

ELISPOT technology: Newer tricks

The BioPark Hertfordshire, Welwyn Garden City, AL7 3AX: 8th July 2008

- 9:00 – 9:45 **Registration**
- 9:45 – 10:00 **Introduction by the Chair:** *Dr Sefina Arif*, King's College London, UK
- 10:00 – 10:30 **Multi color ELISPOT assays for GLP compliant, high throughput measurements of frequencies and effector functions of antigen-specific T cells**
Professor Paul Lehmann, Professor of Pathology at Case Western Reserve University, USA
There is an increasing need for multicolor ELISPOT analysis in T cell immune monitoring. This is because multi-color analysis combines the strengths of ELISPOT assays (high sensitivity, robustness, need for minimal cell material, works with cryopreserved cells) with the need for multiplexing. The major weaknesses of classic ELISPOT assays has been that each assay can detect only one analyte at a time, and that no information is provided on the phenotype of the secreting cell. During this talk I will cover the progress we have made towards the development, optimization, and qualification of Quadruple Cytokine ELISPOT assays (and the obstacles encountered). I will talk about IL-2, IL-4, IL-17 and IFN- γ measurements to assess Thpp, Th2, Th-17 and Th1 effector cell diversity within antigen-specific T cell immunity.
- 10:30 – 11:00 **Dual colour ELISpot: added value against less spots**
Dr John Wijdenes, General Director and Chief Scientific Officer, **Diaclone**.
ELISpot is becoming a familiar technique in the Material and Methods of many papers and has found its place as one of the assays coming closest to the *in vivo* situation. As with other assays it has its imperfections in that not all information can be read from the results but it tells us at least important information about what is secreted by the cell? This unique information should be explored beyond single product detection in multiple colour ELISpot assays. Once the problems with dual colour ELISpot assays are resolved the technique is ready to measure the secretion of cell with special activities.
- 11:00- 11:10 **Speakers photo**
11:10 – 11:30 **Mid-morning break**
- 11:30 – 12:00 **New developments in ELISpot for clinical diagnosis**
Professor Ajit Lalvani, Head, Tuberculosis Immunology Group, National Heart and Lung Institute, UK
- 12:00 – 12:30 **Validation on Elispot Readers.**
Werner Freber, CEO and founder of **BIO-SYS GmbH**, Germany
Elispot is an wide-range variation assay 1) Cell source, treatment, preparation protocol, antibody source 2) Results are hardware and COTS Software combined. 3) Full automatic settings often 'misleading' –observe negative controls plus individual size distribution by trained user. 4) Count full filter— cell concentration at outer region of the filter. **Conclusion:** a) The Elispot Reader system' has to be maintained as a validated System... b) Qualified continuous user training. c) get current - debugged software update. d) Create great relationship to your supplier. e) Apply lifespan model. Document from day one until decommission. f) Create your 'user requirement' specification and choose the supplier matching your requirement.
- 12:30 – 12:50 **Tour of the BioPark**
- 12:50 – 14:00 **Lunch and Poster Viewing**

- 14:00 – 14:30 **Fluorospot - fluorochrome-based detection of cytokine-producing cells**
Dr Niklas Ahlborg, Mabtech AB, Sweden
- 14:30 – 15:00 **The ELISPOT assay; a tricky business**
 Professor *Peter Vander Meide*, Associate Professor Utrecht University and U-CyTech biosciences, The Netherlands
 Two systems can be used to detect cells that release more than one cytokine [i.e. ELISPOT (enzymatic detection) and FluoroSpot (fluorescence detection)]. The FluoroSpot assay is preferred because this system allows the unambiguous detection of two or more cytokines released by the same cell whereas enzymatic detection has significant less discriminative power. However, the technical problems associated with fluorescence detection are more complex and are as yet not fully solved. FluoroSpot assays require specific plates and equipment for spot counting. In our talk we shall go into recent developments in FluoroSpot technology and certain critical aspects of the ELISPOT assay.
- 15:00 – 15:30 **Afternoon Tea/Coffee and Last Poster Viewing**
- 15:30 – 16:00 **Improving detection sensitivity in ELISpot assays**
Dr Roberto Mallone, Hopital Saint Vincent de Paul, France
 Identifying parameters maximizing detection sensitivity in ELISpot assays is important to transfer this technology into the clinical setting for identifying rare Ag-specific CD8⁺ T-cells. We have therefore systematically analyzed different critical variables. Two parameters greatly enhanced detection sensitivity: use of human serum-free vs. serum-supplemented culture medium and addition of low dose IL-7. Incorporating both of these parameters into the ELISpot procedure greatly amplified the low grade CD8⁺ T-cell responses directed against β -cell epitopes of type 1 diabetes patients. Implementation of this ELISpot procedure should expedite development of “immune staging” protocols for autoimmune as well as tumor and infectious diseases.
- 16:00 – 16:30 **A novel trick to identify autoimmune T cell responses**
Dr Huriya Beyan, Queen Mary, University of London, UK
- 16:30- 17:00 **Chairman’s summing up**
- 18:00 **Soiree at *The Best Western Homestead Court Hotel for all the participants**

