

Journal of the Australian Society of Cytology Inc

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Basaloid Tumour of Salivary Gland

A Comparison of APTIMA and Digene Hybrid Capture II HPV Assays

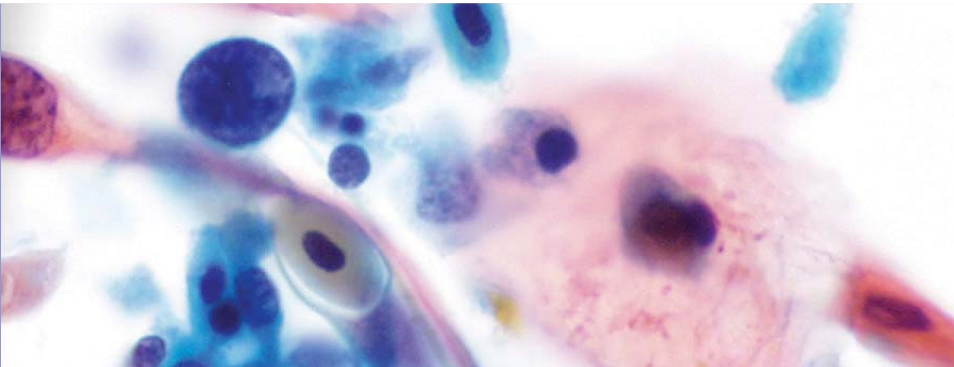
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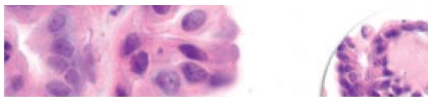
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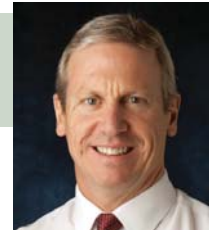
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DEADLINE FOR THE NEXT EDITION: 15 May 2014

The opinions expressed in this Newsletter, including those in the technological and advertisement sections, are not necessarily those of the Editor, the Australian Society of Cytology, Publisher or Printer.

President's Column



Christopher Carter

The Renewal of the National Cervical Screening Program (NCSP), which commenced in November 2011, has progressed to a stage where the Renewal Steering Committee submitted a proposal, based on a systematic review of evidence and economic modelling, to the Medical Services Advisory Committee (MSAC) in November 2013. After a request for further information, MSAC is expected to provide a final recommendation in April 2014. As you will be aware, this recommendation will relate to phase 1 of the Renewal, pertaining to decisions regarding screening tests and pathways, the screening interval, age range and commencement for both HPV vaccinated and non-vaccinated women, taking into account cost-effectiveness. It is MSAC's role to provide recommendations to the Minister for Health on a cervical screening pathway that is safe, effective and cost-effective and that will best enable the NCSP to achieve its objectives into the future. While the Australian Society of Cytology and its members have been provided with opportunities to make submissions to the Renewal, the Society has not been party to the decision-making process.

It is expected that there will be substantial changes to the screening program as it currently exists, particularly in relation to longer screening intervals, a raised age of commencement and likely adoption of HPV testing and liquid-based cytology.

The second phase of the renewal is to explore the operational requirements for transition to a renewed NCSP and an implementation plan is being developed for this purpose by the Renewal Steering Committee. The ASC, together with other pathology organisations (such as the Royal College of Pathologists of Australasia, Pathology Australia, the National Coalition of Public Pathology and Catholic Health Australia) were recently invited to provide commentary on the logistics and other issues related to any potential change in the screening pathway of the NCSP. Items for discussion at this forum included timing, transition, pathology business models, workforce, cytology registers, training and education, communication/resources, NHMRC guidelines and

quality and safety issues including accreditation and performance measures, quality assurance and safety monitoring. It is anticipated that an implementation plan will be prepared by June 2014 and, subject to approval by the Australian Health Ministers Advisory Council, changes will be rolled out sometime thereafter. Integral to this proposed change would be changes to the medical benefits schedule and, presumably, changes to the operations of the Pap test registers and the HPV vaccination registry. Realistically, it is unlikely that any proposed changes will be implemented any earlier than 2016.

There are likely to be many and varied substantial challenges ahead, which we will all need to work through once the outcome of the Renewal is announced.

For further information on the Renewal of the National Cervical Screening Program: <http://www.cancerscreening.gov.au/internet/screening/publishing.nsf/Content/ncsp-renewal>



Please note that following the recent Council and Board of Education decision relating to the distribution of Cytoletter, this edition has been distributed only in hard copy format to those members who replied to a recent request from the National Office to indicate their preference.

If you have not replied to that email you will automatically receive the March edition and all future editions in electronic format only.



Jenny Ross

Welcome to the first edition of Cytoletter for 2014. This year will see significant changes to the Australian cervical screening program due to the Renewal process. As outlined by our President in his column opposite this will have inevitable consequences for cytology laboratories in relation to accreditation, training and workforce issues so it is imperative we are aware and involved in the implementation of these changes over the next few years. The Society has endeavoured to keep members informed via email, our website and FaceBook as these changes are announced, so please ensure you check these regularly.

This edition of Cytoletter includes a number of articles submitted by our members. I am grateful to Frank Musso for another splendid article from his FASC case studies. Frank highlights the difficulty in diagnosing basaloid neoplasm of salivary gland cytologically and highlights the distinction between basal cell adenoma and basal cell carcinoma, and adenoid cystic carcinoma and pleomorphic adenoma. The article includes excellent photomicrographs highlighting the distinguishing features.

Two presentations from the recent Annual Scientific Meeting in Sydney are included in this edition. Anna Santos shares her laboratory's experience in comparing the sensitivity and specificity of the APTIMA HPV Assay with the Digene Hybrid Capture II Assay when correlated with cytology and histology results. The issues raised in the article will be of interest to all those laboratories currently performing these tests as well as those considering implementing HPV testing in the near future. Natalie Hockey has kindly adapted her poster presentation describing a case of ovarian clear cell carcinoma in a Pap smear. Her study illustrates that although this diagnosis is rare in cervical cytology, it should be considered when presented with highly malignant cells in a Pap smear.

Finally, the second part of Ron Bowditch's Michael Drake Fellow presentation "Is Cytology Science? -From Papanicolaou to LBC and HPV" continues in this edition with the emergence of liquid based

cytology and HPV testing into cytology practice. Ron highlights ways to reduce diagnostic error by refining diagnostic criteria, encouraging objective screening practices and minimising biases in judgment. Congratulations once again to Ron on the Society's recognition of his outstanding contribution to cytology practice.

We continue our series in this edition of introducing Board of Education members with profiles on SA members Grant King and Marilyn Betchley. Both Grant and Marilyn have been closely involved with various aspects of the Society over many years and continue to contribute significantly via the Board.

Lastly can I take this opportunity to invite you to attend the 44th Annual Scientific Meeting and Tutorials to be held by the Society for the first time in Darwin, Northern Territory. The organising committee has developed a fantastic scientific program which includes three international speakers - Dr Min En Nga (Singapore) Dr Matthew Zarka (USA) and Dr Margaret Sage (NZ). Non-gynaecological highlights include sessions on EUS FNA, head and neck FNA including lymph nodes and atypical breast cytology. The gynaecological program will focus on issues around screening in remote settings as well as a Renewal update from Professor Ian Hammond and morphology sessions which focus on LBC. We believe that Darwin will offer a unique destination and a great opportunity for our colleagues throughout SE Asia to join the society for our most important scientific activity of the year. We hope you too can join us for what we believe will be a very special society meeting. Register early and take advantage of various hotel and tour options in and around Darwin.



A Difficult Diagnosis: Basaloid Tumour of Salivary Gland

Frank Musso

Anatomical Pathology, Royal Melbourne Hospital

Clinical Presentation

A 56 year old male was referred to a head and neck surgeon for the investigation of a parotid mass that had been slowly enlarging over the last two years. On examination, the patient was found to have a firm mobile mass measuring approximately 2.5cm. The surgeon made one pass into the mass using a 25g needle; 2 wet-fixed smears for Pap stain and 2 air-dried smears for Giemsa stain were made.

Cytological Findings

The smears were hypercellular and consisted of large branching and three-dimensional fragments of cells and abundant bare nuclei in the background. The Giemsa stained smear was helpful in identifying the presence of a mixed two cell population: One cell population consisted of small round-to-ovoid shaped hyperchromatic nuclei with scant cytoplasm (basal cells); the other was larger with ovoid shaped nuclei, small nucleoli, fine chromatin and larger amounts of cytoplasm (luminal cells). The large fragments of cells were difficult to examine on Giemsa stain, however, the Pap stained smears often showed the large fragments of basaloid cells displaying peripheral palisading. Small globules of hyaline material staining magenta on Giemsa stain were seen closely associated with the some of the basaloid cell groups. These globules were densely staining/orangeophilic on Pap stain and easily seen embedded within some of the crowded groups. Many groups and sheets of cells consisted predominantly of the luminal-type cells showing whorling and often keratinisation. Several areas within the smears showed squamous cells, pearl

formation and keratinous debris. Rare mitotic figures were seen. The background contained granular proteinaceous debris. No necrosis was seen.

Cytology diagnosis: Basaloid tumour; differential diagnosis including basal cell adenoma, solid variant of adenoid cystic carcinoma, basal cell adenocarcinoma.

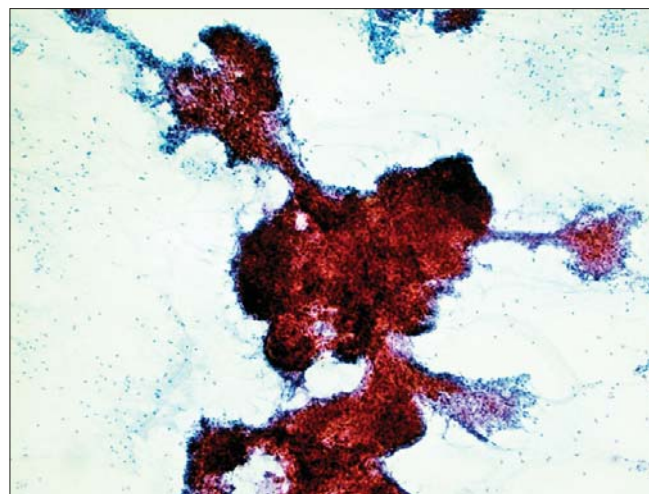
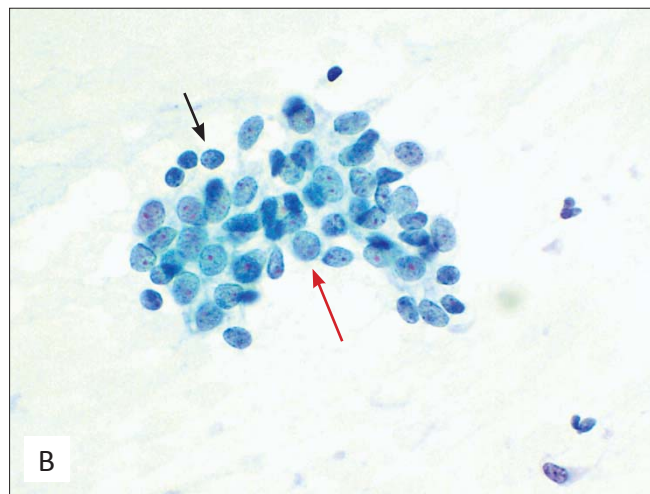
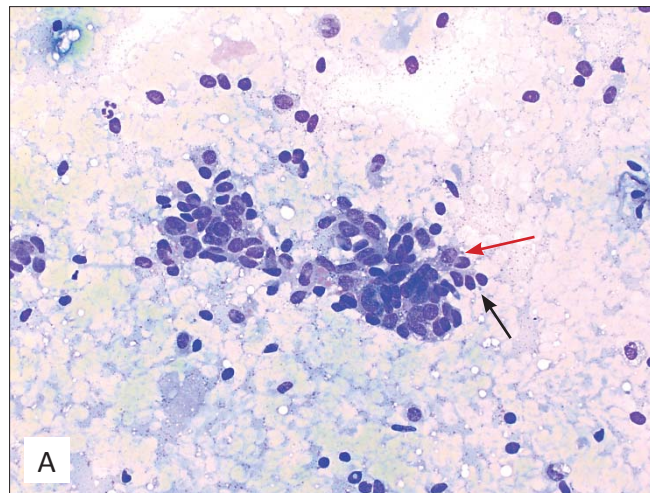


