

# Discussion Workshop: Perfecting the ELISPOT

The Institute, 11 High Road, East Finchley, London, N2 8LL:4<sup>th</sup> March 2011

After our successful **ELISPOT technology: The latest tricks** event which took place in October 2009 we are delighted to announce our follow up event, which will be our 5th event on ELISPOT.

This event is discussion workshop. We have invited 6 experts to discuss their work in an informal lecture setting, discussion and demonstration groups, one2one sessions and panel discussions

Meeting Chair: **Prof. Paul V. Lehmann** - Case Western Reserve University Cleveland, USA

This event has CPD accreditation

On registration please submit your questions to the panel that will be asked by the chair on the day of the event

9:00 – 9:20      **Registration**

9:20 – 9:30      **Introduction by the Chair:** *Prof. Paul V. Lehmann - Case Western Reserve University Cleveland, USA*

## **Talks by Invited Experts:**

9:30 – 9:45      **EliSPOT Standardisation: How do we get the ELISPOT into the Clinic?**

*Dr Sefina Arif*

*Kings College London, UK*

ELISPOT assay is one of the most useful techniques for immunological monitoring of trials and has gained increased application as a measure of specific T cell activation. However there are still issues with standardisation particularly across centres. Multi-centre clinical trials pose the challenge of collecting, shipping and processing samples in a way that ensures consistency and reproducibility hence it is critical to have validated assays that ensure that data is reliable.

9:45 – 10:00      **Developing Multicolor Isotype Revealing Antigen Specific B cell ELISPOT Assay**

*Prof. Daniel Peterson*

*University of Nebraska-Lincoln, USA*

The B cell response to vaccines, commensal bacteria, and pathogens reflect the T cells and inflammatory environment that provide "Help" to differentiating B cells. We are developing a multicolor ELISPOT assay that will measure both the frequency and isotype of antigen specific B cells in the same wells using fluorescent secondary antibodies and the appropriate filters. This will allow us to measure 5-6 isotypes in the same wells, greatly increasing the number of samples that can be analyzed and decreasing the number of B cells from tissue or blood that will be required for the assay. We will describe the progress in this development, using mouse splenic and lamina propria B cells, in the context of oral vaccination of gnotobiotic mice colonized with microbes known to shift the balance of the immune response from Th1, to Th2 T cells.

10:00 – 10:15      **Future of ELISPOT Assays: Lymphocytes and Beyond**

*Dr Alex Kalyuzhny*

*R&D Systems, USA*

ELISPOT assays are traditionally used to study cytokine secretion from immune system cells. However, this type of cell-based assay is quite flexible and can be used for many other cells types including neuronal and glial cells in the central and peripheral nervous system, endocrine and exocrine cells as well as stem cells to mention few. Using ELISPOT for cells other than lymphocytes require adjusting assay conditions and detection chemistry which will be addressed in this presentation.

10:15 – 10:30      **Statistical Analysis of ELISPOT Data**

*Assistant Professor Marcus Dittrich*

*University of Wurzburg, Germany*

The principal goal of most ELISPOT experiments is the reliable identification of a positive antigen response. Different approaches are commonly used, mainly either statistically tests or empirical rules of thumb (e.g. based on the mean spot count difference or ratio between the antigen-containing the negative control wells). Albeit enjoying some popularity empirical rules in general do not have a theoretical justification and provide no measurement of confidence. Instead, the application of solid statistical tests is highly recommended. First data

from cell transfected cell lines indicate that the standard t-test and related statistics should be applicable in most cases.

10:30 – 10:45 **Qualitative and Quantitative Analysis of HIV-1-Specific T-cell Responses**

*Dr Nesrina Imami*

*Imperial College London, UK*

Mechanisms by which immune-based therapies increase T-cell numbers and function in chronically HIV-1-infected treated patients are not fully understood. Our experimental data suggests that by utilising various immunotherapies we can affect T-cell proliferation, survival, development and differentiation and/or maturation and thymic output, all of which lead to enhancement of T cell function. This implies that just as in vitro, in vivo HIV-1-specific T cell defects might be corrected by administration of exogenous stimuli such as cytokines, hormones and/or therapeutic immunisation. Understanding the precise biochemical, molecular and cellular mechanisms involved will be crucial for the optimisation and development of these and other modes of immune-based therapies. Our research work is aimed at carrying out basic research and clinical studies/trials aimed at development of novel immunotherapies. Utilisation of new technologies to assess full functionality of anti-HIV-1 responses combined with expression profiling will be essential in application to human health.

