Medpace Reference Laboratories establishes state of the art Flow Cytometry techniques for flexible approaches to clinical trials across multiple therapeutic areas.

Bryan Eppert, PhD, Associate Director, Medpace Reference Laboratories

Cytometry is the process of measuring the properties of individual cells. These properties may include gene or protein expression, chemical properties, deoxyribonucleic acid (DNA) content, and various cellular functions. The earliest methods of cytometry relied upon light microscopy for the classification and observation of cells and cellular components. Microscopy permitted direct visual observation of cells for the first time, leading to the classification of cells by morphology and insight into cellular functions. However, the time required for microscopic analysis constrains the number of samples or number of cells in each sample that can be examined. Therefore, the utility of microscopy for analysis of rare cells or in situations where sample throughput is a priority is limited. Flow cytometry was developed largely to improve upon these limitations.

Flow cytometry depends upon the ability to pass cells single file in a solution through the path of one or more laser beams. Flowing cells through the path of the laser permits the analysis of thousands of cells per second, but requires that the cells in the sample are in a single-cell, liquid suspension. Therefore, solid tissue samples require dissociation through mechanical or enzymatic processing before analysis. A wide variety of information about the cell can be determined depending upon how the cell interacts with the light from the laser. Detectors that measure the manner in which the cell scatters light can provide information about the size of the cell as well as complexity due to presence of intracellular granules and irregularities in the shape of the cell membrane. On the basis of light scatter properties alone, three distinct populations of leukocytes can be resolved in the peripheral blood: lymphocytes, monocytes and granulocytes. However, the power and flexibility of flow cytometry is fully realized when fluorescent probes that can be excited by the light from the laser are incorporated.

Fluorescent dyes that are activated or quenched based on pH or redox status can provide information regarding the chemical properties within individual cells. Fluorescent dyes that bind DNA can be used to determine the amount of DNA present in the cell. Calcium sensitive fluorescent dyes can be used to identify cellular signaling events that result in calcium flux. Antibodies which bind to cellular proteins can be conjugated to fluorescent molecules and be used to detect expression of specific proteins on individual cells. Flow cytometry can then be used to detect qualitative or semi-quantitative changes in the expression of specific proteins, or it can be used to identify specific cell types defined by the expression of particular proteins. Once a population of interest is identified through protein expression, flow cytometry can then provide both relative and absolute counts of the population or can be coupled with other fluorescent reagents to characterize protein expression, function, or chemical properties of the cells at the individual level.

At Medpace Reference Laboratories, we are able to measure 2 light scatter parameters and up to 10 fluorescent parameters for each cell at a rate of more than 10,000 cells per second using the BD FACSCanto System, the most powerful flow cytometer currently available for assays cleared by the Food and Drug Adminstration (FDA) for in vitro diagnostic (IVD) use.

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Flow cytometry is well established as a research tool due the wide range of cellular parameters that can be measured with fluorescent reagents and the ability to measure as many 20 of these parameters simultaneously.

Clinical applications for flow cytometry began in the mid-1980s with the monitoring of CD4+ T lymphocytes in patients with HIV. Since then the FDA has granted IVD approval for flow cytometric assays that enumerate CD34+ stem cells, identify of HLA-B27 expression, enumerate reticulocytes, and identify deficiency in glycophosphatidylinositol-linked proteins for the diagnosis of paroxysomal nocturnal hemoglobinuria. Applications for flow cytometry in clinical trials go beyond the IVD-approved assays to include immunogenicity assays for peptide or protein based therapeutics, identification of reactive antibodies predictive of graft rejection, monitoring malignancies in circulation, and elucidating mechanisms-of-action for therapeutics that modulate cellular function.

The flexibility of flow cytometry lends itself to a wide variety of therapeutic areas including oncology, infectious diseases, and regenerative medicine.

