

The 2011 Improving Immunohistochemistry Discussion Forum

The Penridge Suite, 470 Bowes Road, London N11 1NL: 28th October 2011

This technical workshop focusses on the technique of immunohistochemistry, from DAB to double staining. The invited experts will be give short overview presentations on their sub-topics, with the majority of the day dedicated to informal question and answer troubleshooting sessions.

Meeting Chair: Dr Will Howat, Head of Histopathology/ISH at Cambridge Research Institute, Cancer Research UK

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 – 9:30 **Registration**

9:30 – 9:35 **Introduction by Meeting Coordinator:** Dr Mickey Ramalho, Euroscicon, London, UK

9:35– 9:55 **Tips for IHC Validation**

Dr Will Howat

Cambridge Research Institute, Cancer Research UK

Validation of a research antibody is a crucial step towards publication and use as a clinical tool, yet there is a huge variation in the quality of published IHC both from the antibody vendors as well as academic groups. This talk will provide a brief overview of the steps that are required to provide a comprehensive validation of an antibody and the results of a large validation study.

9:55 – 10:15 **Breast Biomarkers: Real World & QC Assessment Results; The Continued Need for Improvement.**

Dr Merdol Ibrahim

UK NEQAS ICC & ISH, Hamilton House, Mabledon Place, London, UK

In the UK approximately 45,000 new breast cancers are detected per year, with tissue undergoing immunohistochemistry for HER2 IHC/ISH and breast hormonal markers (ER & PR). Between 2007-2008 UK HER2 positivity rate was on average 15% (range 9-35%). Since 2008 UK NEQAS ICC & ISH has been running a web based breast biomarker database to give insight into National breast biomarker rates in the UK. Furthermore, in 2011 a web based HER2IHC interpretive pilot module has been launched, which give further insights into post analytic phase of interpretation. UK NEQAS ICC & ISH assessment data indicates that the majority of UK laboratories use commercially available standardised HER2 IHC kits, however, no laboratories use a 'standardised kit' for ER & PR, with a 'home-brew' method being the method of choice.

The national audit data shows that the overall HER2 positivity rates has remained at around 15%. Individual ER and PR data show the average positive rates to be 82.3% and 68.7%, respectively. The combined, HER2, ER and PR, breast biomarker status for 2009/10 is 1) 60.5%: ER+/PR-/HER2+ 2) 12.3%: ER-/PR-/HER2- 3) 11.0%: ER+/PR-/HER2- 4) 6.2%: ER+/PR+/HER2+ 5) 5.1%: ER-/PR-/HER2+ 6) 3.1%: ER+/PR-/HER2+ 7) 1.3%: ER-/PR+/HER2- and 8) 0.4%: ER-/PR+/HER2+. Some of the 'real world' data may be explained by false positive/negative hormonal receptor status which is seen in the UK NEQAS ICC & ISH assessments, indicating the need for further optimisation of laboratory protocols and methodologies. The HER2 IHC pilot interpretive module also indicates that, mis-interpretation of previously diagnosed HER2 IHC cases may further account for the false positive/negative results seen in the 'real world' diagnostic results.

The audit database has enabled i) nationwide biomarker positivity rates for the UK and ii) provided testing centres with their individual breast biomarker positivity rates. In some cases the audit has lead to laboratories re-testing their cases. Combining 'real world' data with UK NEQAS assessment results, provides further evidence that such cases of ER-/PR+/HER2- (1.3%) and ER-/PR+/HER2+ (0.4%) may not be genuine but due to an accumulation of factors, from the pre-analytical, analytical and post-analytical testing stages.

10:15 – 10:35

Multiplex IHC: Staining Procedures and Chromogens

Dr Chris van der Loos

Dept. of Pathology, Academic Medical Center, University of Amsterdam, *The Netherlands*

Imaging multiple markers in a tissue section is known as a difficult task. When selecting the right chromogens, co-localization can be observed, but further quantitation is impossible. Basic double staining methods are presented including a new method with a heating step in-between staining sequences for removing the antibodies, but leaving the deposited chromogens in place. This double staining also allows an easy extension from double-staining to triple- and even quadruple-staining coming to 'multimarker tissue analysis'.