10:45 – 11:00 **Fluorospot for Dual and Triple Cytokine Analysis: Applications**

*Associate Prof. Bernt Axelsson*

*Mabtech, Sweden*

Cytokine ELISPOT has become a powerful routine tool for the analysis of disease- as well as vaccine-induced T-cell responses. The method is limited, however, in that only one cytokine at a time is assessed. Fluorospot is a development of the ELISPOT method that facilitates the analysis of single cells secreting several cytokines, e. g. polyfunctional T cells, which are suggested to be of protective importance in various infectious diseases. By detecting each cytokine with a certain fluorophore and analyzing two- or three-colored spots by fluorophore-specific filter systems, spots derived from cells producing single or multiple cytokines are identified. Fluorospot maintains the simplicity and sensitivity of the ELISPOT while taking the analysis a step forward towards multiplex analysis.

11:00– 11:05 **Participant Photo**

11:05 – 11:30 **Mid-morning Break**

11:30 – 12:30 **Question and Answer Session**

12:30 – **Working Lunch (in Exhibition Area)**

**Discussion Groups and One to One Sessions**

- Round table discussion groups will be throughout the afternoon
- Delegates will rotate at 15 minute intervals so that they may participate in all the discussion tables
- All delegates will also be allocated an slot to visit the exhibition stands
- One to one sessions can also be held after lunch (in parallel to the discussion groups)
- Where appropriate delegates will be able to bring their samples to the discussions

12:45 – 13:20 **Discussion Groups (Sessions 1 & 2)**

13:25 – 13:40 **Effect of T-Cell XTend on the Performance of the TSPOT.TB Assay**

**Talk by Dr John Bouwman**

*Med Microbiology & Immunology Diakonessen Hospital, The Netherlands*

Vacutainer CPT tubes are commonly used for collection of whole blood for the TSPOT.TB assay, but require that blood samples are processed within 8 hours. In this study we evaluated the feasibility of T-Cell XTend for isolating peripheral blood mononuclear cells (PBMC). This procedure would allow storage of blood samples for batched processing.

Methods Whole blood specimens from 59 individuals were collected in Vacutainer CPT tubes (CPT) and lithium heparin (LH) tubes. CPT tubes were processed within 8 hours. T-Cell XTend was added to LH tubes after 24 or 48 hours. We measured total white blood cell counts (WBC) and proportions of lymphocytes and granulocytes in the isolated PBMC's. We also evaluated the performance of T-Cell XTend in the TSPOT.TB assay.

**Results** PBMC yields from T-Cell *XTend* treated LH samples did not differ from PBMC yields from CPT tubes, but T-Cell *XTend* had a pronounced effect on the proportions of lymphocytes and granulocytes. The mean lymphocyte percentage in PBMC's isolated with CPT was  $84.31 \pm 1.14$  %, but was decreased to  $52.72 \pm 3.34$  % ( $p < 0.05$ ) in untreated LH blood left to stand for 48 hours. This effect was neutralized by T-Cell *XTend* ( $85.44 \pm 0.74$  %). We observed a similar but opposite effect on granulocytes: The mean proportion in untreated LH blood was increased to  $40.9 \pm 3.67$  % ( $p < 0.001$ ) compared to CPT blood ( $8.26 \pm 0.89$  %). Treatment of LH samples with T-Cell *XTend* (48 hours) restored the proportion of granulocytes to  $8.47 \pm 0.61$  %. Enumeration and analysis of spots in the TSPOT.TB assay demonstrated good agreement between CPT and T-Cell *XTend* results, even after 48 hours.

**Conclusion** T-Cell *XTend* efficiently removes granulocytes from PBMC suspensions and increases the proportion of lymphocytes. TSPOT.TB results from T-Cell *XTend* treated blood samples are at least comparable to the results obtained from the current CPT method.

However, we also observed increased spot counts in control wells of T-Cell *XTend* treated samples, less indeterminate results and a possible increase of positive TSPOT.TB results.

Application of T-Cell *XTend*<sup>®</sup> can be a feasible for ELISPOT purposes, but further research is warranted to investigate the need of (re-)establishing specific cut-off levels for T-Cell *XTend* treated samples.

13:45 – 14:20 **Discussion Groups (Sessions 3 & 4)**

14:20 – 14:45 **Afternoon Break**

14:45 – 15:20 **Discussion Groups (Sessions 5 & 6)**

15:25 – 15:40 **Talk by Associate Prof. Bernt Axelsson**

*Mabtech, Sweden*

Fluorospot for Dual and Triple Cytokine Analysis: Applications: Technical Aspects

15:44 – 16:20 **Discussion Groups (Sessions 7 & 8)**

#### **Table 1: ELISPOT: Challenges and Opportunities**

**Hosted by Prof. Paul Lehmann**, who trained as a T cell immunologist. He introduced and patented image analysis for ELISPOT (United States Patent No 08/577,957) dedicating 40 of his 100 publications to the basics of ELISPOT, including single cell resolution, per cell productivity, cognate vs. bystander cytokine, T cell avidity measurements, determinant mapping etc. In 1998, he founded CTL to assist scientists in ELISPOT analysis. CTL offers GLP-compliant ELISPOT contract research, ELISPOT readers (visible light and UV), PBMC libraries and reference.