Flow cytometry is particularly useful in clinical trials in the area of oncology. A variety of primary and secondary endpoints can be measured using flow cytometry. Monitoring of leukemia and lymphoma in the peripheral blood is less invasive than sampling bone marrow and can provide longitudinal assessment of treatment effects on disease burden. Minimal residual disease can also be identified by flow cytometry for some types of leukemia and lymphoma and is an important predictor of relapse after treatment. For other therapeutic strategies it may be necessary to ascertain the effectiveness of CD34+ stem cell mobilization by enumerating circulating CD34+ stem cells in peripheral blood for subsequent apheresis and autologous transplantation after chemotherapy or radiation therapy. Rates of immune cell reconstitution after chemotherapy or radiation therapy may also be measured by flow cytometry. Circulating tumor cells may be evidence of invasive carcinoma and metastatic potential. Flow cytometry can be used to monitor the level of circulating tumor cells when therapeutic interventions aim to reduce metastasis. Flow cytometry can provide key measurements of critical endpoints in trials of oncology therapies.

Flow cytometry can also measure pharmacodynamics endpoints and biomarkers that might be predictive of therapeutic success in oncology trials.

Based on the ability of flow cytometry to measure a variety of cellular processes at the single cell level, the effects of treatment on the cells of interest can be specifically ascertained. Depending on the mechanism-of-action flow cytometry can monitor treatment effects on the viability of leukemic blasts, cell cycle arrest, and intracellular signaling events. Flow cytometry can identify the expression of specific biomarkers, such as ZAP-70 or CD38, which may predict response to treatment. Flow cytometry can provide valuable information about how the treatment affects the function of individual cells and identify subsets of patients that respond to treatment.

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Flow cytometry has numerous applications in the monitoring of immune system status and function. These applications can provide important measures in clinical trials for therapies for autoimmune disease, infectious disease, and allergy.

Immunotoxicological effects can also be ascertained by monitoring the numbers of specific leukocyte populations throughout a treatment regimen. HIV progression can lead to immunodeficiency due to an insufficiency in the population of CD4+ T lymphocytes. Flow cytometry can monitor the progression of disease and the efficacy of treatment by providing absolute counts of the number of CD4+ T lymphocytes in circulation. In vaccination studies, the efficiency of the vaccination to induce a cell-mediated response can be measured by identifying effector and memory T cell subsets on the basis of cell surface markers or by using tetramers that identify individual T cells that specifically recognize the immunogen and further characterize their expression of inflammatory mediators. Activation markers on specific immune cell subsets can be used as biomarkers to track the effectiveness of therapeutic interventions in allergy and autoimmune disorders that aim to limit immune responses. Flow cytometry is a powerful tool to provide insights into various ways that therapeutic intervention can modulate immune function.

Medpace has extensive experience in the planning and execution of global clinical trials across a variety of therapeutic disciplines including, oncology, cardiovascular and metabolic disease, infectious disease, neurology, and regenerative medicine. Medpace Reference Laboratories has the capacity and global reach to support large scale clinical trials worldwide.

The scientists at Medpace Reference Laboratories have decades of experience in laboratory testing in support of clinical trials and can provide guidance in the best application of flow cytometry for a particular study.

Medpace Reference Laboratories (MRL)

MRL is a global leader in providing customized, high-quality laboratory services to the pharmaceutical and biotech clinical development industries. A full-service central laboratory with locations in Cincinnati, Ohio; Leuven, Belgium; Mumbai, India; and Beijing, China, MRL combines a unique partnering philosophy with state-of-the-art infrastructure. Our team of medical and technical experts has extensive experience in all areas of central laboratory services – including cardiovascular, metabolic, oncologic, neuroscience, nephrology, anti-infectives, and immunology.

About Bryan Eppert, PhD, Medpace Reference Laboratories

Bryan Eppert, PhD is an Associate Director, Clinical Laboratory at Medpace Reference Laboratories. He earned his PhD while researching immunotoxicology at the University of Cincinnati. He has published his research, much of it involving flow cytometry, in such publications as the Journal of Clinical Investigation and the Journal of Immunology.

Contact Information:

Medpace, Inc.
5365 Medpace Way, Cincinnati, Ohio 45227 USA
Toll-free: +1.800.730.5779
Tel: +1.513.579.9911
Fax: +1.513.579.0444
E-mail: info@medpacelab.com