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Committee Page

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BLURB FROM THE BUSH

With winter upon us it also means that Trivia is upon us. Tables are starting to book already for what again promises to be the premier histology social event of the year. There are plenty of spots still available, but don't risk being too late. The trade are already showing their marvellous support of the event, and we hope again to see a few country labs make their way to the big smoke and show the city folk how to consume multiple refreshments responsibly throughout the night.

The committee continues to work on the planning phase of the National Conference with input from the other states also. We hope to be able to release more details by spring when we will start seeking to fill our program.

We have had our last scientific meeting at the old Peter MacCallum. It has served us well, and whilst we venture to St Vincent's Hospital we wait with bated breathe to see what the new Peter MacCallum has to offer. Peter MacCallum also served as the venue for HGV committee meetings until relatively recently. Many a long night was spent laughing around the committee table over cheese and wine and manually packing the newsletter to take down to the post office. Good luck Sue with the move!

Adrian Warmington

HGV President

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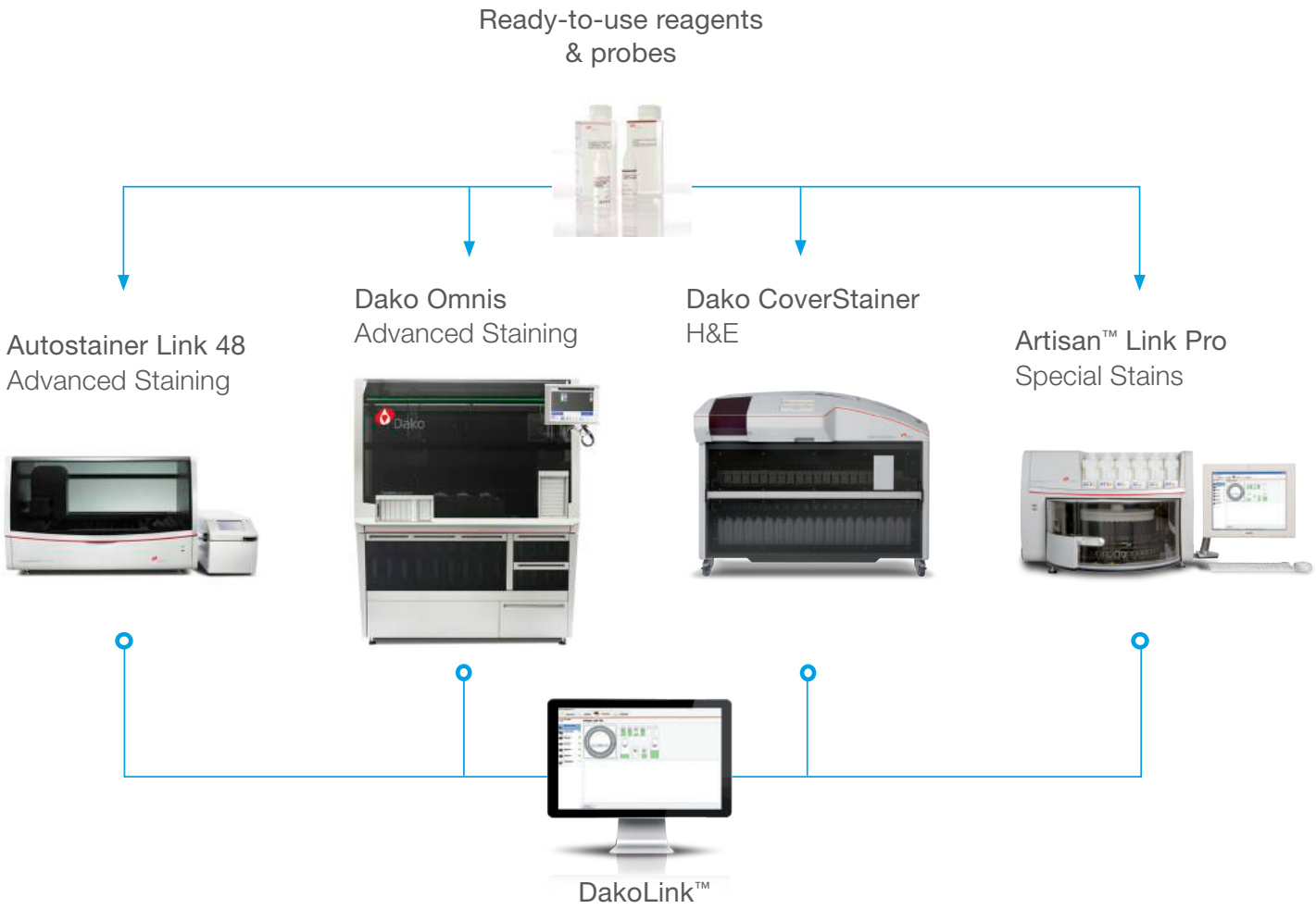

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Review of The Fifth International Workshop in Diagnostic Immunohistochemistry-Part 2

Classification of the Unknown Primary Undifferentiated Tumour (UPT)

A tumour appearing in a metastatic setting without a histologically proven primary tumour is considered to be a metastatic tumour of unknown primary origin. Such tumours constitute an increasing challenge to pathologists due to significant improvements in surgical and oncological treatment options. New, more specific antibodies are continuing to improve the pathologist's diagnostic abilities, accompanied by greater complexity associated with optimising protocols, interpreting staining results and minimising errors. Even so, 10-15% of such tumours remain "UPTs" (unknown primary tumours). Different prognoses lead to different treatment regimes, so the more specific a diagnosis can be, the better. Cost effective, minimally invasive pathology testing is the way of the future. There are multiple websites and publications available for reference such as: www.e-immunochemistry.info

www.Pathology Outlines.com

PathIQ Immunoquery

NordiQC

Primary Panel for the Unknown Primary Tumour

NEOPLASM	CD45	CK	S-100	VIM
Haemato-Lymphoid	+/(-)	-/(+)	-/(+)	+/(-)
Epithelial	-	+/(-)	-/+	-/+
Mesothelial	-	+	-	+
Mesenchymal & neuronal	-	-/(+)	-/+	+
Non-neuronal Neuroepithelial	-	-/(+)	+	+
Germ cell	-	-/+	-/+	+

CD45 (LCA) leucocyte common antigen is a transmembrane protein tyrosin phosphatase essential for haematopoietic signal transduction and cell activation. It is present in a large majority of haematolymphoid cells, lost in maturing erythrocytes, megakaryocytes and plasma cells and 'never' found in non-haematolymphoid cells. More than 90% of lymphomas express CD45.