*This meeting was **organised by Euroscicon** (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), 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*

About the Speakers

Professor Ajit Lalvani, Head, Tuberculosis Immunology Group, National Heart and Lung Institute, UK
Professor Lalvani's team has invented, validated and delivered a genuinely useful new tool into clinical practice. This research has been directly translated from the laboratory bench to the bedside and is now part of public health policy. The latest NICE guidelines on tuberculosis control, as well as several European guidelines, endorse T-cell-based blood testing for latent tuberculosis infection, the first practical advance in this area for over 100 years. The growing impact of his work on global public health was recognised in 2005 by the award of the Scientific Prize of the International Union Against Tuberculosis and Lung Disease. Professor Lalvani's ongoing translational research into TB immunology has great potential to generate further advances in screening, diagnosis and monitoring of TB infection. The Tuberculosis Immunology Group focuses on systematic translation of its cutting-edge scientific biomedical innovations from bench to bedside. The Group has several major international collaborations in Italy, Germany, the USA, India, Turkey, Zimbabwe and South Africa. The Tuberculosis Immunology Group is funded by The Wellcome Trust, The World Health Organization, The Medical Research Council, and Imperial College Healthcare NHS Trust.

Dr John Wijdenes is General Director of Diaclone SAS, a French based company specialised in developing monoclonal antibodies for research, diagnostic and therapeutic purposes. He is also Chief Scientific Officer at **Tepnel, UK**, now the full owner of Diaclone. He received his doctorate in Biology from the Free University of Amsterdam, The Netherlands in 1981. He joined Schering Plough, France in 1983 as Senior Scientist and Head of the Imm. Chem. and Mab laboratories. In 1986 he went to Regional Blood Bank in Besançon, France to set up a laboratory to develop mAbs for therapeutic and diagnostic use and has since then been involved in the development, production and use of therapeutic mAbs.

Dr. Mallone holds a MD PhD degree from the University of Turin, Italy. His research focuses on the autoimmune T cell responses of type 1 diabetes and their characterization by different techniques, including ELISpot. After a postdoctoral period in the Laboratory of Jerry Nepom in Seattle, Dr. Mallone moved to Paris, first as Visiting Associate Professor in Immunology, and then as a Faculty Senior Research Associate of the French INSERM Institute. He has pioneered the analysis of beta-cell-specific CD8+ T lymphocytes through epitope identification and T cell assay development.

Dr Niklas Ahlborg. Niklas Ahlborg is Director of Research and Development at **Mabtech AB**, a Swedish biotech company focusing on the development and production of ELISpot kit and reagents. Background in academic research on parasite immunology, vaccine development and B- and T-cell immunology at Stockholm University and Edinburgh University. Assoc. Professor at Dept of Immunology at Stockholm University.

Professor Paul Lehmann, 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 samples, as well as serum free test media. Prof. Lehmann is the President and CEO of **CTL**.

Mr. Werner Freber was born in 1949 in the historical old City MAINZ on the Rhine River. The history of this part of Germany made him a convinced European. His study years in economy, chemistry and technology did lead to a graduation as a chemical engineer in Darmstadt/Germany in 1972. He did chose, -instead of a academics carrier- to continue his labour in the Industry: Product development and validation of analytical instruments with Grace, Hartman + Braun, Biotronik, Fisher Scientific completed the required background to found its own company BIO-SYS GmbH in 1986. Since then he focussed on Quality management incl Validation/Verification of Systems that count accurately!

Professor Peter Vander Meide, Associate Professor Utrecht University and U-CyTech biosciences, The Netherlands. Studied Biochemistry at Leiden University and got its Ph.D. in 1982 on a study to the role of the elongation factor Tu in E.coli. Was employed at the Dutch Primate research Center as senior scientist from 1982 to 1999. In 1999, he was appointed as department head of cytokine biology unit at Utrecht University. In the same year he founded U-CyTech biosciences. Approximately 50% of his time now he is involved in research aimed at optimizing cytokine ELISA and ELISPOT systems