

# Efficient cytokine detection: The Way Forward

BioPark, Hertfordshire, UK: 3rd July 2009

*Are you measuring or want to measure cytokines ?, trying to optimise your technique or do you want just to make sure you are going about it the right way?*

This meeting aims to summarise some of the options open to you and to discuss which techniques best suit your requirements. A series of presentations from experts working in the academic, clinical and commercial sectors will compare recent developments in technologies ranging from relatively "low-tech" assays such as ELISA, Elispot and bioassays through to the "high-tech" platform technologies of various multiplex cytokine detection systems. Their use in basic science, R&D and translational research will be discussed.

This event has CPD accreditation

- 8:45 - 9:30      **Registration**
- 9:30 - 9:40      **Introduction by the Chair:** *Dr Stephen Thompson*, King's College London, UK
- 9:40 - 10:10     **Cytokine detection in collagen-induced arthritis**  
*Dr Richard Williams*, Kennedy Institute of Rheumatology Division, Imperial College, UK  
Collagen-induced arthritis is a model of rheumatoid arthritis that has been used extensively to validate novel therapeutic targets. The main pathological features of the disease include synovitis, pannus formation and joint erosion. There is a great deal of interest in the development and testing of drugs with the capacity to modulate inflammatory pathways in arthritis. Hence, there is a need to monitor the effect of novel treatments on cytokine expression in vivo. This presentation will focus on the techniques used to quantify changes in cytokine expression following therapeutic intervention
- 10:10 - 10:40   **IL-10, regulatory T cells and respiratory health: the role of the vitamin D pathway**  
*Dr Catherine Hawrylowicz*, Kings College, London  
CD4+Foxp3+ Treg and IL-10 secreting Treg are proposed to play a role in the control of immune homeostasis in the lung and may have therapeutic potential in allergic and asthmatic disease. Our studies are investigating pharmacological protocols to promote these regulatory T cell populations in allergic and asthmatic patients.
- 10:40 - 11:10   **Isolation of live Regulatory, Effector and interleukin-17-producing T cells using cytokine secretion**  
*Dr John Campbell* – Miltenyi Biotec Ltd, Surrey, UK  
IL-17 producing T cells are currently the focus of much interest in the fields of inflammation and immunity. The IL-17-producing phenotype is somewhat plastic, and generation of IL-17-producing T cells in vitro is complex. Here I will demonstrate the detection and isolation of live IL-17-producing T cells direct from blood and spleen using the IL-17 secretion assay system. The phenotypes and functions of natural IL-17-producing cells will be discussed, along with the possibility of splitting the IL-17 and IFN-gamma secreting populations based on two-colour cytokine secretion.
- 11:10 - 11:15   **Speakers photo**
- 11:15 - 11:45   **Mid-morning break**
- 11:45 - 12:15   **Using ELISPOT to detect rare antigen specific T cells**  
*Dr Sefina Arif*, Guys Hospital, London  
There is an inability to access target tissues in many autoimmune diseases and hence one has to focus on peripheral blood in order to get a snapshot of what is occurring in the target tissue. Elispots are highly sensitive assays and can be used to detect low frequency T cells in peripheral blood, In addition, because of the array of cytokines that can be detected, it is possible to gain some insight into the nature of the pathological response and to monitor this response during immunomodulatory therapies.
- 12:15 - 12:45   **Validation of ELISPOT assays for immune monitoring**  
*Professor Paul Lehman*, Case Western Reserve University Cleveland, USA

Assays suitable for monitoring cell-mediated immunity (CMI) need to be sensitive enough to detect the low frequency antigen-specific T cells. ELISPOT assays uniquely meet this requirement being capable of detecting as few as one specific T cell within a million PBMC. In addition, CMI assays need to be suited for formal validation so that regulatory agencies such as the FDA can accept the data generated. Having to test perishable live cell material, validation has however been a major challenge for T cell assays. CTL has succeeded to validate ELISPOT for the NIH and other clients. I will share data illustrating how ELISPOT assays can be validated for monitoring CMI.