Cytokeratins comprise two of the five classes of intermediate filaments. Type 1 are acidic cytokeratins, Type 11 are basic-neutral cytokeratins. Cellular filaments are classified according to size: microfilaments (6nm); intermediate filaments (7-11 nm) and microtubules (23nm). Intermediate filaments are mostly cytoplasmic, forming a meshwork within the cytoskeleton of nearly all cells connecting nuclear and cell membranes. They are often associated with microfilaments and microtubules and are important for mechanical strength and cellular functions. Cytokeratins belong to the most fundamental markers of epithelial differentiation and comprise a large family of subtypes. Different cell types express different patterns of subtypes. Cancers generally express cytokeratin patterns representative of the cells of origin, and metastatic cytokeratin patterns reflect that of the primary tumour.

S-100 protein belongs to a family of calcium-binding proteins 9-13 kDa in size, located in the nucleus, cytoplasm and cell membranes, there are at least 10 α- and one β-chain which create homo- and hetero-dimers. S-100 β-chain is mainly found in melanocytes, glial cells, Langerhan's cells, adipocytes and myoepithelial cells and is detected by polyclonal antibodies.

Vimentin is a 57kDa cytoplasmic intermediate filament present in all mature mesenchymal cells, and present in early developmental stages of all cells, subsequently replaced by other intermediate filaments in most non-mesenchymal cells. Vimentin can be co-expressed with cytokeratin in some epithelia such as endometrium and renal tubules, and also in some non-epithelial cells such as mesothelium.

SECONDARY PANELS FOR CARCINOMA IDENTIFICATION AND SUBCLASSIFICATION.

Cytokeratin subtypes
Oncofoetal proteins
Transcription factors
Neuroendocrine proteins
Hormone receptors
Secretory proteins
Cell adhesion molecules

Breast markers
Lung markers
GI markers
Female genital tract markers
Urinary tract markers
Prostate markers

Squamous cell markers
Mesothelial cell markers
Neuroendocrine cell markers
Liver markers
Adrenal cortical markers
Germinal cell markers

IMMUNOHISTOCHEMICAL CLASSIFICATION OF MALIGNANT LYMPHOMAS (with thanks to Jan Klos)

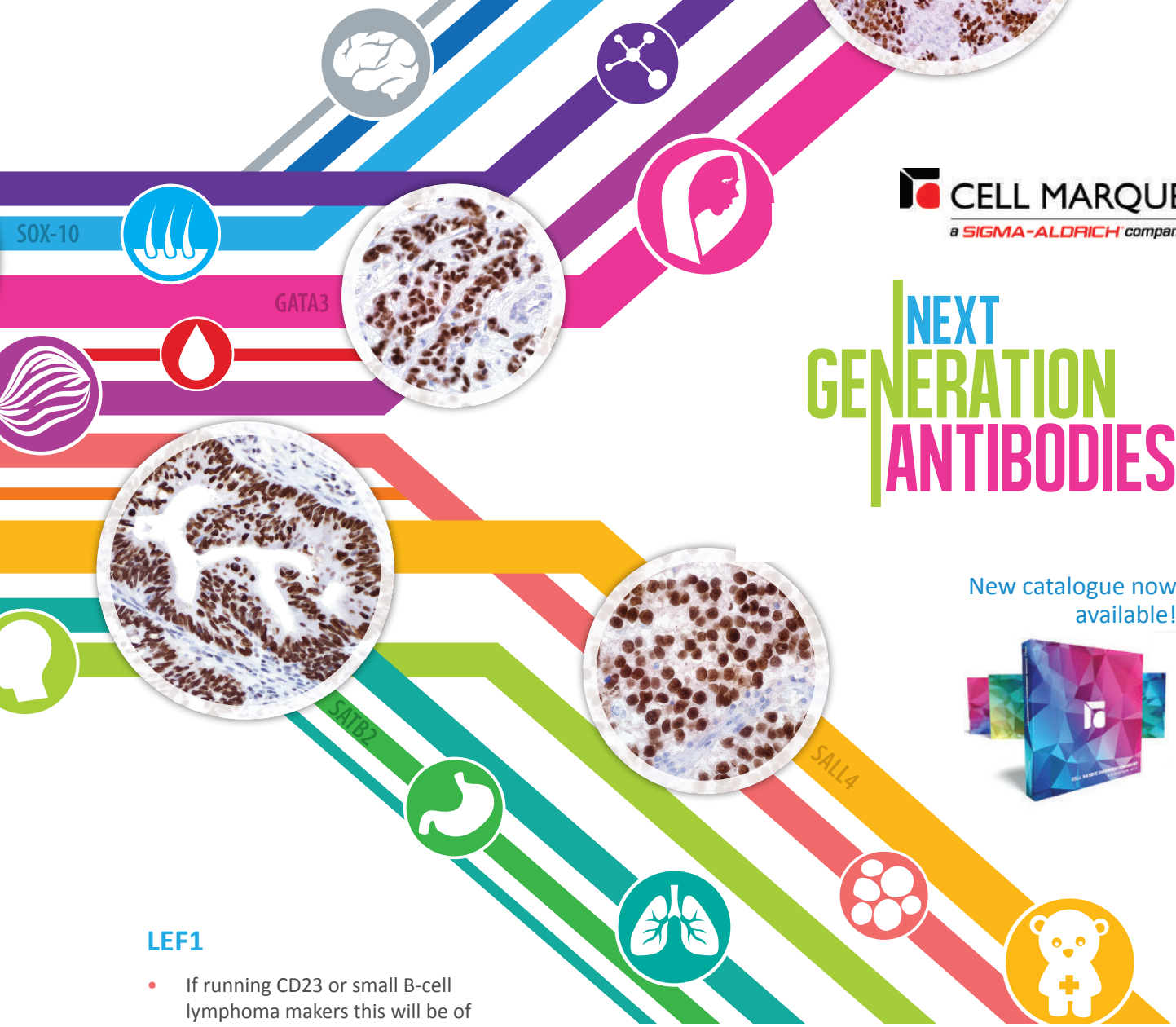
Correct classification of lymphomas distinguishes them from non -haematopoietic neoplasms and reactive lesions and clarifies the prognosis. Neoplasms of lymphoid tissue comprise approximately 5% of all human malignancies.

