75TH ANNIVERSARY ARTICLE

Cytology practice at Wellington Hospital 1996-2020

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Time does not stand still. Technology and methodologies change, with a view to improving quality, adequacy, and diagnosis. In the last 25 years methods of cytology processing and reporting system have undergone major changes. In 1996, laboratory services at Wellington Hospital were operating under Capital and Coast District Health Board [CCDHB] management. At that time Dr Raj Gupta was charge cytopathologist until his retirement in 2003. During his service he had run a training programme for cytotechnologist, registrars, and students from Pacific Islands (PPTC). The laboratory was processing a variety of samples from various body sites such as cervical smears, sputum, urine, CSF, body cavity fluids, bronchoscopy samples, fine needle aspirations including ultrasound and CT guided fine needle aspirations (FNAs), and intra operative FNAs on rare occasions. These were a golden era for the practice of cytology as technology was advancing rapidly and scholars seemed to exude cytology knowledge and observation, and this was manifested in presentations at conferences and journal articles.

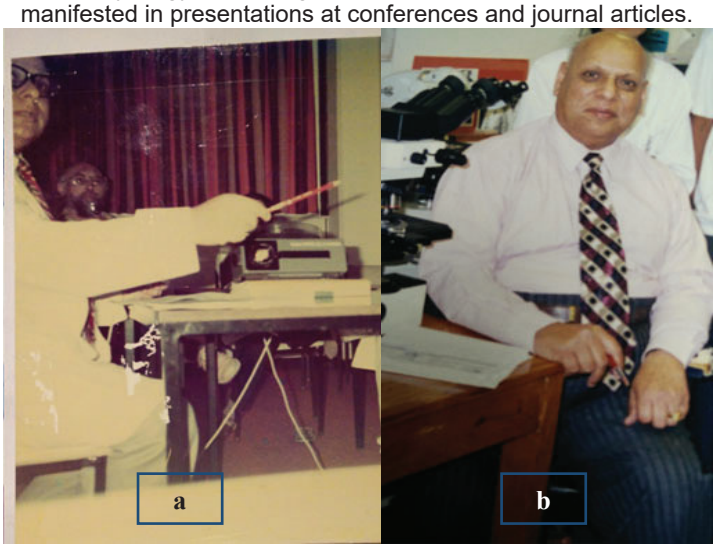

Figure 1 (a): Dr Raj K Gupta teaching gynae and non gynae cytology to the staff using codachrome teaching set on projector as a part of training in 1990's.
Figure 1 (b): Dr Raj K Gupta reviewing daily non gynae cases and abnormal gynae cases on double headed microscope with screener.

The Wellington Hospital cytology laboratory was processing gynaecologic cytology samples, largely from hospital outpatient and colposcopy clinics until 2002 when, as a result of recommendations stemming from the Gisborne Inquiry, the number of laboratories in the country processing gynaecological cytology samples (smears) was reduced from 26 to 8 as a result of the standard that then required laboratories to process a minimum of 15,000 gynaecologic cytology per annum. CCDHB's gynaecological cytology was then contracted to Medical Laboratory Wellington in Courtenay Place, which subsequently became Aotea Pathology and continued operating out of the Courtenay Place premises.

In 1996 gynaecologic [cervical] smears were predominantly "conventional smears" (introduced in 1946), obtained by Cervibroom or cytobrush, spread on glass slides, fixed in 95% ethanol in Coplin jars. In subsequent years, this advanced to fixation by commercially available spray fixative. Slides were stained manually with Papanicoloau [pap] stain, named for the Greek/American physician George Papanicoloau who was a pioneer of cytopathology and early cancer detection by cytologic methods. These glass slides were cover-slipped manually, screened by cytology scientists and rechecked by a second scientist for quality control. Abnormal smears were reviewed and reported by the cytopathologist, often at a double headed microscope with the cytology scientists in attendance for teaching purposes.

Cytology, like other laboratory disciplines, has been in a constant state of evolution over the past 100 years. There were rapid advances in microscopes, discoveries around dyes, staining, cell theories and publishing of colour atlases of cellular changes. An interesting story tells of the discovery of haematoxylin when two inebriated friends returning home from a public house stopped to urinate on a heartwood tree causing it to "bleed" and leading to the discovery of the natural plant pigment haematin used to manufacture haematoxylin, one of the most important dyes in tissue pathology.