Fig 1: Low power view showing a large three-dimensional branching group of cells with single bare nuclei in the background. (Pap x100)

Fig 2: Two cell population consisting of small basaloid cells (black arrow) and larger luminal cells (red arrow). (A: Giemsa x400, B: Pap x600)

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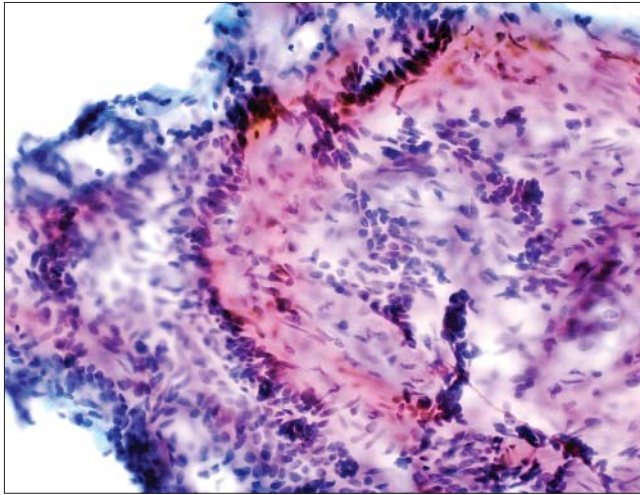


Fig 3: Peripheral palisading of basaloid cells. (Pap x400)

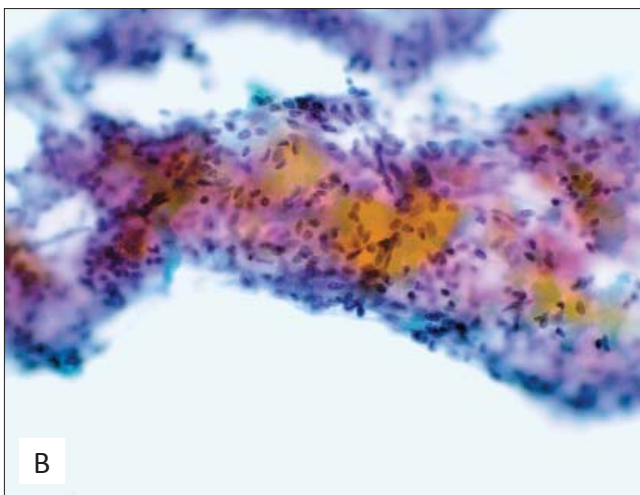
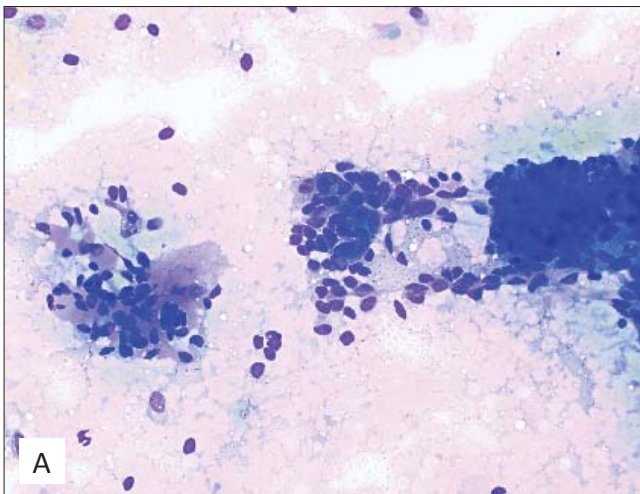


Fig 4: Hyaline globules closely associated with the basaloid cells staining magenta on Giemsa stain and orangeophilic on Pap stain. (A: Giemsa x400, B: Pap x400, original magnification)

Histological Findings

The total parotidectomy specimen revealed a tumour measuring 45mm in maximum dimension. The tumour showed a solid-type growth pattern, consisting of variably sized nests and cords of tumour cells separated by thin bands of fibrous tissue. Focally, small globules and droplets of

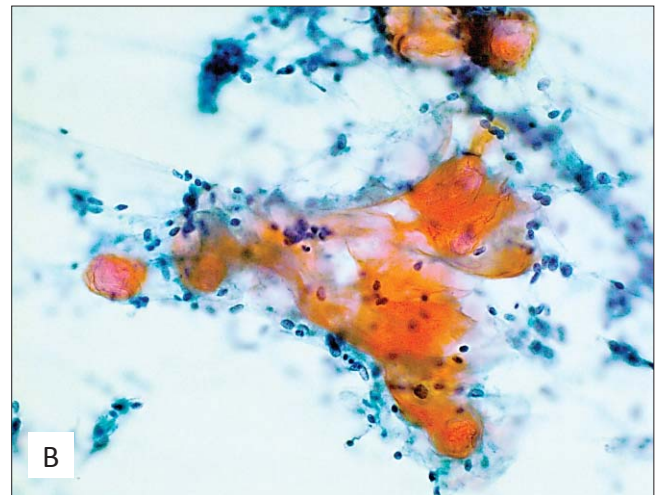
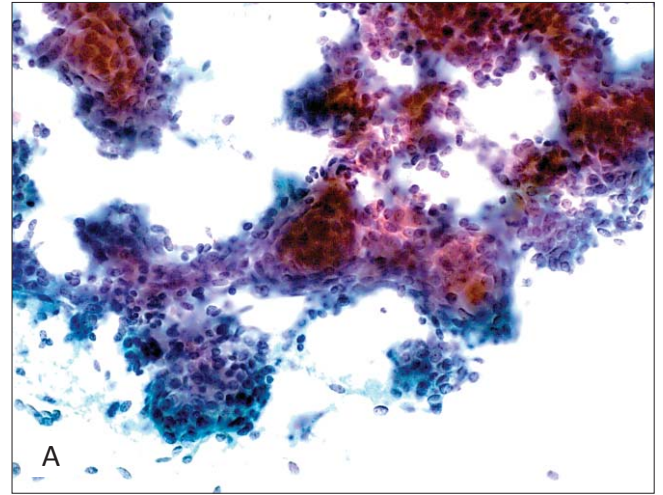


Fig 5: Crowded groups of cells showing whorling (A). Refractile keratinous debris (B). (Pap x400)

eosinophilic hyaline material were seen embedded within these islands of tumour cells. A dual population of cells was often clearly discernible; the larger cells showing open chromatin and small nucleoli (luminal cells) were commonly seen at the centre of the tumour nodules, while the smaller cells with hyperchromatic round-to-ovoid shaped nuclei (basaloid cells) were seen at the periphery and displaying nuclear palisading. Foci of squamous cells, squamous pearls and keratin were seen within the tumour aggregates. Tumour showed malignant features - invasion into the adjacent salivary parenchyma together with vascular and perineural invasion. Occasional mitoses were seen. The tumour was completely excised.

Immunohistochemistry staining for cytokeratins showed strong focal positivity within the tumour nodules while smooth muscle actin (SMA) showed positivity at the periphery of the nodules confirming myoepithelial differentiation.

Histological diagnosis: Basal cell adenocarcinoma.

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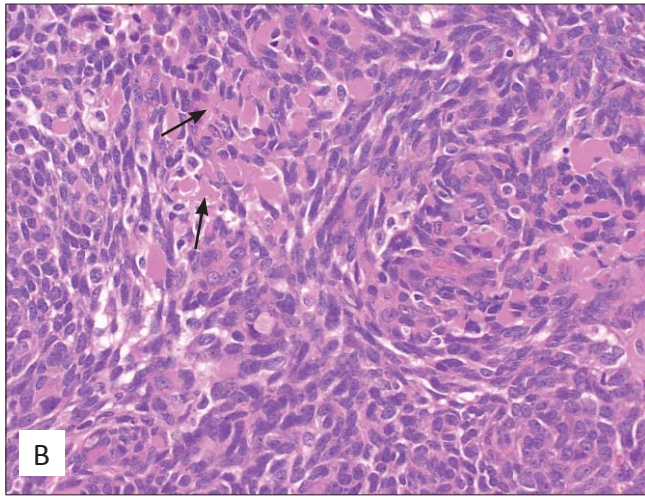
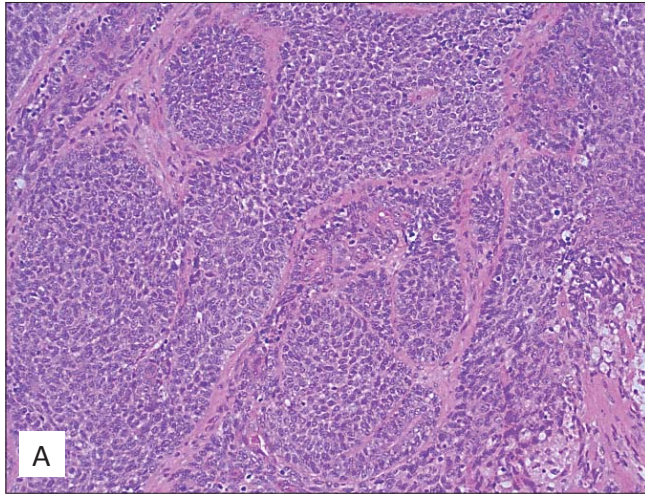


Fig 6: Histological sections showing solid-type growth pattern with cords and nests of basaloid cells (A); small hyaline globules (at arrows) intimately associated with some of the basaloid cells (B). (A: H&E x200; B: H&E x400)

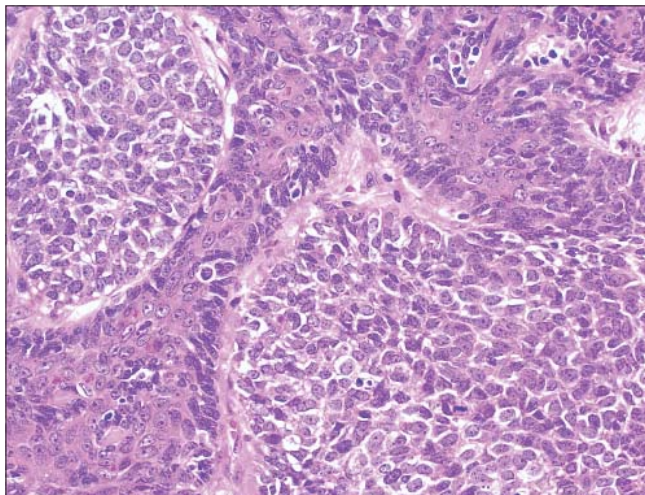


Fig 7: High power view depicting the dual population of cells and peripheral palisading of the basaloid cells. (H&E x400)

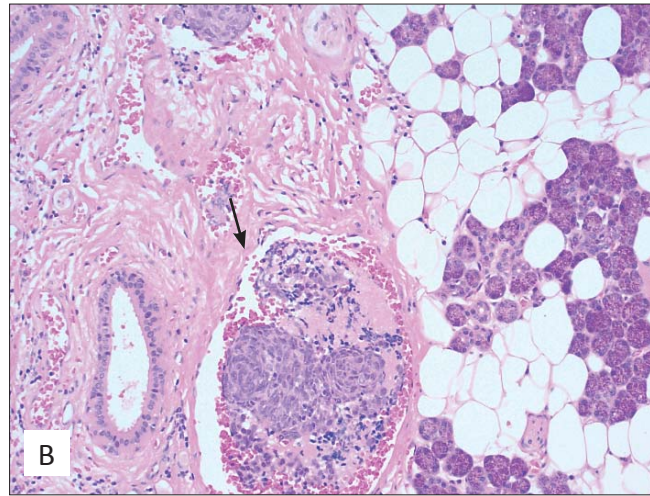
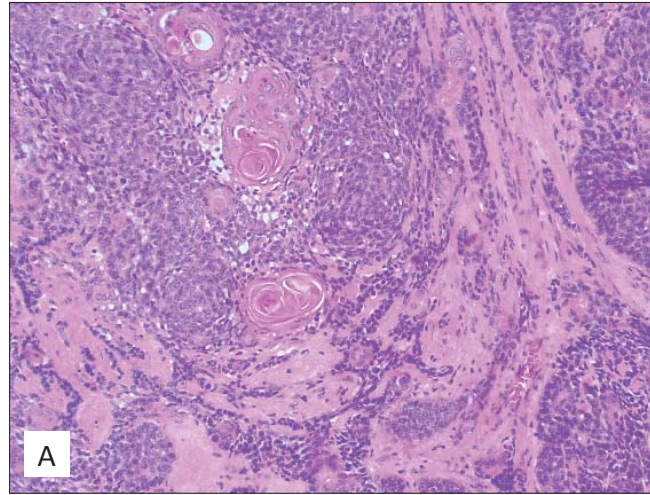


