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Review

The role of flow cytometry in companion animal diagnostic medicine

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Abstract

Flow cytometry is a powerful tool for characterising the composition of complex cell populations. The accuracy and precision of this technology for describing and enumerating cells exceeds traditional methods. The number of diagnostic veterinary laboratories with access to a dedicated machine is increasing, and there is the potential to offer a clinical flow cytometry service.

The improved availability of monoclonal antibodies (mAb) to cell markers expressed by the leukocytes of companion animals, permits the implementation of comprehensive mAb panels suitable for diagnosis of lympho- and myeloproliferative disease. Reticulated erythrocyte and platelet quantification, antiglobulin assays for immune-mediated cytopenias, lymphocyte subset analysis, and immunophenotyping of lymphoma and leukemia, have been validated for companion animal samples on the flow cytometer. It is now timely to consider the role of flow cytometry in diagnostic practice, and the requirement for quality assurance and standardization of testing procedures.

Keywords: Flow cytometry; Immunophenotyping; Leukaemia; Lymphocyte subset

1. Introduction

The flow cytometer has quickly become an indispensable tool for medical research, particularly in the fields of immunology and haematology. Both the physical characteristics and proteins expressed by individual cells can be analysed by flow cytometry, based on the detection of light scatter and fluorescent markers when a laser beam strikes a cell preparation. The technology has been swiftly adopted by manufacturers of haematology analysers and transfigured into a platform that can enumerate erythrocytes, leukocytes, and platelets, and differentiate white cells. In comparison to haematology analysers, dedicated flow cytometers incorporate a number of laser sources, with greater optical capacity, and a more flexible output.

Flow cytometry has the capability to simultaneously measure multiple parameters of individual cells in complex cell mixtures with exquisite sensitivity and specificity. This facilitates the detection of abnormal cells in peripheral blood samples, or cell suspensions prepared from solid tissue biopsies, leading to improved accuracy in the diagnosis of haematological and lymphoproliferative disease. With the increasing specialisation of veterinary practice, a clientele willing to use these services, and the expanding knowledge base in veterinary medicine, there is interest by both researchers and clinicians to use flow cytometry in the diagnosis and investigation of disease.

This review will describe the current and potential use of flow cytometry in veterinary diagnostic medicine. A brief description of the technology and techniques are provided, however the reader is encouraged to refer to the many excellent textbooks covering this topic (Keren et al., 2001; McCoy, 2001; Shapiro, 2003). The technical and diagnostic performance of flow cytometric testing in

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companion animal medicine is discussed, and concerns relevant to the diagnostic laboratory (provider) and clinician (user) are addressed.

2. The technology: the flow cytometer and reagents

Microscopy and electronic particle counters have traditionally been used to define cell populations in blood. Cells are classified by their morphology on stained blood smear preparations, and this information is combined with the enumeration of cells by a particle counter based on size thresholds. Flow cytometry surpasses these methodologies by simultaneously measuring the physical characteristics of each cell, and by way of fluorescent probes to cell markers, objectively identifying the cell and its functional state. Flow cytometry therefore provides a more informative and accurate assessment of the cell populations comprising a given sample in comparison to traditional methods.

The flow cytometer redistributes cells in a solution into a single column by laminar flow and hydrodynamic focusing (Fig. 1). Individual cells are then interrogated by a laser, and the scattered light and fluorescent emission (when using fluorescent probes) of each cell are measured. Low angle deflection of the beam, or forward scatter, provides information on the size of the cell. Reflection and refraction of the beam at high angle, referred to as orthogonal or side scatter, divulge the internal complexity of the cell. The photons are collected by lenses, separated by filters and dichroic mirrors, and detected by photomultiplier tubes (PMT) and photodiodes (Fig. 2). Conversion of photons to analogue voltages takes place in the photodetector, and both the PMT and amplifier permit manual adjustment of the gain (or sensitivity). This facility contributes to the large dynamic range of the instrument (Snow, 2004). The analogue signal is subsequently digitalized into numerical values, and stored as so-called "listmode" files that can be manipulated with computer software.