The relative frequencies of lymphoid malignancies

B-cell	Hodgkin	T-cell
10	3	1

Determining cell lineage in lymphoma

Blasts	TdT+	CD10+	CD34 +/-	CD1a+(T-blasts)	
B-cell	CD20+	PAX5 +	CD79a +		
Plasma cells	CD 138	Kappa/Lambda +	CD79a +/-		
T-cell	CD 3	TCRαβ/λδ+	CD 2+	CD 5+	CD 7+
NK cell	CD 2+	CD 56+	c-CD3ε+		
Hodgkin/Reed - Sternberg cells	CD 15+	CD 30+			



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- If running CD23 or small B-cell lymphoma makers this will be of interest!
- LEF1 is a nuclear transcription factor that helps distinguish CLL/SLL from mantle cell lymphoma
- It works similarly to CD23, but is a nuclear stain and is considered a good addition to the small B-cell lymphoma panel

PHOX2B

- A useful antibody for paediatric pathologists and neuropathologists
- PHOX2B is a nuclear marker that is used in the diagnosis of neuroblastoma
- It helps differentiate undifferentiated neuroblastomas from other small round blue cell tumors in pediatrics
- Highly sensitive for peripheral neuroblastic tumors and is also expressed in paragangliomas and pheochromocytomas

New antibodies include:

- Arginase-1 (EP261)
- EGFR (EP22)
- LEF1 (EP310)
- LEF1 Control Slides
- MUM1 (EP190)
- Myosin, Smooth Muscle (EP166)
- PHOX2B (EP312)
- PHOX2B Control Slides
- SOX-2 (EP103)
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TdT- Terminal deoxynucletidyl transferase (TdT) is quite specific for lymphoid cells acting as DNA polymerase in B- and T-cell precursors. TdT is present in cortical thymocytes and some precursor cells in the bone marrow. Acute lymphoblastic leukaemia and lymphoblastic lymphoma are TdT +ve, as are 30% of blast crises in chronic myeloid leukaemia.

CD34 is a transmembrane glycoprotein with a cell adhesion function present on 1-4% of bone marrow cells and endothelial cells. CD34 is expressed in most AML and B-ALL, and is rarely expressed in T-ALL and many solid tumours.

CD20 is a membrane protein virtually specific for all B-cells involved in cell activation, proliferation and differentiation. CD 20 is expressed in the vast majority of B-cell leukaemias and lymphomas.

CD79a is involved in signal transduction in B-cells. It is a highly specific pan B-cell marker expressed at all stages of B-cell differentiation including ~50% of plasma cells. (recommended for CD20-ve cases).

PAX-5 (B –cell specific antigen or BSAP) is a transcription factor highly specific for B-cells present from the early stages of differentiation to mature B-cells but not plasma cells.

BCL-2 is an anti-apoptotic protein present on nuclear and mitochondrial membranes and smooth endoplasmic reticulum. It is present in many cells including mature B- and T-lymphocytes but is down-regulated in germinal centre cells. BCL-2 is useful in the differential diagnosis of follicular lymphoma versus reactive hyperplasia.

BCL-6 is a transcription regulating nuclear phosphoprotein expressed in the nuclei of germinal centre B-cells and subpopulation of T-cells. It is expressed in all follicular lymphomas, but does not differentiate between follicular lymphoma and hyperplasia.

CD10 is a membrane-bound endopeptidase expressed in bone marrow and haematolymphoid stem cells, immature B-cells and follicular centre cells. It is useful in diagnosing B-cell neoplasia and angioplastic T-cell lymphoma. >90% B-cell ALL and 20% of T-cell ALL are positive. CD10 is also positive in AML, neutrophils and many epithelial and mesenchymal tumours.

Cyclin D1 is a cell cycle protein expressed in the G1/S phase, and is present in nearly all mantle cell lymphomas and some plasma cell myelomas. Endothelial cells are also positive. Cyclin D1 can be present in non-lymphoid solid tumours.

Ki-67 is a nucleoprotein present in the G1-M phase of the cell cycle but not the G0. It is indicative of the percentage of proliferating cells and often correlates with prognosis, as well as classifying lymphomas in to low grade (<20%) and high grade (>40%). If close to 100% of cells express Ki-67 Burkitt's lymphoma, ALL or or Burkitt-like DLBCL are suspected.

Kappa and Lambda Light chains –one or the other is present in all immunoglobulin molecules and are specific B-cell markers occurring first on the membrane then in the cytoplasm as cells mature towards plasma cells. They are present in the cytoplasm of many lymphomas, and all immunoblastic, plasmablastic lymphomas and myelomas. Immunostaining is associated with a great deal of background, and demonstrating membrane staining in paraffin sections is problematic.

CD 138 is a transmembrane adhesion molecule important for cell growth, differentiation and adhesion expressed in some types of epithelial cells, early pre-B lymphocytes and plasma cells. It is positive in many carcinomas and early precursor B-ALL.

MUM-1 is a nucleoprotein regulating the development and maturation of B-cells to plasma cells present late in B-cell development after CD10 and BCL-6. Mum-1 protein is expressed in the nuclei of plasma cells and a small percentage of germinal centre B-cells, activated T-cells, Reed-Sternberg cells and a wide spectrum of haematolymphoid neoplasms derived from these cells. Anti-Mum-1 is useful in the sub-classification of lymphoid malignancies.

T-cell and NK-cell lymphomas.

These are rare neoplasms, frequently found in Asian populations, related to HTLV-1 infections. Symptoms can include hypercalcaemia and haemophagocytic syndromes. The prognosis and response to therapy for these neoplasms is usually worse than for other lymphomas. Morphologic features are broad-ranging and often over-lapping, abnormal antigen expression or loss of reactivity for typical T-cell antigens are common immunohistochemical features. As with many neoplasms, diagnosis relies on the big picture: clinical findings, morphology, immunophenotype, cytogenetics and molecular profile.

CD 3 is a transmembrane polypeptide which functions as a component of the TCR complex specific for T-cells and is expressed in the cytoplasm of NK-cells. Membranous expression is specific for T-cells. 90% of T-cell and most NK-cell lymphomas express CD 3. It can be difficult to distinguish membrane and cytoplasmic staining of T-cells from purely cytoplasmic staining of NK-cells and immature T-cells.

CD5 is a transmembrane glycoprotein expressed on T-cells and more weakly in a sub-population of B-cells, but is used as a fairly specific pan T-cell marker. CD 5 expression is lost in some T-cell lymphomas, and is negative in NK-cell lymphomas.

ALK is a growth hormone receptor present normally only in the cytoplasm of a few cells in the CNS. It is expressed in ALK+ ALCL, rare cases of ALK+DLBCL, 50-70% of inflammatory myofibroblastic tumours and some solid tumours.