12:45 – 14:00 **Lunch**

14:00 – 14:30 **Testing of Cryopreserved PBMC Reference Samples Shows Unexpectedly Low Intra- and Inter- Laboratory Variability of ELISPOT Results.**

*Dr Wenji Zhang, Cellular Technology Limited, Germany*

While T cells play a critical role in most immune processes, reliable measurements of antigen-specific T cell responses ex-vivo remain a challenge. In conjunction with a novel cryopreservation protocol, we demonstrated that the T cell recall response in cytokine ELISPOT assays provided identical results with fresh and thawed PBMC, regardless of whether peptide or protein antigen were tested. The ability to work with different frozen aliquots of PBMC from the same blood draw allowed us to further test the very same cell material repeatedly to assess the intra- and inter- lab variability of antigen-specific ELISPOT assays.

14:30 – 15:00 **Immunohistochemical visualization of cytokines and other small molecules**

*Dr Chris van der Loos, Academic Medical Center, Univ. of Amsterdam, The Netherlands*

The use of post-fixed cryostat tissue sections has been widely accepted as 'gold-standard' for testing primary antibodies in immunohistochemistry (IHC). However, IHC staining of unbound small molecules like cytokines, may result into false-positive plasma cells, whereas specific staining is lacking. Leaking of the cytokine molecule during post-fixation is most likely the cause of this problem. In contrast, pre-fixation does retain the small molecules much better. Based on these findings suggestions are made for positive controls from cells and the use of optimally formalin-fixed paraffin tissue sections. Furthermore, testing of more than one primary antibody is highly recommended.

15:00 – 15:30 **Afternoon Tea/Coffee**

15:30 – 16:00 **Cytokines in anti-infective drug discovery**

*Dr. Deborah O'Neil, NovaBiotics Ltd, UK*

16:00 – 16:30 **The use of X Map Luminex technology for multiple cytokine detection**

*Dr Ciaran Sewter, Millipore, France*

During this presentation we will describe the principles and uses of the Luminex xMAP technology. As the original partners of Luminex Corporation, Millipore (through Upstate and Linco) have developed one of the most extensive ranges of multiplex protein assay menus available - the Milliplex assays. Here we will describe how the use of these assays, together with the Luminex xMAP technology, can be used to simultaneously detect up to 75 cytokines in a single, small volume sample

16:30 – 17:00 **Differing multiplex cytokine analysis platforms**

*Dr Gendie Lash, Institute of Cellular Medicine, Newcastle University*

Multiplex cytokine analysis technologies have become readily available in the last seven years. Two main formats exist: multiplex sandwich ELISA and bead based assays. While these have each been compared to individual ELISAs, there has been little direct comparison between the two formats. I will discuss the comparison between two multiplex sandwich ELISA assays (FAST Quant and SearchLight) and a bead based assay (UpState Luminex).

17:00 **Chairman's summing up & close.**

#### About the Chair:

**Stephen Thompson** received his B.Sc. in Cellular Pathology from the University of Bristol in 1984. He received his PhD in 1989 for studies characterising the role of House Dust Mite allergens in children with asthma and eczema. After these studies he began working on murine models of inflammatory arthritis, most notably pristane-induced arthritis, where he and his group were one of the first to characterise the immunopathology of this disease. Through the award of Arthritis Research Campaign post-doctoral fellowships (both

in the UK and at the Southwestern Medical Center in Dallas, USA) he developed his interests in the role of stress proteins as inducers or targets of regulatory T cells and their potential use as anti-inflammatory immune modulators. He is currently based at Kings College London, Department of Rheumatology at Guys Hospital where he and his colleagues continue translational research to evaluate novel immunotherapeutic strategies for the treatment of rheumatoid arthritis

#### About the Speakers

**Richard Williams** is a Senior Lecturer at the Kennedy Institute of Rheumatology Division of Imperial College London. He has been working for many years towards the development of novel therapeutic strategies for rheumatoid arthritis using collagen-induced arthritis as an animal model. His work contributed to the successful development of anti-TNF therapy and introduced the concept of combination therapy, targeting both TNF $\alpha$  and T cells.

