

Monitoring of Cell Mediated Immunity – By Dr. Magdalena Tary-Lehmann, Cellular Technology Limited (CTL)

ELISPOT Assays Offer Unique Qualification for Cytokine-Based Immune Monitoring and Standardization Strategies

Assessing immunogenicity is a challenge in the biopharmaceutical industry as an increasing number of new drugs and vaccines aim to elicit a response from the cellular components (e.g. T cells) of the immune system. Some of the challenges include measurement of a vaccination's effectiveness, and early detection of adverse effects. There is also a need for representative biomarkers and establishment of correlates of protection for novel vaccines aiming to elicit cellular immunity.

Cell Mediated Immunity (CMI) is a critical component during immunological responses; involved in infectious diseases, cancer, and autoimmunity. Research into correlates of protection has exposed limitations of vaccine approaches relying solely on antibody responses to confer protection against pathogens (e.g., HIV, Smallpox, etc.). Immune monitoring and biomarker screening can be implemented during all pre-clinical and clinical phases of drug and vaccine development. To increase chances of successfully detecting biomarkers for use in future clinical studies, the readout system must have high resolution and must be compliant with Good Laboratory Practice (GLP) regulations.

What is a biomarker? A biomarker is a nonclinical endpoint used to predict a clinical course or response, which can be molecular, cellular, or a radiological readout. Secretion of cytokines by T cells could be used as a biomarker. An assay for monitoring CMI via such biomarkers should perform identically with fresh or previously frozen samples. Further, it should be standardized, enabling inter-study comparisons of data in multi-center or large clinical trials. High throughput testing is necessary to accommodate the needs of clinical studies, involving high-volume testing of hundreds of samples using automated data read-out equipment.

ELISPOT is an ideal assay system for immune monitoring of cytokines secreted by T cells. The ELISPOT assay establishes the quality of an immune response (i.e., the type of products T-cells secrete in response to a vaccine candidate or drug), allowing the differentiation between Th1/Th2/Th17 (CD4⁺ T-cells) and Tc1/Tc2 (CD8⁺ T-cells) based on cytokine signatures. It establishes the quantity of the secreted cytokine product in response to an antigen or vaccine and reveals the magnitude (clonal sizes) of the response. ELISPOT is extremely sensitive, with routine detection limits of 1 in 500,000 cells or better. This is orders of magnitude more sensitive than ELISA, CBA, ICS, etc. Direct *ex vivo* T cell frequency responses are measured.

ELISPOT further assesses the functional characteristics/differentiation states of T cells (per cell cytokine productivity and frequency, and quality of T cell immunity) and their changes under physiological stimulation conditions (T cell affinity versus anergy). The robustness of the assay allows use of cryo-preserved samples, facilitating high throughput testing (450 samples per week) of batched samples collected at different time-points, which is a great advantage in clinical trials. ELISPOT assays can be validated under GLP-compliance with multiple cytokines and test systems.

Cellular Technology Limited (CTL) specializes in GLP-compliant ELISPOT testing for pre-clinical and clinical trials involving the measurement/characterization of T cell responses in various species. For example, ELISPOT is ideal for Th1/Th2 analysis, measurement of per cell cytokine production and direct *ex-vivo* cytotoxicity assays. Testing for simultaneous secretion of one or more products e.g. cytokines, T cell functional avidity studies, or epitope mapping, are just a few areas where ELISPOT assays are used.

CTL's GLP Laboratory has high throughput capability, allowing us to significantly accelerate the development process for drugs and vaccines entering the clinical testing phase, as well as subsequent immune-monitoring. CTL also provides laboratory services for processing whole blood and cryo-preservation of lymphocytes for later functional assay testing. CTL has been providing contract research services to government laboratories, major pharmaceutical companies, and academic institutions for over a decade in a regulated or non-regulated environment. In its relatively short history, CTL has been chosen repeatedly to serve as a reference laboratory for ELISPOT, including the Immune Tolerance Network, and most recently, the Sabin Vaccine Institute's Cancer Vaccine Consortium. CTL has been awarded multiple IDIQ contracts by the U.S. National Institute of Health (NIH) for development and validation of human and animal models of infectious diseases and bio-defense applications. Furthermore, CTL has optimized, qualified, and validated test methods for its clients in various test systems: human, mouse, monkey, and pig, among others. This has provided CTL a wealth of expertise encompassing a wide range of assay types (ELISPOT, ELISA, and Flow Cytometry) in a regulated and non-regulated environment.

CTL not only has a GLP-compliant laboratory operation, but also specializes in developing and manufacturing image analysis-based high throughput 21 CFR Part 11 compliant instrumentation and software. For ELISPOT assays, we have developed the ImmunoSpot[®] Analyzer line. Our scientists built and patented the first ELISPOT reader at Case Western Reserve University, and CTL has since become the leading manufacturer of ELISPOT instrumentation and analysis software. In recent years, CTL further customized and optimized our instruments, introducing the BioSpot Analyzer for various applications and platforms, e.g., viral plaque assays and bacterial colony counting. This has allowed us to enter into entirely new markets while advancing the research in these fields. With our expertise in both ELISPOT testing and instrumentation, CTL is uniquely qualified to be at the forefront of cytokine-based immune monitoring and standardization strategies.

If you have questions or comments regarding any information in this article, please feel free to contact us at info@immunospot.com or call us at (216)791-5084.

Executive bio for Magdalena Tary-Lehmann, M.D. Ph.D., Chief Scientific Officer, Cellular Technology Limited (CTL)

Dr. Magdalena Tary-Lehmann is Co-Founding Scientist and Chief Scientific Officer for Cellular Technology Limited (CTL). Dr. Tary-Lehmann received her M.D. and Ph.D. in Immunology. Her postdoctoral training in Immunology was at the University of California, Los Angeles. She moved thereafter to Case Western Reserve University, where she was awarded tenure and appointed as Associate Professor in the Department of Pathology. As Chief Scientific Officer for CTL, she provides guidance and oversight for technical operations, ensuring the ongoing scientific excellence of the company. Over the past ten years, she has worked with clients and regulatory agencies to develop and validate reference samples and controls for use in regulated immune monitoring assays.