Hodgkin Lymphoma Markers

CD 30 is a non-specific membrane bound activation marker expressed in activated immunoblasts, Reed-Sternberg cells, most ALCL, primary cutaneous CD30 + T-cell lymphoproliferative disorders and embryonal carcinomas. Only membrane and Golgi staining is accepted as positive.

CD15 is a carbohydrate-derived haematopoietic differentiation antigen expressed in mature granulocytes, myeloid and monocytic cells, many carcinomas and CMV infected glandular cells. It is used to differentiate classic Hodgkin lymphoma from most of its mimics, and also to sub-type acute leukaemias. 5% of classic HL express T-cell markers.

Other antibodies

Granzyme B, TIA-1 and perforin are the most common cytotoxic proteins present on CD8+ T-cells and NK cells.

Oct-2 and BOB.1 are both present in all Ig-producing B-cells.

Other useful antibodies include CD21, EBV, HHV-8.

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IMMUNOHISTOCHEMICAL CLASSIFICATION OF THE UNKNOWN PRIMARY CARCINOMA- THE CYTOKERATINS

The cytokeratins are a highly complex family of intermediate filaments, with more than 50 distinct types. The most diagnostically relevant are CK1 - CK20. Class 1 (type A-acidic) are CK9 – CK20. Class 11(type-B basic/neutral) CK1 - CK8. Cytokeratins occur in pairs of one CK class 1 and one CK class 11, with the CK class 1 in the pair being ~ 8 kDa smaller than the class 11 CK.

Cytokeratin types and cell types

Neutral/ Basic (B, class 11)	**1**	**4**		**5**				**7**		**8**
Acidic (A, class 1)	**10**		**13**		**14**	**17**	**19**		**20**	**18**
SQUAMOUS EPITHELIA:										
-suprabasal, keratinising	+++			+	++	(+)				
-suprabasal, non-keratinising	+	+++	+++	+	++	(+)				
-basal cells (tonsil, mucosa)				+++	+++	(+)	(++)	(+)		(+)
TRANSITIONAL EPITH: superficial cells							+++	+++	++	+++
-intermediate/basal cells		(+)	+++	(+++)		(++)	+++	+++	(+)	+++
MESOTHELIUM				++	++	+	+++	+++		+++
Bronchus, breast, prostate, cervix										
-basal/ myoepithelial cells				+++	++	+++	++			
-luminal cells				+	+	+	+++	+++		+++
Biliary/pancreatic ducts, lung alveoli, Endometrium, renal collecting ducts							+++	+++		+++
Stomach, intestine							+++	(+)	+++	+++
Hepatocytes, pancreatic acini, proximal renal tubules										+++
Endocrine cells(Merkel, thyroid)							(+++)	(++)	(+++)	
Smooth muscle(vascular, myometrium), myofibroblasts, vessel endothelia							+	(++)		++

Cytokeratins in epithelial neoplasia

Neutral/ Basic (B, class 11)	**1**	**4**		**5**				**7**		**8**
Acidic (A, class 1)	**10**		**13**		**14**	**17**	**19**		**20**	**18**
Squamous cell carcinoma	(+)	+	+	++	++	(+)	(+)	(+)		(+)
Transitional cell tumour		+	+	+	++	+	++	++	++	++
Malignant mesothelioma				++	++	+	++	+		++
Adenocarcinoma: Complex epithelia (bronchial, breast, prostate)				(+)	(+)	(+)	++	++		++
Adenocarcinoma: biliary tract, pancreas, endometrium, ovary				(+)	(+)	(+)	++	++	(+)	++
Adenocarcinoma: stomach							++	+	+	++
Adenocarcinoma: intestine							++	+	++	++
Hepatocellular carcinoma, renal carcinoma								+		++
Endocrine tumours: carcinoids							+	+		++
-Merkel cell carcinoma							+		++	++
-Thyroid carcinoma							+	++		++

	CK20+	CK20 AMB	CK20-
CK7+		Lung MBAC Oesophagus Biliary tract Pancreas Ovarian mucosa urothelium	Lung Breast (ductal, lobular) Thyroid (pap, foll) Endometrial Cervix Ovary (non-mucous)
CK7 AMB	Appendix Small intestine Rectum	Stomach	Neuroendocrine Germ cell Chromophobe renal
CK7 -	Colon Merkel cell	Carcinoid Liver cell ca Renal cell ca	Adrenal cortex ca Prostate

Coordinate CK7/CK20 expression

The easiest way to summarise the vast amount of information presented during this session was to reproduce the three preceding tables directly from Mogens' presentation. By referring to the NordiQC website, information regarding the best performing antibody clones and protocols can be obtained.

IMMUNOHISTOCHEMICAL CLASSIFICATION OF BREAST CARCINOMAS

Breast markers for unknown primary tumours

Oestrogen receptor	**Predictive**
GCDFP-15	
GATA-3	
Mammaglobin	
Cytokeratins	**Subclassification**
E-Cadherin	**Subclassification**
Smooth muscle markers	**Subclassification**
HER-2	**Predictive**

Oestrogen sensitive tissues include breast, ovary, fallopian tube endo- and myometrium and uterine cervix. To a lesser extent thyroid, pituitary, pancreas, sweat and salivary glands, prostate and liver also exhibit sensitivity. > 80% of breast carcinomas are ER +ve.(>1% ER staining) It should be noted that there can be quite a difference in staining results depending on the antibody clone being used, out of 92 lung adenocarcinomas 8%, 14% or 27% demonstrated ER receptor staining depending on which antibody clone was used!!(Gomez-Fernandez et al 2010).

Gross cystic disease fluid protein-15 is a prolactin-induced glycoprotein localised in apocrine glands in the axilla, vulva, eyelid and ear canal, and in the apocrine metaplastic epithelium lining breast cysts. Anti-GCDFP-15 is the most specific marker for breast carcinoma, over 70% of breast carcinomas exhibit positive staining.

Mammaglobin is a mammary-specific member of the uteroglobin family, identified in a substantial proportion of primary and metastatic breast carcinomas. Anti-mammaglobin has a higher sensitivity and lower specificity for primary and metastatic breast carcinoma than GCDFP-15

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GATA-3 is a transcription factor that recognises the G-A-T-A nucleotide sequences in target gene promoters, directing cell proliferation, development and differentiation in many different cell types such as T-cells, breast epithelium, urothelium, kidney, skin and trophoblasts. GATA-3 is primarily seen in breast and urothelial carcinomas, as well as skin cancers such as BCC and SCC, and yolk sac tumours.

