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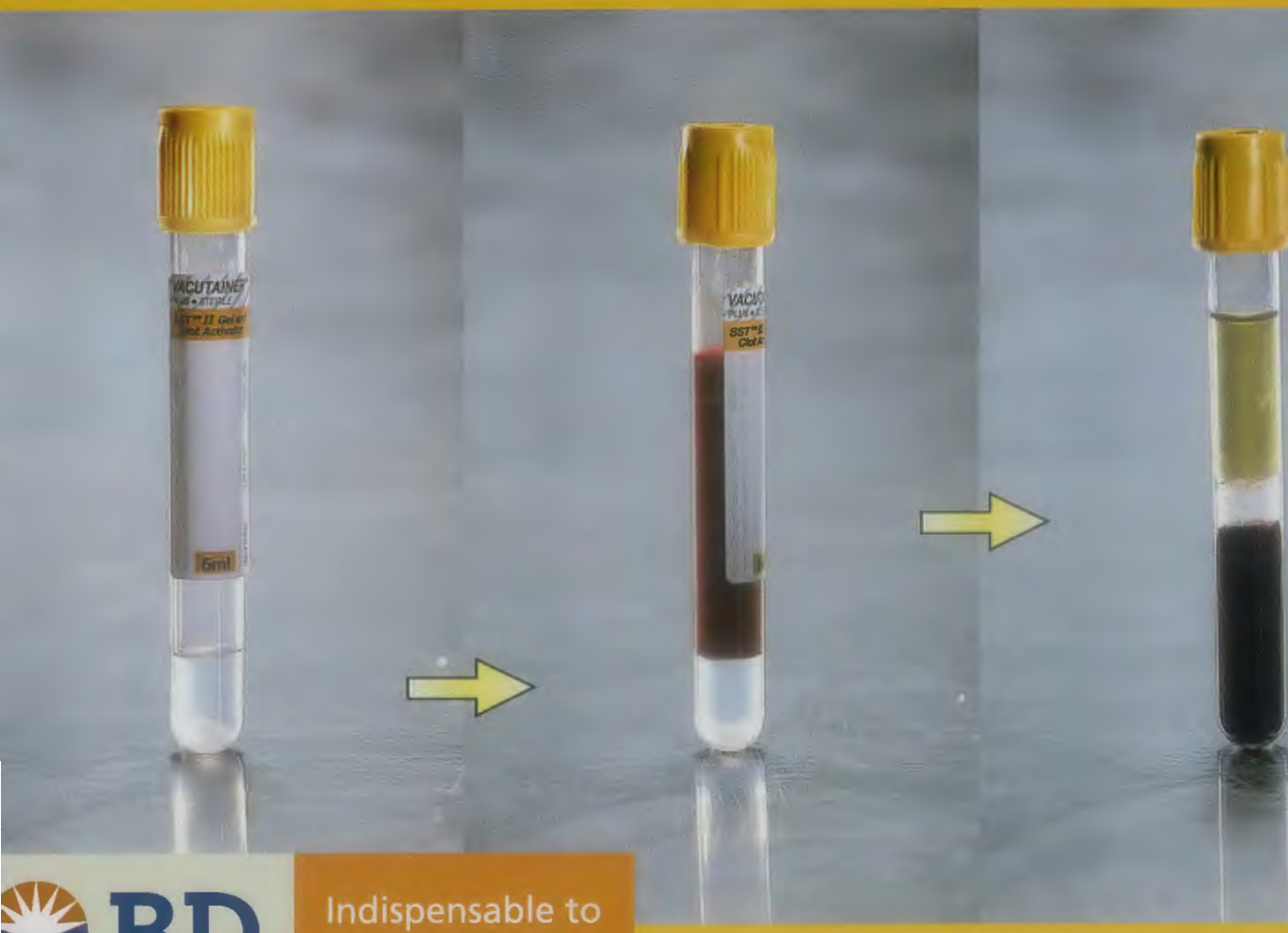

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Are we going cyclic?

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The many changing faces of the laboratory have shown that a cycle does take place. Let me explain as to how. When I first started in the laboratory as a young trainee approximately 30 years ago there were four main disciplines, biochemistry, haematology/blood banking, microbiology, and histology/cytology. With the advent of the New Zealand Certificate of Science (NZCS) as part of the training, along with day release or block courses, trainees simultaneously worked in the laboratory and trained through the educational institutes. When they successfully passed NZCS, they undertook a further two years of study, in one or two disciplines, to gain registration with the Medical Laboratory Technologists Board. This then allowed the newly registered person to undertake greater responsibility within the laboratory and even start thinking of a career path.

At the same time laboratory assistants were employed and through the NZIMLT, as our Institute was known then, were able to sit the Institute run exams after two years in a discipline to become a qualified technical assistant (QTA). They had the choice to sit exams in all the major and minor disciplines, and also a general exam. This general exam was usually for laboratory assistants from smaller public hospital laboratories as there was a requirement for general knowledge of at least three of the major disciplines, n.m. biochemistry, haematology, and microbiology. The smaller hospital laboratory, and also private laboratories, required staff to cover these areas because of the "stat" capabilities.

Major changes took place over a few years, laboratory staff became more specialised, and smaller laboratories started to disappear at an alarming rate. The need to produce general QTA's now became defunct. New thinking started to be born out of "how do we do this now?" approach. Larger laboratories were looking to create core laboratories, because of automation and ease of operation, to produce results in a timely matter to the referrer. Over the last ten years these core laboratories have come into existence and staffing has changed once again in that core laboratories are nothing more than a small laboratory within a larger laboratory, with a change only in geography of location.

The question now is, how do we educate laboratory assistants to accomplish the tasks at hand with a general knowledge required of at least two of the major disciplines, n.m. biochemistry and haematology? Does the NZIMLS now go back to resurrecting the general QTA exam, or expect laboratory assistants to sit two separate exams? Have we gone full circle to as it was in the beginning with a general QTA exam being required, or are we being stupid and reinventing the wheel? People's skill level has, I would summize, increased, but the act of giving and taking has not, only location. We need to be a bit more innovative if we think we can create something from nothing, or do something new that has not already been done. The challenge is now to the profession to provide an appropriate qualification to those laboratory assistants working in core laboratories.

The applications of microwave heating in histological techniques and its potential in pathology service

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Abstract

In the last few decades the use of microwave heating in histotechnology has gained some popularity in the histopathology laboratory. The heat energy generated from microwave irradiation is almost instantaneous and homogenous compared with conventional heating. Investigators have found that the application of microwave heating in processing tissue at higher temperature reduced the amount of time needed in histological processes such as in fixation and staining without compromising quality. The most rapid development occurred in immunohistochemical staining, where microwave heating is primarily used for the process of antigen retrieval technique.

The modern domestic microwave oven is user-friendly and safe to use. It is affordable and most medical laboratory owns a microwave oven or has ready access to it. However, microwave heating in histological techniques is still not widely accepted for routine use.

This review explores the past and current literature for the applications of microwave heating in histological techniques that can decrease turnaround time for specimen processing when compared with conventional techniques. In addition, to highlight the evidence of the potential uses of microwave heating in histopathology laboratory for a faster and cost-effective service than its present use. Suggestions for the implementation of microwave heating for routine use in the medical laboratory are discussed and included are simple quality control procedures for calibration and standardisation for the use of the microwave oven in histological techniques.

Keywords: heating, histological techniques, immunohistochemistry, microwaves, staining

Introduction

Medical laboratory technologists and scientists in healthcare organisations are working in a climate of continued change. They are required to improve techniques, to reduce the costs and to improve turnaround time without compromising quality and standard of service. In histopathology this has led to the development of techniques aimed at reducing the amount of time needed for the processing of specimens.

The histological processing of a routine specimen consists of fixation, macroscopic examination, tissue sampling, tissue processing, embedding, sectioning and staining, all of which are performed in a sequential order. This is a long process that may take approximately 6-24hr or longer to complete and produce microscopic slides for microscopy. The literature search has revealed that the duration for the processing of specimen is reduced if controlled heating is utilised at certain stages of processing with no or minimal detrimental changes to the quality of processing.

Historically, in 1898 Ehrlich and Lazarus first proposed using heat as an accelerator in fixation of blood smears (1). In the last three decades there has been a rapid increase in interest in the use of microwave treatment in fixation, tissue processing, drying sections, staining and antigen retrieval techniques. Mayers in 1970 described and used microwaves generated from a physiotherapy-heating device as a means of tissue fixation to obtain satisfactory fixation of 1 cm cubes of human, rabbit and mouse tissues (2). With the advent of the microwave oven the use of microwave heating as a method of tissue fixation was further investigated. Some investigators used 'dry' microwave fixation (3,4) while others indicated that microwave heating must take place in a fluid medium in order to obtain artefact-free tissues (5-7).

Bernard in 1974 investigated 'dry' microwave fixation on whole carcasses of hairless mice and concluded that the quality of staining depended on the type of tissue and the temperature generated within the tissue (3). His investigations also revealed that the central area within the microwave oven chamber had the most uniform power fluxes. The work of Login in 1978 discussed that saline microwave fixation was superior to formalin microwave fixation of surgical and autopsy specimens (7). He constructed microwave-heating curves for various volumes of fluid to control the temperature achieved to optimise microwave fixation. Other investigators who applied microwave fixation for light and electron microscopy (8-10) confirmed his findings. Brinn in 1983 reported the first success of metallic staining of tissue section using microwaves (11). Subsequently, encouraging reports of time saving techniques using microwave heating for rapid staining in histochemistry (12,13), immunohistochemistry (14) and immunofluorescence (15) were published.

In 1991 Shi and colleagues pioneered a novel microwave technique for antigen retrieval in immunohistochemical staining (16). They showed that it was possible to retrieve antigens in formalin-fixed, paraffin-embedded tissues by boiling the sections in heavy metal solutions in a microwave oven. Recently, microwave heating has been successfully applied in molecular histological studies e.g. DNA in situ hybridisation (DISH) (17,18) to improve the turnaround time and quality of DNA staining. In a short time the use of the microwave oven for heating has had a profound impact in histotechnology.

This is attributed to the modern domestic microwave oven, which is highly sophisticated in terms of design, and has software features that enhance the efficiency and quality of the instrument. It is user friendly, programmable and affordable. The microwave oven provides an alternative source of instantaneous heating, compared to conventional heating such as water-bath and oven, to hasten chemical reactions occurring in specimen processing. There are now many documented histological techniques that utilise microwave heating, which also demonstrates the benefits of using the microwave oven as a tool in nearly all stages from fixation of tissue, tissue processing and staining of frozen, paraffin, and resin sections and smears for cytopathology.

Aims and objectives of the review

As described earlier, the use of the microwave oven for microwave heating to speed up specimen processing is not a new concept. A compilation of research and clinical applications of microwave technology 1970-1992 was reviewed by Login and Dvorak (19). A detail account of the physics and the applications of microwave technology and methods of histological techniques using microwave heating can be found in textbooks (20,21). However, the use of microwave heating in histological techniques is still not widely applied for routine processing of specimens.

The intent of this review is to explore the literature and experimental evidence for the applications of microwave heating in histopathology. The review focuses particularly on applications in specimen processing that can decrease turnaround time. In addition, the review includes simple procedures of calibration and standardisation for the use of microwave oven in histological techniques.

Microwaves and the microwave oven

What are microwaves?

Microwaves are short waves of the electromagnetic spectrum that occur between the frequencies of 300MHz and 300GHz (22). Similar to light waves, microwaves exhibit diffraction and interference. The frequency of a microwave remains the same through different medium such as air and water, but the wavelength of that microwave change when propagated through these media (23). Natural environmental sources of microwaves are from the sun and cosmic rays, while man-made sources are from telecommunications and radar antenna. In therapeutic applications microwaves are used in physiotherapy and for treatment of hypothermia, and some times in conjunction with chemotherapy and radiotherapy for the treatment of tumours.

The effect of microwave irradiation at molecular level

During microwave irradiation the microwave energy penetrates into a substance to induce dipolar molecules within the substance, such as water molecules and polar side chains of proteins, to continuously oscillate through 180° (dipolar moment), thus causing random collisions with the adjacent molecules and producing heat. The larger the dipolar moment of a molecule the faster the heating process occurs in the substance.

The origins of the microwave oven

In 1945, Percy Spencer conceptualised the use of microwaves for the purpose of cooking (24). He adapted the mass production of microwave generators called magnetrons during World War II as cooking devices. The early magnetrons consisted of oscillator tubes to generate microwaves. In 1951 the first microwave oven was patented in America (22) and by 1954 the precursor of today's domestic microwave oven had established itself as essential commercial equipment in the kitchens of hospitals, hotels, and restaurants for thawing and re-heating prepared meals.

The modern domestic microwave oven is compact, with programmable features designed for the purpose of heating, thawing and electronic cooking. All domestic microwave ovens are designed to produce microwaves with a frequency of 2.45GHz, which corresponds to a wavelength of 12.2cm in vacuum (23,25). A device known as the magnetron that consists of a tungsten cathode element emits the microwaves in the cavity of the microwave oven. A step-up transformer converts the 240V AC to a high voltage of 3-4kV DC to power the magnetron. Located within the cavity of the oven are field stirrers that function as radiation reflectors to create a uniform microwave intensity and there are metal fans for ventilation.

Microwave heating versus conventional heating

In conventional heating, that is 'external heating' with an oven or a water-bath, heat energy is transferred by conduction from heating surface to specimen surface, which eventually heats the interior of the specimen. This form of 'external heating' is not ideally suitable in histological techniques of specimen processing because heat conduction through biological material is poor. In microwave oven heating, the microwave energy is delivered directly into the specimen being heated. The same reasoning applies to heating solutions in which the heating takes place within the solution without conduction from the heating source. The heating process is more rapid, and has fewer problems due to erratic heating from direct flaming and element heating. The major advantage of using a microwave oven for heating is that there is no exposure to naked flames and heating elements, these being potential fire hazards in the laboratory. Besides, it is relatively easier to control the amount of heat delivered and the length of time over which it is delivered merely by the push of a button. The heating process is almost instantaneous and the increase in temperature is homogenous. Microwave ovens designed for laboratory use have features for precise timing, temperature, and power cycle controls, and some microwave ovens have a pumping mechanism for fluid agitation, but these are more expensive than domestic kitchen microwave ovens. A comparative study between a domestic microwave oven and a commercial laboratory microwave oven revealed no significant difference of morphological details in microwave fixed frozen sections (26). Some of the top range of domestic microwave ovens have similar features to the laboratory microwave oven and are equally suitable for microwave heating in the laboratory. The use of temperature probes and rotary trays during microwave heating permits greater control of the heating process through a load.

Health and safety for microwave heating

The effect of exposure to microwave irradiation on the biological systems of the body is uncertain. Microwaves operated at 2-3 kHz can penetrate about 2cm into human tissue (25). Studies on microwave interactions and biological effects of microwave irradiation on animal models and some human models (reported cases of military personnel exposed to microwave irradiation from radar communication sets and screens) have been documented. But none are conclusive to indicate whether microwave irradiation has ionising or non-ionising effect on the body. It is speculated that microwaves, unlike X-rays, (and (rays, are not sufficiently energetic and too small an energy level to effect ionisation in matter (24). Likewise, it cannot be said whether microwave irradiation has an effect on biological molecules of proteins with dielectric properties.