**Dr Gendie Lash** obtained her undergraduate and PhD degrees in Biochemistry from University of Otago, Dunedin, New Zealand, completed in 1997. She then did Post-Doc jobs in Obstetrics and Gynaecology, University of Nottingham and Department of Anatomy and Cell Biology, Queen's University, Kingston, Ontario, Canada where she held a Canadian Hypertension Society Post-Doctoral Fellowship. In 2002 she moved to Newcastle University where she has been ever since and currently is a Newcastle University Faculty of Medicine Research Fellow. Her research focuses on maternal adaptations to pregnancy, with particular interest in regulation of trophoblast invasion and spiral artery remodeling. More specifically her research involves studying the role of uterine natural killer cell-derived cytokines and growth factors in both non-pregnant endometrium and early pregnancy decidua in relation to vascular development.

For the past few years **Dr Sefina Arif** has been studying T cells in type 1 diabetes with the current focus on the detection of Th17 cells in type 1 diabetes using the ELISPOT assay. She has extensive experience of the ELISPOT assay including the use of this assay to monitor patients undergoing immunotherapy and to discriminate between patients and controls in blinded studies

**Dr John Campbell** joined Miltenyi Biotec Germany in 2001 as project leader in T cell immune therapy, formerly lecturer in tumour Immunology at Glasgow University. Director of Clinical Immunology 2005-2006 USA. Now managing all aspects of clinical trials and applied immunology for MB UK. Long standing interest in flow cytometry, particularly rare cell analysis; use of MHC-Antigen complexes; measurement of cytokine production; analysis of patient samples; isolation of cells from complex mixtures

**Dr Chris van der Loos** trained as histotech and prepared a PhD thesis in 1992 as first in the AMC. Major interest in the technical issues of IHC: fixation, tissue handling, IHC detection techniques, multiple staining and spectral imaging.

**Dr Deborah O'Neil** founded NovaBiotics in August, 2004. An immunologist by training with over a decade's experience in the field of natural antimicrobials, Deborah studied at University College London and then worked in internationally acclaimed laboratories in San Diego and Ghent before moving to Aberdeen. It was here where, in order to fully develop the commercial potential of novel antimicrobial peptide therapies, she created NovaBiotics. A clinical phase company with a lead antifungal product set to compete in a \$5 bn global market, NovaBiotics is at the leading edge of novel peptide anti-infective drug design and development.

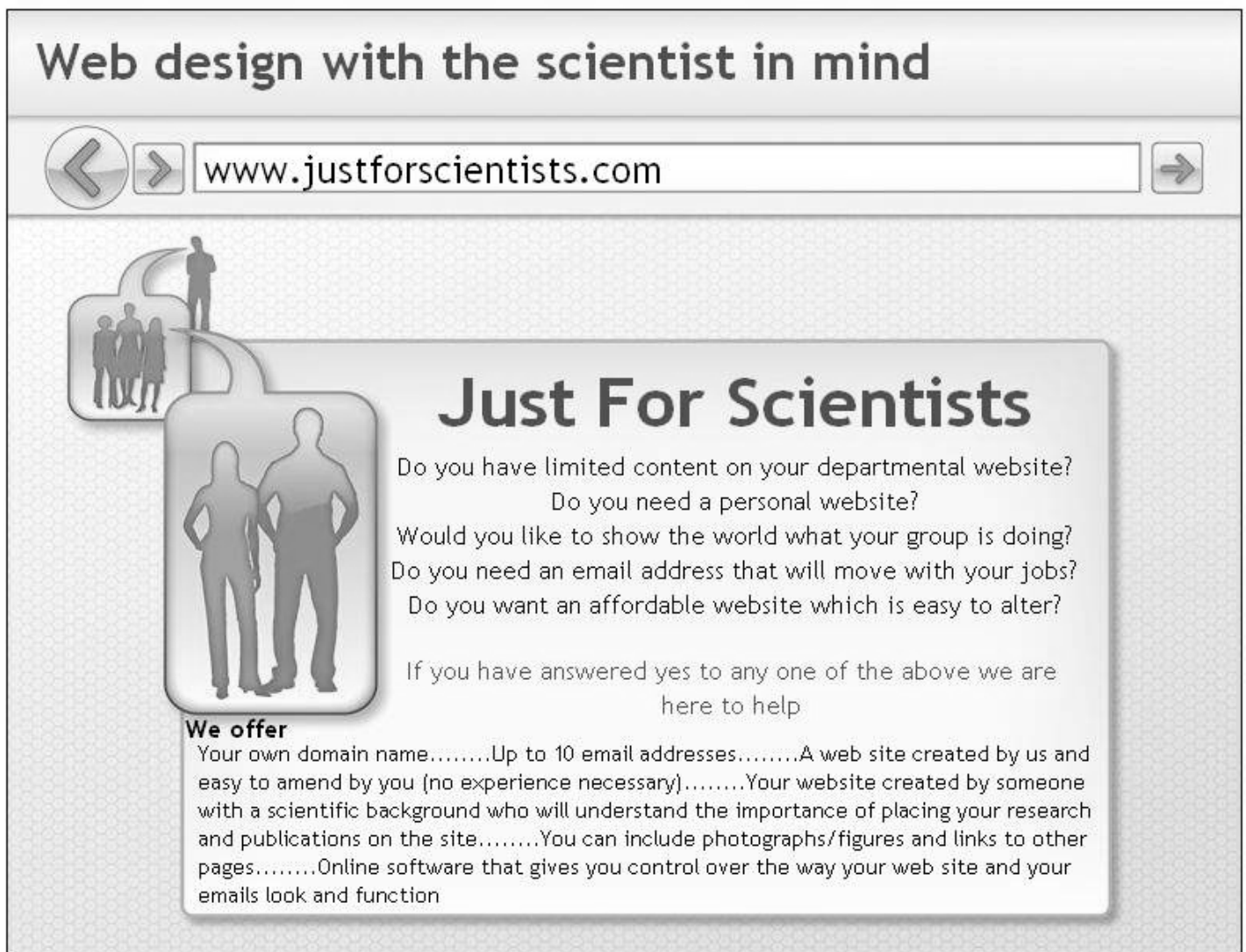
**Dr Hawrylowicz's** research interests focus on immune regulation in allergic diseases, including asthma. Her lab is investigating ways to promote Foxp3+ and IL-10+ regulatory T cells in patients for therapeutic benefit.