Breast Tumour Agenda

• Immunohistochemical biomarkers for
– Invasive vs. non-invasive lesion

• Benign Hyperplasia and Atypical Ductal Hyperplasia
• Ductal Carcinoma in Situ and Lobular Carcinoma in Situ
• Carcinoma In Situ and Invasive Carcinoma

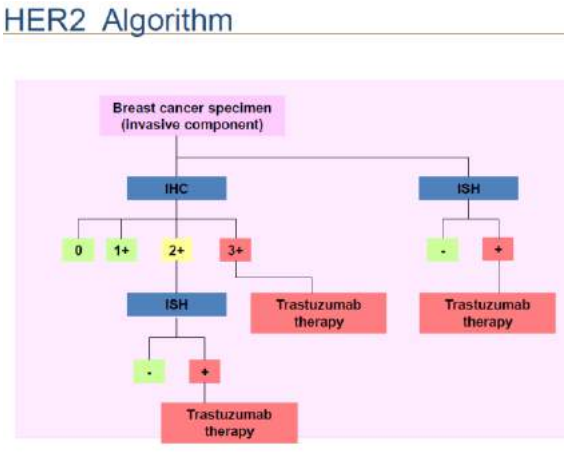
– Invasive Carcinoma
• Histological subtypes
• Prediction/Prognosis
– Estrogen Receptor
– Progesteron Receptor
– HER2
– Ki67
– Intrinsic subtype classification by surrogate biomarkers

Diagnostic markers

Luminal epithelial markers	**Myoepithelial/stromal markers**
CK-LMW(8/18, 7,19)	Smooth muscle myosin, (Smooth muscle actin, Calponin)
E-Cadherin	CK5/14
Mammaglobin	P63/P40
GCDFP-15	Basal membrane collagen IV
GATA-3	
Androgen receptor	
Nestin	

Ductal	~80%
Lobular	5-14%
Tubular	2-8%
Mucinous	2-4%
Apocrine	1-4%
Papillary	1-2%
Other	

Histological sub-types of invasive breast cancer



(copied from Mogens Vyberg's presentation)

HER-2 targeting has changed the natural history of HER-2 positive advanced breast cancer.

Tumour hetergeneity accounts for significant variation in staining patterns for both hormone receptors and HER-2 within the same tissue sample.

In summary below, also taken directly from Mogens' presentation:

American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of:
Estrogen and Progesterone Receptors (Arch Pathol Lab Med. 2010;134:e48-e72) and
HER2 (ahead of print at www.jco.org on October 7, 2013) in Breast Cancer
()

- **Preanalytical standardization**
  - Fixation
- **Analytical standardization**
  - Antibody/Antigen Retrieval/Detection Systems
  - Control samples
- **Postanalytical standardization**
  - Interpretation
  - Cut-off level
  - Internal quality control
  - Tissue / Material
  - Image Analysis
- Participation in quality assurance programs

IMMUNOHISTOCHEMICAL CLASSIFICATION OF LUNG CARCINOMAS AND MESOTHELIOMAS

Lung cancer derives from stem cells in the lung epithelium. It is usually detected by radiological imaging, and diagnosed from a bronchial biopsy. Lung markers include TTF-1, Napsin A and p63/p40.

Thyroid transcription factor-1 (TTF-1) in the thyroid regulates thyroglobulin, thyroperoxidase and thyrotropin receptors, and in lung regulates surfactant proteins and Clara cell secretory protein. It is expressed in thyroid follicular cells and C-cells, type II pneumocytes, Clara cells and is also found in the brain, pituitary and parathyroid.

There is variability of expression in various lung tumours depending on the antibody clone used.

% positive	**SPT24**	**8G7G3/1**
Lung adenocarcinoma	80	70
-large cell carcinoma	50	40
-small cell carcinoma	90	90
-carcinoid	60	20
-squamous cell	15	0
Non-lung small cell	20-40	?
Thyroid ca (non-anaplastic)	100	100
Non-lung carcinomas	0-5	0-5
Non-lung carcinoids	~0	~0

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Napsin A is an aspartic proteinase expressed as a single chain protein MW 38 kDa. It can be demonstrated in Type II pneumocytes, proximal and convoluted renal tubules and pancreatic acini and ducts. Napsin A is +ve in 80% lung adenocarcinomas, 0-30% lung SCCs and 0-70% renal cell carcinomas.

p63/p40 nuclear proteins and transcription factors belonging to the p53 gene family, and play a significant role in the growth and development of many epithelial types. They are expressed in squamous epithelium, urothelium, basal cells/ myoepithelial cells, lung, breast, sweat glands, salivary glands and prostate.

Lung cancer panel

	Napsin A	TTF-1	P63	CK5/6
Squamous cell carcinoma	-	-	+	+
Adenocarcinoma	+	+	-	-

3-6% of lung adenocarcinomas have a driver mutation causing a gene fusion which gives rise to the oncogene ALK-EML4 fusion protein. A kinase inhibitor, crizotinib is approved for treatment of ALK mutated lung cancers. ALK can be demonstrated by IHC, FISH and PCR. Around 36% of IHC positive ALK cases are found to be FISH +ve.

MALIGNANT MESOTHELIOMA as we know is an aggressive neoplasm resulting from asbestos exposure. Mesothelial markers include Calretinin, podoplanin (D2-40) and WT1.

Calretinin is a calcium-binding protein related to the S-100 proteins. It normally occurs in neurons, mesothelial cells, steroid-producing cells (adrenal cortical cells, testicular Leydig and Sertoli cells, and ovarian theca interna cells and surface epithelium), neuroendocrine cells and breast glands. In neoplasms, calretinin is expressed in adrenocortical carcinomas and adenomas, mesotheliomas, some soft tissue tumours and sex cord stromal tumours.

Podoplanin is a 38kDa transmembrane mucoprotein recognized by the D2-40 antibody. It is expressed in lymphatic endothelium, fibroblasts, osteocytes, smooth and striated muscle cells, renal glomerular podocytes…..and a lot of other things! Both podoplanin and WT1 are expressed in mesotheliomas and many other tumours but rarely adenocarcinomas, hence their usefulness in pleural tumour pathology.

……

Mogens Vyberg is a senior consultant pathologist at the Institute of Pathology, Aalborg Hospital, Denmark; Professor of Clinical Pathology at Aalborg University Hospital (AAUH); Head of the Laboratory for Immunohistochemistry –Research and development at AAUH; and co-founder and director of Nordic Immunohistochemical Quality Control (NordiqQC) which was established in 2003. His final presentation for the meeting was entitled **External Quality Assurance in IHC the NordiQC experience.**

"NordiQC is an international academic proficiency testing program primarily aimed at assessing the analytical phases of the laboratory IHC quality". NordiQC is an independent not-for-profit organization.