The conclusions from animal model studies cannot be extrapolated to humans due to differences in weight, size, surface-area to volume ratio, density of tissue, and the capacity to absorb radiation (22). The effects of microwave irradiation on the unborn human foetus are unknown. Microwave ovens are designed to specifications strictly regulated by government agencies, such as the Ministry of Health and Occupational Safety and Health, to control emission of microwaves. This is to protect the general public and professionals routinely exposed to electromagnetic radiation, which may be harmful. The exposure to microwave irradiation decreases rapidly with increasing distance from the oven, the operating manual recommends a location or standing distance of 1m from the oven, when in use. The microwave oven should be checked periodically for radiation leakage around the door seal.

The major health hazard is injury by scalding from hot fluid, steam, or the container. It should borne in mind that the container feels less hot on removal from the oven, but becomes very hot on standing on the bench. When microwave heating aqueous solutions of chemicals, there may be release of flammable and noxious fumes from evaporation, which can be harmful to the user through inhalation. This can be avoided by locating and operating the oven in a fume hood, or connecting to a fume extraction system. Microwave-safe containers should be used to prevent cracking and meltdown of material. The build-up of vapour pressure during heating in a tightly sealed screw-capped container can cause an explosion. Materials like metal and graphite from pencil lead can cause sparking, and paper can catch fire. The

most common incident or injury with the use of microwave oven in the laboratory is caused by using non-microwave-safe materials, incorrect selection or setting of temperature and duration of heating, that results in over heating to excessive boiling, causing spillage, and even explosion.

Implementations of microwave heating in a routine histopathology laboratory

Microwave heating accelerates diffusion, penetration, and exchange of reagents. The distinct advantage gained in utilising microwave heating in histological techniques is a considerable reduction in the time necessary for completion of specimen processing. Many investigators have documented that microwave fixation and microwave histological staining techniques are faster, reproducible, and the results obtained are comparable, or even better than conventional specimen processing procedures (8-15). Morphometric analysis of nuclear size in fixed tissue has revealed excellent preservation of morphology, with no significant changes in nuclear size between microwave fixed and conventional formaldehyde-fixed specimens (27,28). Microwave fixed liver tissue in phosphate buffered saline (PBS) showed absences of liver glycogen streaming artefact, which is usually seen with conventional formaldehyde fixation (8).

Immunofluorescence studies of microwave-fixed tissue culture cells of human lung revealed no associated loss of protein (antigens) in the cells (29). Immunohistochemical studies have demonstrated good preservation for a wide range of tissue antigens in microwave fixed tissues (8,30,31,32). The immunohistochemical staining results of microwave fixed tissue are superior to formaldehyde fixed tissue, and in the latter the prolonged exposure to formaldehyde fixative has an adverse effect on many antigens, resulting in loss of antigens in tissue sections (33).

In the author's laboratory microwave heating is used to accelerate fixation of small biopsy tissues from the gastrointestinal track, liver and kidneys, to enable rapid processing of specimen for urgent reporting within the same day. This is accomplished by allowing the fresh specimen to fix for a minimum of 30-60min in 10% Neutral Buffered Formaldehyde (10% NBF), followed by microwave heating the tissue in 20ml of 10% NBF to 60(C for a duration of 90sec in a laboratory microwave oven (Energy Beam Sciences) at maximum power level. The specimen is subsequently auto-processed on a shorter tissue processing cycle of 180min from 70% alcohol omitting formaldehyde fixation step on the tissue processor. The processed tissue blocks are sectioned and stained, all within the same day. Therefore, the combination of microwave fixation technique and rapid tissue processing enables the provision of a diagnosis the same day the specimen is surgically removed. For larger blocks of tissue less than 3mm thickness, they are allowed to fix for 2hr before microwave fixation at 60(C for 180sec, and then subsequently tissue processed on an overnight cycle. Microwave fixation did not show any detrimental effects to nuclear detail and morphology of tissue and the quality of Haematoxylin and Eosin stain (Figure 1).

The use of microwave heating for drying paraffin sections and in both metallic silver and enzyme-histochemical staining, helps speed up the staining process thus reducing turnaround time by 60-90mins without compromising the quality of staining, e.g. Jones methenamine silver for basement membranes, Grocott-Gomori methenamine silver for fungi micro-organisms (Figures 2&3), or enzyme histochemical stains such as Chloroacetate Esterase (Figure 4).

Microwave heating is also used for heat-induced antigen retrieval in immunohistochemistry. It has been found that it is best to heat a solution of 10mM sodium citrate buffer pH 6.0 to boiling point first. Then to simmer the sections in the preheated buffer at above 95(C for 20min, instead of continuous vigorous boiling of the citrate buffer. The heating temperature of above 95(C is one of the critical factors for consistent reproducible staining in immunohistochemistry.

The procedure is as follows: 2.3L citrate buffer pH 6.0 in a 3L microwave cook pot is microwave heated to boiling point using a domestic 650W Philips microwave oven set at high (100%) power setting. Without delay, the hydrated sections are transferred into the pre-heated citrate buffer and re-heated at a lower (80%) setting for 20min. The sections are then allowed to remain in the heated buffer to cool down for 20min at room temperature before incubation in reagents.

This protocol ensures that the microwave oven magnetron is pre-warmed before use, enabling 60 slides to be treated for antigen retrieval at any one time, and that there is reduced detachment of tissue sections from the slides due to the continuous vigorous boiling action. The staining procedures are automated using DAKO immunostainer (DAKO Corporation, Carpinteria, USA), and with immunohistochemical staining using the labelled streptavidin-biotin and horseradish peroxidase method (DAKO LSAB2/HRP kit K0675), according to the manufacturers instructions, and visualised with development in DAB chromogen (DAKO Liquid DAB kit K0365).

Good consistent reproducible results (Figures 5-8) were achieved for automated immunohistochemical stains with the DAKO LSAB2/HRP-DAB system. The use of an autostainer apparatus has made it feasible to carry out two 'immuno runs' (maximum 48 slides per run, each run taking less than 3hr to complete) during the day, and it is very convenient to include a third run overnight to meet the demands for immunohistochemical stains.

Here are some suggestions on how the implementation of microwave heating in a routine histopathology laboratory can be beneficial, and improve the speed of pathology service.

Microwave fixation

Fixation is a process to stop autolysis and decomposition, to preserve the cellular details and morphological structure of a tissue to as near life-like as possible. It is a chemical process that stabilises the tissue protein by converting the proteins in gel-state into a coagulated or cross-linked state to facilitate diffusion of chemicals into tissue (34), and to enable subsequent tissue processing, embedding, sectioning, staining, and storage. Therefore, fixation is an important process in combination with tissue processing that determines the end result of a well-cut and well-stained section.

The usual method to fix small biopsy specimens is to fix in a formaldehyde fixative, such as 10% NBF for a minimum of 4hr, larger specimens may take longer to fix. To achieve adequate fixation, larger specimens, such as bowel, are required to be cut open and pinned out, while whole organs, such as breast, kidney, liver, and spleen are thin sliced, stuffed with paper towel in between slices, and allowed to fix in fixative for at least 12-24hr. Therefore, specimen fixation is a lengthy time-consuming process that affects the turnaround times for specimen processing.

The application of microwave heating in the fixation process will significantly reduce the turnaround times for specimen processing. Small specimens fixed with microwave fixation in normal saline (7,9,32) or in Tris buffer (28) yield adequate fixation, with minimal shrinkage artefact and preservation of antigenic sites in tissue, compared to conventional formaldehyde fixation for light microscopy studies. The use of saline with microwave heating eliminates noxious fumes, such as from formaldehyde fixatives. To be safe, it is recommended that fresh specimens be collected in a formaldehyde fixative, sampled and fixed for at least 1hr before microwave fixation (9), while others consider a 4hr fixation in formaldehyde fixative necessary for consistent results

(34). Reports differ as to the optimal temperature for microwave fixation, varying between 50-74°C. However, as a guideline, tissue blocks measuring about 2cm x 2cm of 2mm thickness in plastic cassettes with metal lids (it is safe to use metal lids provided they are fully submerged in fluid) in normal saline microwave heated to a temperature of 58°C are adequate for fixation and preservation of tissue antigens (9,35). The entire process of set-up and microwave fixation takes no more than 10min. The tissue blocks are then auto-processed, starting from 70% alcohol, omitting the additional fixation step on the tissue processor, thus reducing the tissue processing time, and there is less exposure to noxious formaldehyde fumes from the tissue processor.

However, with larger biopsy specimens, e.g. bowel, an initial microwave fixation of the entire specimen followed by a second microwave fixation of the sampled tissue blocks completes the fixation process in about 20min. The initial microwave-heating step hardens the tissue, allowing dissection and sampling of the specimen, and in addition, lymph nodes become opaque and firm pink-tan coloured for easier identification and sampling (36). This is accomplished by submerging the entire specimen in a volume of normal saline and microwave heating to 67-74°C. The tissue blocks are subsequently microwave fixed in normal saline and tissue processed, or stored in fixative until processing. This protocol is applicable to all specimens, with the exception of tissue blocks from large pieces of skin and dense tissues like uterus, which require a 30min formaldehyde fixation before microwave fixation (37). A variation of the above double microwave fixation technique is to retain the specimen in the heated solution for 10min to continue fixation. This protocol has been successfully applied to fix specimens of human whole eyes in 10% NBF fixative at 51-54°C (38). Likewise, the injections of 100ml of 10% NBF fixative into a fresh whole prostate specimen at multiple sites, followed by double microwave fixation, ensured complete fixation of the specimen (39).

Hence, microwave fixation is a useful alternative to current conventional procedures of specimen fixation. The procedure is simple and helps to reduce the exposure to noxious formaldehyde fumes in the 'cut-up' room. It is a rapid and time saving procedure for handling gross specimens that omits the need for pinning-out and slicing of specimen and enables processing of such specimen on the same day as receipt. In addition, prolonged fixation of specimen in formaldehyde fixative is avoided; thus antigen preservation in tissue sections is improved.

Rapid automated tissue processing

Tissue processing involves the procedures of dehydration with alcohol (or substitute), clearing with xylene (or substitute), and impregnation with paraffin wax (or its substitute, such as resin). It is another time consuming process, where the average duration of an overnight automated tissue-processing cycle is approximately 11-12hr, and the rapid cycle is about 3-5hr during the day. This limits the number of tissue processing runs within a working day.

The basic principle of each of these steps is molecular diffusion. It has been shown that rapid paraffin tissue processing is possible with microwave heated reagents at all stages, enabling paraffin sections to be produced within 1hr of receipt of surgical specimens (40), and less than 30min required for tissue processing in a microwave oven (41). Microwave heating accelerates diffusion, penetration and exchange of reagents, thus reducing the dehydrating, clearing and impregnating times. But the use of microwave heating methods for tissue processing is not practical for processing large numbers of specimens in a microwave oven because microwave heating is less effective in large baths needed to process a large volume of tissue (42).

By integrating microwave fixation with rapid automated tissue processing, it is possible to carry out several 'short' cycles of about 3 hr duration each during the working day, enabling rapid preparation of good quality diagnostic sections with superior antigen preservation, compared to conventional formaldehyde fixation (37). The step of post formaldehyde fixation on the automated tissue processor is eliminated, and the total time of the rapid processing cycle is 175min, compared to 12hr for the conventional method of tissue processing. For endoscopic biopsies, an even shorter processing cycle of 80min has been described (37).

Thus, microwave fixation with rapid tissue processing is a useful alternative to conventional methods of specimen processing, which are time consuming. Furthermore, the omission of formaldehyde step on the tissue processor eliminates the exposure to noxious formaldehyde fumes. It is now possible to issue a histopathology report the same day, since sections are available on the same day as receipt of specimens.

Microwave drying of paraffin sections

In most laboratories, the common practise is to dry paraffin sections in a 60°C oven for 30min to 1hr or more, to ensure section adhesion to the slide. Other laboratories dry paraffin sections of bone and brain tissue overnight at 60°C, or keep sections over the weekend at 60°C. Some laboratories use unconventional heating sources, such as a hot paint-stripper or a hair-dryer to dry sections. It has been known and speculated that prolonged drying at 60°C may be deleterious to some antigenic sites, such as for oestrogen and progesterone receptors (unpublished observations). It is also known that prolonged drying at higher temperatures may contribute to excessive blue staining of nuclei and other parts of tissue.

Alternatively, microwave heating can be used to rapidly dry paraffin sections before deparaffinisation and hydration (43,44). Paraffin sections of 4μm thickness were dried for 3 minutes at high setting in a 650W microwave oven, or for 4min at power level 6 in a 600W microwave oven. The slide racks used are plastic or 'microwave save' slide racks, and section adhesives are not required for routine staining. Adhesive coated slides, such as poly-L-lysine and silanized (3-aminopropyltri-ethoxysilane) are highly recommended for microwave staining and heat-induced antigen retrieval procedure respectively. Microwave oven drying of paraffin sections did not affect the quality of histochemical stains (Figures 1-4) or antigenic sites for immunohistochemical staining (Figures 5-8). Therefore, the use of microwave heating will reduce the drying time of paraffin sections from 30min to a mere 3-4min, another time saving process in specimen processing, decreasing results turnaround time.

Microwave staining

The quality and result of histochemical staining is influenced by many variables, such as dye-substrate affinities, diffusion and penetration of dyes, and technical variables, such as pH, temperature of staining solution, staining time, concentration of dyes, and others, such as fixation and nature of the solvent used (45). In immunohistochemical staining, other factors, which also influence the quality of staining and result, are specificity and sensitivity of antibodies to antigens, the preservation and availability of antigenic sites in tissue, and the variability in antigen retrieval procedures.

In smaller laboratories, manual staining is still the preferred option, due to cost of instrumentation. Automated staining will reduce the cost of manpower, usage of reagents, and maintain a consistent quality of staining between batches for high output. But, automated staining does not necessarily decrease the turnaround time for staining, and in some instances it may even increase the turnaround time to achieve the desired quality of staining, thus becoming a disadvantage.

Since the pioneering work of Brinn in 1983 for rapid metallic silver staining (11), many histochemical staining techniques have been modified with use of microwave heating to accelerate staining in frozen sections

(46), paraffin and plastic sections (13,47,43,48,49), as well as smears (12). Conventional histochemical staining techniques that require many hours to complete can be completed within a few minutes. For example, Grocott-Gomori methenamine silver nitrate for fungi and Fontana-Masson ammonical silver nitrate for melanin stains is completed in less than 30min with microwave heating steps. A literature search revealed that all microwave staining techniques documented, not only had a significant reduction in staining times, but also produced bright preferential staining with decreased background precipitation, compared to conventional staining (12,25,47).