10:35 – 10:55

Antibody Labelling in Thick Skin Sections

Dr Carrie Ambler

Durham University, UK

The skin is a dynamic organ in which the outer layers are continually shed and replaced by stem cells. My lab is characterising how the interplay between the epithelia and underlying supporting cells regulates these skin stem cells. We found activation of Notch signalling in the basal epithelial layer dramatically alters the organisation and composition of both the epithelia and the underlying dermis. Currently, we are investigating how the Notch signalling network is transmitted in skin cells by looking for expression of Notch pathway genes in the skin and hair follicles. Additionally, my lab is using a combination of proteomic and genomic approaches to investigate how epithelial-mesenchymal interactions regulate skin stem cells in vivo with a view to understanding their role in skin homeostasis and disease.

10:55 – 11:15

Mid-morning Break

11:15 – 11:35

Substrates: 'Any Colour as Long as it's Brown?'

Dr Tony Warford

Managing Director, Warford Technology, UK

The use of Horse radish peroxidase (HRP) with diaminobenzidene has become synonymous with paraffin wax IHC. The enzyme/substrate combination is easy to use and when employed with sensitive detection systems provides a crisp, intense and permanent brown precipitate. However, it has never been number one for chromogenic ISH where alkaline phosphatase (AP) combined with NBT/BCIP provides significant amplification for simple detection systems. Furthermore for delicate frozen section and cell preparations several HRP and AP enzyme/substrate combinations can provide excellent results without risk of comprising morphology or cytology. Accordingly, the aim of this presentation will be to stimulate discussion as to choice of IHC colour card.

11:35 – 11:55

Introducing BOND-RX; Leica's Fully Automated IHC/ISH Research Platform

Mr David Roche

Leica Microsystems

The Leica BOND RX builds on our previous research offering and Leica BOND-III benefits, delivering improvement across three major areas: Software, Reagents & Detection, Speed

11:55 – 12:15

Cell by Cell – Image Analysis on Aperio Whole Slide Images with Definiens Tissue Studio®

Dr. Thomas Einert,

Definiens, Germany

Co-sponsors with Aperio

Definiens Tissue Studio® 3.0 is the solution of choice for biomarker and morphological profiling in translational research because it is easy to use, precise, robust, powerful and fast. Regions of interest are accurately detected and cells as well as sub-cellular objects can be distinguished within these target regions – fully automatically. Morphology and expression profiles per individual cell or cell compartment are readily measured, helping to solve your most challenging biological questions. Whether you are working with bright field or immunofluorescence images; on whole tissue slides or microarrays; Definiens Tissue Studio® 3.0 is your ultimate resource for comprehensive digital pathology image analysis. Definiens Tissue Studio® 3.0 is perfectly complemented by Aperio Spectrum. The value of automated IHC analysis and of the integrated workflow for digital pathology provided by Definiens and Aperio will be presented.

12:15 – 12:40

Working Lunch

Please collect your lunch and take it to your discussion table (Session 1)

12:40 – 14:10

Discussion Group Sessions 1 - 4

- Round table discussion groups (20 minutes each) will be held throughout the afternoon
- Delegates will rotate so that they may participate in all the discussion tables
- All delegates will also be allocated a session for visiting the exhibition stands
- Where appropriate delegates will be able to bring their samples to the discussions
- See end of agenda for description of discussion tables

14:10 – 14:30

Getting 'more' out of Morphology; Permission to Multiplex!

Ms Roslyn Lloyd

Caliper Life Sciences

Multiplex IHC images analysed by spectral unmixing allows quantitation of individual markers and co-localisation of up to six chromogens in a tissue section. An innovative, learn-by-example, image analysis software allows quantitation up to the level of individually stained cells.

14:30 – 15:35

Discussion Group Sessions 5 - 7

15:35 – 16:20

Question and Answer Session

This session will include summing up of the discussion tables and questions submitted both prior to the meeting and throughout the day

16:20 – 16:30

Chairman's Summing Up and Feedback Prize Draw

Round-table Discussion Sessions:

Table A: Tips for IHC validation

Hosted by Dr Will Howat, who graduated with a BSC (Hons) in Immunology & Pharmacology from the University of Strathclyde, before gaining a PhD in Pathology from the University of Southampton. After two post-doctoral positions in Southampton, he moved to the Wellcome Trust Sanger Institute in Cambridge as the leader of Research & Development for the Immunohistochemistry group of the Atlas of Protein Expression project. He is now with Cancer Research UK as the head of the Histopathology/ISH facility at the Cambridge Research Institute