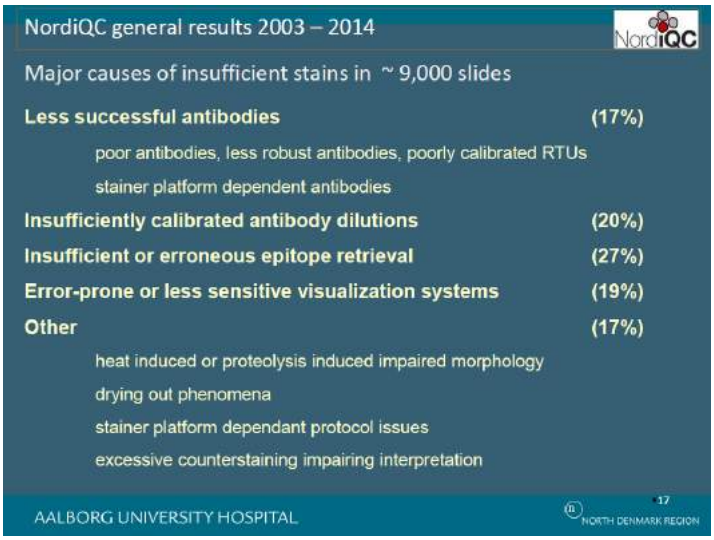
If you get one beneficial piece of information from this very lengthy report, let it be this:

www.nordiqc.org

A great deal of Mogens' presentation described the activities of the organization, and rather than have them reproduced here, I thoroughly recommend a visit to the website. There are 3 modules for annual assessment: general (15-18 different markers) 3 runs per year; Breast cancer IHC (Her-2,Er/Pr,Ki67/E-Cad) 2 runs per year; Her-2 ISH (BRISH,FISH) 2 runs per year.

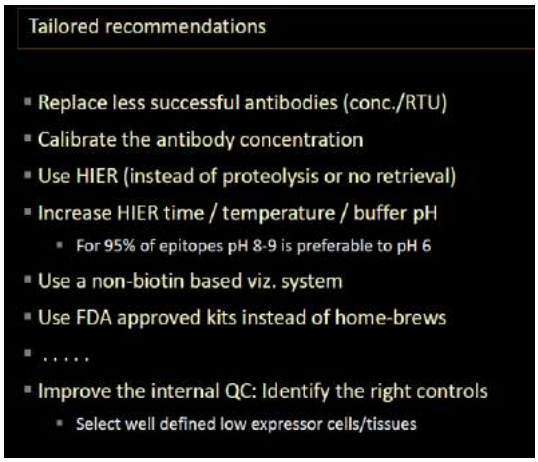
NordiQC : assesses the quality of the immunohistochemical assay based on standard processed tissues distributed for testing; identifies optimal and insufficient results correlated to antibodies, protocols and staining platforms; publishes the general results on the website and in Journals; gives directions for improvement, individually tailored if requested. Around 90 markers have been tested up to 15 times.

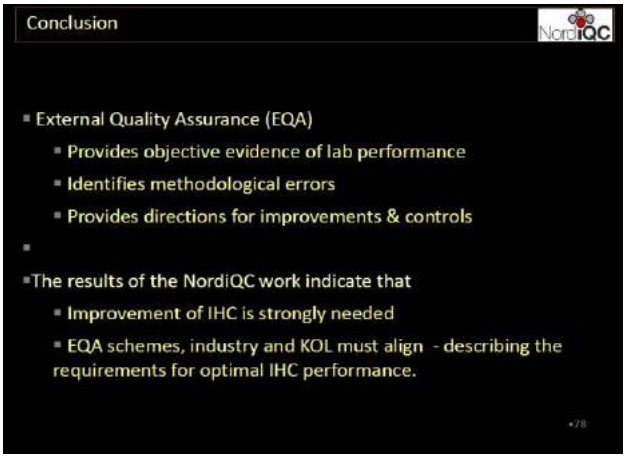
Scores are either optimal (perfect), good (totally acceptable but protocol could be optimized), borderline (insufficient due to weak, negative or false positive staining) or poor (very insufficient due to false negative staining of most samples or significant false positive staining).



Several examples of the causes for insufficient results as shown above (copied from Mogens' presentation) were discussed. Also discussed were some of the best and worst antibodies and clones (but you had to be there……!!!!)

I have included 4 slides from the presentation that pretty much sum it all up.





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See page U5, cat. no. UM-EC2800

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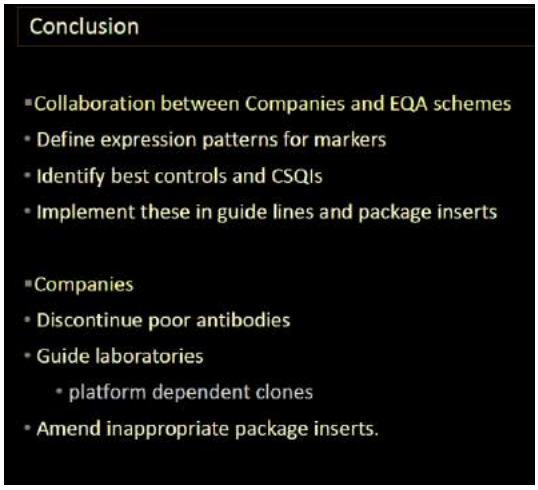

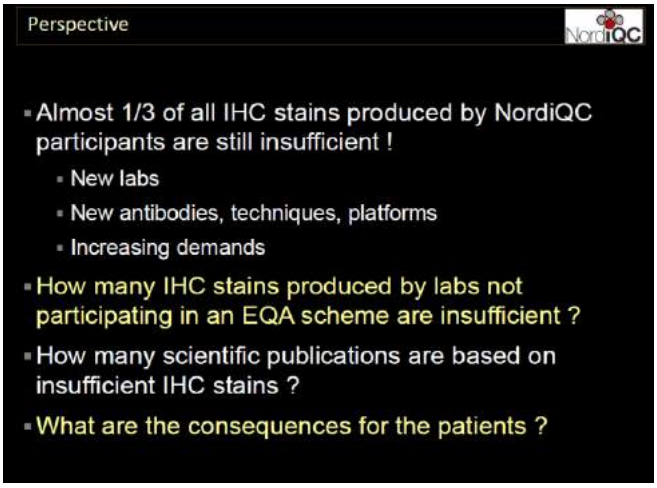

A good deal of the preceding report has been reproduced from Mogens Vyberg's presentations which were available on line to attendees at the meeting, for which I am very grateful for without access to these, my report would have been sadly lacking.