After completing PhD from Cambridge University in Obesity Research, **Dr Ciaran Sewter** worked for a number of Pharmaceutical and Biotechnology companies including GSK, Icen Biodescovery and 3D Molecular Sciences. Research focused on antibody production and assay development. Following a number of years working at the bench, Ciaran joined Upsate (now part of Millipore) as a Business Development Manager for Multiplex Assays and the Luminex Technology.

**Prof. Paul V. Lehmann** trained as a T cell immunologist. He has patented image analysis for ELISPOT assays (United States Patent No 08/577,957) and has dedicated more than 40 of his over 100 publications to establishing the basic aspects of ELISPOT analysis (see [www.immunospot.com](http://www.immunospot.com), for his ELISPOT-related publications by theme). In 1998 Prof. Lehmann founded CTL to assist scientists in performing ELISPOT analysis with the highest degree of accuracy and sophistication. CTL offers GLP-compliant ELISPOT contract research, ELISPOT readers (visible light and UV), PBMC libraries and reference samples, as well as serum free test media. Prof. Lehmann is the President and CEO of CTL.

**Dr. Wenji Zhang** is a senior staff scientist at CTL. She has dedicated the last five years to ELISPOT standardization. Since serum is a major viable in ELISPOT assay performance, she has developed serum free PBMC freezing and thawing protocols, as well as test media. She developed human PBMC Reference Samples with defined antigen reactivity levels and cytokine signatures. Moreover, she has been a key player in defining the user requirements for the SmartCount function in CTL's ImmunoSpot software that automatically establishes scientifically validated counting parameters thereby eliminating subjectivity from ELISPOT analysis

*This meeting was **organised by Euroscicon** ([www.euroscicon.com](http://www.euroscicon.com)), a team of dedicated professionals working for the continuous improvement of technical knowledge transfer to all scientists. Euroscicon believe that they can make a positive difference to the quality of science by providing cutting edge information on new technological advancements to the scientific community. This is provided via our exceptional services to individual scientists, research institutions and industry. The event was hosted by **BioPark** ([www.biopark.co.uk](http://www.biopark.co.uk)), a research and development centre in Welwyn Garden City providing specialist facilities and support for bioscience and health technology businesses to grow, and to develop new products and technologies*



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