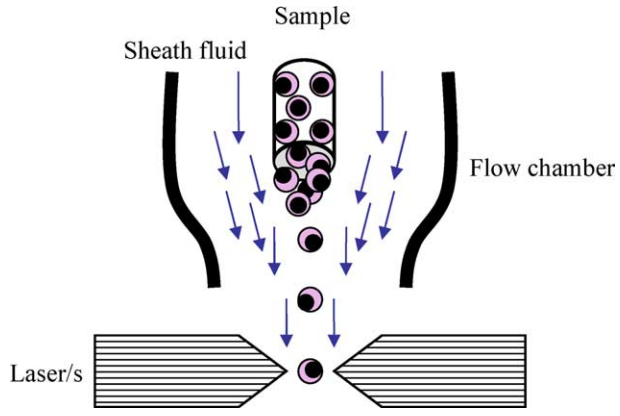

Fig. 1. Hydrodynamic focusing in the flow cytometer. The sample, a suspension of cells in fluid, is focused into a column of single cells by gradually restricting the diameter of the coaxial, laminar streams of sheath fluid and sample.

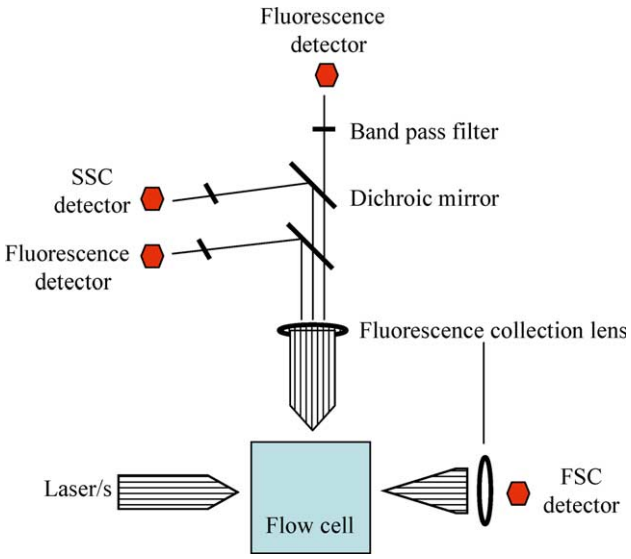

Fig. 2. Flow cytometry optics. Individual cells are interrogated by laser light in the flow cell. The scattered light is collected at low (forward scatter, FSC) and high (side scatter, SSC) angles to measure the physical properties of the cell. The high angle light also contains fluorescent emission from cells prelabelled with florescent markers, which is then separated into different wavelengths by filters (dichroic mirrors and band filters) for detection.

The generation of monoclonal antibodies (mAbs) to antigens expressed by lymphocytes and haemopoietic cells has led to major advances in the fields of immunology and haematology. The antigens recognized by mAbs have been refined into cluster of differentiation (CD) antigen groups, and the expression of these molecules by blood cells is demonstrated by a technique known as immunophenotyping. Differentiation of cell lineage and subsets is now predicated on the antigens expressed on the cell surface, and subsequently, intracytoplasmic and nuclear markers.

Flow cytometry has been at the forefront of immunophenotyping. By coupling fluorochromes to mAbs, the specific binding of antibody to antigen can be detected by the fluorescence emitted by the cell as it passes through the laser. The ability of the flow cytometer to rapidly process millions of cells, at a nominal rate of 5000 s^{-1}, and store the physical and fluorescence details of each cell in listmode data, illustrates the capacity of this technology to characterise constituent cell populations. Initially, the light source was restricted to an argon-ion laser and the choice of fluorochromes was limited. Analysis was therefore constrained to one mAb/flurochrome combination (i.e. colour) for each assay. Subsequently, the advent of new fluorochromes with different emission spectra, and multiple lasers on one platform has resulted in the simultaneous analysis