In conclusion, the 5th International Workshop in Diagnostic Immunohistochemistry was as usual a world-class meeting, held in maginificent surroundings at Coolangatta/Tweed Heads. Congratulations and many thanks to Eugen Petcu and the organising committee of the Australasian Immunohistochemistry Society and the sponsors.

Judy Brincat
Peter MacCallum Cancer Centre.

****

Under the Microscope with Andrew Kung

Anatomical Pathology Scientist, The Alfred Hospital.
Surgical Cut Up Scientist, Anatpath Services Pty Ltd.

1. What was your first part-time job?
My very first part time job was after completing my RMIT Bursary Year at St. Vincent's Pathology, Melbourne. They gave me a part-time casual job at Central Specimen Reception, dealing with Pathology Data entry, as well as sorting and preparing various pathology specimens for all areas of Pathology.

2. How long have you worked in histology?
As of date, I have been an Anatomical Pathology Scientist since 1998, a total of 18 years (including this year). I prefer to use Anatomical Pathology as to just Histology. After all, I am trained in both disciplines (i.e. Histopathology and Cytopathology). I also have worked as a Phlebotomist, a pathology courier, and specimen collector in Hobart. My career in Anatomical Pathology had taken me to the United Kingdom apart from just Victoria and Tasmania. I also returned to RMIT University to look after Laboratory Medicine Students between 2005-7 and I am working with some of them now!

3. When people ask, "So, what do you do?" How do you explain Histology?
I can be a bit long winded when I attempt to explain that. I had worked at the Deli Section of Coles when I was in between Pathology Jobs and had cut sections of ham, and customers did request thinly sliced ham (which I almost asked them how many microns would you like). I use that to bring an analogy of microtomy.

For more than half of my Anatomical Pathology career, I have been trained by Anatomical Pathologists to do surgical cutting up. For that I told friends that I make human sushi and sashimi.

4. What is a skill you'd like to learn and why?
Over the years, I seemed to have picked a bit of Korean, German, French and Spanish. I love to be able to find the time to learn as many languages as possible. It is through the learning of various languages that you will unknowingly open up to many other customs and cultures between people and countries.

5. If you had won the lottery, what would you do?
First things first, pay off all my mortgages, and bills – not just me personally, but my parents, and younger sister (that is if they still have outstanding bills to pay). Find myself a beach front property, and live happily ever after. Never fly economy class again!

6. Who do you most admire in life?
From my career stand point in Anatomical Pathology, I admired Dr. Norman Sonenberg, who was the Medical Director of Gippsland Pathology Services. He was the one who trained me to do all kinds of Surgical Cut up, and I was his Senior Scientist in charge at that time.

My professional experience sky rocketed because I had the opportunity to learn from him about Pathology. My understanding of Pathology and Pathology Services was taken to a whole new level. I was known as a Pseudo Registrar, because in the country, we do not always have Registrars.

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At the moment, I admire Professor Catriona McLean, who is the current Medical Director of Anatomical Pathology at the Alfred Hospital. I think she is one of a kind. There is no other Anatomical Pathologist like Professor McLean.

7. If you could witness any event of the past, present or future, what would it be?
I like to see where Boeing and Airbus further their developments in creating magnificent Aircraft models, such as the current A380s, A350s, B787s, and if there would be another supersonic aircraft like the Concorde. Definitely should make a trip to Seattle, USA visiting the Boeing Factory and Toulouse, France for the Airbus factory.

8. What is the best conference you have ever attended?
It had been such a long time since I attended any specific conferences, the past 6-7 years had been mostly working and moving around. I will have to get back to you on this one.

9. If you could only keep five possessions, what would they be?
This would have been different if I was asked 10-15 years ago. I think I want to make sure that I continue to have good health, good food, good company, good job, and above all do the things that keep me going and stay happy.

10. What is your dream holiday destination and why?
I like to visit St. Maarten, which is an island country in the Caribbean. It has the most amazing beach, called the Maho Beach, famous for being at the end of a runway, where you can see planes really close. I am sure by now you realise I do like aeroplanes!!!!

Reported by: Kellie Vukovic

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Date: Friday 29th July

Time: 6.30pm-10.30pm

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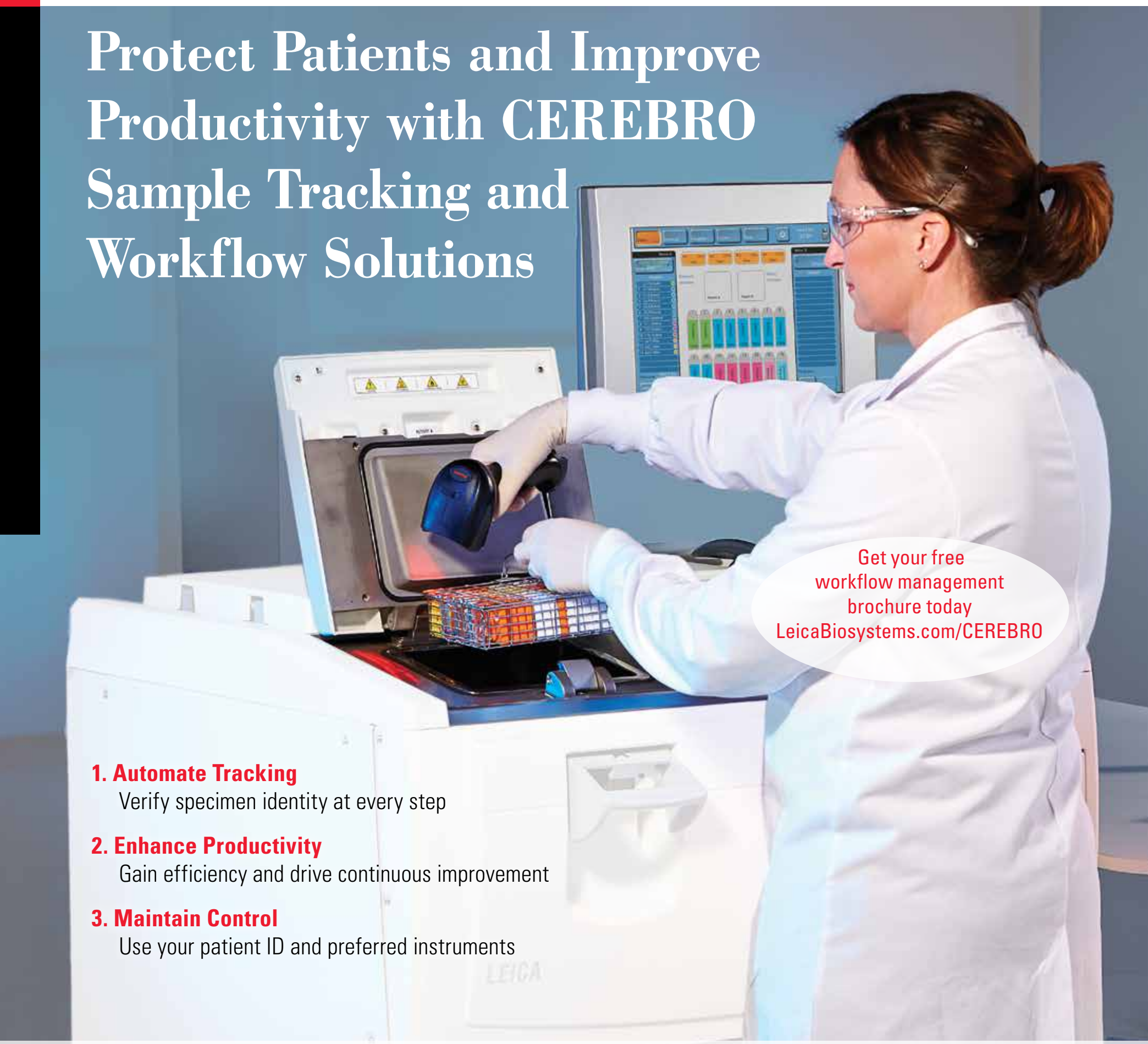

