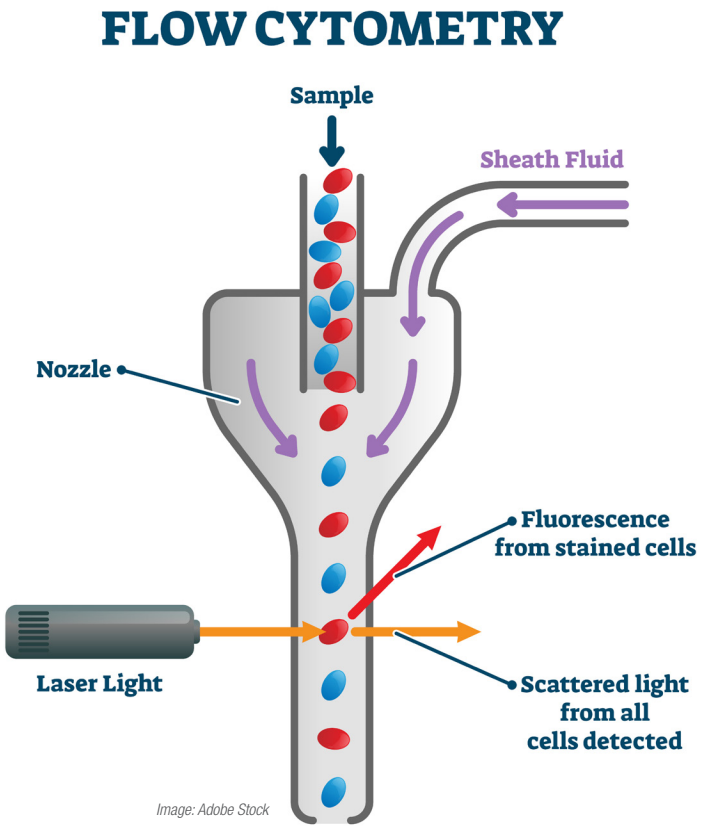
UTCVM IMMUNOLOGY LAB

Immunophenotyping by Flow Cytometry

A TOOL TO IDENTIFY INSIGHTS INTO THE IMMUNE RESPONSE

The immune system is a complex network of cells, tissues, and organs that work together to defend the body against foreign invaders. The cells of the immune system are highly specialized and perform a wide range of functions, from identifying and destroying pathogens to regulating the immune response. Immunophenotyping is a technique that involves the use of antibodies that recognize specific cell surface markers unique to each type of immune cell. The antibodies are labeled with fluorescent dyes that allow them to be detected by a flow cytometer, which is a specialized instrument that measures the fluorescence emitted by each cell.

Sample requirements for canine immunophenotyping vary depending on the specific test or technique used. At UTCVM, we perform immunophenotyping of blood, bone marrow, body cavity fluids (peritoneal, pleural), and solid tissue aspirates, such as lymph nodes. This test is currently validated and available for samples from dogs and cats.



Types of cells that can be identified using immunophenotyping

There are several types of immune cells that can be identified and characterized through immunophenotyping. These include T cells, B cells, natural killer cells, and various subsets of each of these cell types. T cells are responsible for cell-mediated immunity and are involved in the recognition and destruction of infected cells. B cells are responsible for the production of antibodies and play a key role in the humoral immune response. Natural killer cells are a type of lymphocyte that is capable of killing infected or abnormal cells.

In addition to these major cell types, there are various subsets of each of these cell types that can be identified using immunophenotyping. For example, within the T cell population, there are several different subsets, including helper T cells and cytotoxic T cells.

Applications of immunophenotyping

Immunophenotyping has a wide range of applications in both research and clinical settings. In research, it is used to investigate the immune response to various pathogens and to study the mechanisms of immune cell activation and differentiation. For example, immunophenotyping can be used to study the response of T cells to viral infections or to investigate the mechanisms underlying autoimmune diseases such as multiple sclerosis.

In clinical settings, immunophenotyping is used for the diagnosis and classification of different types of cancer in animals. By analyzing the expression of different surface markers on cells, veterinarians can differentiate between different types of cancer. This information is

UPDATED 05/11/2023

essential for developing effective treatment plans and improving the prognosis of animals with cancer.

Immunophenotyping can also be used to monitor the response of tumors to chemotherapy or other treatments. By analyzing the expression of certain surface markers on tumor cells, clinicians can determine whether the treatment is effective and adjust the treatment plan as necessary.

Immunophenotyping by flow cytometry is an incredibly useful diagnostic tool for identifying different cell populations and gaining insights into the immune response. However, it is important to remember the results of an immunophenotyping assay should never be interpreted in isolation. This is because immunophenotyping is just one piece of information that needs to be considered in the context of the patient's clinical history, physical exam, and other diagnostic tests. It is also important to note that immunophenotyping is not a substitute for cytology. Cytology provides valuable information about the morphology and structure of cells, which can aid in the interpretation of immunophenotyping results. In addition, cytology can help to identify any other abnormalities or underlying conditions that may be present in a sample. Cytology is often the first step in the diagnosis of cancer, while immunophenotyping can provide more specific information about the cells present in a sample. Both techniques are complementary and should be used together to provide the most accurate and comprehensive diagnosis possible.

Sample collection and handling for immunophenotyping:

Proper sample handling and storage are crucial for accurate canine immunophenotyping by flow cytometry. The following guidelines should be followed to ensure the integrity of the sample and reliable results:

For peripheral blood, 3 ml of whole blood should be collected using sterile techniques to avoid contamination. Blood samples should be collected using EDTA as an anticoagulant. Mix the sample gently by inverting the tube several times to ensure proper anticoagulation of the blood.

For body cavity fluids, 2-3 ml of fluid should be collected using sterile techniques to avoid contamination.

For lymph node aspirate, a 22-gauge needle is typically used for aspirating lymph nodes. The needle should be sterile and disposable. A minimum of 2-3 and up to 6-8 needle aspirate biopsies should be collected into sterile saline. Adding 10% autologous serum from the patient can help stabilize a lymph node sample for 2-day shipping.

Samples should be shipped overnight on a cold pack. It is important that the sample is analyzed within 48 hours of collection.

It is important to note that while stabilizing the lymph node aspirate sample with autologous serum can help maintain cell viability during transport, it may not completely prevent cell death or degradation. For this reason, it is generally recommended to transport samples as quickly as possible, and to use appropriate shipping conditions (e.g. cold packs) to minimize temperature fluctuations that could further impact cell viability.

There are several reasons why a sample may be rejected for flow cytometry analysis. Some of the most common reasons include:

Insufficient number of cells:
If the sample contains too few cells, it may not be possible to perform accurate and reliable flow cytometry analysis.

Poor sample quality:
If the cells are dead, it may not be possible to obtain accurate and reliable results. This can be assessed by checking for proper preservation and viability of cells.

Inappropriate anticoagulant or fixative:
Using the wrong anticoagulant or fixative can interfere with the accuracy and reliability of flow cytometry analysis.

For more information please visit our website
https://vetmed.tennessee.edu/vmc/dls/immunology/immuno-flow-cytometry/

Dr. Mohamed A. Abouelkhair, DVM, MS, Ph.D., DACVM, CABMM,
Assistant Professor and Director of the Virology Lab
The University of Tennessee | College of Veterinary Medicine
Email: *mabouelk@utk.edu*