Fig 8: Squamous cells and keratin (A). Intravascular deposit of tumour at arrow (B). (H&E x200)

Discussion

Both basal cell adenocarcinoma (BCAC) and basal cell adenoma (BCA) are classified as basaloid tumours and are thought to arise from the basal cells of the striated ducts.¹ They share common demographic, clinical and histological features. Both tumours most commonly occur in the parotid gland,¹ with the malignant counterpart reported to occur exclusively in the parotid gland.² Most patients are in their sixties with no sex predilection for the malignant tumour,³ however, BCA shows a slight female predominance.^{1,3} BCA represent less than 2% of all salivary neoplasms while BCAC makes up about 1%.² Most BCAC cases are believed to arise de novo (about 77%) while the remainder arise from a pre-existing BCA.⁴

Histologically, both BCA and BCAC share identical growth patterns; solid, trabecular, tubular and membranous.¹ The features which are expected for malignancy include an invasive outgrowth, perineural and vascular involvement, necrosis and

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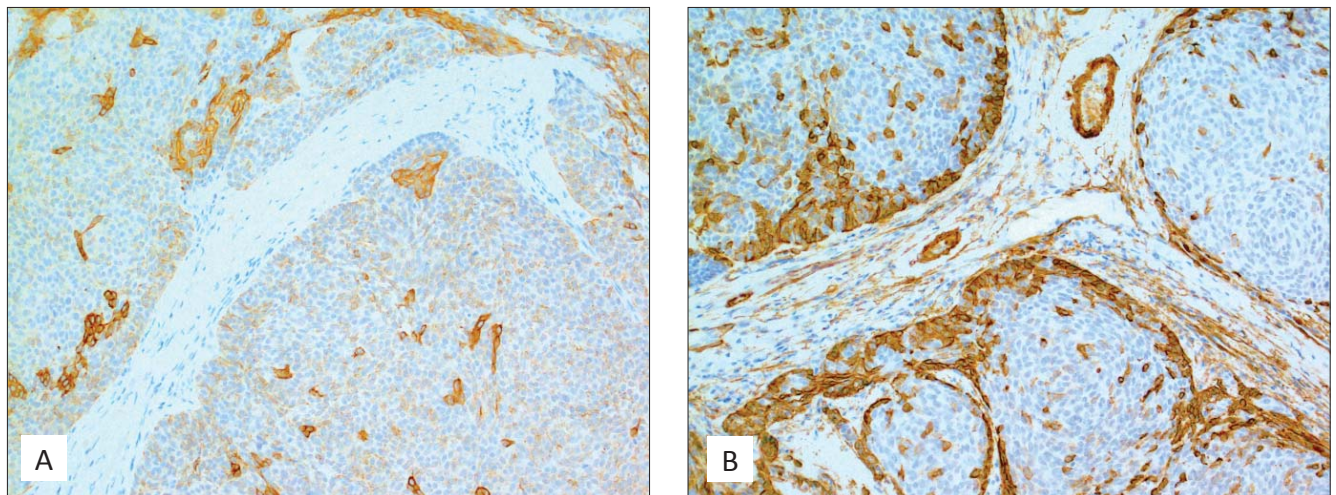


Fig 9: Scattered cells showing cytokeratin positivity (A). Cells showing myoepithelial differentiation located mainly around the periphery of tumour nodules. (A: AE1/3 x200, B: SMA x200)

mitoses⁵ – characteristics that separate BCA from BCAC. The degree of nuclear atypia is minimal in both tumours and is therefore not a reliable criterion for malignancy.³ In fact, the WHO define BCAC as a malignancy that has the cytological characteristics of BCA but morphological growth pattern (invasiveness) indicative of malignancy.⁴ BCAC is considered a low grade malignancy with a high recurrence rate but good prognosis.⁵ The solid-type pattern is the most common histological presentation¹ and in this case was the predominating pattern with focal areas also displaying a membranous-type pattern. It is common to find different growth patterns in the same tumour.³

Basaloid neoplasms also include the solid variant of adenoid cystic carcinoma (SVACC) and cellular (epithelial-rich) pleomorphic adenoma (CPA). The broad definition of a basaloid tumour is the presence of crowded ovoid cells exhibiting a high N:C and devoid of chondromyxoid matrix (which is characteristic of pleomorphic adenoma).² Cytologically, there are a number of challenges faced when a basaloid neoplasm is encountered; for example, whether the tumour represents malignancy (BCAC/SVACC) or a benign entity (BCA/CPA), and the specific tumour type. These tumours have overlapping cytomorphological features which even make a distinction between benign and malignant very difficult. This was clearly demonstrated in this case, as a diagnosis of malignancy was only able to be made on histology. The common cytological characteristic of BCA

and BCAC is the presence of two cell populations, as shown in this case. Both these cell types are referred to as basaloid and described as either dark (small cell type) or pale staining (larger cell type).^{1,6,7} Recent text² refers to the small dark cells as basal, while the larger pale cell as luminal. The recognition of these different cell types is critical in better understanding the nature and cytological features of these tumours. However, sometimes there is no reference to these different cell types in the cytological description of these tumours.⁸ Other distinctive cytological features of BCA and BCAC include peripheral palisading of basaloid cells (Fig 3), intercellular globules and droplets of homogeneous densely staining hyaline matrix (Fig 4), and a peripheral band of hyaline material (not represented in this case). A common feature also seen is whorling of cells and squamous differentiation (Fig 5).^{2,6,7} When BCAC presents with large three-dimensional clusters, mitotic figures, necrosis and marked atypia of individual cells, a diagnosis of malignancy can be made on cytology.^{2,9} However, it is rare for BCAC to show all these features and in most cases BCA and BCAC cannot be reliably distinguished by cytology alone.⁶ It is important to note that even in cytologically benign cases, a malignancy can never be excluded as 23-25% of BCACs arise from a pre-existing BCA.^{4,9} With reference to the large three-dimensional clusters of cells, as seen in this case, a review of 12 basal cell adenomas by Kawahara et al¹⁰ found three-dimensional clusters to be a common occurrence in this benign entity, reinforcing that this feature alone would be

insufficient for a diagnosis of malignancy.

A major differential diagnosis of BCA/BCAC is the solid variant of adenoid cystic carcinoma (SVACC). The distinction of these different tumours is said to be the most difficult in all of cytology.¹ The most common and classical presentation of adenoid cystic carcinoma (ACC) is the cribriform and tubular pattern.^{1,6} It is suggested that the cytological features seen in these patterns of ACC should not be difficult to differentiate from BCA/BCAC.¹¹ Cytologically, these common variants of ACC show a monotonous population of round to oval basaloid cells with dark, often angulated nuclei. The cellular arrangement varies from branching clusters, cup-shaped fragments, rosettes, and sieve-like formations corresponding to the cribriform pattern seen histologically.¹² The matrix material is also very characteristic in these variants of ACC, presenting as metachromatic spheres (globules), cylinders and linear branching structures. The matrix material stains magenta on Giemsa stain while it appears light green-blue to almost colourless/invisible on Pap stain.^{6,7} This is in direct contrast to the densely staining matrix material present in BCA/BCAC (Fig 4B). The presentation of the globules of matrix material is also unique in ACC; occurring isolated, in groups and in association with peripherally surrounding basaloid cells.^{2,12} The globules are often large and show variability in size.¹² The cell-stroma/matrix interface, as reported by Stanley et al¹³ occurs differently in ACC and BCA/BCAC. They reported that the matrix material seen in BCA interdigitates with adjacent cells (this would also apply to BCAC, refer to Fig 4A), whereas in ACC, the interface between matrix and cells has sharp smooth borders. This aspect of the cell-stroma interface seen in ACC and proposed to be the distinguishing feature from that seen in BCA/BCAC was not corroborated in the reviewed literature. Instead, there was much more emphasis placed on the broad differences seen with the cell-stroma relationship, i.e., the stromal matrix in BCA/BCAC often surrounds the cells (intercellular), whereas in ACC the cells surround the matrix.⁷ An exception to this was put forward by Auclair et al¹ who mentioned that desmoplastic stroma associated with invasion in ACC can mimic the cell-stroma relationship seen in BCA/BCAC.

Gupta et al¹⁴ reported on a case which they believed had all the classic features of ACC;

namely, globules of matrix surrounded by basaloid cells and cylinders/tubular structures of hyaline matrix. The subsequent histology revealed a basal cell adenoma, membranous type. They acknowledged the report by Stanley et al¹³ and on retrospective review found the suggested features to be unhelpful in making a correct diagnosis. The cytologist is however very reliant on observing this matrix material and its presentation when considering a diagnosis of BCA/BCAC vs classic ACC. The problem encountered with SVACC is that the matrix material is absent or scant and smears are made up predominantly of basaloid cells.^{2,7}

SVACC is also known as poorly-differentiated adenoid cystic carcinoma and considered to be the most aggressive form of ACC.^{2,6} The cytological distinction of BCA/BCAC and SVACC becomes a matter of examining the cellular component rather than the matrix material. The differences between these tumour types are that SVACC often shows at least moderate nuclear atypia, apoptotic cells,⁶ frequent mitotic figures and necrosis.^{2,15} SVACC lacks a dual cell population of dark and lightly staining cells, peripheral palisading⁶ and squamous differentiation/basosquamous whorling^{6,16} seen in BCA/BCAC.

CPA can also pose a problem in a similar fashion to SVACC because the characteristic fibrillary matrix material is sparse or absent.^{2,6} A case series review of 35 histologically proven BCAs found that this tumour has the greatest similarity with CPA.¹⁷ A diagnosis of CPA can be at least favoured if plasmacytoid myoepithelial cells are identified and if a careful search reveals characteristic chondromyxoid fibrillary matrix.^{2,6,17} Failing this, a descriptive diagnosis stating the differential diagnosis is necessary. It is important to note that squamous metaplasia and keratin pearls can sometimes also be seen in CPA.⁶

Metastatic neoplasms that should be considered in the differential diagnosis of BCAC (showing cytological features of malignancy) include cutaneous basal cell carcinoma (CBCC), basaloid squamous cell carcinoma (BSCC) and small cell carcinoma. Stanley et al¹⁸ reported three cases of CBCC metastatic to parotid (2 cases) and submandibular gland (1 case). They reported the cytological features as being very similar to basal cell adenocarcinoma - basaloid cells occurring in three-dimensional clusters and isolated bare

Cont...

nuclei, occasional mitotic figures and necrosis. Matrix material was seen in the three reported cases, however, it was found to be fibrillary in texture and similar to that seen in pleomorphic adenoma. Clinical history is imperative in making a correct diagnosis, accompanied by clinical examination of orbital and skin areas.^{9,16,18} Muller and Barnes⁴ concluded that a distinction between BCAC and CBCC can even be impossible on histology; immunohistochemical demonstration of myoepithelial differentiation is needed for BCAC. BSCC should be distinguished from BCAC by the lack of any matrix material¹⁶ and again clinical information is important, with most patients having a history of head and neck squamous cell carcinoma.² Small cell carcinoma can be distinguished by the significant nuclear pleomorphism, moulding and smearing artefact.²

The treatment of BCA and BCAC is complete surgical resection with negative margins. The overall prognosis for this patient, despite having perineural and vascular involvement, is excellent.² Local recurrence is the primary concern and a 6

month CT scan of this patient showed no evidence of recurrence.

Conclusion

This case shows the difficulty encountered in making a definite diagnosis when faced with a basaloid neoplasm of salivary gland. While there are many overlapping cytological characteristics, a number of features can be found in basal cell adenoma and basal cell carcinoma which enable their distinction from solid variant of adenoid cystic carcinoma and cellular pleomorphic adenoma. These include the presence of a dual cell population of dark and light staining cells, peripheral palisading and squamous differentiation. The matrix material can show peripheral band formation and the globules present are small, intercellular and densely staining on Pap stain. Unless there are features of malignancy (atypia, necrosis, mitoses) a diagnosis of basal cell carcinoma cannot be made on cytology. In this instance and when other basaloid tumours cannot be excluded on cytology, a descriptive diagnosis outlining the differential diagnosis may be necessary.