Table B: External Quality Control of Immunocytochemistry - Optimising Protocols

Hosted by Dr Merdol Ibrahim, who has been the manager of the (UK NEQAS ICC) since 2004. He obtained his PhD from the University of London and concentrated on immunohistochemical and morphological analysis of CNS myelination. From 1997 to 2001, he was in Switzerland, within the department of Histology in Fribourg then with Novartis Pharma (Basel), within the department of Toxicopathology. From 2001 to 2004, he was a research scientist at the Institute of Psychiatry (London), studying mechanisms of brain plasticity. Merdol was appointed the manager of the UK NEQAS-ICC in 2004, where he oversees the quality of immunocytochemistry produced 600 clinical laboratories, from 54 countries.

Table C: Multiplex IHC Strategies

Hosted by Dr Chris van der Loos, a Histotech involved with many different topics at the Academic Medical Center, Dept. of Pathology since 1974. In 1992 wrote a PhD thesis on multiple staining methods as first technician in the AMC.

Table D: Antibody Labelling in Thick Skin Sections

Hosted by Dr Carrie Ambler, who received her doctoral training from the University of North Carolina at Chapel Hill in vascular development. As a post-doc she joined the lab of Fiona Watt at Cancer Research UK from 2003 to 2007 before starting her own laboratory at Durham University. Carrie Ambler's research both past and present focuses on the development, maintenance, and differentiation of epithelial stem cells both during embryonic skin development and in adult skin homeostasis.

Table E: Substrates: 'Any Colour as long as it's Brown?'

Hosted by Dr Tony Warford, whose expertise is in molecular histopathology. He has set up and managed laboratories in the UK health service, academic institutions, biotechnology and Pharmaceutical companies. Technology developments he has spearheaded include the introduction of diagnostic immunohistochemical methods, validation of antibodies for use as biomarkers, production of probes and methods for in situ hybridisation and supervision and interpretation of GLP tissue based safety studies of potential therapeutic antibodies. Concurrently he has championed quality assurance programmes in histopathology and automation of immunohistochemistry coupled with image capture and analysis. He has also run laboratory safety and human bio-banking programmes. He has published in these fields and shared experience with fellow scientists by organising wet workshops, chairing symposia and lecturing in many countries.

Table F: From Tissue to Data – Hands on the Integrated Digital Pathology Workflow by Aperio Whole Slide Imaging Systems, Spectrum Plus and Definiens Image Analysis.

Hosted by Mr Brian McClintock, who is a European Sales Manager with Aperio, with a BSc. J Hons. in Biochemistry/Physiology. He has worked in clinical laboratories and lectured in Clinical Chemistry in Papua New Guinea and Tonga, as well as achieving FIBMS, by examination at the Royal Berkshire Hospital, Reading. He moved to sales, twenty five years ago, selling Laboratory instrumentation and LIMS. He joined Aperio eight years ago, when they had thirty people worldwide. They now have more than 850 systems in over 30 countries, including more than 550 systems in hospitals and reference laboratories. The company's customers also include the 13 largest pharmaceutical companies as well as numerous biotechnology and government organizations.

About our Invited Speakers:

Mr David Roche, who since leaving clinical histology laboratory work in 2003, has spent the last 8 years in various posts within Leica and affiliated companies, including; product development, clinical testing, applications support, sales & account management. His main area of interest for all of this time has been IHC and ISH applications, specifically the BOND automated IHC/ISH systems, Novocastra antibodies and research applications.

Dr Thomas Einert is working as a Field Application Scientist at Definiens AG in Munich being a specialist for image analysis and digital pathology applications. Prior to that, he completed his doctorate in Biophysics at the Technische Universität München, Germany, focusing on theory of bio-polymers folding.

Ms Roslyn Lloyd is a multidiscipline scientist with several years experience working within multispectral imaging.

Keywords:

IHC, immunohistochemistry, multimarker tissue analysis, chromogens, spectral imaging, enzymes, substrates, enhancers, quality assurance, double staining, technical, troubleshooting, skin, epidermis, dermis, antibody labelling, HER2 IHC, oestrogen receptors, progesterone receptors, breast biomarkers, digital pathology, image analysis, biomarker development, cell morphology, automated IHC/IF algorithms, ISH, Leica, BOND, research, autofluorescence removal, automation, multiplex, colocalisation, unmixing.

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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.

Registration Web Site: <http://www.regonline.co.uk/WorkshopIHC2011>