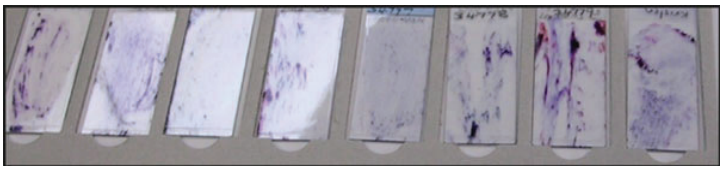

Figure 2: Conventional PAP stained cervical smears

From 1996 to 2012, the Wellington Hospital laboratory was processing non-gynaecological samples traditionally and manually using a very labour-intensive filter preparation technique. Sartorius filters were used through a filtration method to capture cells, using a cytosieve filter holder. The samples were either fresh or fixed with 30 % ethanol in saline. The prepared filters were stained using the pap stain and cover-slipped manually using Eukits and at a later date using EZ mount. The stained filters were screened by cytology scientists and preliminary handwritten reporting crafted on the request form, reviewed with the cytopathologist. The final reports were typed by secretarial staff, authorised by the cytopathologist on printed reports and then manual mailed. There was very little opportunity for database formation at the time and only crude manual card systems were maintained with very little opportunity for audit or complicated statistical analyses of trends. The samples were registered manually in a daybook which replaced by computer system in 2012.

RCPA QAP (Royal College of Pathologists of Australasia, Quality Assurance Programs were performed on provided glass slides until 2016, changed to USB stick and currently performed through virtual on-line images.

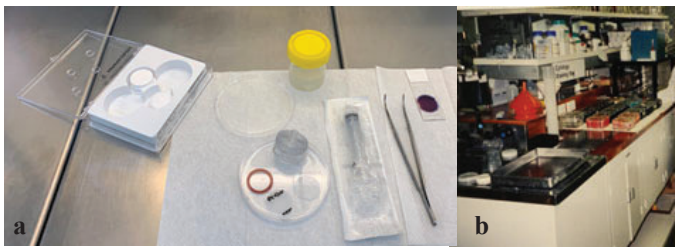

Figure 3 (a): Manual filter preparation of non gynae samples until 2012
Figure 3 (b): Manual PAP staining battery for gynae smears and filter preparations of non gynae samples

Cytospin preparations were done for low cellular samples, such as CSF specimens, using a Shandon cytocentrifuge (Shandon single disposable cyto funnel, sample chamber attached to metal chambers with white filter card).

The most profound development in cytology to occur at the end of the last century was the development of systems for liquid based cytology that allowed the mechanised preparation of evenly spread thin layered samples onto glass slides direct from sample vials. With advances in technology, FDA approved Liquid Based Cytology (LBC) techniques (ThinPrep and SurePath testing) were introduced to replace conventional cytology to improve adequacy, preservation, and to achieve a representative sample. ThinPrep (Hologic) was introduced in 1996 and SurePath (BD) in 1999.

These two LBC systems remain currently in use throughout the country for gynaecological and non-gynaecological sample processing. In 2012 Thinprep LBC was introduced at Wellington Hospital cytology lab. The samples were collected in cytolyt solution and slides were prepared using a TP 2000 machine. TP slides were stained on the automated Leica ST 5020 staining machine and covers lipped on the automated Leica CV 5030 cover slipper from 2013. This resulted in a significant reduction in specimen preparation time compared to the older manual filter methods previously employed.

Figure 4 (a): Hologic ThinPrep 2000 machine
Figure 4 (b): ThinPrep cytolyt vial for collection of non gynae samples, non gynae Transcyt blue filter and TP slide
Figure 4 (c): PAP stained ThinPrep slide

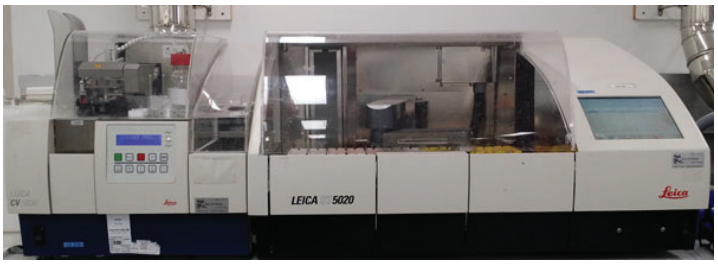

Figure 5: Automated stainer (Leica ST 5020) on right and automated coverslipper (Leica CV 5030) on left

During the first two decades of this century there has been enormous developments in laboratory information systems and CCDHB employed a number of these systems with increasing sophistication and functionality during this time. The database and search functions of these systems allowed for better analysis and quality control of results and greatly improved the scope and speed of histology-cytology correlation activities.

Following a DHB contractual tendering process for laboratory services, Wellington SCL was formed on the 1st of November 2015, which saw the provision of lab services across three DHB's (CCDHB. Hutt Valley DHB and Wairarapa DHB) and included processing of both community and hospital work. Aotea Pathology was incorporated into the new entity, based primarily on-site at Wellington Hospital. A decision was made that all gynaecological cytology would then be processed and reported at SCL Dunedin. Wellington SCL now operating across two floors of new purpose-build laboratory space in the CSB Building at Wellington Hospital. This new laboratory then processes and reports on all the regions non-gynaecological samples that are processed and stained using the BD PrepStain processor. The samples are collected in BD SurePath preservative fluid.