		BCA	BCAC	SVACC	CPA
Cellular features	• Dual cell population (dark and light)	+	+	-	-
	• Peripheral palisading	+	+	-	-
	• Squamous differentiation	+	+	-	+/-
	• Nuclear atypia	-	+/-	+	-
	• Necrosis	-	+/-	+	-
	• Mitotic figures	-	+/-	+	-
	• Plasmacytoid myoepithelial cells	-	-	-	+/-
Matrix characteristics	• Peripheral band of hyaline material	+	+	-	-
	• Hyaline globules (intercellular, small)	+	+	-	-
	• Cylindrical/tubular shape	-	-	+/-	-
	• Chondromyxoid/fibrillary	-	-	-	+/-

Table 1: Comparison of cellular features and matrix characteristics of basaloid salivary gland neoplasms. (+, common; +/-, rare; -, absent)

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FLASHBACK

2005

The Conference dinner at the Melbourne Museum, 2005. How many people do you recognise?

I invite you to send your own 'historical' photos of past meetings or workshops to share with Cytoletter members. Editor

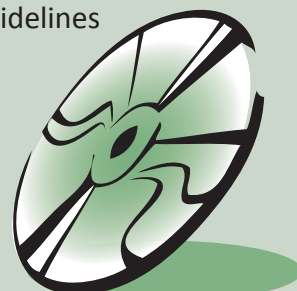
CD-ROM AUTHORS WANTED

The ASC Board of Education is looking for expressions of interest in authorship of CD-ROM titles in the ASC Continuing Education Sets. We require titles in Gynaecological, Non-gynaecological and Fine Needle Aspiration Cytology. For further information, (written guidelines are available) please contact:

Grant King, CD-ROM Coordinator

Australian Society of Cytology
283-287 Sir Donald Bradman Drive
BROOKLYN PARK SA 5032

Phone: 08 8222 6708 **Email:** grant.king@health.sa.gov.au



A Comparison of APTIMA HPV Assay with Digene Hybrid Capture II Assay when Correlated with Cytology/Histology Diagnosis.

Anna L Santos,¹ Sally Dubedat,² Lyndal Anderson.¹

Department of Tissue Pathology and Diagnostic Oncology, Royal Prince Alfred Hospital, Camperdown
Department of Microbiology, Royal Prince Alfred Hospital, Camperdown

Introduction

The conventional Pap smear has long been the most effective and cost efficient screening tool for cervical neoplasia. Australia has the second lowest incidence of cervical cancer in the world amongst countries with comparable cancer registration systems.¹ Whilst the Pap smear has significantly reduced the incidence of cervical cancer since its introduction in the mid 1960's, morphological interpretation alone is not able to predict if a patient will progress to cervical cancer.² Conventional screening methods are influenced by many factors including sampling of the transformation zone, fixation, interpretation issues and staining problems, all of which may lead to false negatives and false positive diagnoses. Conclusions regarding the performance of liquid based screening and conventional Pap smears against HPV assays have been varied and difficult to interpret due to the complexity of cervical screening.^{2,3} In February 2012 the Microbiology Department at Royal Prince Alfred Hospital evaluated the PANTHER system to test for the presence of HPV mRNA from ThinPrep samples using the Gen-Probe APTIMA HPV (AHPV) test⁴ and Digene Hybrid capture II HPV assay (HC2). Concurrently the Department of Cytology sought to assess AHPV versus HC2 findings against morphology interpretation on cytology and histology specimens. Clinical data on the Gen-Probe HPV assay which targets E6/E7 viral messenger RNA suggests that this method can predict infections likely to progress to cervical cancer.^{2,3,5} Our laboratory is the first in Australia to trial the APTIMA HPV assay. Sensitivity, specificity and predictive values were calculated against morphology results. Both AHPV and HC2 showed good specificity with morphology interpretation 89 and 83% respectively. The AHPV was more sensitive 88% compared to HC2 84%. PPV for both tests were AHPV 80% compared to 69% for the HC2 assay and NPV 94% compared to 92%. Whilst the sample size for this report is limited, we describe our experience comparing the APTIMA HPV assay against the standard Digene Hybrid capture II DNA analysis with consideration of morphological analysis.

Table 1.	Gen-Probe AHPV Assay	Digene HC2 Assay
High Risk Types Detected	16/18/31/33/35/39/45/51/52/56/58/59/66/68	16/18/31/33/35/39/45/51/52/56/58/59/68

Methods and Results

The Royal Prince Alfred Hospital Cytology Department examined the diagnostic accuracy of high-risk genital HPV abnormalities detected by the APTIMA HPV assay and Digene Hybrid Capture II HPV DNA assay when compared to detection by screening analysis of Thin Prep slides, cytology slides and histology. Table 1 shows the high risk HPV types detected by each assay:

Evaluation of cytology results was not limited to detection of high-grade squamous intraepithelial lesions (HSIL) alone, but also included reports of possible low-grade squamous intraepithelial lesions (LSIL), low-grade squamous intraepithelial

lesions (LSIL) and PHSIL (possible high-grade squamous intraepithelial lesions). The inclusion of all squamous intraepithelial lesions on cytology was thought to be relevant as it is well established that morphology interpretations on Pap smears, Thin Prep samples and histology slides alone are subjective and cannot predict if a patient has a "high-risk" HPV type, hence a LSIL report on Cytology/Histology does not exclude progression to cervical cancer and a high risk diagnosis will not confirm progression to cancer. In addition, not all cytology cases had a corresponding histology report. Patients reviewed in this study were mostly part of a non-screening population.

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Table 2. Number of Assay Cases Reviewed with Insufficient Cells or with an Equivocal Report			
Total Cases 201 Insufficient and Equivocal Results		Positive	Negative
Insufficient cells HC2	8	6	2
Insufficient cells AHPV	0	0	0
Equivocal HC2	1		
Equivocal AHPV	1		

All discordant cytology cases (including discordant negative cytology) were rescreened. The AHPV assay was performed on a fully automated enclosed PANTHER system. The Digene HC2 assay was performed by a manual hybrid capture system. All Pap smears and prepared Thin Prep slides were fixed in 95% alcohol and stained on an automated Papanicolaou staining machine. Both the Pap smear and Thin Prep slides were reviewed by two cytologists and abnormal smears were sent to a pathologist for reporting.

The AHPV assay, HC2 assay and ThinPrep slides were run in parallel with sampling taken from the same ThinPrep vial for each patient according to manufactures procedures. Specimens were thoroughly mixed prior to the removal of the aliquot for HPV testing. The APTIMA HPV assay required 1mL of aliquot to be placed directly into an APTIMA specimen transfer tube. The sample was then sent to the microbiology department for processing. The collection tube has a pierceable foil cap, thus no further handling of the specimen was necessary. Tubes were labelled with a bar code, generated by our laboratory information system (LIS). The PANTHER has a bi-directional host-query interface to the laboratory LIS. Samples and reagents were loaded onto the PANTHER with minimal operator input, and no further input is required until the assay has been completed. The Digene HC2 assay required 4 mL of aliquot which was placed into a sterile 10 mL centrifuge tube and sent to the microbiology department for processing.

All assay results with insufficient cells (refer to table 2) yet still testing positive for HPV were included in the report as true positive results. All AHPV and HC2 assays results were independently validated by the molecular department and all cytology and histology reports were examined by cytology. The data was then tabulated and reviewed.

In total 201 APTIMA and Digene results were run in parallel and examined against corresponding

cytology and histology cases. Two microbiology cases were removed from the study due to invalid reports. If there were insufficient cervical cells present, determined by the presence of a deposit during the sample conversion step, the negative results were not issued. A report was issued stating that there were insufficient epithelial cells present, and a repeat sample was requested. Positive results obtained from a sample with insufficient cells were issued.

The results from the study analysis are tabulated as follows (Refer to table 3 & 4). Other comparisons were also reviewed, these are tabulated in table 5.

Discussion

The sample size for this study is small with only 201 samples run in parallel and 199 valid results used in the final study analysis. Hence, only limited comparisons should be drawn from the data. This sampling size was attributed to cost and time restraints in a public hospital setting.

The results show both AHPV assay and HC2 assay both exhibit good specificity with 89% and 83% respectively when correlated with the morphology results. Since both assays are specific and most HPV infections can be cleared by the patient's immune system, HPV DNA assays can provide proof of cure and help avoid unnecessary invasive treatments. High specificity may also indicate that both HPV assays may be potentially suitable as a screening test.

The AHPV assay showed better sensitivity compared to the HC2 assay. It must be noted though, not all results had a corresponding histology report which is considered to be the "gold standard" for morphological diagnosis. However, all abnormal cases were reviewed by two separate cytologists and a pathologist before reporting, so a positive result on cytology is likely to be a true positive result. This may suggest that the HC2 when compared to screening has a higher

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Table 3. Comparison AHPV and HC2 Assays Against Conventional Pap Smears, Liquid Based Cytology & Histology.			
Number of cases excluding equivocal results = 199	Cytology Report +/- Histology (Includes ThinPrep and Conventional slides)	Gen-Probe's AHPV	Digene HC2
52	+	+	+
8	+	+	-
7	-	+	+
0	+	-	+
3	-	-	+
15	+	-	-
1	-	+	-
113	-	-	-

Table 4. Performance against Histology/Cytology	AHPV	HC2
Sensitivity	88%	84%
Specificity	89%	83%
PPV	80%	69%
NPV	94%	92%

Table 5. Other Comparisons		
Comparisons	AHPV	HC2
Cost per test without controls	\$15.60	\$23.84
Time spent per test	1 hour	8 hour shift
Amount of material	1 ml	4ml
Reduced transcription error	No transcription errors	Increased risk transcription errors

proportion of false negative reports compared to the AHPV assay. Better sensitivity of the AHPV assay may be contributed to reduced operator error as no further manipulation of the sample is required once it is placed into the collection tube, compared to HC2 assay where multiple steps are required for processing.

Whilst the predictive values were similar for both HPV assays, the AHPV still performed better compared to the HC2 assay (PPV 80%vs 69% and NPV 94%vs 92% respectively).

The fully automated testing process for the AHPV assay also significantly reduced the time required by staff to perform the tests compared the HC2. The Digene HC2 assay is a manual assay, there are several incubation steps and processors required and it takes one staff member in microbiology nearly a full shift to complete. Increased turnaround times were also achieved with the automated AHPV assay.

The chance of operator error and transcription

errors is also significantly less with the automated AHPV compared to the HC2 assay as the specimen does not require further manipulation once a sample has been placed on the PANTHER and results are up loaded automatically onto the LIS. The HC2 assay results are manually entered into the LIS, which can lead to potential transcription errors.

The cost of the Aptima reagents are significantly less than the Digene HC2 assay, not taking into consideration labour costs, and control costs which would add even further savings.

Whilst not noted during this study, additional post implementation advantages have been seen with fewer insufficient Thin Prep slides be reported by cytology. This is likely attributed to the requirement of 1 mL of aliquot form the Thin Prep vial for the AHPV assay compared to the 4 mL required for the HC2 assay.

Conclusion

The Gen-Probe APTIMA HPV assay from our

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experience showed better correlation with morphology and is more sensitive with fewer false negative reports compared to the Digene Hybrid Capture II assay. Whilst both tests showed good specificity, the AHPV had many other advantages, including fewer transcriptions errors, reduced turnaround times and improved cost effectiveness. In addition, due to the amount of aliquot required from the Thin Prep vial being significantly less in comparison to HC2, it was noted that there was a reduction in the number of insufficient Thin Prep slides in cytology with the introduction of the AHPV assay.

From the data reviewed the APTIMA HPV assay is the preferred viral detection method and may be a suitable stand-alone screening test.

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Cytoletter Guidelines for Contributors

The success of Cytoletter depends significantly on the contributions of members. Please consider submitting Original Articles, Case Studies, Technical Reviews, Letters to the Editor and Book Reviews. Speakers at Branch Meetings could be encouraged to share their presentations with the wider Cytology Community by submitting them to Cytoletter.