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Scientific Meeting Review-Mysteries of Molecular Pathology

We were fortunate to have Dr Suzanne Svobodova from the Molecular Diagnostics Laboratory , Austin Health speak at the HGV April meeting.

Suzanne reminded us that DNA is a long polymer of sugar and phosphate groups and that molecular pathology is the ability to define the genetic sequences of either human or microbial species. Some of its applications include looking at viral load, inherited disease, looking at the likelihood of developing cancers, diagnosis, prognosis and prediction of disease and defining cancers at the molecular level in order to match the drug treatment.

It was only in 1973 that 24 base pairs sequencing became available. In 1987 the first automated sequencing became available and shortly after in 1990 the next generation sequencing came into play some including Pyro, Solexa and SoLid. The Next,Next, Next generation are not on the market yet, but involve nanopore and microfluidics among many others. It is a rapidly expanding diagnostic tool.

The first human chromosome sequence was done using Sanger Sequencing (invented by Frederick Sanger in 1977) and is the most widely used method of DNA sequencing based on the selective dideoxynucleotides by DNA polymerase. It currently takes 1 instrument, 1 week to do a whole genome and there are some limitations. When looking at gene incorporation of chain terminating mutations you need to look at the whole exome and you can't pick up gene amplification, translocation or insertions.

Drug companies are putting large amounts of money into oncology drugs for targeted therapies, but it is difficult to get drugs through the system and on to the market. This is in part due to survival figures from drug trials, so if you can select patients through molecular studies to go into drug trials, you can increase the survival rate. This means targeted drugs can get onto the market sooner. There is a significant risk of missing out on a good drug if using unpredicted patients in the initial drug trial. Her2 testing is a familiar example to us, but we are currently focusing on lung cancers. We are looking at biomarkers ALK, EGFR, BRAF. With the EGFR mutations, some mutations are drug sensitive while others are resistant and there has been some good survival indicators. It is important to remember that cancer has heterogeneity, so some cancer cells are different to others, so sampling is critical.

The Next Gen Sequence workflow involves the use of FFPE samples with a high percentage of tumour. The tumour is hand selected, the DNA is extracted, followed by hybridization of the oligo pool, the removal of the unbound oligos and then the MiSeq is loaded. 3 days later the 10µl obtained can load the flow cell. It can take 24 hrs to read the end product DNA sequence or several days for bacterial analysis. If dealing with a whole exome there is a lot of data generated , so the software gives you a good read out and shows a clear mutation. It is easy to get false results however, bioinformatics , which involves methods and software tools for understanding biological data, can assist for sequence alignment, and is constantly undergoing changes.

Suzanne briefly spoke of the clinical issues with the Next Gen Sequencing around the need to communicate better, the incidental findings and the legal implications. The Austin is not doing whole genome sequencing, however the Melbourne Genomic Alliance is doing whole exome sequencing for $8000. There are insurance issues with the whole exome. Whole genome sequencing has come down to $2500 in the US and the costs are coming down. The molecular pathology field and the use of this powerful technology to define cancers at the molecular level for the ultimate 'personalized medicine' is an ever expanding and exciting area to work in.

Review by Kerrie Scott-Dowell

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Key Note Speaker:

Dr Joe McDermott
Technical Head
Anatomical Pathology,
LabPlus
New Zealand

Topic: Team Development

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- Digital Pathology
- Paediatric Tumours
- Forensic Pathology
- Biobanking & Misconceptions
- 21st Century Learning for Laboratory Personnel

Workshops:
Friday 30th September 2016
Registration closes on **31st July 2016**

- **Workshop 1**: IHC Validation by Tony Henwood, CHW, NSW
- **Workshop 2**: Presentation Skills by Dr Joe McDermott, LabPlus, NZ

Workshop Prices:
Members: $50 Non-members: $65
Limited places only

Conference Information

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Conference Dinner Venue: Cassegrain Winery

764 Fernbank Creek Road, Port Macquarie, NSW 2444

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1. **Two-day conference registration** - Sat 1st and Sun 2nd October
2. **Opening Ceremony** - Friday 30th September
3. **Wine tasting** - Saturday 1st October
4. **Conference** - Saturday 1st October

Conference Package Prices:

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~~Fifth International Workshop in Diagnostic Immunohistochemistry~~
~~Venue: Mantra Twin Towns, Coolangatta-Tweed Heads Qld/NSW~~

~~**Thursday 18th st February**~~
~~Scientific Meeting-RIMT Student Project Presentations~~
~~Venue: Peter Mac~~

~~**Thursday 14th April**~~
~~Scientific Meeting: Basic Molecular~~
~~Venue: Peter Mac~~

Thursday 16th June
Scientific Meeting: Haematoxylin Blues and other Jazz
Venue: St. Vincent's

Friday 29th July
Trivia night
Venue: Metropolitan Hotel
263 William St.
Melbourne VIC 3000

Thursday 15th September
Scientific Meeting: The New Peter Mac tour
Venue: Peter Mac

September 16-21
NSH Symposium/Convention
Longbeach, California
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September 30- October 2
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Joint State Conference
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