However, there is a trade off between speed and temperature. Too high a temperature may cause precipitation and decomposition of dyes and chemicals, thus affecting dye-substrate interactions, and result in poor staining. It has been reported that the optimal temperature for most non-metallic stains is 55-60°C, whereas the optimal temperature for metallic stains is 75-80°C (25).

The use of microwave heating in immunohistochemistry has two different applications. First, it is used to enhance immunohistochemical staining, by exposing antigenic sites that have been masked or rendered inaccessible by formaldehyde fixation. The microwave heat-induced antigen retrieval technique (MHIAR) was first described by Shi and colleagues (16), and subsequently improved with the use of 10mM citrate buffer pH 6.0 for retrieval of the Ki-67 antigen in formalin fixed paraffin sections (50), and later successfully applied to demonstrate a wide range of tissue antigens (51,52). This technique, when applied on paraffin sections pre-digested with proteinase-K enzyme, revealed improved DNA in situ detection of apoptotic cells (53). In contrast to enzyme induced antigen retrieval techniques (e.g. trypsin and pepsin enzyme-digestion methods), the MHIAR technique is a simple technique, easy to perform, inexpensive, and permits higher working dilutions of primary antibodies.

The MHIAR technique has less background staining of endogenous avidin-biotin in tissue compared to pressure cooker technique for antigen retrieval in paraffin sections. There is evidence of heat damage to cellular/nuclear detail in paraffin sections (very pale or no nuclei counter-stain) treated with the pressure cooker antigen retrieval technique, compared with sections treated with the MHIAR technique. However, the pressure cooker technique offers even greater working dilutions for some antibodies, and does not require a 'cool down' step after antigen retrieval.

Secondly, it has been demonstrated that the use of microwave heating during the immunohistochemical staining process can reduce the duration of incubation in reagents, thus speeding up the staining process, and produces results comparable to conventional procedures within 10-20min (14,54,55). These methods may be useful when an urgent immuno-diagnosis is warranted. However, the implementation of microwave immunohistochemical staining methods on a larger scale may be unsuitable for routine use. Some of the disadvantages associated with this technique are that higher concentration of antibodies are required (54), over heating can destroy the antibodies, the number of slides placed in an oven is limited, and the procedure may take a longer technical time for preparation and staining. Thus it may affect the cost, turnaround time and reproducibility of the immunohistochemical stains.

Other reports have shown that microwave incubation steps shorten and improve stains for immunogold silver staining (56), for DISH (DNA *in situ* hybridisation) technique using alkaline phosphatase and immunogold silver (17), and for indirect immunofluorescence staining for immunoglobulins and compliments in renal biopsies (15).

Therefore, microwave histological staining techniques could be a useful option, where necessary, to reduce turnaround times for special stains in the laboratory. It is highly recommended for metallic silver staining techniques that normally take longer to complete. In immunohistochemical staining, the use of an autostainer is the preferred option on a larger scale, compared to the speed of microwave immunohistochemical staining. However, the implementation of these techniques depends on the workload, the demand for special stains, the degree of urgency for each test, and the effective use of technical time and resources.

Other uses of microwave heating

Frozen sections are required for rapid diagnosis and for enzyme histochemical stains. These sections are usually of lesser quality than their paraffin section equivalents. It has been demonstrated that microwave fixed frozen sections had superior quality of morphology compared to conventional procedures (57,58). The adhesion of sections to the slide is enhanced with microwave heating. Another advantage would be for rapid immunohistochemical staining of frozen sections.

Microwave heating has applications for electron microscopy studies. Good preservation of ultra-structural details of membranes and sub-cellular structures has been obtained with microwave fixation with glutaraldehyde fixative (9), or a mixture of glutaraldehyde-formaldehyde fixative (10). Tissue processing steps of washing, post-staining, and partial dehydration performed on the microwave oven had significantly shortened the specimen processing times in electron microscopy (59).

Microwave irradiation effectively destroys microorganisms like *Staphyloccus aureus* and *Salmonella typhi*, compared to conventional cooking (22). In addition, microwave irradiation can be a useful means of decontaminating some infectious bacteria (60) and parasites (61) in specimens and glassware in the clinical laboratory.

Hence, the microwave oven is a useful tool in the laboratory that has a wide range of potential applications and benefits to offer in histological techniques.

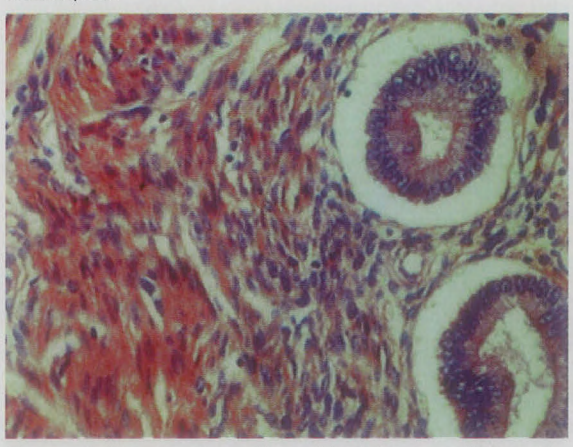

Figure 1. Human uterus tissue block from partially unfixed specimen was fixed for 2hr in 10% NBF, followed by microwave fixation in 10% NBF, and tissue processed on a 13hr overnight cycle. Microwave dried paraffin sections stained with Harris's Haematoxylin and Eosin. Note typical swollen looking nuclei associated with formaldehyde fixation, and distinct nuclear membrane and nucleolus staining with almost unstained nucleoplasm (X125)

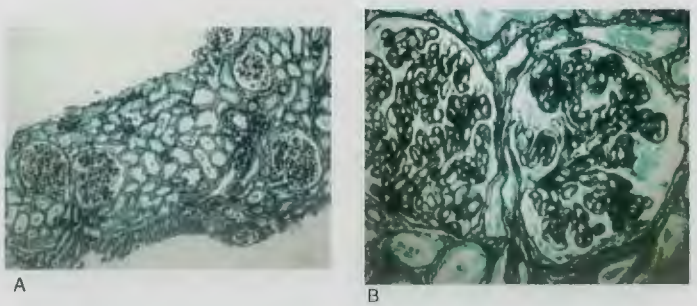

Figure 2. Microwave dried paraffin section stained with microwave-heating Jones methanamine silver staining of renal glomerular basement membranes. The staining process was completed within 30min. (**A**) the quality of silver staining is bright with clean background (X50) (**B**) basement membrane and mesangial matrix structures are stained black. (X125)

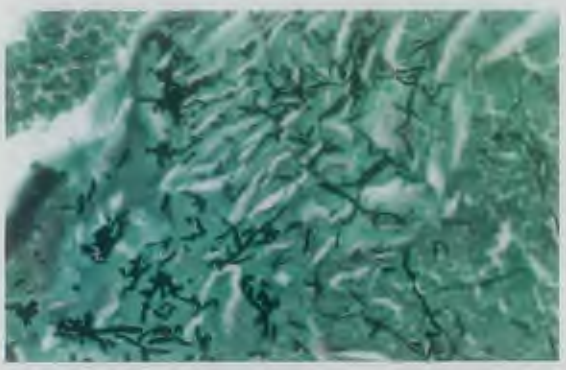

Figure 3. Microwave dried paraffin section stained with microwave-heating Grocott-Gomori methanamine silver staining for fungi. The staining process was completed within 30min. Note the spores and hyphae structures are stained black. (X125)

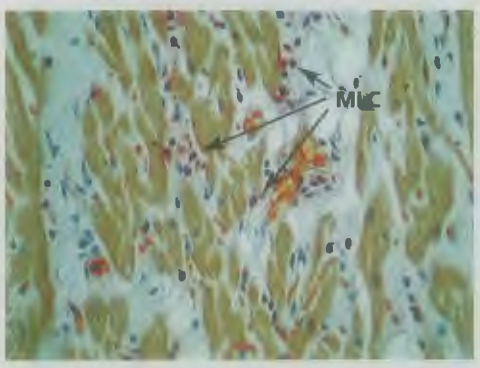

Figure 4. Microwave dried paraffin section stained with Chloroacetate Esterase stain demonstrating myeloid leukaemic cells (MLC) infiltrating myocardium. Microwave heating was applied to maintain optimal temperature for incubation in reagents. (X50)

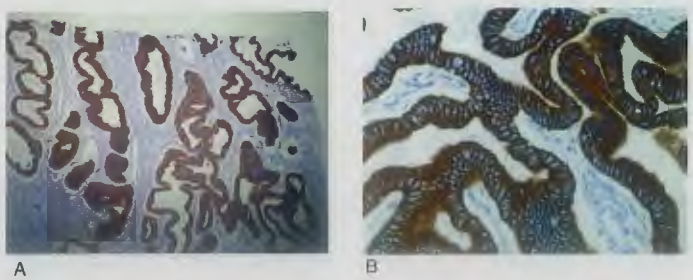

Figure 5. Human uterus stained with Cytokeratin AE1/AE3 (DAKO) antibody showing staining of
(A) epithelium of endometrium (X50) and **(B)** intense cytoplasmic staining of cytokeratin
(B) intermediate filament proteins in epithelium (X125). Microwave dried paraffin sections were
(C) pre-treated with microwave heat induced antigen retrieval technique and
(D) immunohistochemical staining with DAKO LSAB2/HRP-DAB system.

Figure 6. Human tonsil stained with CD3, NCL-CD3-PS1 (Novocastra) antibody showing staining of **(A)** T-lymphocytes predominantly in parafollicular region (X50) and **(B)** intense membrane staining of T-lymphocytes (X125). Microwave dried paraffin sections were pre-treated withmicrowave heat induced antigen retrieval technique and immun histochemical staining with DAKO LSAB2/HRP-DAB system.

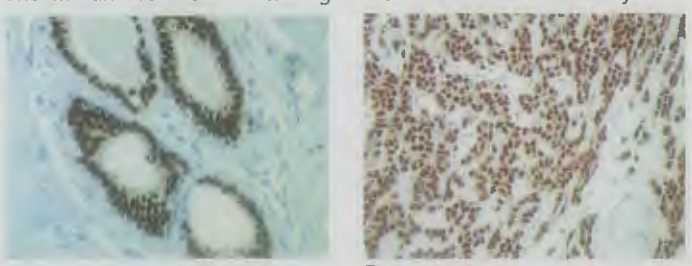

Figure 7. Breast carcinoma stained with Estrogen Receptor, NCL-ER-6F11 (Novocastra) antibody showing intense nuclear staining of **(A)** ductal epithelium (X125) and **(B)** tumour cells (X50). Microwave dried paraffin sections were pre-treated with microwave heat induced antigen retrieval technique and immunohistochemical staining with DAKO LSAB2/HRP-DAB system.

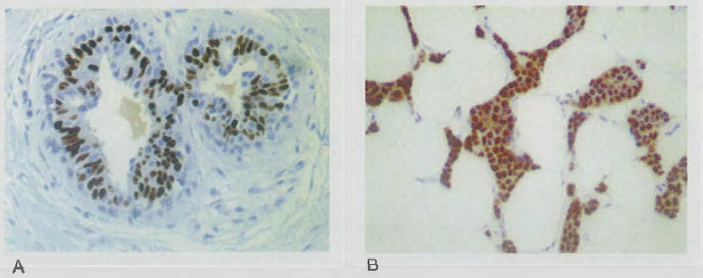

Figure 8. Breast carcinoma stained with Progesterone Receptor, PgR 1294 (DAKO) antibody showing intense nuclear staining of **(A)** ductal epithelium (X125) and **(B)** tumour cells infiltrating fatty tissue (X50). Microwave dried paraffin sections were pre-treated with microwave heat induced antigen retrieval technique and immunohistochemical staining with DAKO LSAB2/HRP-DAB system.

Calibration, standardisation, and instrument quality control

The microwave field intensity within the oven cavity varies from one oven to the other and the variation is intensified when the oven is loaded for 'microwaving'. There are so called 'hot' and 'cold' spots or regions of high intensity and low intensity fields respectively within the microwave field. Microwave ovens manufactured specially for laboratory use allow the selection of different power wattage settings; whereas changing the power settings of a domestic microwave oven does not affect the wattage, where the time that the microwaves are emitted is altered.

Calibration

The microwave oven is calibrated with calibration tools called the neon bulb array and agar-saline-Giemsa (ASG) tissue phantoms to identify regions of 'hot' and 'cold' spots (62). The neon bulb array consists of neon bulbs inserted in a polystyrene base that is used to map out regions of high and low power in a microwave oven. Neon bulbs glow when in contact with high microwave field. It can also be used as a simple test for leakage of microwave irradiation around edges, hinges and catch on the door of the microwave oven (24). The ASG tissue phantom consists of agar stained with Giemsa dyes, which simulates the tissue used, to determine if the microwave oven irradiation conditions would uniformly heat a tissue. Ideally, the ASG tissue phantom should show a uniform colour change, provided the same standardisation protocol for microwave heating tissue samples is followed.

Standardisation

The results of microwave histological techniques are reproducible provided the domestic microwave oven is calibrated for use, and the following basic parameters are standardised (23,62).

a) **irradiation time -** prolonged heating of tissue samples are more likely to show thermal artefacts, dissociation, and evaporation of chemical reagents. To optimise the heating process, the use of microwave heating curves (Figure 9) helps to predict the duration of microwave heating needed to heat a volume of solution to any given temperature.

b) **volume of solution -** the measurement of fluid volume is critical to enable the determination of precise time requirements for microwave heating various volumes of solution to attain final temperature.

c) **water load volume -** a procedure used to reduce the energy density in sample and solution, thus slow down the microwave heating process. A 250ml beaker of water at room temperature is located at the right rear corner of the oven during 'microwaving' to absorb some of the microwave irradiation intensity.

d) **initial and final temperature -** monitor and control temperatures to prevent boiling and evaporation of chemicals. Solution baths must be heated from the same initial temperature (room temperature) to yield consistent fixing and staining times. Check the temperature in between and after heating, and reheat again to ensure desired temperature is attained. Multiple short periods of heating will have a negative effect on the tissue but overshooting the temperature will damage the specimen. A fast reading digital thermometer is handy for rapid measurements of temperature.

e) **power levels -** operate the microwave oven at low power levels to allow more time for dissipation of heat. Intentional under heating is a reasonable safe guard against over heating.

f) **placement within oven cavity -** place the container of contents in the centre of the oven where the microwave flux is considered the most uniform within the oven cavity. The use of plastic sheet ruled with co-ordinates enables reproducible placement of specimens in the oven cavity.

g) **microwave oven magnetron -** pre-heating a water load helps to warm up the magnetron before use. The use of microwave heating curves will help to monitor the ageing of the magnetron that leads to a lowered heat production.

h) **container shape and size -** containers with curved surfaces can show a focusing effect contributing to maximum heating occurring in the centre, called centre-heating (23). It has been suggested that using rectangular containers in place of cylindrical types may decrease or eliminate centre heating. Plastic ware is ideal for 'microwaving'. Glassware absorbs microwave energy in varying degrees, causing differing temperatures from one solution to another.

i) **fluid agitation -** the temperature of the heated solution is not uniform throughout the Coplin jar. The top portion of the solution is warmer than that near the bottom of the jar. Therefore, in order to equalise the temperature of the solution, the slides are dipped up and down to agitate the solution. This ensures uniformity of staining throughout the tissue section.