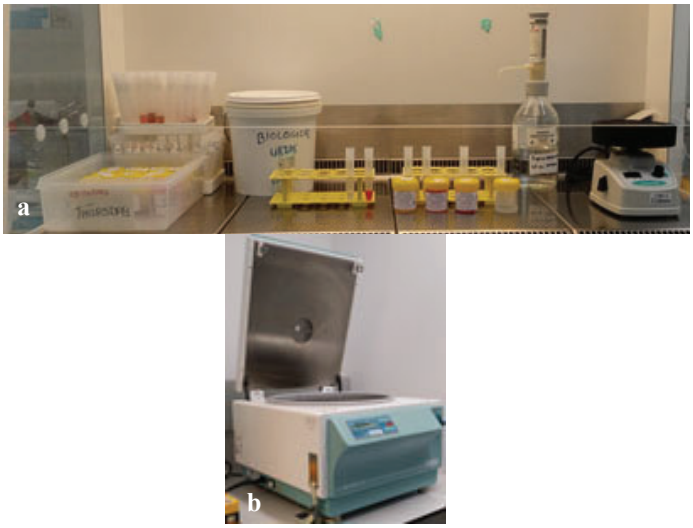

Figure 6 (a): SurePath preparation in Biosafety cabinet
Figure 6 (b): **C**entrifuge (Hettich)

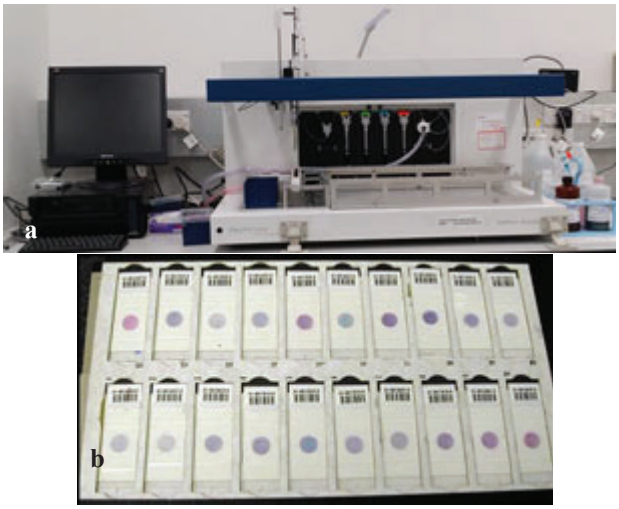

Figure 7 (a): BD SurePath processor
Figure 7 (b): PAP stained SurePath slides

In addition to the process and structural changes outlined above, the past 25 years have witnessed major changes in the demand, role, and scope of non-gynaecological cytology in this region. In 1996 one of the laboratories most common specimens were from fine needle aspiration of breast masses. However, the development of excellent thin core needle biopsy instruments combined with the development of a national breast screening programme have seen an 80% decline in the number of breast FNA samples submitted. Despite these changes in diagnostic fashion, demands for cytology services continue to grow annually. FNA has now become the triage tool of choice for assessment of thyroid nodules, and combined with the easy availability of portable ultrasound equipment, thyroid FNA's have numerically replaced breast FNA's over the past 25 years. In addition, increasing radiologist skills combined with better cross-sectional imaging and the development of endoscopic ultrasound have greatly increased the range and number of deep sites that are now routinely sampled by FNA.

At the end of the last century, the role of cytology was essential that of a screening or triage tool that would usually be followed up by tissue biopsy for histological confirmation in many instances. However, changes in medical practice over the past 25 years, combined with more sophisticated sampling techniques, and the exponential expansion of immunohistochemical antibodies have moved cytology to being more commonly the primary and only tissue diagnostic procedure performed, with treatment decisions made directly from cytology findings. These changes, in combination with the increasing use of molecular studies and flow cytometry, have meant that there are increasing testing demands being made on often limited cytology samples. Nowadays the cytology scientist and cytopathologist must not only provide diagnostic services but have become the curators of these limited samples and are involved in triage decisions about how these samples are best used.

The past 25 years have been exciting and challenging for the cytology service at Wellington Hospital. It has been sad to see the gynaecological cytology specimens be transferred out of region, but at the same time the scope and importance of the non-gynaecological cytology service has grown considerable and cytology is now longer the diagnostic Cinderella service of the past. It is our hope that the late Dr Gupta would be both surprised and pleased by the developments that have occurred in the nascent cytology department he helped to establish.

What of the next quarter-century? The future is much harder to predict than the past is to describe. We can make some reasoned guesses; there will be enormous changes in cervical cytology with the move to primary HPV testing that will likely see significant reductions in the laboratory screener workforce. Artificial intelligence technologies, which has been heavily applied to screening of cervical cytology specimens, will become more mainstream for all cytology specimens. We can be sure that the future will see much more molecular testing; the dire predictions from the early 2000's that molecular pathology would replace the need for routine morphologic assessment have not come to pass. However, the near future will see next generation sequencing on cytology samples rapidly translate from the research laboratory into routine diagnostic practice.

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