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Flow cytometry using FACS

The abbreviation FACS stands for fluorescence activated cell sorting. It is a flow cytometry method with which fluorescence labelled cells can be measured and analyzed.
The cells flow through a laser beam and cell size, infrastructure, and the relative intensity of the fluorescence are determined. Fluorescence labelled antibodies that target the immunophenotypical surface antigens (cluster of differentiation, CD) are available for multiple cell types, particularly lymphocytes. Cells can be studied and identified using these CD-markers. Cytotoxic T cells, for example, are CD3 and CD8 positive and CD21 and CD4 negative.

These assays are used routinely in hematological diagnostics. Since the sample material must be in a fluid form, blood samples are particularly appropriate. In order to obtain reliable results, it is important that the cells are intact. It is therefore necessary that testing be carried out as quickly as possible, within 3 days at the latest. At Laboklin, the FACS machine is used for a variety of important tests.

1. Anti-thrombocyte antibodies
Increased amounts of immunoglobulins (mostly IgG) on the surface of thrombocytes leads to premature phagocytosis of the thrombocytes by macrophages, which causes thrombocytopenia. This immune mediated thrombocytopenia (IMT) can be primary or secondary. The rare primary form in caused by autoantibodies against thrombocyte specific epitopes. Secondary immune mediated thrombocytopenia, on the other hand, is associated with various drugs, infectious agents (bacteria, viruses, protozoa, or helminths), neoplasia, and other immune moderated diseases.

Primary IMT is mostly seen in dogs, twice as often in females as in males, in all age groups, but most commonly in middle aged dogs. Cocker Spaniels, Miniature and Toy Poodles, Old English Sheepdogs (Bobtails), Golden Retrievers, and German Shepherds are predisposed.

At 30 g/l, the number of thrombocytes measured is usually lower in cases of primary thrombocytopenia than in the secondary form.

Diagnosis can be carried out using FACS and an antiplatelet antibody test. For this, a double incubation is done to detect IgG bound to thrombocytes. Antibody levels of <15% are considered negative, while >30% are considered positive for IMT. The sample material (EDTA whole blood) cannot be more than 3 days old in order to allow an interpretable immunostaining. The test must be carried out before beginning immunosuppressive therapy, since this leads to false negative results. Other diagnostic options are a diagnostic therapy with cortison as well as detection of megakaryocytic hyperplasia in the bone marrow.

2. Cellular immune status
The cellular immune status includes a large blood profile as well as the determination of the relative and absolute numbers of peripheral lymphocytes: B cells, T cells, total CD4+ (T helper cells) and CD8+ (cytotoxic T cells) cells.

Immunophenotyping of the blood is based on the selective identification of cell surface antigens by fluorescence labelled monoclonal antibodies by flow cytometry (FACS). Testing of the cellular immune status should be carried out when the clinical signs suggest a primary or secondary disorder of the cellular immune system.

In dogs, the immune status can be helpful for the diagnosis of pyoderma, demodecosis, systemic lupus erythematosus, and

leishmaniasis, as well as congenital T cell defects. Serial testing can be carried out during medicinal treatment for control purposes and to optimize dosing.

In horses, this method is used to clarify repeated and prolonged infections. Determining the immune status is of particular importance in infections with the **feline immunodeficiency virus (FIV)**.

This virus causes chronic-persistent infections with a progressive course of disease. Infection of immunocompetent cells leads to an increasing depletion of the immune system. The virus has a clear tropism for T cells and macrophages. The T cell functions are most strongly affected by the damage, particularly quantitative and qualitative damage of the T helper cell population. At first, an increase in the number of CD8+ cells leads to a percentage decrease of CD4+ cells. The number of CD8+ cells remains high during the asymptomatic period of infection and drops relatively shortly before clinical signs are seen. At first, mostly CD4+ cells are infected, and this is where the highest viral load is found. During the course of infection, an absolute reduction in the number of CD4+ cells leads to a drop in the CD4+/ CD8+ ratio.

The function of the humoral immune system is only damaged relatively late in the infection. A drop in the number of B cells is usually only detected in the final stages. The amount of virus in the blood increases substantially, but no antibody response occurs.

Determining the immune status can therefore help evaluate the prognosis in cases of primary or secondary immune deficiency.

3. Leukemia differentiation

Leukemia differentiation can be carried out using FACS in cases of lymphocytosis of >30 g/l or lymphoproliferative diseases (leukemia, stage V lymphoma) with >5 g/l leukocytes that have been confirmed by clonality testing (PARR). Using CD markers to identify the cells, this assay allows a differentiation between acute and chronic leukemia (CD34: stem cell marker). This can be helpful to determine the prognosis and for the choice of therapy. In addition, neoplastic cells can be divided into B and T lymphocytes and subtypes.

Differentiation of the myeloid leukemias using FACS has now been established at Laboklin and can be used especially in cases with atypical blasts or monocytic cells. The diagnosis of acute myeloid leukemia is also carried out using the CD34 marker. Similar to the lymphatic form, myeloid leukemias also carry a poor prognosis (<6 months). Since clonality testing is limited to lymphoproliferative disease, differentiation of leukemias using FACS is the only minimally invasive test method for myeloid leukemias in peripheral blood.

However, in the case of a so-called leukemoid reaction, consisting of an extreme left shift, neutrophilia, and monocytosis, with a total white blood cell count of >50 g/l, a clinical exam is important for the detection of pyogenic infection (e.g. pyothorax, pyometra, peritonitis), immune mediated disease (e.g. glomerulonephritis, IMHA), neoplasia, tissue necrosis, or hyperestrogenism.