Instrument quality control

A periodically scheduled quality control program and instrument maintenance history of microwave oven should be documented for accreditation. The instrument should be kept clean at all times, annual check-ups for electrical safety and radiation leakage performed, and a quality control program to monitor the magnetron power output is recorded. The procedure for monitoring the magnetron power output follows:

Equipment required: plastic beakers 500ml x2, digital thermometer, flat stirrer.

Method:

1. Fill each beaker with 500ml of distilled water at 10(C (2(C.
2. Check and record the precise temperature of water, note as starting temperature.
3. Place a beaker of water in the centre of the oven cavity.
4. Heat the water at full power for 62sec (allowing 2sec for the magnetron to warm-up from cold).
5. Remove the beaker from the oven and immediately stir the water, within 30sec of removing the beaker from the oven measure the water temperature again and note as final temperature. Repeat the steps 2-5 for the second beaker of water.
6. Average the two starting temperature readings.
7. Average the two final temperature readings.
8. Subtract the average starting temperature from the average final temperature to obtain an average temperature rise.

9. Multiply the average temperature rise by 70, and then multiply by 1.15.
10. The resulting figure is the power output in Watts I.E.C. (International Electrotechnical Commission). A significant drop in power wattage indicates the magnetron is not performing at optimal level.

(This information was supplied courtesy of Electrical Dept., Waikato Hospital, Hamilton).

Besides thermal heating, it is not clear whether microwave irradiation accelerates a reaction process by mechanisms of increase in reaction rate, or a faster diffusion of the reagents through cells and tissue (63), and a separate non-thermal microwaves effect of histological staining (64). In microwave fixation, it has been suggested that heating the tissue causes proteins to coagulate (7), and the heat denatures disulphide bond formation, resulting in decreased insolubility of proteins (8), while in microwave staining, internal heating accelerates diffusion of dye molecules into tissue, and thus enhances dye-substrate binding (25). The theory of a separate non-thermal effect of microwave irradiation on histological stains could not be confirmed (64).

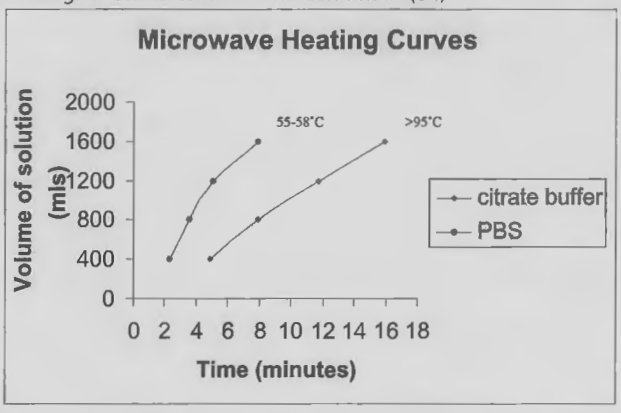

Figure 9. Graph shows the relationship between varying volumes of solutions and duration of microwave heating for final temperature. A 650W domestic microwave oven was used at high (100%) power setting to heat various volumes of solutions in a 2L plastic beaker.

Conclusions

In summary, the domestic microwave oven is suitable for most histological techniques where heating is required. In fact, it is a lot cheaper and more affordable, compared to the laboratory microwave oven. The microwave oven provides an alternative source of heating, without the use of naked flames and heating elements in the laboratory. Microwave heating in histological techniques could successfully be used routinely in the laboratory for microwave fixation of tissue and for gross specimen dissection, microwave drying of paraffin sections, microwave staining, and for immunohistochemistry. The advantages of microwave heating techniques are speed and/or improved quality. Employing these methods saves time in specimen processing. In the future, microwave heating may be incorporated in automated tissue processors for fixation and tissue processing, and in automated staining machines to dry sections and perform staining at higher temperatures to speed up the staining process.

The use of microwave oven and microwave heating in histotechnology is a relative new development, compared to conventional histotechnology. However, the applications of microwave heating could be an alternative for increased productivity and efficiency. The initial process of experimentation and set-up of microwave histological techniques in the routine laboratory can be time consuming, laborious, and requires careful standardisation, but once completed, has tangible benefits, particularly in turnaround times and the quality of processing and staining.

Acknowledgements

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References

1. Ehrlich P, Lazarus A. Wien:Abt Holder. *Die Anaemie* 1898; 1.
2. Mayers CP. Histological fixation by microwave heating. *J Clin Pathol* 1970; 23: 273-5.
3. Bernard GR. Microwave irradiation as a generator of heat for histological fixation. *Stain Technol* 1974; 49: 215-24.
4. Petrere JA, Schardein JL. Microwave fixation of fetal specimens. *Stain Technol* 1977; 52: 113-4.
5. Zimmerman GR, Raney IA. Fast fixation of surgical pathology specimens. *Lab Med* 1972; 3: 29-30.
6. Gordon HW, Daniel EJ. Preliminary report: microwave fixation of human tissues. *Am J Med Technol* 1974; 40: 441-2.
7. Login GR. Microwave fixation versus formalin fixation of surgical and autopsy tissue. *Am J Med Technol* 1978; 44: 435-7.
8. Hopwood D, Coghill G, Ramsay J, Milne G, Kerr M. Microwave fixation: its potential for routine techniques, histochemistry, immunocytochemistry and electron microscopy. *Histochem J* 1984; 16: 1171-91.
9. Leong AS, Daymon ME, Milios J. Microwave irradiation as a form of fixation for light and electron microscopy. *J Pathol* 1985; 146: 313-21.
10. Login GR, Dvorak AM. Microwave fixation provides excellent preservation of tissue, cells and antigens for light and electron microscopy. *Histochem J* 1988; 20: 373-87.
11. Brinn NT. Rapid metallic staining using the microwave oven. J Histotechnol 1983; 6:125-9.
12. Hafiz S, Spencer RC, Lee M, Gooch H, Duerden BI. Use of microwaves for acid and alcohol fast staining. *J Clin Pathol* 1985; 38: 1073-6.
13. Moorlag HE, Boon ME, Kok LP. Microwave methods for reducing staining time to seconds. *Stain Technol* 1987; 62: 357-60.
14. Leong AS, Milios J. Rapid immunoperoxidase staining of lymphocyte antigens using microwave irradiation. *J Pathol* 1986; 148: 183-7.
15. Chiu KY, Chan KW. Rapid immunofluorescence staining of human renal biopsy specimens using microwave irradiation. *J Clin Pathol* 1987; 40: 689-92.
16. Shi SR, Key ME, Kalra KL. Antigen retrieval in formalin-fixed, paraffin -embedded tissues: An enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. *J Histochem Cytochem* 1991; 39:741-8.
17. Van den Brink WJ, Zijlmans HJ, Kok LP, Bolhuis P, Volkers HH, Boon ME, et al. Microwave irradiation in label-detection for diagnostic DNA-in situ hybridization. *Histochem J* 1990; 22: 327-34.
18. McMahon J, McQuaid S. The use of microwave irradiation as a pretreatment to in situ hybridization for the detection of measles virus and chicken anaemia virus in formalin-fixed paraffin-embedded tissue. *Histochem J* 1996; 28: 157-64.
19. Login GR, Dvorak AM. Methods of microwave fixation for microscopy. A review of research and clinical applications: 1970-1992. *Prog Histochem Cytochem* 1994; 27: 1-127.
20. Kok LP, Boon ME. Microwave Cookbook for Microscopists, 3rd ed. Coulomb Press Leyden, Leiden, 1992.
21. Login GR, Dvorak AM. The Microwave Tool-book: A Practical Guide for Microscopists, Beth Israel Hospital, Boston, 1994.

22. Thuery J. Microwaves: Industrial, Scientific and Medical Applications. Artech House, Norwood, USA, 1992.
23. Kok LP, Boon ME. Physics of microwave technology in histochemistry. *Histochem. J* 1990; 22: 381-8.
24. Copson DA. Microwave Heating. 2nd ed. AVI publishing Co., Connecticut, USA, 1975.
25. Suurmeijer AJH, Boon ME, Kok LP. Notes on the application of microwaves in histopathology. *Histochem. J* 1990; 22: 341-6.
26. Reed W, Erichsen A, Roald B. Rapid supplementary fixation in frozen sections: microwave versus conventional fixation. A double-blind comparative study. *Pathol Res Pract.* 1991; 187: 824-7.
27. Kayser K, Stute H, Lubcke J, Wazinski U. Rapid microwave fixation - a comparative morphometric study. *Histochemical J* 1988; 20: 347-52.
28. Van de Kant HJG, De Rooij DG, Boon ME. Microwave stabilization versus chemical fixation. A morphometric study in glycomethacrylate and paraffin-embedded tissue. *Histochemical J* 1990; 22: 335-40.
29. Patterson MK Jr, Bulard R. Microwave fixation of cells in tissue culture. *Stain Technol* 1980; 55: 71-5.
30. Login GR, Schnitt SJ, Dvorak AM. Rapid microwave fixation of human tissues for light microscopic immunoperoxidase identification of diagnostically useful antigens. *Lab Invest* 1987; 57: 585-91.
31. Leong AS-Y, Milios J, Duncis CG. Antigen preservation in microwave-irradiated tissues: A comparison with formaldehyde fixation. *J Pathol* 1988; 156: 275-82.
32. Moran RA, Nelson F, Jagirdar J, Paronetto F. Application of microwave irradiation to immunohistochemistry: preservation of antigens of the extracellular matrix. *Stain Technol* 1988; 63: 263-9.
33. Leong AS-Y, Gilham PN. The effects of progressive formaldehyde fixation on the preservation of tissue antigens. *Pathology* 1989; 21: 266-8.
34. Boon ME, Gerrits PO, Moorlag HE, Nieuwenhuis P, Kok LP. Formaldehyde fixation and microwave irradiation. *Histochem J* 1988; 20: 313-22.
35. Leong AS-Y. Microwave techniques for diagnostic laboratories. *Scanning* 1993; 15: 88-98.
36. Leong AS, Duncis CG. A method of rapid fixation of large biopsy specimens using microwave irradiation. *Pathology* 1986; 18: 222-5.
37. Leong ASY. Microwave fixation and rapid processing in a large throughput histopathology laboratory. *Pathology* 1991; 23: 271-3.
38. Margo CE, Saxe S, Grossniklaus HE. Microwave-stimulated chemical fixation of whole eyes. *Ophthalmology* 1992; 99: 1117-22.
39. Ruijter ET, Miller GJ, Aalders TW, van de Kaa CA, Schalken JA, de Bruyne FM, et al. Rapid microwave-stimulated fixation of entire prostatectomy specimens. *J Pathol* 1997; 183: 369-75.
40. Ni C, Chang TC, Searl SS, Coughlin-Wilkinson E, Albert DM. Rapid paraffin fixation for use in histologic examinations. *Ophthalmology* 1981; 88: 1372-6.
41. Boon ME, Kok LP, Ouwerkerk-Noordam E. Microwave -stimulated diffusion for fast processing of tissue: reduced dehydrating, clearing, and impregnating times. *Histopathology* 1986; 10: 303-9.
42. Kok LP, Visser PE, Boon ME. Histoprocessing with the microwave oven: an update. *Histochem J* 1988; 20: 323-8.
43. Kennedy A. Routine use of microwave technology in Scotland: Retic, Mucin, Myelin and Fungi stainings. *Eur J Morphol* 1991; 29: 74-6.
44. Miller RT, Kubier P, Reynolds B, Henry T, Turnbow H. Blocking of endogenous avidin-binding activity in immunohistochemistry. The use of skim milk as an economical and effective substitute for commercial biotin solutions. *Appl Immunohistochem Mol Morphol* 1999; 7: 63-5.
45. Bancroft JD, Stevens A. Theory and Practice of Histological Techniques. 3rd ed. Churchill Livingstone, UK.
46. Kayser K, Bubenzer J. Microwave-assisted staining procedures in routine histopathology. *Histochem J* 1990; 22: 365-70.
47. Leong AS, Gilham P. A new, rapid, microwave-stimulated method of staining melanocytic lesions. *Stain Technol* 1989; 64: 81-5.
48. Boon ME, Kok LP, Moorlag HE, Gerrits PO, Suurmeijer AJ. Microwave-stimulated staining of plastic embedded bone marrow sections with the Romanowsky-Giemsa stain: improved staining patterns. *Stain Technol* 1987; 62: 257-66.
49. Horobin RW, Boon ME. Understanding microwave-stimulated Romanowsky-Giemsa staining of plastic embedded bone marrow. *Histochem J* 1988; 20: 329-34.
50. Cattoretti G, Becker MHG, Key G, Duchrow M, Schluter C, Galle J, et al. Monoclonal antibodies against recombinant parts of the Ki-67 antigen (MIB 1 and MIB 3) detect proliferating cells in microwave-processed formalin-fixed paraffin sections. *J Pathol* 1992; 168: 357-63.
51. Leong AS-Y, Milios J. An assessment of the efficacy of the microwave antigen retrieval procedure on a range of tissue antigens. *Appl Immunohistochem* 1993; 1: 267-74.
52. Cuevas EC, Bateman AC, Wilkins BS, Johnson PA, Williams JH, Lee AH, et al. Microwave antigen retrieval in immunocytochemistry: a study of 80 antibodies. *J Clin Pathol* 1994; 47: 448-52.
53. Strater J, Gunthert AR, Bruderlein S, Moller P. Microwave irradiation of paraffin-embedded tissue sensitizes the TUNEL method for in situ detection of apoptotic cells. *Histochem Cell Biol* 1995; 103: 157-60.
54. Leong AS, Milios J. Accelerated immunohistochemical staining by microwaves. *J Pathol* 1990; 161: 327-34.
55. Vitarelli E, Sippelli G, Tuccari G, Barresi G. The use of microwave irradiation for immunohistochemistry: a new methodological proposal. Histol *Histopathol* 1995; 10: 35-8.
56. Jackson P, Lalani EN, Boutsen J. Microwave-stimulated immunogold silver staining. *Histochem J* 1988; 20: 353-8.
57. Kok LP, Boon ME, Suurmeijer AJH. Major improvement in microscopic-image quality of cryostst sections. Combined freezing and microwave-stimulated fixation. *Am J Clin Pathol* 1987; 88: 620-3.
58. Kennedy A, Foulis AK. Use of microwave oven improves morphology and staining of cryostat sections. *J Clin Pathol* 1989; 42: 101-5.
59. Wagenaar F, Kok GL, Broekhuijsen-Davies JM, Pol JM. Rapid cold fixation of tissue samples by microwave irradiation for use in electron microscopy. *Histochem J* 1993; 25: 719-25.
60. Latimer JM, Matsen JM. Microwave oven irradiation as a method for bacterial decontamination in a clinical microbiology laboratory. *J Clin Microbiol* 1977; 6: 340-2.
61. Conder GA, Williams JF. The microwave oven: a novel means of decontaminating parasitological specimens and glassware. *J Parasitol* 1983; 69: 181-5.
62. Login GR, Leonard JB, Dvorak AM. Calibration and standardization of microwave ovens for fixation of brain and peripheral nerve tissue. *Methods* 1998; 15: 107-17.
63. Horobin RW, Flemming L. Trouble-shooting microwave accelerated procedures in histology and histochemistry: understanding and dealing with artefacts, errors and hazards. *Histochem J* 1990; 22: 371-6.
64. Feirabend HKP, Ploeger S, Kok P, Choufoer H. Does microwave irradiation have other than thermal effects on histological staining of the mammalian CNS. A light microscopical study of microwave stimulated staining under isothermal conditions in man and rat. *Eur J Morphol* 1992; 30: 312-27.

