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An Overview of Flow Cytometry and Its applications in Veterinary Science

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Measurement of the physical and chemical characteristics of cells is called cytometry (Erotocritou *et al.*, 2007). Flow cytometry is a simple and rapid method for the quantitative and qualitative analysis of single-cell suspensions. Flow cytometry is a laser-based technology whereby cells are suspended in a fluid stream and stimulated by a laser, the cells scatter and/or emit light which is then detected by photomultiplier-based tubes (PMTs) (Papenfuss, 2017). All parameters measured can be divided into two main groups: 1) those related to light scattering, which mainly reflects the size of the cell and its internal complexity and 2) those related to fluorescence. These are associated with the presence of one or more fluorochromes inside the cell or attached to the cell surface membrane, either naturally (autofluorescence) or artificially (e.g., using fluorochrome conjugated monoclonal antibodies) (Erotocritou *et al.*, 2007).

Flow cytometers are composed of three main systems: fluid system, optical system and electronic system. The fluid system takes the sample and directs the cells to the interrogation point. In order for cells to be best illuminated, they must pass one by one through the center of the laser beam. The fluidic system works on the principle of hydrodynamic focusing where a sheath fluid, usually a buffered saline, compresses the specimen core into small area. Cells are forced to pass singly through the focal point of the laser, which is crucial for collecting accurate information about each cell in the specimen (Sanchez-Torres *et al.*, 2022).

The optical system is composed of two systems, the excitation system (laser beams) and the signal collector. When the laser encounters a cell, it is scattered depending on the physical properties of the cell, in particular its size and internal complexity. The scattered light is captured by a front-end detector (forward scatter = FSC), and the value reported is proportional to the cell surface or size of the illuminated particle. On the other hand, the laterally scattered light is captured by another detector located at 90° to the laser (side scatter = SSC), in this case, the value generated is proportional to the internal complexity of the cell or particle. Cells being illuminated at the analysis point scatter light or, if labelled with suitable fluorescent probes, emit light at specific wavelengths (Reggeti and Bienzel, 2011). A fluorescent compound absorbs light energy over a range of wavelengths that is characteristic for that compound. This absorption of light causes an electron in the fluorescent compound to be raised to a higher energy level. The excited electron quickly decays to its ground state, emitting the excess energy as a photon of light. This transition of energy is called fluorescence (Robinson, 2022). Commonly employed fluorescent probes include fluorescein isothiocyanate (FITC), phycoerythrin (PE), and allophycocyanin (APC) (Papenfuss, 2017).

The electronic system is responsible for converting the optical signals into proportional electronic signals or voltage pulses. Light signals are generated as each cell passes through the laser beam. These light signals are transformed into electronic signals by photodetectors and, based on their intensity, are assigned a relative value on a

scale (Sanchez-Torres *et al.*, 2022).

Flow cytometry data analysis has been considered as the most critical parameter for biological experiments. The major principles of data analysis are to selectively show the cells of interest. This method is called ‘‘gating’’ in flow cytometry. Gating can provide an opportunity to eliminate results from the unwanted particles such as dead cells and debris. The data can be displayed by several different plot types. These range from histograms to 2-D plots such as dot plots, contour and density plots, to 3-D plots such as a tomogram plot (Adan *et al.*, 2017).

There are two different types of flow cytometry – named as non-sorting and sorting. Non-sorting type can perform light scattering and fluorescence emission while the sorting type has the ability to sort particles as well. Fluorescent activated cell sorters (FACS) are flow cytometers that have the capacity to sort fluorescent-labeled cells from a mixed cell population (McKinnon, 2018).

With the flexibility of flow cytometry, and the reliability of results, this technology has become an essential veterinary research instrument with important clinical diagnostic capabilities, particularly in the fields of immunology and hematology (Thomason *et al.*, 2014). It is possible to perform functional tests, biological evaluations of compounds, purify cell populations even when they are present in a low proportion in the sample. The most common use of flow cytometry is in the identification of markers on cells, particularly the immune system or immunophenotyping. It is one of the most popular and versatile techniques for studying apoptosis. It can be applied as a rapid, precise and objective tool for routine cell viability measurement that overcome the problems associated with the traditional haemocytometer and trypan blue exclusion method. It can also use to evaluate cell proliferation, metabolic activity and intracellular signalling (Papenfuss, 2017). Flow cytometry has been extensively used to study mammalian sperm in the areas of reproductive toxicology (to monitor effects from environmental, occupational and therapeutic exposures), veterinary science (to preselect the gender of offspring by sorting X- and Y-chromosome-bearing sperm) and clinical andrology (to assess individual fertility potential) (Cordelli *et al.*, 2005). Flow cytometry is most often applied in veterinary oncology to diagnose lymphoid neoplasia and leukemia, and can also be used to assess neoplastic and non-neoplastic marrow conditions (Thomason *et al.*, 2014).

In Conclusion, Flow cytometry is a technology that provides rapid multi-parametric analysis of single cells in solution. Flow cytometers utilize lasers as light sources to produce both scattered and fluorescent light signals that are read by detectors such as photodiodes or photomultiplier tubes. These signals are converted into electronic signals that are analysed by a computer. Flow cytometry is a powerful tool that has applications in immunology, molecular biology, pharmacology, cancer biology and infectious disease monitoring.

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