Please note:

- Articles must be submitted in electronic format.
- If the article is too large to be submitted by email, contact National Office for an alternative.
- Text must be in Microsoft Word format or equivalent.
- Photomicrographs/photos **should be submitted separately** and not embedded in the text. .jpg files are preferred. Image size should be double the size it will be reproduced at and the resolution should be 300dpi.

- Tables and/or diagrams should also be submitted separately.
- Referencing should be in Vancouver style, numbered in the order in which references appear in the text (see instructions for Authors in the journals Cytopathology or Diagnostic Cytopathology for details).

Quarterly reports from Branch Secretaries are most welcome and as a guide should include:

- Summary of speakers and topics at meetings held since the last news.
- Proposed future meeting dates, speakers and topics.
- Changes to committee membership, State Councillor, etc.
- Notice of AGM.
- Other news items of particular interest to Branch members.

Email submissions to:

jennifer.ross@rcpaqap.com.au

Grant King - Webmaster

I graduated in 1977 with a BSc majoring in Biochemistry intending to be a science teacher. I eventually found my way, via Histopathology, to Cytology in 1981 where I have remained.



My professional qualifications include the CT(ASC) and CFIAC.

I joined the ASC in 1983 and have served on state branch committees and was on the National Executive from 1993 to 1997. I have been a member of the Board of Education since 1999 including a term as Chair from 2001 to 2005. My role as Webmaster commenced in 1996 when I developed a very basic website for the Society to establish a presence on the internet. This became more formalised when a position on the Board of Education was created for the Webmaster.

I have always been a strong supporter of the educational aspects of Cytology through teaching Cytology in the B Lab Med at University of SA and the coordination of the CD-ROM, Study Guide and National Syllabus for Cytologists projects. I also was the Convenor of the ASC Tutorial for 7 years. I believe the role of the ASC in education and professional development are the key to its success in the past and its bright outlook for the future.

Marilyn Betchley

I have been a member of the Board of Education since March 2013 so I am a "newby" to the Board, however not so for my Cytology career.

Rewind to 1970 when I began at the Royal Melbourne Hospital and was seconded to VCS for training in cytology under Dr Michael Drake and senior staff. I sat first IAC exam in Australia in 1973 – long before there was an ASC exam, which I sat in 1991. In between this time, I worked in four other countries including Saudi Arabia where I was their first cytotech!

Cytology became an immense part of my life, especially in regard to continuing education and teaching, whereby I have mentored cytotechs, re-written and updated gynae and non gynae lecture notes, and held workshops in prep for ASC exam. I became particularly interested in endocervical cells about which I have spoken at local and national meetings, the ASC Tutorials and an International conference. I co-authored the ASC Continuing Educational CD-ROM Set on Endocervical Pathology and attained the fellowship of IAC in 2002.

I have been State Counsellor for SA, Assistant Secretary for the National Executive and part of the organising committee for a number of ASMs. Since 2006, my added passion has been assisting with the Bhutanese cervical screening program in a voluntary capacity. Over five visits much has been achieved, and some of this has been published in Cytoletter. We are privileged in Australia to have expertise, high standards and a sense of community in our field of Cytology.





Australian Society of Cytology Inc

44th Annual Scientific and Business Meeting

Darwin Convention Centre
Darwin, NT, Australia

3-6 October 2014

'Cytology on the Frontier'

featuring Dr Min En Nga (Singapore), Dr Margaret Sage (NZ),
Dr Bastiaan de Boer (Australia) and Dr Matthew Zarka (USA).

Tutorial 7-10 October 2014

Guidelines for proffered papers & posters

1. Abstract Guidelines

For all presentations an abstract must be submitted for consideration by the deadline and must adhere to the following guidelines. Abstracts will appear in the conference booklet and be published in Cytoletter.

All abstracts must be:

- Submitted as a Microsoft Word file.
- Use Times New Roman font, size 12, single line spacing, aligned left.
- A maximum of 300 words in length, excluding references.
- Specify all abbreviations in full at first use, followed by the abbreviation in parentheses. Thereafter only abbreviations should be used.
- Checked thoroughly for spelling and grammar.
- References should be limited to a maximum of five. They should be numbered consecutively in the order they appear in the text and follow the Vancouver style.

- Structured as follows:

a. *Original research:*

Title: in bold

Authors: The principal author should appear first. Underline the name of the author who will be presenting the paper/poster (may be different to principal author). Use forename, initials and surname and omit degrees and titles. Include affiliations for each author. Use superscript numbering after the authors name to indicate affiliations.

Objective: The purpose of the study; hypothesis tested.

Methods: Brief description of materials, subjects and methods used.

Results: The main findings of the study. Do not include tables, graphs or diagrams.

Conclusion: The main outcomes and implications of the study.

b. *Case studies:*

These should follow the same guidelines as for original research but with the following headings in the body of the abstract:

Clinical presentation: Relevant presenting clinical/radiological findings

Cytological findings: Results of confirmatory tests/clinical outcome

Discussion: Consideration of differential diagnoses and important points illustrated by the case.

Disclosure of interest statement:

The Society recognises the need for transparency of disclosure of potential conflicts of interest by acknowledging these relationships in publications and presentations. If your abstract is accepted, any financial support or sponsorship relevant to your presentation must be stated in your presentation or poster.

Selection criteria

Abstracts will be favourably reviewed if they are novel and incorporate original data of high quality that extends existing knowledge in the discipline of Cytopathology.

In balancing the program, the organising committee may request authors to present their work in an alternate format eg poster rather than platform presentation.

Abstract submission

Abstracts must be submitted prior to the closing date either by e-mail (national.office@cytology-asc.com) or on CD to:

Cheryl Edgton

Australian Society of Cytology Inc

283-287 Sir Donald Bradman Drive

BROOKLYN PARK SA 5032

All abstracts must be accompanied by the Abstract Submission form available on the ASC website or from the national office.

Closing date for abstract submissions: 30 July 2014

By submitting an abstract all authors agree to the Society publishing the abstract in the conference booklet and Cytoletter and in so doing certify that the abstract is original work. If the abstract does not conform to the guidelines detailed above it will be returned to the submitting author to revise.

Prizes

Prizes will be awarded in the following categories:

- Best oral presentation by a non-medical cytologist.
- Best case study poster by a non-medical cytologist.
- Best research poster by a non-medical cytologist.
- Best poster or oral presentation by a Registrar.

Only current financial members of the Society are eligible for prizes.

These guidelines have been recently reviewed by the Board of Education, full guidelines are available from the website or the ASC national office (email).

Ovarian Clear Cell Carcinoma in a Pap Smear.

Natalie Hockey

Cytology Department, QML Pathology, Brisbane, Queensland.

Clinical Presentation

A 66 year old female presented to her doctor with post menopausal bleeding. She had a history of a high grade squamous intraepithelial lesion of the cervix histologically confirmed by a cone biopsy six months previously. Ascites was found and a conventional Pap smear and cervical polyp biopsy were taken. CEA and CA125 serum analysis were also performed due to the ascites.

Cytological Findings

The Pap smear was satisfactory for assessment and contained groups of atypical epithelial cells, some in papillary arrangements, in a background of abundant blood. These cells had enlarged nuclei with irregular nuclear membranes, coarse and hyperchromatic chromatin and prominent centrally located macronucleoli. The nuclei were crowded and overlapping with marked variation in nuclear size and shape. There were also many clear cells present with vacuoles in the cytoplasm distending the nucleus. Sparse squamous epithelial cells were present and no endocervical component was detected. The smear was

reported as 'Adenocarcinoma – Papillary groups of malignant glandular cells consistent with adenocarcinoma. It is not clear whether this is a cervical or endometrial primary'.

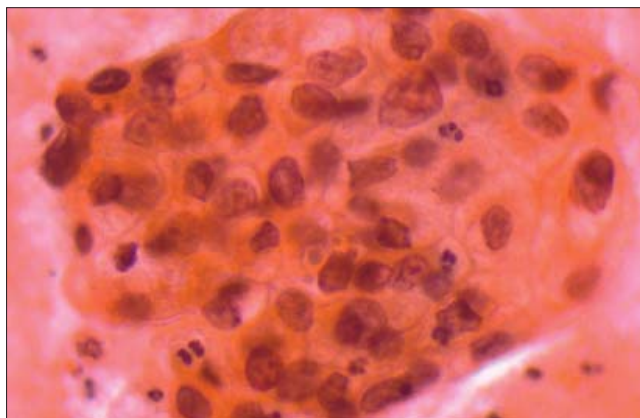


Fig 3: Papanicolaou stain, x400

Pap smear showing crowded group of malignant cells with variable size and shape.

Additional to the Pap smear, a cervical polyp biopsy was taken along with CEA and CA125 tumour marker tests. CEA was within normal limits but CA125, an ovarian tumour marker, was 200 U/ml which is well above the standard range of less than 30U/ml. The cervical polyp biopsy was reported as, 'Adenocarcinoma favouring papillary variant of clear cell carcinoma'.

Follow-Up Studies

The patient underwent a total hysterectomy, bilateral salpingo-oophorectomy with attached bladder and peritoneum and histological specimens were taken from the omentum, pouch of douglas, paracoli, and appendix. Peritoneal washings were also collected for cytology analysis. The definitive diagnosis was an ovarian clear cell carcinoma FIGO stage IIIc.

The histological specimens contained the distinctive clear cell features with hyaline cores of papillary clusters surrounded by hobnail clear cells. These are often referred to as raspberry bodies.¹

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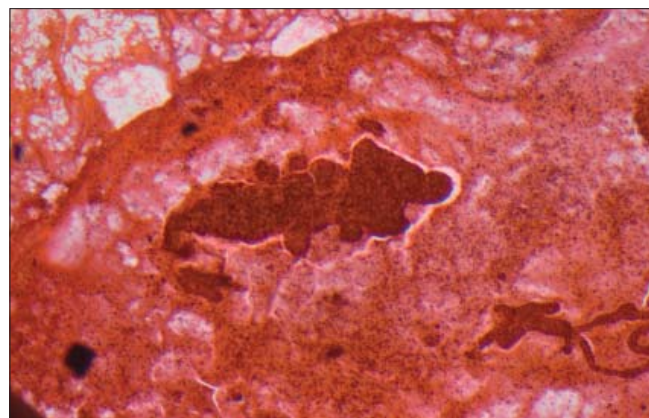


Fig 1: Papanicolaou stain, x40

Pap smear containing malignant cells showing papillary architecture with abundant blood in the background.

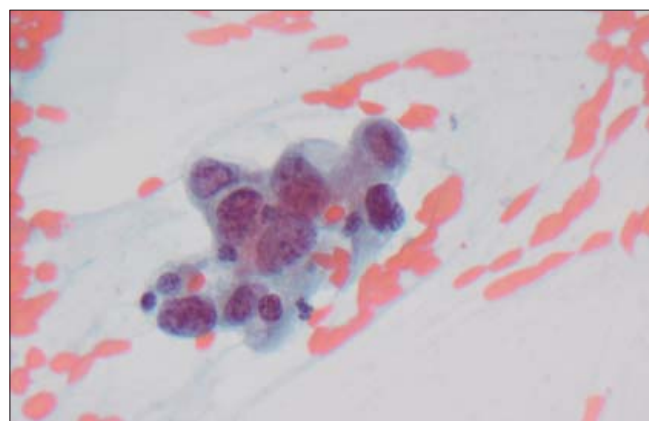


Fig 2: Papanicolaou stain, x400

Pap smear showing malignant cells with vacuoles in the cytoplasm.

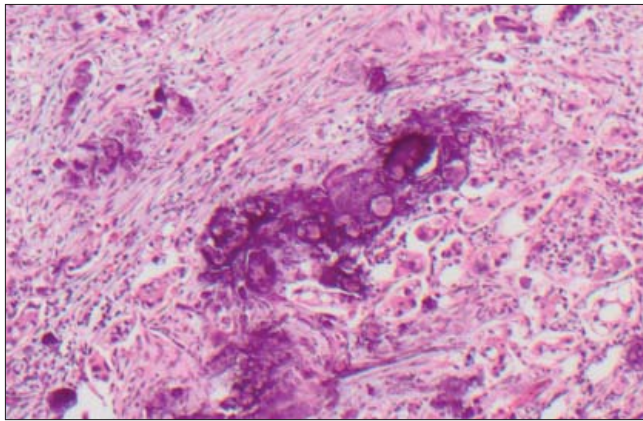


Fig 4: H&E stain, x100

A histology section from the cervix showing metastatic ovarian clear cell carcinoma with hyaline cores.