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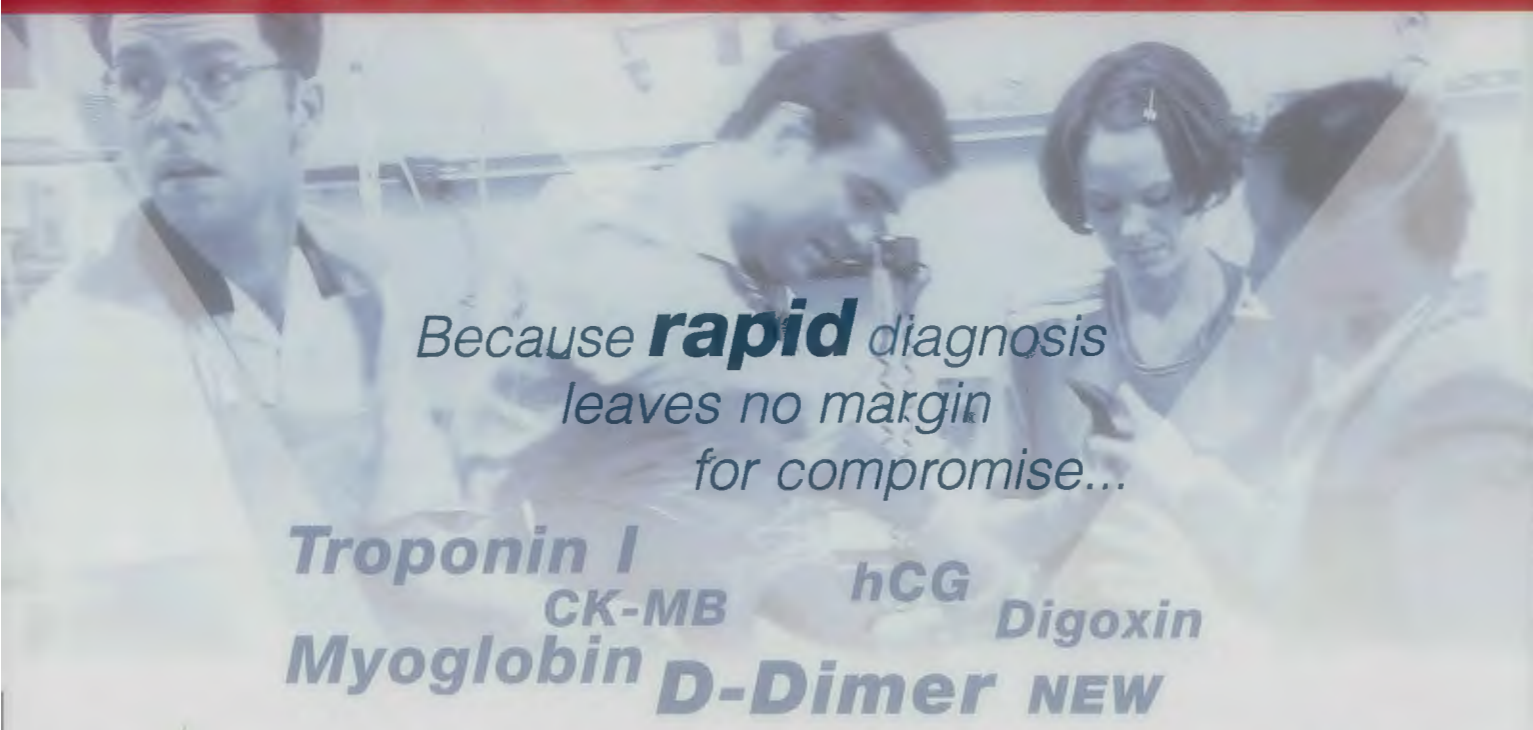

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D-dimer testing in the investigation of venous thromboembolism

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Introduction

D-dimers are formed by breakdown of fibrin clots. They act as markers of venous thromboembolism (VTE), including deep vein thrombosis (DVT) and pulmonary embolism (PE). A number of different methods exist for D-dimer measurement. These methods have differing degrees of sensitivity and specificity, and are therefore suited for use in different circumstances. When combined with clinical assessment, the D-dimer test is a useful tool in the evaluation of VTE. In this context, a test with high sensitivity and negative predictive value is desirable.

Evaluation of suspected venous thromboembolism

Patients presenting with possible VTE are evaluated using a combination of clinical assessment and radiological investigation. A diagnosis of DVT or PE cannot be reached from clinical assessment alone. In both conditions, further investigations are required to either prove or exclude the presence of thrombus (1).

In the case of suspected DVT, evaluation is relatively straightforward. Lower limb compression ultrasonography (CUS) in symptomatic individuals is usually sufficient to demonstrate the presence of proximal thrombus. In cases of doubt, ultrasound study is repeated after three to seven days or may be supplemented by further imaging of the deep venous system. Traditionally this has been accomplished using venography with intravenous contrast, but in future this technique is likely to be replaced by newer imaging methods such as magnetic resonance direct thrombus imaging (MRDTI) (2).

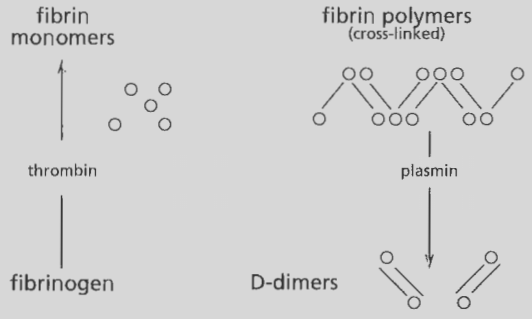
Evaluation of suspected PE is not so simple. Radiological investigations alone may not provide a clear 'Yes' or 'No' answer to the question of whether thrombus is present in the lungs. Ventilation-perfusion (V/Q) scans frequently demonstrate an "intermediate probability" of PE, particularly in those with other underlying lung disease (3). Likewise, although rapidly achieving widespread use, helical computed tomography of the chest with pulmonary angiography (CTPA) may not provide sufficient evidence to form the sole basis of a diagnosis due to limitations in scanning resolution (3). The 'gold standard" investigation, pulmonary angiography, is now rarely performed due to it's invasive nature, perceived possible adverse events and inter-observer variability.

Clinicians must judiciously combine information from both clinical assessment and radiological investigation in order to reach a positive or negative diagnosis. Following clinical evaluation, a sequence of investigations is obtained until there is sufficient evidence to tip the balance of probability either for or against the presence of thrombus.

What are D-dimers?

D-dimers are the product of fibrin degradation. Thrombus formation involves lysis of fibrinogen (by thrombin) to form fibrin monomers, which then become cross-linked to form a fibrin polymer. Subsequent breakdown of the fibrin clot (by plasmin) produces a number of fibrin degradation products, including D-dimers.

Figure 1. Formation of D-dimers from fibrin breakdown



Whenever thrombus formation occurs in the body, fibrin breakdown is also activated. This results in production of various fibrin degradation products. When large amounts of thrombus are being formed there is a corresponding increase in fibrin breakdown, and thus in levels of fibrin degradation products. D-dimer levels are therefore elevated in any situation where there is increased formation of fibrin clot. Such situations include VTE, disseminated intravascular haemolysis, post-operative states, post-trauma states, malignancy, and pre-eclampsia.

The role of D-dimer testing in suspected venous thromboembolism

Until recently, laboratory investigations have had little to contribute to the assessment of suspected DVT or PE. In recent years, however, the plasma D-dimer test has emerged as a potential tool in the evaluation of VTE.

In assessing a patient with possible DVT or PE, a D-dimer level may help the clinician decide whether or not there is sufficient clinical evidence to proceed to radiological investigation. A patient presenting to the emergency department with possible DVT or PE is initially assessed through review of the history, followed by physical examination. If there is suspicion of a DVT or PE, the patient then goes on to have further investigations. Traditionally, these have been in the form of radiological studies - usually serial compression ultrasonography in the case of suspected DVT, and V/Q scanning, CTPA or pulmonary angiography for suspected PE. These scans are expensive and take time to organise, often necessitating admission of the patient so that treatment can be started while scans are arranged, undertaken and reviewed. The addition of a D-dimer assay to this clinical work-up has the potential to save both time, money and patient exposure to unnecessary investigations (4).

In a patient with low or moderate clinical suspicion of DVT or PE, a negative D-dimer test effectively excludes the presence of significant thrombus. Such patients can be confidently discharged from the emergency department without requiring radiological investigations (1,3,5). However, if a normal D-dimer level (or 'negative' test result) is to be used in this way as the sole test of exclusion, the assay must have very high sensitivity (6). Van Beek and colleagues estimate that "for every 2% decrease in sensitivity, one per 1,000 evaluated patients (with

clinically suspected PE) would die as a result of inadequately treated pulmonary embolism." (7).

Patients with a negative D-dimer and high clinical suspicion, or a positive D-dimer test require further investigation in order to establish or exclude a diagnosis of DVT or PE. D-dimer levels are elevated in a number of situations, so a positive test is not specific for VTE; in other words, an elevated D-dimer level may be caused by a number of conditions, and does not necessarily indicate a diagnosis of DVT or PE. Radiological confirmation of VTE is usually required before a positive diagnosis can be reached.

Thus, in terms of reaching a diagnosis, a negative D-dimer test is much more useful than a positive result. Figure 2 demonstrates how a quantitative D-dimer assay may assist in evaluating suspected PE, using a test with high sensitivity. A negative test result (e.g. D-dimer level <500ng/mL) significantly decreases the post-test probability for PE (see lower curve). In contrast, the curve for a positive D-dimer result (e.g. >500ng/mL) is close to unity and thus produces only a small change from the pre-test probability.

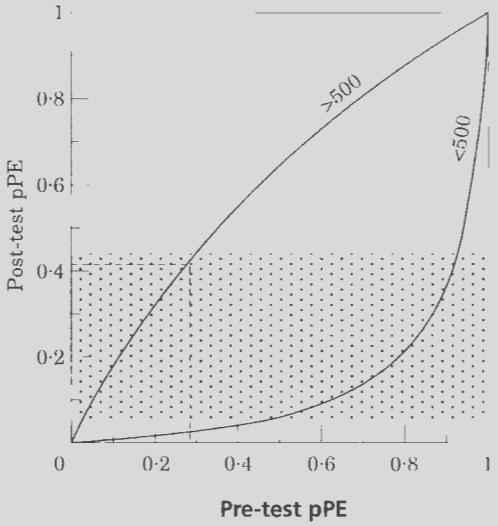


Figure 2. D-dimer testing in suspected PE - relationship between pre- and post-test probability ©

The horizontal axis represents a patient's pre-test probability for PE. The vertical axis gives the patient's probability of PE following D-dimer testing. The dotted area represents the range of indeterminate probability - i.e. where there is insufficient evidence to either positively diagnose or confidently exclude PE (6% to 44% according to the model proposed by Perrier and Junod).

For example, a patient with a pre-test probability of around 28% who is found to have a D-dimer level <500ng/mL has a post-test probability of around 2% - sufficient to exclude a diagnosis of PE. If the same patient had a D-dimer level >500ng/mL, the post-test probability would be around 41%, and further investigations would be required to reach a diagnosis.

This graph is based on a quantitative D-dimer assay using 500ng/mL as the threshold for a positive result.

© Reprinted from Resp Med 1995; 85:241-51. Perrier A, Junod AF. Has the diagnosis of pulmonary embolism become easier to establish? Page 244 (copyright by permission of the publisher, WB Saunders).

Measuring D-dimer levels

Detection of D-dimers in plasma samples is carried out using monoclonal antibodies specific for antigens on the D-dimer fragment. There are several different methods using this technique.

1. Traditional enzyme-linked immunosorbent assay (ELISA)

This uses a membrane coated with anti-D-dimer antibodies. Plasma is added to the membrane, and D-dimers in the plasma bind to the membrane antibody. A second antibody labelled with a fluorescent substance is added. The amount of labelled antibody bound to the membrane can be measured; this indicates the level of D-dimer present in the plasma sample.

The traditional ELISA method is very accurate, and is used as the reference standard for measuring D-dimer concentration. However this traditional ELISA technique is labour-intensive, expensive and slow (results may not be available the same day). For this reason it is not of practical use in the diagnosis of VTE in individual patients.

2. Manual latex agglutination assay

Monoclonal antibodies specific for D-dimer are coated onto latex particles. When D-dimer levels are elevated, macroscopic latex agglutinates are seen. This method produces a simple yes/no (i.e. qualitative) outcome, rather than a numerical D-dimer level. It is inexpensive and fast, giving an almost immediate result. However, it is less accurate than the ELISA assay and has fairly low sensitivity.

3. Manual whole blood agglutination assay

This is another qualitative method. Two monoclonal antibodies are conjugated together, one specific for D-dimers and the other specific for erythrocytes. Unlike other D-dimer assays, which use plasma, this method uses whole blood. If D-dimer levels are elevated in the blood sample, antibody-antigen binding leads to visible agglutination of erythrocytes (a 'positive' result). This technique is very fast (taking less than two minutes), and relatively inexpensive. However, the result relies on visual evaluation of the test (making it interpreter-sensitive), and lacks the accuracy of the ELISA assays.

4. New rapid ELISA assays

These use the same principle as the traditional ELISA assay, but are very much faster.

a) Qualitative methods

These give a simple positive/negative result, based on detection of D-dimer levels above a certain concentration (e.g. 500ng/mL). They produce a result within 10 minutes, often seen as a change in colour on a test card. Studies have shown this method to be much more sensitive than the manual latex agglutination assay (8-11). There is an element of operator-dependence in their use, as they rely on visual interpretation of the test card.

b) Quantitative methods

These use a rapid ELISA assay to give a numerical D-dimer result (i.e. an actual concentration). This method is more time-consuming than the qualitative ELISA assay (taking around 35 minutes), but has been shown to deliver more sensitive results (3, 8-12). In fact, the sensitivity of some of these tests is comparable to that of the traditional ELISA assay. This makes the quantitative rapid ELISA assay a suitable test for excluding VTE in a clinical setting.

There is growing evidence that quantitative assessment of D-dimer may also predict thrombus burden and the extent of pulmonary vascular bed occlusion (14,15). In addition, serial D-dimer assessment by this manner may aid in predicting those individuals most at risk of recurrance, in whom prolonged anticoagulation may be indicated (16,17).

