

Functional characterization of Largemouth bass (*Micropterus salmoides*) soluble FcγR homolog in response to bacterial infection

In the manuscript reviewed by the authors, no reference to the comments I have made has been included, I send them again.

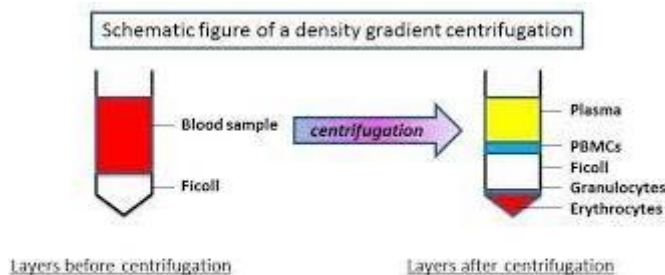
Show the detection histograms of leukocyte populations, when the cells are extracted with Ficoll by centrifugation, the cell layer contains lymphocytes and monocytes, the population of neutrophils sediments together with the erythrocytes.

In phagocytosis experiments, the only population that phagocytoses is that of monocytes, therefore the value that is observed is a value of monocytes. This problem can be solved in several ways

1 Lying peripheral blood with commercial erythrocyte lysing solution, in that case FSC vs SSC morphology will clearly separate lymphocytes, monocytes and neutrophils.

2 Separating the population with Ficoll but adding a pan-leukocyte antibody such as CD45.

Example of separation of populations with Ficoll



You can work with whole peripheral blood using a buffer that lyses erythrocytes and leukocytes remain intact for functional studies of oxidative burst, in this case information on lymphocytes, monocytes and neutrophils is obtained.