The peritoneal washings contained both single and large clusters of malignant cells. The cells showed eccentrically placed nuclei with coarse chromatin and prominent macronucleoli and vacuolated cytoplasm. The background contained reactive mesothelial cells, histiocytes, lymphocytes and blood.

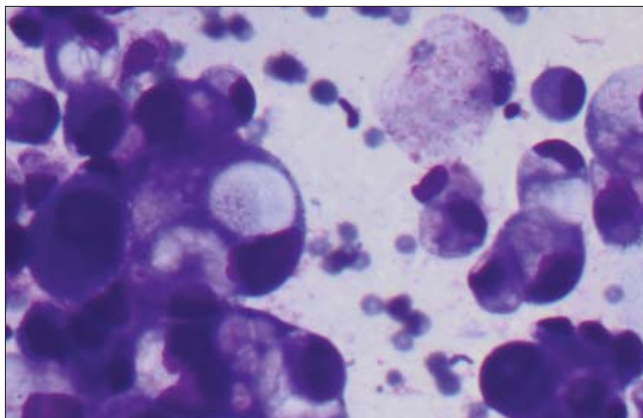


Fig 5: Diff-quick stain, x400

Peritoneal washings showing clear cells with vacuolated cytoplasm.

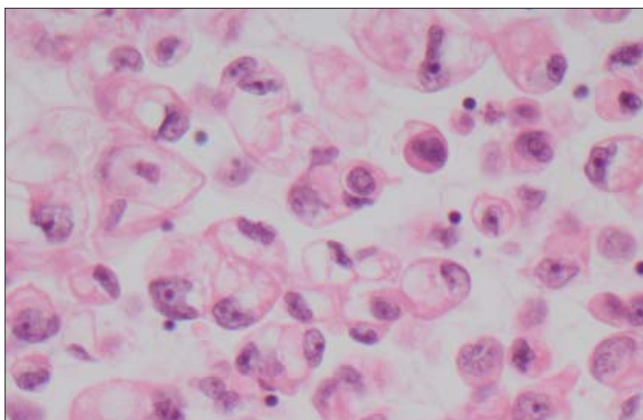


Fig 6: H&E stain, x400

Cell block made from peritoneal washings showing the prominent nucleoli and vacuolated cytoplasm of the malignant cells.

Discussion

Ovarian carcinoma is the sixth most common cancer in women worldwide² and ovarian clear cell carcinoma accounts for less than 5% of all ovarian carcinomas.³ Detecting ovarian cancers in a Pap smear is rare and most patients are asymptomatic at the early stages of the disease. Just over half (58%) of all ovarian cancers are not diagnosed until metastases have occurred at stage III or IV². It is uncommon to find metastatic malignant cells in a Pap smear without prior knowledge of a primary site. The literature describes the prevalence of malignant ovarian cells in the vagina as being anywhere between 4.6% to a much higher 30%⁴.

Cytologically it was difficult to determine the primary site of the malignant cells in this case so the differential diagnosis included endocervical and endometrial adenocarcinoma due to the glandular features of the cells. The clinical history of the patient and the papillary architecture of the cells helped us to consider the likelihood of a metastatic carcinoma. Clear cell carcinoma in a Pap smear looks cytologically the same no matter the primary site so the differential diagnosis also includes primary sites such as renal cell carcinoma, female genital tract clear cell carcinoma, particularly those women with DES exposure, and PEComa³.

A panel of immunohistochemistry stains could have been used in determining the correct diagnosis of ovarian carcinoma which usually stains positive to PAX8, p21, CK7 and cyclin E while WT-1 and CK20 are negative.³ In this case it was not necessary as the histological presentation is quite distinctive.

This case illustrates that cells from an ovarian carcinoma can present in a Pap smear, and although a rare finding, it should be included in the differential diagnosis when highly malignant cells are detected.

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seems). Advanced Anatomical Pathology 2012; 19:296-312.

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2001; 80: 659-660.

Acknowledgements

Dr Jason Stone, Gwenda Lawrence and Terese Boost

Third Sydney Advanced FNB Cytology Tutorial

St Vincent's Hospital and Garvan Institute

Monday 5 to Friday 9 May 2014

This third tutorial will focus again on FNB cytology with new speakers in some topics and new approaches in other topics, and will add sessions on brain squash cytology by Dr Matt Zarka, pleural/pericardial/ascitic fluid cytology by Dr Elizabeth Salisbury, and live by Professor Pitman. Professor Pitman will present the new Papanicolaou Society of Cytology approach to pancreatic cytology reporting. Professor Ali will present a new session on EBUS/lung.

The faculty will include:



Associate Professor Andrew Field

St Vincent's Hospital

(breast, algorithmic approach to reactive and infectious lymph nodes)

Professor Syed Ali

John Hopkins Hospital, Baltimore

(thyroid and EBUS/lung)



Professor Martha Pitman

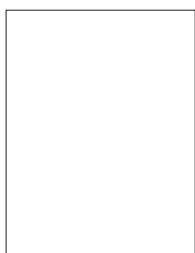
Massachusetts General Hospital, Boston

(EUS/pancreas/liver)

Dr William Geddie

University Health Network, Toronto

(algorithmic approach to lymphomas, FNB technique)



Associate Professor Elizabeth Salisbury

Prince of Wales Hospital, Sydney

(fluid cytology)

Dr Matthew Zarka

Mayo Clinic, Scottsdale

(salivary gland, brain squash cytology)



Each topic is covered by a didactic lecture followed by case based tutorials and a videomicroscopy session.

It is hoped to have several visiting pathologists travelling to the tutorial on bursaries from funds raised at the first two tutorials.

Please send expressions of interest to Dr Andrew Field, who will provide registration information, at afield@stvincents.com.au and contact him 0414 243 130 for further information.

Expressions of Interest

APPRENTICE TO THE CEC REGISTRAR

Expressions of interest are sought for the position of Apprentice to the CEC Registrar of the ASC CEC Scheme, commencing 2014. Experienced current financial non-medical ASC members with an interest in continuing education are encouraged to apply.

The position has been created to facilitate a smooth transition to undertaking the duties and responsibilities of the Registrar on expiry of his term on the Board of Education. Initially the appointee will be required to provide support to the Registrar in addition to participating in other activities of the Board. The duties and responsibilities of the Registrar, which the apprentice will finally undertake, are as follows:

- Participate as a member of the Board of Education (appointment for a 5 year term).
- Responsible for the overall design and content of the Scheme in liaison with the Board of Education.
- Liaise closely with the ASC National Office Manager and the ASC Executive to ensure content is up-to-date and relevant issues are covered.
- Liaise with the ASC National Office Manager; the ASC Executive and the Society's IT contractor on content changes require significant programming effort.
- Investigate and promote the use of new initiatives in the operation of the Scheme and strategies to improve compliance.
- Mentor the Registrar-Elect in a jointly run "hand over".

Applicants for the position should be experienced cytologists with a keen interest in continuing education and with good communication skills. Other desirable attributes include experience with web-based applications and teaching.

APPRENTICE TO WEBSITE MANAGER

Expressions of interest are sought for the position of Website Manager of the ASC website, commencing 2014. Experienced current financial non-medical ASC members with an interest in continuing education are encouraged to apply.

The position has been created to facilitate a smooth transition to undertaking the duties and responsibilities of the Website Manager on expiry of his term on the Board of Education. Initially the appointee will be required to provide support to the Website Manager in addition to participating in other activities of the Board. The duties and responsibilities of the Website Manager, which the apprentice will finally undertake, are as follows:

- Participate as a member of the Board of Education (appointment for a 5 year term).
- Responsible for the management of the ASC website in liaison with the Board of Education.
- Liaise closely with the ASC National Office Manager and the ASC Executive to ensure content is up-to-date and relevant issues are covered.
- Liaise with the ASC National Office Manager; the ASC Executive and the Society's IT contractor on content changes require significant programming effort that extends beyond routine website maintenance.
- Investigate and promote the use of new initiatives in the delivery of ASC services via the website.
- Mentor the Website Manager-Elect in a jointly run "hand over".

Applicants for the position should be experienced cytologists with a keen interest in educational activities of the ASC and some experience in website management. Other desirable attributes include good communication skills.

The Board of Education currently has two face-to-face meetings per year prior to Council meetings to review activities and plan new initiatives.

Please send expressions of interest to:

Dr Jane Twin, Chair of the ASC Board of Education jane.twin@capitalpath.com.au, with a copy to Cheryl Edgton, Office Manager, ASC national office, national.office@cytology-asc.com. An appointment will be considered at the October 2014 meeting of the Board.

DEADLINE FOR APPLICATIONS: 31 July 2014.

YOU ARE INVITED TO JOIN US AT THE 2014 ASC TUTORIAL IN DARWIN



7-10 OCTOBER 2014, at the Darwin Convention Centre.

Join us for '**Cytology...beyond the microscope**' with Guest Speakers:



Dr Min En Nga a Senior Consultant Pathologist, at National University Health System, Singapore. Dr Nga is a Fellow of the IAC and a Fellow of both the Royal College of Pathologists of Australasia and the United Kingdom.

In 2007, Dr Nga completed a Cytology Fellowship at PathWest, QEII Medical Centre, under the mentorship of Assoc Prof Greg Sterrett and Dr Felicity Frost. Dr Nga has a keen interest in undergraduate and post graduate teaching and her speciality areas include Thyroid, Lymph node and Salivary gland cytology. Dr Nga's publications include a book chapter on Gastrointestinal stromal lesions with journal articles focusing on thyroid, pancreatic and Hodgkin's lymphoma. Dr Nga will share with us her experience in Lymph Node cytology.



Dr Matthew Zarka a Consultant Pathologist and Director of Cytopathology at the Mayo Clinic Arizona. He is a member on the Papanicolaou Society of Cytopathology, College of American Pathologists, American Society of Cytopathology, Australian Society of Cytology and a member of the World Health Organisation's Technical Advisory Group on comprehensive cervical cancer control. Dr Zarka is an advocate for humanitarian pathology programs in the developing world and has taught pathologists in Tanzania and has served as medical Director of Grounds for Health, a non-profit organisation promoting cervical cancer screening in rural Mexico and Central America. We look forward to Dr Zarka's presentation on Bone and Soft tissue Cytology at the ASC Tutorial 2014, with the use of Aperio images.



Dr Felicity Frost a Consultant Pathologist in Histology and Cytology and is Head of the Cytology department at PathWest QEII Medical Centre, Nedlands. Under her guidance the laboratory provides a comprehensive teaching program for Pathology registrars and cytology scientists with a continuing national profile in research and development. Dr Frost has played an integral role in establishing the national guidelines on breast core biopsy and Fine Needle Aspiration for the National Breast Cancer Centre. Currently Dr Frost is the Medical Advisor, Pathologist on the Advisory Committee of the Western Australian Cervical Cancer Prevention Program. Dr Frost's presentations at the 2014 ASC Tutorial will include the 'New thyroid FNA guidelines' and 'Gynaecological squamous cytology'.

Is Cytology Science?

From Papanicolaou to LBC and HPV



Ron Bowditch

Continued from the December edition

Just as cytology was really getting its act together, and cervical cancer rates and deaths were falling in screened populations, two major changes to the screening world emerged, both related to cytological practice and technique - LBC and HPV: LBC as a major thread in the practices-and-techniques strand, and HPV, not just for its place in understanding of the disease, but for its role in screening and diagnosis, and also, through vaccination, its impacts on the entire context of cervical cancer screening.