5. Automated latex assay

This is a latex-enhanced photometric immunoassay. Unlike the manual latex and whole blood agglutination techniques, it produces a numerical (quantitative) result. Some automated latex assays demonstrate sensitivity comparable with the rapid quantitative ELISA assays (12,15,20). They also offer the advantage of being fully automated, and can be performed on many standard coagulation analysers.

Table 1. D-dimer assays in the diagnosis of deep venous thrombosis - comparison of performance

Assay type	Cut-off (mcg/L)	Sensitivity	Specificity
Traditional ELISA assay			
Asserachrom D-dimer	43	100%	33%
Enzygnost D-dimer micro	50	100%	44%
Fibrinostika FbDP	500	100%	36%
Manual latex agglutination assay			
Minutex D-dimer	-	80%	90%
Manual whole blood agglutination assay			
SimpliRED D-dimer	-	80%	94%
Rapid ELISA assays			
a) Qualitative			
INSTANT I.A. D-dimer	-	94%	63%
b) Quantitative			
VIDAS D-dimer	500	100%	41%
Automated latex assays			
Turbiquant D-dimer	250	98%	40%
IL Test D-dimer	130	100%	47%
LIAtest	350	100%	33%
TINAquant D-dimer	500	100%	39%

Adapted from reference 3. van der Graaf, et al. Exclusion of deep venous thrombosis with D-dimer testing. *Thromb Haemost 2000; 83: 191-8.*

The most extensively studied ELISA assay is the VIDAS system (Biomerieux, Paris, France) which has consistently demonstrated high sensitivity and negative predictive value in comparative studies (see Table 2) and in a large prospective trial (5,19). Despite the seemingly similar performance of other assays (8,20), few have received rigorous evaluation in prospective studies assessing longer term outcome in those subjects with negative results. Whilst the choice of testing platform in individual laboratories will be determined to some extent by local experience and laboratory resources, given the current level of evidence, the VIDAS system represents the "gold standard" by which other assays must be compared.

Study	*Patient Population*	*Number*	*Prevalence DVT*	*PE*	*Reference Standard**	*Cut-off (ng/mL)*	*Sensitivity*	*Specificity*	*Positive Predictive Value*	*Negative Predictive Value*
van der Graaf (8) 2000	outpatients	99	51%	-	venogram	500	100%	41%	63%	100%
Freyburger (9) 1998	inpatients	100	43%	7%	venogram PA	550	100%	38%	62%	100%
Legnani (12) 1997	outpatients	87	48%	-	venogram	500	95.2%	55.3%	70.2%	91.3%
Janssen (10) 1997	outpatients	132	67%	-	US	500	100%	19%	72%	100%
Elias (11) 1996	17% outpatients 83% inpatients	171	44%	-	US	400	97%	26%	51%	93%
de Moerloose (13) 1996	outpatients	195	-	24%	V/Q scan PA	500	100%	38%	33%	100%

*'venogram' = lower limb venography; 'PA' = pulmonary angiography; 'US' = lower limb ultrasonography; 'V/Q scan' = ventilation-perfusion lung scan

Table 2. Studies evaluating the VIDAS D-dimer assay in investigation of venous thromboembolism.

Clinical experience with the D-dimer assay

To date, only one large prospective study has evaluated the use of high sensitivity quantitative D-dimer assays as the primary initial test for excluding VTE. Perrier and colleagues (5,19) assessed 918 patients with clinically suspected venous thromboembolism. D-dimer testing (with the VIDAS assay) was used as the first investigation in their diagnostic algorithm, and the sole basis of a negative diagnosis in 31% of subjects. Of all subjects with D-dimer levels < 500ng/mL, only two subsequently had DVT demonstrated on ultrasonography. (In one of these cases, D-dimer measurement had been carried out more than a week after the onset of symptoms.) Prevalence of VTE in the study group was 23%; thus the VIDAS D-dimer assay had a sensitivity of 99.1% and negative predictive value of 99.3% in the hands of these investigators.

Prospective studies assessing algorithms that incorporate standardised clinical scoring systems, D-dimer assessment and newer imaging modalities in the evaluation of suspected VTE are curently being undertaken. Several studies have already assessed the use of the whole blood agglutination test (SimpliRED, Agen, Australia) in combination with a clinical scoring system in both DVT and PE (1). However, significant concerns regarding the sensitivity of this testing method and limitations of the scoring system have limited it's widespread acceptance.

Figure 3 (over the page) presents a recently developed algorithm tailored to the requirements of two tertiary hospitals in New Zealand, and demonstrates the systematic approach to the investigation of VTE that should be considered, given current international data. There is now good evidence to support the use of such approaches in emergency department evaluation of DVT (21,22), and preliminary data in the evaluation of PE suggests similar results. A number of hospital emergency departments are now adopting D-dimer assays as part of their clinical work-up of suspected DVT or PE.

However, it is important to note that clinical assessment forms the first step of such an algorithm, and must be undertaken before any decisions can be made about the need for laboratory or radiological investigation. Patients with high clinical suspicion for DVT or PE should always proceed to radiological investigation, and the role of quantitative D-dimer in this situation is to aid the prediction of the extent of thrombus formation and possible prognosis. In patients with low or moderate clinical probability of VTE, the role of a D-dimer test is to determine those with a negative result, in whom with-holding anti-coagulation without further investigation can be performed safely.

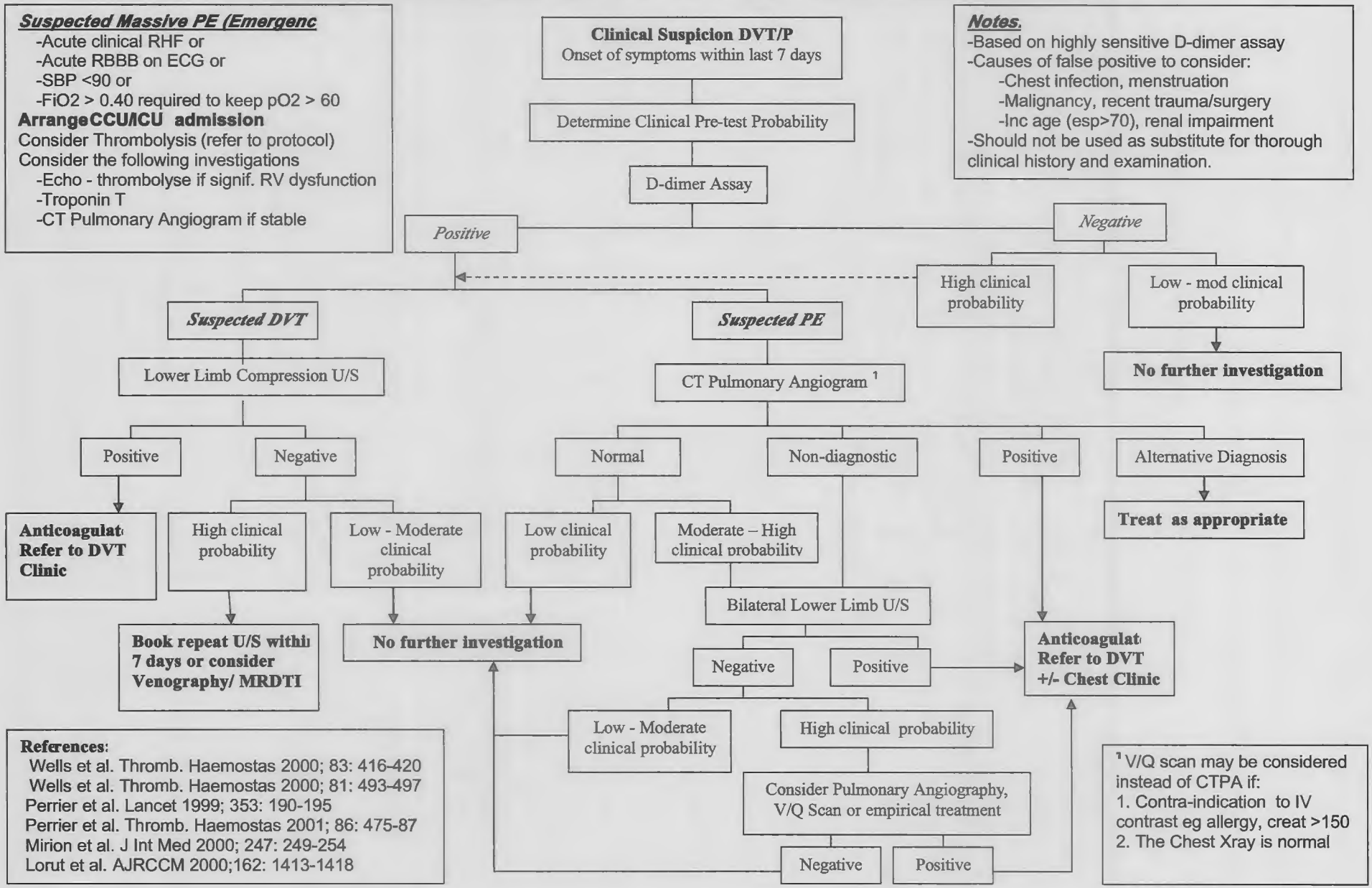
The VIDAS D-dimer assay is also being used as part of a study being undertaken in New Zealand. The New Zealand Air Travellers' Thrombosis Study (NZATTS) is a large prospective study currently in progress to determine the incidence of VTE in long-distance air travellers. Investigators are using the VIDAS D-dimer assay as a screening tool to determine which study participants may have developed a thrombus during their journey. Subjects with a negative D-dimer assay (<500ng/mL) are assumed to be thrombus-free; those with a positive assay (>500ng/mL) go on to receive radiological investigations to exclude or prove the presence of DVT or PE. Results from this study should become available within the next few months (Hughes R, Hill S, Hopkins R, van de Water N, Nowitz M, Milne D, et al. The incidence of venous thromboembolism in low to moderate risk long distance air travellers. The NZATT Study. Submitted for publication).

Limitations of the D-dimer assay

Age-related variability

Serum D-dimer levels tend to increase with advancing age. This appears to be a normal physiological pattern, and probably reflects multiple factors [including higher fibrinogen concentrations in the elderly,

Suspected Venous Thrombo-embolism Algorithm



slower urinary excretion, and more frequent fibrin generation (23)]. A study of D-dimer levels (using the VIDAS assay) in healthy volunteers found that the mean D-dimer level in individuals over the age of 70 was 528ng/mL (23) - greater than the 500ng/mL threshold normally used for a 'positive' test result.

This means that the specificity of the D-dimer test is lower in older age groups. In other words, older subjects often have a D-dimer level >500ng/mL in the absence of any pathological process. This limits the use of D-dimer testing in older age groups, since a 'positive' result is unlikely to be significant. It has been suggested that a higher "cut-of" for D-dimer be used in such a population, but the safety of such an approach remains to be assessed.

Low specificity

While the D-dimer test has a high sensitivity for VTE, its specificity is generally low (around 12-40%). A positive D-dimer test must always be followed up with appropriate radiological investigations in order to establish a diagnosis of VTE.

D-dimer levels are elevated in a number of situations, including infection, inflammation, post-operative and post-traumatic states, cardiac failure, renal impairment, malignancy and vascular disease (23,24). Such conditions are more prevalent in hospitalised patients; thus the specificity of the D-dimer test is particularly low in a hospital setting. As a result, the D-dimer test is most suitable for use in an outpatient or emergency department setting.

Timing of sample collection

D-dimer levels fall gradually as clot burden diminishes, and may return to the normal reference range around 14 days after the acute thrombotic event (9). Blood for D-dimer testing should therefore be taken as soon as possible after the onset of symptoms suggesting VTE. Where blood for D-dimer testing is obtained more than two weeks after the onset of symptoms, the test is likely to be less sensitive and may give rise to false negative results.

However, once collected in citrated tubing, D-dimer is stable for up to 24hr without further processing (25). Therefore, samples can be reliably transported for non-urgent testing if a high sensitivity assay is not immediately available.

Summary

Plasma D-dimer levels provide a potentially useful tool in evaluating patients with suspected venous thromboembolism. In order to be used safely to exclude VTE, this test must have very high sensitivity and negative predictive value. It must also be sufficiently fast and inexpensive to be used in the evaluation of large numbers of patients.

Several different methods exist for measuring D-dimer levels. The new rapid quantitative ELISA assay (e.g. VIDAS) offers the advantages of high sensitivity and negative predictive value, and is faster than the traditional ELISA assay. Many of the automated latex assays also demonstrate high sensitivity, and have the further advantage of being fully automated and faster than even the newer ELISA assays.

Use of the D-dimer assay is somewhat limited by its low specificity, especially in older age groups. The physiological increase in serum D-dimer levels with advancing age may mean that effective use of this test is limited to younger individuals. While a negative D-dimer effectively excludes VTE, a positive result may be due to a variety of conditions including inflammation, infection and trauma, as well as DVT or PE. The D-dimer test is best suited for use in the evaluation of suspected DVT or PE in an outpatient setting where the symptoms are of recent onset.

As with other laboratory tests, D-dimer evaluation should always be interpreted in the context of clinical assessment. Clinical algorithms that include standardised clinical assessment, sensitive D-dimer measurement, and a systematic approach to imaging modalities are likely to form the cornerstone of the investigation of suspected VTE in the foreseeable future. At present, D-dimer testing is most useful in the case of an appropriate patient with low to moderate clinical probability for VTE, where a negative test result may be used to reach a negative diagnosis. Fortunately, this group represents the majority of patients presenting for assessment. In cases where there is a high degree of clinical suspicion, it is probably inappropriate to use the D-dimer evaluation as a sole test for exclusion of suspected VTE.

References

1. Wells PS, Anderson DR, Ginsberg J. Assessment of deep vein thrombosis or pulmonary embolism by the combined use of clinical model and noninvasive diagnostic tests. *Semin Thromb Hemost 2000*; 26: 643-56.

2. Fraser DG, Moody AR, Morgan PS, Martel AL, Davidson I. Diagnosis of lower-limb deep venous thrombosis: a prospective blinded study of magnetic resonance direct thrombus imaging. *Ann Intern Med* 2002; 136: 89-98.

3. Lorut C, Ghossains M, Horrelou MH, Achkar A, Fretault J, Laaban JP. A noninvasive diagnostic strategy including spiral computed tomography in patients with suspected pulmonary embolism. *Am J Respir Crit Care Med* 2000; 162: 1413-8.

4. Perone N, Bournameaux H, Perrier A. Comparison of four strategies for diagnosing deep vein thrombosis: a cost-effectiveness analysis. *Am J Med* 2001; 110: 33-40.

5. Perrier A, Desmarais S, Goehring C, de Moerloose P, Morabin A, Unger PF, et al. D-dimer testing for suspected pulmonary embolism in outpatients. *Am J Respir Crit Care Med* 1997; 156: 492-6.

6. Perrier A, Junod AF. Has the diagnosis of pulmonary embolism become easier to establish? *Respir Med* 1995; 89: 241-51.

7. van Beek EJ, Schenk BE, Michel BC, van den Ende B, Brandjes DP, van der Heide YT, et al. The role of plasma D-dimers concentration in the exclusion of pulmonary embolism, *Br J Haematol* 1996; 92: 725-32.

