ezgi Ergen[1], Bilge Acar Bolat[2], Mukaddes Özcan[1]

1. Istanbul University, Veterinary Faculty, Department of Physiology, 34320, Avcilar, Istanbul, Turkey **2.** Istanbul University, Faculty of Business Administration, Department of Quantitative Methods, University of Istanbul, 34320, Avcilar, Istanbul, Turkey

Abstract

The aim of this study is to modify the flow cytometric methods for avian heterophil that used to analyze neutrophil functions. Within the aim of the project, we tested the amount of blood for acquiring the heterophil and the storage duration of the blood before the analysis and the time of centrifugation. Also, we tested the amounts of cell suspension and dihydrordamine-123 (DHR-123) during the flow cytometric analysis. We reviewed the amount of porbol miristat asetat (PMA) used to stimulate the oxidative burst and the amount of formyl methionyl-leucyl-phenylalanine (fMLP) used to stimulate chemotaxic activity. Experiments on the incubation temperature and incubation duration were also performed. The results showed that 0.5-3 ml of blood could be used to detect heterophil functions and it would be ideal to study in fresh blood samples. However, it also showed that the stored blood can be used for a maximum of 8 hours at +4 degrees. In order to isolate the cells, centrifugation of blood samples for 30 minutes would be sufficient, and it would be appropriate to use 30µL from the cell suspension. DHR-123, which is used as a chemical probe to measure heterophil functions, had to be used in 2µL, and when used excessively, it affected the heterophil functions negatively. In addition, it was seen that using 2µL each of fMLP, which is used as an oxidative burst stimulant, and PMA as a stimulant of chemotaxic activity was sufficient. It was concluded that the incubation at 41 ° C for 5 minutes after stimulating the heterophil would also be sufficient. As a result, it was thought that this study could be used to isolate heterophil and to analyze with flow cytometry and to contribute further research and clinical studies in poultry.

Keywords: Heterophil, flow cytometry, phagocytic activity, oxidative burst, chemotaxic activity

*Corresponding Author: Erdal Matur
E-mail: mature@istanbul.edu.tr

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