Liquid based cytology (LBC) emerged in the early 1990s and was quickly accepted as a major improvement in technique. LBC standardises the specimen, reduces subsampling error and removes obscuring and distracting elements from the smear, and reduces unsatisfactory specimens. LBC rapidly replaced the conventional smear in many developed countries, but not in Australia, where its role is as an optional extra. Computer assisted primary screening of LBC slides was introduced a decade after the introduction of LBC. Studies conducted in Colin Laverty's laboratory by Jenny Roberts and colleagues showed that LBC offered advantages in sensitivity for significant lesions, of the order of 12% to 21% for squamous lesions – the advantage mostly from reduction of subsampling errors because the examined sample is drawn from a thoroughly mixed sample. The trials, however, raised doubt about the technology regarding detection of glandular abnormalities. These studies identified the need for adjustment of cytological criteria and of screening practices, initially for the change to ThinPrep LBC and then again to adapt to the different screening regime using the Imaging technology. Productivity gains were less than anticipated.^{23,24} More recently, SurePath LBC has been reintroduced in Australia, with its own computer assisted screening technology, FocalPointGS. Once again, the need for adjustment of diagnostic criteria and screening

practice has been identified as essential to realise the full potential of the technology,²⁵ and the great white hope that a technically improved sample would make cytologists less likely to make human errors has been shown again to be false.

There are several ways to approach the problem of human error in cytology. One way is to make technical improvements, for example to make the sample clearer for visual interpretation; this is one goal of LBC and it is very successful, removing obscuring blood and leukocytes, and displaying a clear, well and consistently fixed specimen. A second approach is to concentrate the abnormal cells within the specimen to be examined. This can be done in two ways. The first is by physically separating the specimen into a more and a less abnormal fraction, and discarding the less abnormal. Both the LBC technologies (ThinPrep and SurePath) do this by removing irrelevant cells such as blood and leukocytes, but neither preparation markedly concentrates the abnormal fraction amongst the remaining epithelial cells. Indeed, the ThinPrep slide process may leave many larger abnormal cell groups behind in the vial.²⁶ This is particularly disappointing for me personally, because I have spent so much time and effort refining the diagnostic criteria for crowded sheets, and consider that they present a superb opportunity for accurate diagnosis. The second way LBC technologies seek to concentrate the specimen is indirect; the computer assisted technologies present a restricted number of microscope fields, selected as potentially abnormal by the computer, to be reviewed by the screener. I am quite sceptical about the contribution to accuracy offered by this aspect of the technologies. The manufacturers claim that abnormal cells will be somewhere in the selected fields, although not necessarily centred in the field and not necessarily including the most diagnostic cells present. This is not a very demanding standard.

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To explore this, I propose an experiment anyone can do, with some geometry, an afternoon reviewing 50 high grade abnormal slides, a calculator, and appreciation of the union of independent probabilities $P=1-(1-p)^n$ (where P = Probability of at least one abnormal cell being present in a seen field, p = probability that any one abnormal cell will be in a seen field (ie proportion of slide covered by seen fields) and n = total number of abnormal cells on the slide): Attempt to falsify this hypothesis: A random selection of fields (22 for ThinPrep or 10 for SurePath) will achieve an 'at-least-one-abnormal-cell-present-in-selected-fields' rate comparable to imaging/computer-assisted technology. I will leave you to do that simple experiment amongst yourselves.

So LBC helps. But in my opinion Imaging technologies don't help much, if at all. Mistakes are still made by cytologists and cytopathologists. Purveyors of computer assisted screening machines take no responsibility for these errors. My personal preferred way to reduce errors has been threefold, all aiming, through education, to increase the ability of cytologists to avoid errors by:

1. Refining and clarifying diagnostic criteria, making them more definite, less fuzzy and ambiguous, and more soundly based - related to the actual goings on at the biological level (eg criteria for crowded sheets reflect the histology and incorporate understanding of aneuploidy and the resulting differences between cells)
2. Discouraging the process of snap intuitive judgement and encouraging screening practices that put more objective handles on interpretation processes (eg breaking the task of interpreting a crowded sheet into several manageable, more objective steps, such as looking at nuclear sizes, then chromasia variation, then the range of nuclear structures, then patterns of polarity, and a search for mitoses)
3. Minimising biases in perception and judgement, through understanding visual perception and judgement, when and why they fail, and how to avoid the traps (eg making the target of screening not abnormal cells but high risk cellular patterns - increasing the prevalence of targets and forcing closer

evaluation, and introducing debiasing practices such as deliberately seeking disconfirming information, ie contrary to your decision, before finally deciding)

However, as the results of our LBC and computer assisted screening trials show, errors still occur, even after years of experience and training according to these principles. The reliance of all cytological screening technologies on the perception and judgement of the cytologist, and the intractable problem of errors inherent in human judgement, gives impetus to the call for more objective screening tests. HPV DNA testing is emerging as such a test. One aspect of LBC is that the liquid specimen is also available for testing for HPV DNA. HPV DNA testing either by target amplification (PCR) or signal amplification (Hybrid Capture) has long since left the research lab and has become important in the diagnosis and management of cervical lesions. HPV DNA testing is used in Australia for its value as a test of cure, but is increasingly used elsewhere, eg in the USA, for triage of cytology-detected abnormality. HPV DNA testing is promising as a replacement for cytology for primary screening, because it is a more sensitive test with a higher Negative Predictive Value than cytology. Primary screening by HPV DNA has commenced in Europe, and widespread extension of this mode of screening merely awaits the results of large trials. Numerous trials already confirm its efficacy, eg the POBASCAM trial.²⁷ Meta-analysis of trials so far suggests that optimum screening would comprise HPV DNA at extended (5-yearly) intervals, with triage to cytology to select cases for colposcopy.²⁸ This would reduce the need for cytology to about 10-15% its current level, and the focus would shift from pure screening to a more diagnostic emphasis, as in non-gynae cytology. The conservatism of Australian regulators with regard to direct to vial LBC and HPV DNA triage suggests that this change might be a way off, but you never know. The newly converted can be the most ardent enthusiasts.

The second great change for screening emerging from the science of HPV is vaccination. It was first adopted in 2007 in Australia as a population measure, vaccinating young adolescents. The vaccine appears to provide women who are HPV-naïve at vaccination the prospect of very long

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lasting immunity from cervical cancer caused by subsequent infection by the two included oncogenic HPV types (16 and 18). Despite the efficacy of the vaccine, approximately 30% of cervical cancers are caused by other HPV types, so screening is still necessary. The implications of vaccination for screening have not yet fully emerged, as the first vaccinated cohort is only now entering the age at risk. One anticipated impact is a significant reduction in the proportion of cytologically abnormal cases in the cytologists' routine daily workload. Unknowns include i) whether non-vaccinated subtypes will emerge as a greater source of oncogenic HPV infections and cancer and ii) whether many vaccinated women, believing themselves protected, will not participate in screening.

Other tests, based on molecular markers such as p16^{INK4a}, Ki-67 and E6/E7mRNA are either under development or under trial, and these could ultimately impact on screening practice.²⁸ Eg could a HR-HPV. p16, Ki-67 and E6/E7mRNA automated panel work?

As in the 1940s and the 1980s and the 1990s, the world of cervical screening is changing, and who can know where it will lead?

I started with a tip of the hat to Karl Popper, philosopher of science, who would argue that cytology is not science. But cytology works. Not absolutely, and not always. But it works. With or without HPV testing. With or without LBC. It has saved many lives. Cytology may not fit Popper's definition of science, but it is not weird religion either. Perhaps there really is another type of science, one that does not fit the Popperian hard science definition, but is legitimately described as science anyway – a pragmatic 'successful science' according to Pierce, whose strength comes from its many threads. Such sciences are difficult rather than hard. They operate in more uncertain realms, like biology and medicine, where phenomena are not so black and white, and answers are not so clear cut. There is an irreducible subjective element, and inherent uncertainty, but the principles of seeking evidence, constant revision of understanding and practice in the light of new evidence, accountability to the evidence, and ultimately practical success are the measures. Gynaecological cytology is indeed such a successful science, and it has been a privilege to be a part of

it and to be recognised in this award by the ASC, an organisation so thoroughly dedicated to the pursuit of excellence in that most difficult science.

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New Members

December 2013 - February 2014

New South Wales

Mrs Diana Thandar Maitland

Mrs Amanda Nellist

South Australia

Dr Amardeep Gilhotra

Queensland

Ms Patricia Anne Cullen

Mr Garry Joseph Robinson

Mrs Danielle Catherine Turner

Tasmania

Ms Carol Briscoe

Victoria

Ms Joanne Romano

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Branch News

New South Wales

The NSW Branch AGM was held on 21 August 2013 in combination with a trivia night presented by Michelle Blackwell at the Ranch Hotel in North Ryde. New office bearers were elected unopposed during the AGM and are as follows:

Councillor: Dr Lyndal Anderson (Staff Specialist in Charge of Cytology Department, Royal Prince Alfred Hospital)

Secretary: Anna Santos (Senior Scientist in Charge, Royal Prince Alfred Hospital)

Treasurer: Michelle Blackwell (Product Manager – Diagnostic ANZ, Hologic Australia)

We would like to thank the outgoing committee for their amazing efforts and hard work over the last few years. It has been greatly appreciated by all NSW ASC members.

The NSW Branch committee introduced the NSW Branch Bulletin in 2013. The bulletin is issued monthly to all NSW ASC members and provides members with important upcoming events, interesting cytology information, along with cytology games, trivia and news. The bulletin was received with “mixed reviews” with some members finding the inclusion of recipes and other non-cytology related content unnecessary. The committee was delighted to have sparked interest (if not some controversy) in the cytology community and invited all members through a voluntary survey to tell us what topics they wished to include in the bulletin. Whilst some members liked the inclusion of recipes, a majority of members preferred the bulleting to contain cytology only content. This was also a great opportunity for the committee to gain insight into what members were looking for in up-coming meetings in 2014.

The final NSW meeting for 2013 was held on 4 December at Laverty Pathology. Ron Bowditch presented a wonderful talk about “Change in Cytology”. The points raised included the various forms of change, how people respond/preserve change and the outcomes to change. He focused these points on different stages through cytology evolution and raised important issues regarding the current proposed changes to the National Cervical Screening Program Renewal. Overall, the night was enjoyed by all and we wish Ron all the best for his retirement.

The Branch Meeting held on 19 February 2014 was presented by Dr Tracey Bessell, Director of Screening/Cancer and Palliative Care Branch/Department of Health and Professor Ian Hammond, Chair Renewal Program. The meeting focussed on “The National Cervical Screening Program Renewal”.

Anna Santos

NSW Branch Secretary

Australian Capital Territory

2013 was a busy year for cytology in the ACT and several entertaining branch meetings were held. In March Dr Jane Twin presented “Oropharyngeal Cancer in the Third millennium”, during which she discussed the contributing factors to oral cancer including the link between HPV and tonsillar cancer and the role the HPV vaccine may play in reducing the incidence of this disease.

In May Dr Tracey Lu presented “Fine Needle Aspirate (FNA) diagnosis of Hepatoid variant of yolk sac tumours of the ovary.” This talk focused on a rare tumour sometimes found in young women and the steps involved in differentiating it from other diagnoses such as hepatoid adenocarcinoma and metastatic hepatocellular carcinoma.

The AGM was held in August in conjunction with a urine cytology workshop conducted by Dr Jane Twin. The workshop was based on her experience at the urine workshop held at the IAC conference in Paris last year. The main outcome from this talk was the importance on cytologists identifying high grade urothelial changes and that low grade changes may be better left to Histology. The 2014/15 branch committee was also appointed at this time and is as follows:

Councillor – Sonja Boehm

Secretary – Tony Bell

Treasurer – Olivera Milenkovic

Committee Members – Meredith Harrigan, Monika Stanczew, Linda Beckett, Dr Jane Twin and Dr Tracey Lu.

The final branch meeting for 2013 was held in November. Brett Matthews presented “A Cervical Registry for Nepal”, where he gave a brief rundown on his continuing efforts to start a cervical registry from scratch in the small Asian nation.

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Brett Matthews giving his presentation in traditional Nepalese garb!

A massive thank you to all 2013 presenters for taking the time out to give such interesting and informative talks. The first meeting for 2014 is tentatively planned for mid-late March. Here's looking forward to another exciting year!

Tony Bell

ACT Branch Secretary

Queensland

The committee of the Queensland Branch met on 13 January to organise the Scientific Program for 2014. The following is an outline of what has been planned:

3 May Pre-exam workshop at QML with presenters Dr Jason Stone (QML) and Terese Boost (QML).

13 May Branch Meeting at SNP: "Cervical Cytology - Histological Correlations with a Learning Point". Presenters include Dr Jennifer Borowsky, Dr Sarah Sim and Dr Edwin Tan. This meeting will incorporate a friendly presentation competition with Dr Michaela Lee (consultant gynaecologist and obstetrician, The Wesley Hospital) as our invited judge for the evening.