8. van der Graaf F, van den Borne H, van der Kolk M, de Wild PJ, Janssen GW, van Uum SH. Exclusion of deep venous thrombosis with D-dimer testing - comparison of 13 D-dimer methods in 99 outpatients suspected of deep venous thrombosis using venography as a reference standard. *Thromb Haemost* 2000; 83: 191-8.

9. Freyburger G, Trillaud H, Labrouche S, Gauthier P, Javorschi S, Bernard P, et al. D-dimer strategy in thrombosis exclusion. Thromb Haemost 1998; 79: 32-7.

10. Janssen MC, Heebels AE, de Metz M, Verbuggen LH, Wollersheim H, Janssen S, et al. Reliability of five rapid D-dimer assays compared to ELISA in the exclusion of deep venous thrombosis. *Thromb Haemost* 1997; 77: 262-6.

11. Elias A, Aptel I, Huc B, Chale JJ, Nguyen F, Cambus JP, et al. D-dimer test and diagnosis of deep vein thrombosis: a comparative study of 7 assays. *Thromb Haemost* 1996; 76: 518-22.

12. Legnani C, Pancani C, Palareti G, Guazzaloca G, Fortunato G, Grauso F, et al. Comparison of new rapid methods for D-dimer measurement to exclude deep vein thrombosis in symptomatic outpatients, *Blood Coagul Fibrinolysis* 1997; 8: 296-302.

13. de Moerloose P, Desmarais S, Bounameaux H, Reber G, Perrier A, Dupuy G, et al. Contribution of a new, rapid, individual and quantitative automated D-dimer ELISA to exclude pulmonary embolism. *Thromb*

Haemost 1996; 75: 11-3.
14. Galle C, Papazyan JP, Miron MJ, Slosman D, Bounameaux H, Perrier A. Prediction of pulmonary embolism extent by clinical findings, D-dimer level and deep vein thrombosis shown by ultrasound. *Thromb Heamost* 2001; 86: 1156-60.
15. Sijens PE, Oudkerk M, Berghout A, van Ingen HE, Kemperman H. Comparison of a quantitative latex and a quantitative ELISA plasma D-dimer assay in the exclusion of segmental and subsegmental pulmonary embolism. *Thromb Haemost* 2001; 86: 1580-2.
16. Andreescu AC, Cushman M, Rosendaal FR. D-dimer as a risk factor for deep vein thrombosis: the Leiden Thrombophilia Study. *Thromb Haemost* 2001; 86: 1156-60.
17. Palareti G, Legnani C, Cosmi B, Guazzaloca G, Pancani C, Coccheri S. Risk of venous thromboembolism recurrence: high negative predictive value of D-dimer performed after oral anticoagulation is stopped. *Thromb Haemost* 2002; 87: 7-12.
18. Dempfle CE, Zips S, Ergul H, Heene DL. The Fibrin Assay Comparison Trial (FACT): evaluation of 23 quantitative D-dimer assays as basis for the development of D-dimer calibrators. *Thromb Haemost* 2001; 86: 1204-9.
19. Perrier A, Desmarais S, Miron MJ, de Moerloose P, Lepage R, Slosman D, et al. Non-invasive diagnosis of venous thromboembolism in outpatients. Lancet 1999; 353: 190-5.
20. Gardiner C, Mackie I, Machin S. Evaluation of a new automated latex particle immunoassay for D-dimer: IL Test D-dimer. *Lab Haematol* 2000; 6: 147-50.
21. Cornuz J, Ghali WA, Hayoz D, Stoianov R, Depairon M, Yersin B. Clinical prediction of deep vein thrombosis using two risk assessment methods in combination with rapid quantitative D-dimer testing. *Am J Med* 2002; 112: 198-203.
22. Dryjski M, O'Brien-Irr MS, Harris LM, Hassett J, Janicke D. Evaluation of a screening protocol to exclude the diagnosis of deep vein thrombosis among emergency department patients. *J Vasc Surg* 2001; 34: 1010-5.
23. Hager K, Platt D. Fibrin degeneration product concentrations (D-dimers) in the course of aging. *Gerontology* 1995: 41: 159-65.
24. Currie MS, Rao MK, Blazer DG, Cohen HJ. Age and functional correlations of markers of coagulation and inflammation in the elderly: functional implications of elevated crosslinked fibrin degradation products (D-dimers). *J Am Geriatr Soc* 1994; 42: 738-42.
25. Caliezi C, Reber G, Lammle B, de Moerloose P, Wuillemin WA. Agreement of D-dimer results measured by a rapid ELISA (VIDAS) before and after storage during 24h or transportation of the original whole blood samples. *Thromb Haemost* 2000; 83: 177-8.

Continuing Professional Development (CPD)

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This is a summary of a powerpoint presentation to the South Island NZIMLS seminar held at Lake Tekapo Saturday 16th March, 2002.

Introduction

The present CPD programme presently in operation had its beginning some years ago in what was then termed MOLS. Initially MOLS was introduced and run by the Medical Technologists Board, until revised as CPD and organised and run by the NZIMLS. At present the CPD programme is free to financial members of the NZIMLS. This presentation is aimed at confirming beliefs already held, or to stimulate thought and offer some new perspectives on the subject. If as it seems CPD will have to be a legal employment and registration component (1), then the NZIMLS membership had better design a CPD programme to meet all needs, or one will be imposed on the them.

My interest in CPD

With the every increasing number of professional bodies able to be joined, I had let my membership to the NZIMLS lapse as I felt I could get better value from other societies more directly related to Biochemistry. However, I continue to follow events in the Institute and have compiled my own ongoing CPD register. Now that CPD is being seen as an integral component of the HR side of laboratory employment, I decided to see how our CPD scheme compares with other schemes world wide. I was of a mind that while MOLS and now CPD were reasonably efficient, they were dated and designed more for the senior laboratory personnel rather than for today's composite work force of new graduates, laboratory assistants, scientific officers or part timers.

The three why's

To get to the core reason for any proposal, ask why. To that answer ask a second why and to that answer ask a final and third why and the true reason usually appears.

1. So, why embrace CPD?
   To keep up with the play (knowledge / development).
2. Why keep up with the play?
   It will be a legal requirement for employment and registration.
3. Why will it be a legal requirement?
   For the protection of the public, i.e. "in the Publics' best interests".[2]

So it will not be for personal warm fuzzies, it will be necessary to gain employment and fulfil legal registration requirements, so that Joe or Judy Public can have confidence in medical laboratory services.

Who directly befits from CPD? :

The direct beneficiaries from a CPD programme are the employer and employee for the following reasons:

Employer	Employee
Part of IANZ requirements of workforce	Formal recognition for previously competence voluntary efforts.
Aids recruitment/retention of staff	New knowledge and skill acquired
Improves staff morale Reduces stress	Increased job satisfaction Evidence of continuing competence
Enhances quality of service	Job security
Customer assurance	Valuable for performance appraisal purposes Addition to CVs Keeping up with, or ahead of colleagues

As there is mutual benefit to both parties, there will have to be some of the routine working period devoted to achieving the CPD criteria.

International CPD situation

There is a wide divergence world wide as to CPD programmes and requirements they contain. From an internet query the following information was obtained:

Country	CPD Requirements
1. Australia	No formal CPD programmes implemented (3)
2. New Zealand	CPD on a voluntary basis.
3. USA	Employment and registration requirements in some states, not all (4,5)
4. Canada	Similar to the USA, as programmes seemed to be provincial in nature (6-8)
5. UK	Run under the auspices of the IBMS (9)

In the USA and Canada CME points or a set number of educational hours are the norm.

New Zealand CPD categories

There are thirteen categories in the present CPD scheme, covering employment, scientific meetings and publications, study, peer reviews (IANZ) and community service/extension. I am not quite sure what community extension is. My feeling is that a lot of these thirteen categories are unattainable for the majority of laboratory employees, and that the scheme is in need of a major overhaul. In particular, I would like to see the employment category replaced with job related components of

lesser value but which together add up to the same value, but force people to actively complete tasks etc. rather than reward someone for turning and vegetating.

What essential qualities should a CPD programme have?

- It must have specific objectives. What does it want to achieve and how are these met.
- Activities must have demonstrable measurable values. What and how are the values obtained and allocated for the activities.
- Programme equally reflects full time and part time employees.
- Programme reflects today's and, hopefully tomorrow's challenges.
- Most of all, the programme must be attainable.

What is missing from the present CPD scheme

There are many aspects of modern laboratory work that are not represented in the present scheme. A few of them are:

- Membership of scientific societies.
- Journal reading.
- Use of the internet for journal / article searches.
- CPD credits via the internet.
- Authorization to perform new techniques/procedures within a discipline.
- QA assessment, both internal and external programs.
- Mentoring.
- Health and safety issues.

My proposal for a CPD scheme

After considering the issues, the following is my suggestion for a CPD scheme.

- Membership of a scientific society.
- Self assessment programmes via the internet.
- Seminar / lecture attendance.
- In-house or other lecture / talk presentation.
- Journal reading.
- Internet use for article / subject searches.
- Case history participation via the internet or scientific journal.
- Supervision and tutoring staff and or BMLS students.
- Quality assurance reviews.
- Post graduate or other studies (work related).
- Scientific meeting attendance.
- Community service for the promotion of medical laboratory sciences.

I have left peer reviews, book reviews etc. out of this programme as they affect a limited few in the industry.

My scheme is just a starter, but one group that could benefit from this is those ex-laboratory workers now employed in the laboratory trade and supply sector. Many of these keep their registration current, but would find it hard to qualify for registration if the present scheme was made mandatory.

Other considerations

To add a new dimension, I would like to float the following idea of making some categories compulsory. Perhaps some 60 - 70% of the annual CPD credits are to be earned from a "compulsory" list, and the additional 30 - 40% credits attainable from an "optional" list. As I have said previously I would like to see the employment component replaced by a larger series of employment related tasks.

Conclusions

The future of the existing CPD rests in our hands. It is up to the NZIMLS membership (me included as I have rejoined) to put views forward, and better still solutions to problems. Better we do it than have someone outside the industry impose a solution on us. With that in mind, let your views be known and make use of your local council and branch representative (or as a letter to the Journal)) to create a strong robust and practical CPD programme for the future.

References

1. Health Professionals' Competency Assurance Bill. Complaints and discipline provisions. http://www.executive.govt.nz/minister/king/cabinet 10-01/hpca.htm
2. Phil Saxby. Medical Laboratory Technologist Board, NZ, personal e-mail correspondence.
3. Paul Martin. Perth, WA, personal e-mail correspondence.
4. Cheryl R Caskey. Christushealth, USA, personal e-mail correspondence.
5. Debbi Tiffany. Swedish American Hospital, Rockford Illinois, USA, personal e-mail correspondence.
6. Janet L. Kingston. New Brunswick, Canada, personal e-mail correspondence.
7. Lisa Denesiuk (ACMLT President 2002, Canada) personal e-mail correspondence.
8. Professional Development Program, New Brunswick. http://www.nbsmlt.nb.ca/html/pdp.html
9. IBMS scheme. http:/www.ibms.org/00/public/content/scheme.php3

Editor's note: The above two articles are by invitation of the Editor for the start of a new series in the Journal, entitled "For debate". The aim of this new series is to present different views on topics of interest to the profession. It is hoped that these will generate lively discussion, preferably as Letters to the Editor. In a later Journal issue the topic of PSA screening - is it justifiable?, will be debated. I welcome suggestions of topics (and possible debaters) from the profession.

The NZIMLS Continuing Professional Development Programme

C. J. Kendrick, MNZIMLS, Dip Sci, MSc (Dis.)
Senior Lecturer. Massey University, and Convenor of the NZIMLS CPD programme.

Introduction

The first three year period of the NZIMLS Continuing Professional Development (CPD) programme will be completed on the 30th March 2003. The programme was developed following the demise of the Maintenance of Laboratory Standards (MOLS) programme that was run by the Medical Laboratory Technologist's Board (MLTB) and was introduced in 2000. During the development phases the NZIMLS looked at a number of continuing education (CE) programmes run by other MLS professional organisations worldwide as well as that used by the Royal College of the General Practitioners in NZ. The purpose of providing this service was to provide a formal means by which members of the NZIMLS could demonstrate competence to practise as a Medical Laboratory Scientist (MLS) and to augment the already well established SIG activities of the Institute.

Participation

The CPD programme is available to members of the NZIMLS registered with the MLTB and is voluntary. The programme provides a means whereby members gain points for attendance at CPD accredited scientific meetings, CE seminars and for a range of other professional related activities (see below). The goal is to achieve a minimum of 300 points over a three year period. Each year members are requested to forward their claims which are reviewed and audited and the points collated to include previous years activities. Records are kept with membership details at the NZIMLS office. Following completion of the three year period participants will be issued certificates that reflect their CE activities.

Current programme

There are seventeen categories in the current CPD programme handbook.

1. Laboratory employment.
Fifty points have been allocated to Technologists/Scientists employed in either a diagnostic medical laboratory, the NZ Blood Service or other medical laboratory related fields. These are available only to those who are required to hold a current NZ MLTB practicing license, irrespective of the hours of work each week. This allocation recognises the professional development that accompanies both part-time and full-time laboratory employment. Activities recognised under this include laboratory and section management, use of scientific technique, the practise of occupational safety, quality assurance, staff development, student and staff training, the performance of research & development (outside of post graduate study) and the reading of professional and scientific literature.

2. Laboratory peer review.
Laboratory review for IANZ, or other agency(s).

3. Scientific meeting attendance (approved meeting).
Meetings considered suitable for CPD points are listed in this handbook (see later). This list includes SIG meetings, NZIMLS and AIMS annual scientific meetings, user group meetings and others. Meetings of other societies/organisations may also be suitable for CPD points so you should provide some evidence to support its suitability (eg. programme).

Definitions:	*Half day meeting*	(2 - 4 hrs.) 35 points.
	1 day meeting	(6 - 8 hrs. including meetings of similar duration spread over 2 days) 75 points.
	2 day meetings	(> 8 hrs. spread over two or more days) 125 points.

3. Post graduate study.
Study while enrolled in an appropriate post-graduate tertiary qualification and/or NZIMLS Fellowship. Appropriate: medical science, management, technology, quality, occupational safety and health etc.

5 & 6
Scientific paper publication.
Publication of a scientific article, either as primary or co-author, in a refereed or non-refereed journal.

7. Oral or poster presentation.
Oral or poster presentation at a scientific meeting (primary presenter only).

8. Self-assessment programmes.
Both hard copy and web based programmes are eligible.

9. Publication of a MLS book review.

10. Seminar/lecture attendance.
Does not include laboratory tutorials and excludes bench-related training. Currently limited to 5 points per attendance with a maximum of 20 points / year.

11, 12.
Seminar, lecture or clinical review presentation "in house".
Does not include "at bench" instruction or discussion but includes formal tutorials given to MLS students, technical assistants, nursing and other medical staff.