#### **Table 2: Quality Control and Multi Centre Validation**

**Hosted by Dr Sefina Arif**, who did her PhD in Immunology at King's College London focusing on the identification of autoantigens in type 1 diabetes. Since then she has continued in this field specifically working on the role of T cells in type 1 diabetes characterising autoreactive T cells in both patients and healthy controls specifically T cells producing interferon- $\gamma$  in patients and cells making IL-10 in healthy controls Her recent studies focus on the role of Th17 cells in this type 1 diabetes.

#### **Table 3: Developing Multicolor Isotype Revealing Antigen Specific B cell ELISPOT Assay**

**Hosted by Prof. Daniel Peterson**, who grew up outside of Lincoln NE and attended the University of Nebraska, studying Animal Science graduating in 1993. From there he went to Washington University in St Louis to pursue a MD/PhD in Immunology with Emil Unanue. He then went to Switzerland to study human immune responses to the Cancer Melanoma. Following internship in Internal Medicine at Case-Western Reserve University Hospitals of Cleveland, he returned to Wash U to complete a Residency in Clinical Pathology and a postdoctoral Fellowship with Jeffrey Gordon. In 2008, he moved back to Lincoln NE, and set up his laboratory focused on the specific immune response to gut microbes. Since his arrival at UNL he has established a germ-free gnotobiotic facility and immune monitoring core with Flow Cytometry, ELISA and ELISPOT capabilities in his laboratory.

#### **Table 4: Future of ELISPOT Assays: Lymphocytes and Beyond**

**Hosted by Dr Alex Kalyuzhny**, who gained a Ph.D. in embryology and histology in 1988, in 1998 set up immunocytochemistry and ELISPOT assays department at R&D Systems, Inc. and manage it till now. Developed more than 50 ELISPOT assays which are currently manufactured and sold by R&D Systems, Inc. worldwide. A faculty at the department of Neuroscience at the University of Minnesota. Author of 27 peer-reviewed publications, 16 chapters in textbooks, editor of 3 books including both 1st (published in 2005) and 2nd (will be published in 2011) editions of the

Handbook of ELISPOT (Humana Press). Professional interest: developing multiplex ELISPOT assays for veterinary and human diagnostics.

#### **Table 5: Statical Analysis of ELISPOT Data**

**Hosted by Dr Marcus Dittrich**, who studied Medicine at University of Tübingen. From there he went to the Case Western Reserve University in Cleveland (Ohio) where he did his MD thesis in the Lab of Prof. Paul V. Lehmann. After internships at the University Hospital of Zürich he joined the MD/PhD program in Würzburg where he graduated in bioinformatics about novel systems biological approaches to the functional analysis of cellular networks. Today he leads a bioinformatical research group at the University of Würzburg and mainly works on the integrated statistical analysis of multivariate high-throughput data (e.g. microarray, proteomics) in the context of biological networks.

#### **Table 6: Design of Assessment of HIV-1-Specific T-cell Function in HIV-1 Infected Individuals and Vaccine Recipient: Concomitant Assessment of Perforin, IFN-gamma, IL-2 and IL-4 Secretion**

**Hosted by Dr Dr Nesrina Imami**, who is a Reader in Immunology and Fellow of the Royal College of Pathologists, qualified in medicine, microbiology and immunology. She further specialised in viral immunology, in particular HIV-1, and focused on cell mediated immunity and immunotherapeutic development. She has published primarily on the immunology of T cells and their responses to viral infection, was awarded a University of London PhD in 1992 and after a subsequent period of postdoctoral work was awarded a Wellcome Trust Fellowship. She embarked on a long-term detailed programme of research to assess HIV-1-specific T-cell immune responses, mechanisms of non-responsiveness/anergy and to evaluate the effects of drugs, cytokines and other immunomodulators such as vaccines on these responses. The Wellcome Trust Fellowship enabled her to establish her research group at the forefront of international research into the immunopathogenesis of HIV-1 infection and immune reconstitution in HIV-1 disease, and to continue her work in an area that integrates basic biological science with clinical science, with direct application to human health. She has completed and set up novel immunotherapy and drug clinical trials for which she has been awarded the prestigious MRC Experimental Medicine Award.

#### **Table 7: Fluorospot**

**Hosted by Associate Prof. Bernt Axelsson**, who gained a PhD in immunology at Stockholm University (SU) 1984 under late prof. Peter Perlmann. He continued as a lecturer and researcher in immunology at SU to 1995, mainly engaged in mechanisms of T cell activation (35 peer reviewed articles). He then went to the swedish biotech company Biovitrum AB as a manager engaged in immunological aspects of obesity and diabetes type II. Since 2006 he has been at Mabtech AB developing the Fluorospot method.

16:30 – 17:00 **Expert Panel's Summing Up**

17:00 **Chairman's Summing Up**

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Meeting Web Site: [www.regonline.co.uk/workshopELISPOT2011](http://www.regonline.co.uk/workshopELISPOT2011)