16 July Oral Presentation Competition & Annual General Meeting at SNP with Dr Ben Kroon (director of Eve Health) as our invited judge for the evening.

30 August Half Day Conference at Brisbane Convention & Exhibition Centre: "FNA of Head &

Neck" - A combined meeting with the Australian Sonographers Association (ASA). Presenters still to be confirmed.

8 November Business Breakfast at Eves on the River, Teneriffe. Topic and presenter still to be confirmed.

This year, the committee have made a few changes to freshen up the program for 2014. Instead of our usual Saturday morning tutorial and dinner meeting, we will be hosting an afternoon conference and a breakfast meeting. I hope all our members are as excited as we are about these new events and pencil the above dates into their diaries. I look forward to seeing you at our next meeting at SNP on 13 May.

Lee Cadoo

Queensland Branch Secretary

South Australia

The first meeting of the year kicks off with the Registrar State Based Competition. It will be held at SA Pathology (RAH) on 18 March. Registrars can apply by submitting a synopsis to Associate Professor Chris Carter. Remember, only financial ASC members can claim the prize. We look forward to seeing your presentations.

The next branch meeting will be held in May at Adelaide Pathology Partners followed by the state branch AGM in August. The Scientists State Based Competition will be held in November.

Kimberly Wojtkowiak

SA Branch Secretary

Victoria

The Victorian Branch held an End of Year Trivia Night at the Geelong Polo Club on Thursday, 21 November. Many thanks to our hosts Myfanwy Blyth and Rebecca Steer who did a wonderful job and entertained with many Cytology-related questions.

Our next meeting will be held on Thursday, 13 March at the Royal Dental Hospital of Melbourne. Further details of our meetings for 2014 will be sent out at a later date.

Elizabeth Shao

Victorian Branch Secretary

Meeting Calendar

2014

23-25 May	NUHS Cytopath 2014 Diagnostic Cytology Workshop	www.med.nus.edu.sg/path/cytopath2014	Singapore
30 May - 1 June	International Academy of Pathology Australasian Division ASM	http://www.iap-aus.org.au/	Brisbane
20-25 August	International Papillomavirus Conference	http://www.hpv2014.org/	Seattle
30 Aug - 1 Sep	European Congress of Pathology	http:// esp-pathology.org/	London
21-26 September	Congress of the International Academy of Pathology	http://www.iapcentral.ca/meetings.htm	Bangkok
27-30 September	European Congress of Cytology	http://www.efcs.eu/	Geneva
3-6 October	Australian Society of Cytology Annual Scientific Meeting	http://www.cytology-asc.com/	Darwin
7-10 October	Australian Society of Cytology Tutorials	http://www.cytology-asc.com/	Darwin
5-10 October	International Academy of Pathology Congress	http://iap2014.com/	Thailand
3-5 November	Eurogin	http://www.eurogin.com/	Florence

2015

27 Feb - 1 Mar	RCPA Pathology Update	http://www.rcpa.edu.au	Melbourne
4-7 June	Asia Pacific International Academy of Pathology Conference		Brisbane
23-27 October	Australian Society of Cytology Annual Scientific Meeting	http://www.cytology-asc.com/	Brisbane
28/31 October	Australian Society of Cytology Tutorial	http://www.cytology-asc.com/	Brisbane

2016

28 May - 1 June	19th International Congress of Cytology	www.cytologyjapan2016.com	Yokohama
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CEC: Coordinator.....Head of Cytology

IAC SLIDE SETS

1	Problems in Cytodiagnosis Female Genital Tract
2	Aspiration Biopsy Cytology of head & neck
3	Aspiration Biopsy Cytology of the breast
5	Cytology of normal epithelia and benign proliferative reactions of the uterine cervix
6	Cytology of Dysplasia, Carcinoma in situ and adhesive carcinoma of the uterine cervix
7	Human chromosomes and chromatin
8	Hormonal Cytology
10	Cytology criteria for differentiation of benign, dysplastic and malignant changes of uterine cervix
11	Cytology of uterine adenocarcinoma
12	Look-alikes in Gynaecologic Cytology
13	Gynaecologic Endocrinopathies
14	Oral Cytology
15	Pulmonary Cytology
16	Cytology of Effusions and its Histologic Basis
17	Cytology and Histology of ionising radiation on the female genital tract
18	Cytologic specimens obtained by the brush technique (respiratory tract)
19	Respiratory Cytopathology
20	Self-evaluative test in Cytology for Pathologists
21	Cytology of the Urinary Tract and its histologic basis
22	Testing program and self-assessment exercises for Cytotechnologists
23	Gastric Cytology
24	Cytology of the Cerebrospinal Fluid and its histologic basis
25	Look-alikes in the Cytology of the respiratory tract and serious effusions
26	Cytologic diagnosis of opportunistic infections: Fungi and higher bacteria

27	Phase contrast microscopic Cytologic diagnosis: A Gynaecological procedure
28	Cytopathology of soft tissue and bone tumours
29	Condylomata of the Uterine Cervix: A human PAPILLOMAVIRUS infection - Cytology, Histology, Ultrastructure, Immunocytochemistry & Differential Diagnosis
30	Cytology of inflammatory reactions, effect of IUD, contaminants and microbiologic classification including chlamydial organisms (female genital tract)
31	Techniques for electron microscopy in human tissues
32	Cytology in pregnancy
33	Endometrial aspiration and other Cytologic techniques for the detection of endometrial cancer and its precursors
34	Needle aspiration biopsy Cytology of the head and neck 1. Lymph nodes and salivary glands
35	Needle aspiration biopsy Cytology of the head and neck 2. Thyroid, soft tissue, bone and miscellaneous conditions
36	Fine needle aspiration of the prostate gland
37	Vulvar cytology
38	Cytopathology of Aids
39	Paediatric cytopathology
40	Bronchoalveolar lavage in diagnostic cyto
41	Basic colposcopy
42	Cytology of tissue repair & differential diagnosis
43	Atypical Squamous & Glandular Lesions of the Cervix
44	Thyroid Fine Needle Aspiration Cytopathology
45	Thin Layer Slides in the Laboratory Routine for Pap tests

New Purchases

The Society has received new copies of the following sets. They are all volumes which have been updated since the sets were originally produced. VOLUMES 10, 11, 12, 15, 18, 22, 25, 28, 30

KODACHROME NOTES FROM THE 1991 ASM

A	Cytology in the Detection and Monitoring of Bladder Neoplasms: William M Murphy, MD
B	Fine Needle Aspiration of Lymphoma and Reactive Hyperplasia: Ruth L Katz, MD
C	Fine Needle Aspiration of Adrenal, Kidney and Retroperitoneum: Ruth L Katz, MD

CD ROM

Body Fluids: C Kjeldsberg and Knight, 1996

The Art & Science of Cytopathology: RM DeMay

BOOK

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3	Normal and Benign Cervical Cytopathology <i>by Deborah Reich, Dr Gabriele Medley and Grant King</i>		\$45.45	\$4.55	
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5	Cervical Cytology Self Assessment Quiz <i>by Paul Shield, Bernadette Tanner and Grant King</i>		\$45.45	\$4.55	
6	Fine Needle Aspiration of Breast <i>by Paul Shield and Diane Cominos</i>		\$45.45	\$4.55	
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ABN 76 001 239 606

APPLICATION FOR MEMBERSHIP 2013-14

(Please use block letters)

TITLE (Dr/Mrs/Ms/Miss/Mr):		SURNAME:	
GIVEN NAMES:		DOB: / /	
LABORATORY:			
PREFERRED ADDRESS:			
			POSTCODE:
OTHER ADDRESS:			
			POSTCODE:
BH PHONE:		FAX:	MOBILE:
EMAIL:			
QUALIFICATIONS <i>Please attach copies to your application</i>		INSTITUTION	YEAR
CYTOLOGY EXPERIENCE:			

PROPOSER AND SECONDER (must be either Medical or Non-Medical financial members of the Society).

PROPOSER:	SIGNATURE:
SECONDER:	SIGNATURE:

APPLICATION AND DECLARATION

I apply for the following class of membership (please tick):

MEDICAL	Registered medical practitioners who engage in the practice of Cytology. Specialist Total \$290.00 (entrance fee \$50, annual subscription \$213.64, GST \$26.36) Registrar Total \$239.50 (entrance fee \$50, annual subscription \$167.73, GST \$21.77)
NON MEDICAL	Graduates of a degree course in Medical Laboratory Science (or its equivalent) from a recognised tertiary institution <u>or</u> persons who hold the CT(ASC) or an equivalent qualification, who are not registered medical practitioners but who engage in the practice of Cytology. Total \$239.50 (entrance fee \$50, annual subscription \$167.73, GST \$21.77)
ASSOCIATE	Persons interested in Cytology not eligible to be Medical or Non-Medical members. Associate members do not have the right to vote in the affairs of the Society, but may participate in all other activities of the Society. Total \$175.00 (entrance fee \$50, annual subscription \$109.09, GST \$15.91)

SIGNATURE:	DATE: / /
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PAYMENT DETAILS: Enclose Cheque or Money Order made payable to Australian Society of Cytology Inc. **OR**

Debit my ___ Visa ___ Mastercard (*Please tick*) Card Verification Code ___/___/___ For \$ _____

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Return this form to: Australian Society of Cytology Inc or national.office@cytology-asc.com
283-287 Sir Donald Bradman Drive fax: 08 8238 3456
BROOKLYN PARK SA 5032

UPDATED JUNE 2013

Applicants from overseas are not required to pay GST.

Diagnostic Cytopathology Workshop



Singapore
23 - 25 May 2014

**Organized by: The Department of Pathology,
Yong Loo Lin School of Medicine, National University of Singapore and
National University Hospital, National University Health System**

EARLY BIRD REGISTRATION DEADLINE 31ST MARCH 2014

This workshop is suitable for pathologists, cytologists, cytotechnologists and residents. Clinicians with special interests may also find this workshop educational

This three-day workshop covers gynecological and non-gynecological cytopathology. Our guest faculty is Dr Dina Mody from Houston, Texas. Dr Mody has 30 years of experience and practices in Pathology, Cytopathology, Anatomic Pathology and Clinical Pathology, and Pathology Recertification. The local faculty comprises experienced pathologists from several hospitals in Singapore.

Invited Speaker



Dina R. Mody, M.D
Medical Director, Cytopathology
Department of Pathology and Laboratory Medicine
The Methodist Hospital Physician Organization
Professor of Pathology and Laboratory Medicine
Weill Cornell Medical College of Cornell University, USA

This workshop aims to:

1. Provide an in-depth and updated review of important areas in gynecological and non-gynecological cytopathology through lectures, slide demonstrations, case presentations, interactive small group breakout sessions, slide self-review and MCQ self-assessments. The focus will be on recognition of diagnostic problems and pitfalls, approaches to resolving differential diagnostic dilemmas, and best practices in diagnostic cytopathology.
2. Foster opportunities for research and international collaboration.
3. Provide a forum for networking and exchange of experience and co-operation for training and CME activities.

Participants are encouraged to present their work through a poster platform. Best poster awards will be given in three categories according to the category of registration. The Call for Abstracts is available on the website.

We are also introducing for the first time an art competition entitled "Art in Cytopathology". Please refer to the website for details.

Aileen Wee, MB,BS, FRCPA, FRCPath
Course Director
Professor & Senior Consultant

Qasim Ahmed, MB,BS, Diplomate ABPath, IFCAP
Course Director
Senior Consultant & Head of Cytopathology Section



Yong Loo Lin School of Medicine
Faculty of Dentistry
Saw Swee Hock School of Public Health



**For enquiries, please contact Siew Hong or Erika at: Email: cytoconf_sec@nuhs.edu.sg
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www.med.nus.edu.sg/path/cytopath2014**

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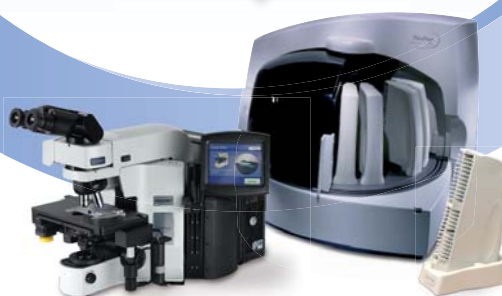
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