13. Formal lecture given at University or Polytechnic.
Includes lectures given as part of a structured programme of teaching at a University or Polytechnic.

14. Examiner and/or moderator.
Examinations conducted on MLS related subjects on behalf of the NZIMLS, MLTB, NZ Universities or Polytechnics and other related organisations.

15. Service to the profession.
The following activities are considered to be appropriate:

- Individuals involved on the organising committees for the NZIMLS annual scientific meeting.

- Administration of the profession through the NZIMLS council and includes sub-committee co-opts.
- MLTB representation and sub-committee co-opts.
- Convenors of NZIMLS Special Interest Groups.
- Representatives on Board's of Study (or equivalent) of tertiary teaching Institutions.
- Preparation of SIG workshops etc.

16. Community service/extension.
The promotion of the profession of MLS through representation at career's events, or addresses to community groups about medical science related subjects.

17. Others
Claims not covered in other sections of this schedule.

Programme review

At the end of this year the NZIMLS is to seek submissions on the structure of the current programme and comment on operation of the first three years. To date we have received a number of comments as interest in the CPD programme continues to grow. The following are examples that have been received to date:

- "Increase the allocation of points for attendance at seminars". (currently limited to 20 points per year)
- "There are thirteen categories in the present CPD scheme, covering employment, scientific meetings and publications, study, peer reviews (IANZ) and community service/extension. My feeling is that a lot of these thirteen categories are unattainable for the majority of laboratory employees, and that the scheme is in need of a major overhaul".
- "I would like to see the employment category replaced with job related components of lesser value but which together add up to the same value, but force people to actively complete tasks etc. rather than reward someone for turning and vegetating".
- "An increase in the points allocation for laboratory peer review".
- "Allocation of points for tutoring MLS trainees".
- "Will the Lab Assistant group be catered for in the future?"
- "Can the MLS supply industry representatives participate in the programme?"

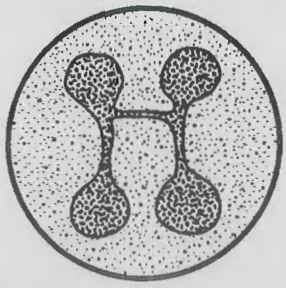
As part of the consultation process the NZIMLS will consider any suggestions for change received in written submissions. It is hoped that the release of the HPCA Bill will coincide with this review process allowing us to consider its impact as part of the review. At this stage it is planned to complete the process by the end of 2002 ready for introduction of the modified programme in April 2003. The NZIMLS is therefore interested in receiving comments to help with this process so here is your chance to have a say. It is hoped that through open discussion and debate that the CPD programme will be ready to meet the needs of the profession and the future regulation of our profession.

Please forward submissions to:

NZIMLS Executive Office
PO Box 505
Rangiora
or email: nzimls@exevents.co.nz

Haematology

Special Interest Group



STANDARDISED REPORTING OF HAEMATOLOGY LABORATORY RESULTS 3rd EDITION 1997

This booklet provides guidelines for basic blood cell morphology reporting and includes photographic images with concise text. It is a revision by the Auckland Haematology Special Interest Group (HSIG) of recommendations originally made by the Haematology Charge Technologist Group in 1998 to standardise blood film morphology reporting. Ideally suited for trainees and students of haematology.

Now available at a reduced price $20.00 each

Contact Jacquie Case (HSIG committee) at Haematology Laboratory, Middlemore Hospital, Otahuhu, Auckland. Ph (09) 2760044 xtn 8515 or e-mail jcase@middlemore.co.nz

HSIG journal based learning - questionaire

Blacklock HA, Royle GA. Idiopathic erythrocytosis - a declining entity. British Journal of Haematology 2001; 115: 774-81.

1. What biological measurement is used in the definition of absolute polycythaemia?
2. What are the three historical categories of absolute polycythaemia?
3. What has revealed more heterogeneity within the categories and prompted a need for reclassification of the polycythaemias?
4. Why is it important to correctly identify the cause of polycythaemia?
5. Polycythaemia Vera is characterised by hyperplasia of the marrow erythroid lineage.

TRUE / FALSE

6. 50% of patients with polycythaemia vera have splenomegaly.

TRUE / FALSE

7. The NAP score is a useful test in the diagnosis of polycythaemia vera.

TRUE / FALSE

8. Endogenous = spontaneous erythroid colonies?

TRUE / FALSE

9. Absence of spontaneous erythroid colonies in peripheral blood excludes a diagnosis of polycythaemia vera.

TRUE / FALSE

10. State the vulnerability of the erythroid colonies assay.
11. Polycythaemia vera is characterised by a low serum erythropoietin level.

TRUE / FALSE

12. What is the specific cytogenetic abnormality in polycythaemia vera?
13. Cytogenetic changes are present in all cases of polycythaemia vera.

TRUE / FALSE

14. Name the most frequently found cytogenetic abnormality in polycythaemia vera.
15. Erythroid progenitors in polycythaemia are independent of erythropoietin.

TRUE / FALSE

16. What two substances stimulate erythroid burst formation "in vitro" in polycythaemia patients?
17. What protein may play a role in the pathophysiology of polycythaemia vera?
18. Name the anti-apoptotic protein expressed in polycythaemia vera patients to a greater extent than normal individuals.
19. An assay of which substance would provide a more specific test for polycythaemia vera?
20. Diagnosis of polycythaemia vera can be difficult due to a co-existing morbitity, the most common is.....?
21. List the thrombohaemorrhagic problems common in polycythaemia vera.
22. Name the three groups of familial/congenital polycythaemia.
23. PFCP has autosomal inheritance.
24. What mutations have been found in pedigrees of PFCP?
25. To which familial polycythaemia group do increased oxygen affinity haemoglobinopathies belong?
26. Chuvashian polycythaemia is characterised by autosomal recessive inheritance.

TRUE / FALSE

27. List the causes of congenital secondary erythrocytosis.

For a copy of this journal article, contact Jacquie Case at Haematology Dept., Middlemore Hospital, Otahuhu, Auckland., or e-mail jcase@middlemore.co.nz

Answers on Page 70

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HSIG journal based questionaire - answers

1. Body red cell mass.
2. Primary, secondary and idiopathic.
3. The ability to define underlying cellular and molecular defects.
4. Early recognition of polycythaemia vera can prevent serious or fatal vascular events. Prognosis can be determined.
Identification of secondary causes avoids inapprpriate myelosuppression treatment.
5. False.
6. False.
7. False.
8. True.
9. False.
10. Technical problems.
Assay limited by low number of erythroid progenitors in most peripheral blood samples.
Some individuals without polycythaemia vera can have spontaneous erythroid colonies.
11. True.
12. There are no specific cytogenetic abnormalities in polycythaemia vera.
13. False.
14. Deletion of long arm of chromosome 20 (del 20q).
15. True.
16. IGF-1 and insulin-like growth factor binding protein.
17. PRV-1
18. Bcl-X_L
19. Mp1
20. Haemorrhage.
21. Increased viscosity and low blood flow.
Thrombocytosis and platelet dysfunction.
Altered vascular reactivity.
22. i) Primary familial and congenital polycythaemia (PFCP).
ii) Non-PFCP disorders.
iii) Chuvashian polycythaemia.
23. False.
24. Erythropoietin receptor mutations (EPOR).
25. Group ii) non-PFCP disorders.
26. True.
27. Chuvashian polycythaemia
Mutant high oxygen affinity Hb's
Methaemoglobinaemia.
Rbc 2,3-BPG deficiency.

Quality issues in haematology

HSIG seminar held in Auckland May 17 & 18 2002

This seminar was aimed to suit technical supervisors and experienced staff with formal responsibilities for quality assurance, and 75% of the attendees were of this calibre. Topics included Quality Assurance with an update from IANZ on the latest ISO 15189 International Medical Standard, a current perspective on the Waikato Survey programme, a review of the discontinued HSIG Morphology survey; Quality Control with particular emphasis on establishing reference ranges and the use of controls, a statistician provided a purer approach to the use of numerical data, Equipment and method evaluation, Haemostasis - preanalytical variables, changing lot numbers of APTT reagents/controls, the use of controls; and a review of D-Dimer kits.

85% of attendees rated the seminar as very good to excellent. All presentations were well received, the most popular were the sessions on Equipment & Method Analysis, and Quality Control.

On the Friday evening many attended the dinner held at the EPO (how appropriate is that!!) restaurant in the Ellerslie township close to the seminar venue. A most enjoyable evening was had by all, and for some....we know who you are.....it was just the beginning of a long, expensive, fun-filled night. Our thanks to the Auckland HSIG committee for another excellent event, keep up the good work and we look forward to the next.

THE Pacific WAY

Microscopes, laboratories and Pacific islands

A small pre-fab building in the Wellington hospital Level E car park has been playing a vital part in the development of Pacific Island medical laboratories for 21 years. Laboratory technicians from 22 different island nations come here, to the Pacific Paramedical Training Centre (PPTC), for four or five weeks at a time to build up their expertise.

The director of the PPTC, John Elliot, says many of the countries have hospital laboratories that are equipped to about the standard of New Zealand labs in the 1960s. Some have more recent equipment, but once it breaks down, there's generally no one to fix it until a manufacturers representative calls, often months later.

And the technicians themselves have little opportunity for formal training to use the equipment. Only Fiji's School of Medicine offers a relevant diploma course. To obtain a degree, students must travel even further, to either a New Zealand or Australian university. Most learn their skills on the job and at the courses held in Wellington at the PPTC.

The philosophy of the training centre is to provide development training and technical assistance to the health laboratory services of the Pacific which is appropriate, affordable and sustainable."This means that our courses are tailored to match Pacific Island levels of resources, equipment and needs" says John Elliot.

The centre's work is largely funded by the Ministry of Foreign Affairs, which pays the operating costs while it's overseas development agency finances the travel and study costs of most of the students. The World Health Organisation also funds some students and some of the consultancy work carried out by John Elliot in the Pacific. This includes his visits to island countries to maintain the laboratory quality assurance programme that is run by the PPTC. John says it also gives him a chance to catch up with some of his students and see how they're progressing.

The Wellington hospital laboratory, the School of Medicine and the New Zealand Blood Transfusion Service also support the programme, helping with some of the tutoring and explaining the intricacies of their equipment.

Over the years, around 600 laboratory technicians from the Pacific have undergone training at the training centre. Students, such as Jim Wamdi of Papua New Guinea who works at a mission hospital, say the training given in Wellington is extremely useful and often means they return home able to carry out a broader range of tests. Others like Geoffrey Wuatai of the Cook Islands says they have good equipment but rely on New Zealand biomedical technicians to come and fix the automated analysers when they break down.

Many of the students on the current blood morphology course have already attended two or three previous courses. And some, like Jim Wamdi says they'll go back, envious of the extensive and modern equipment they've seen in the Wellington hospital laboratories.

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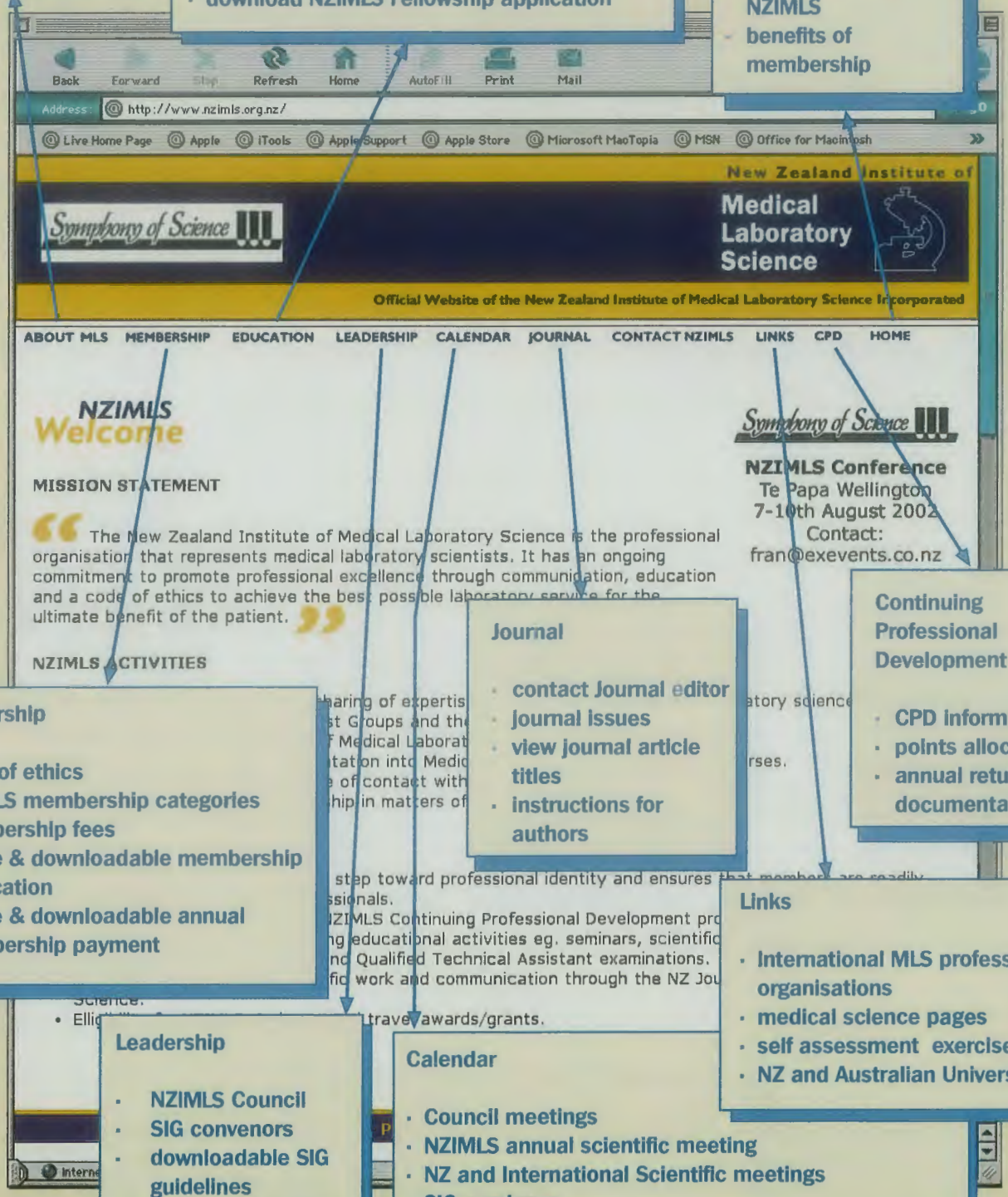

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Calendar

- Council meetings
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- NZ and International Scientific meetings
- SIG seminars
- application deadline dates for NZIMLS examinations
- Fellowship and QTA examination dates

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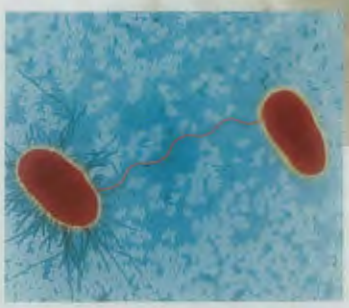

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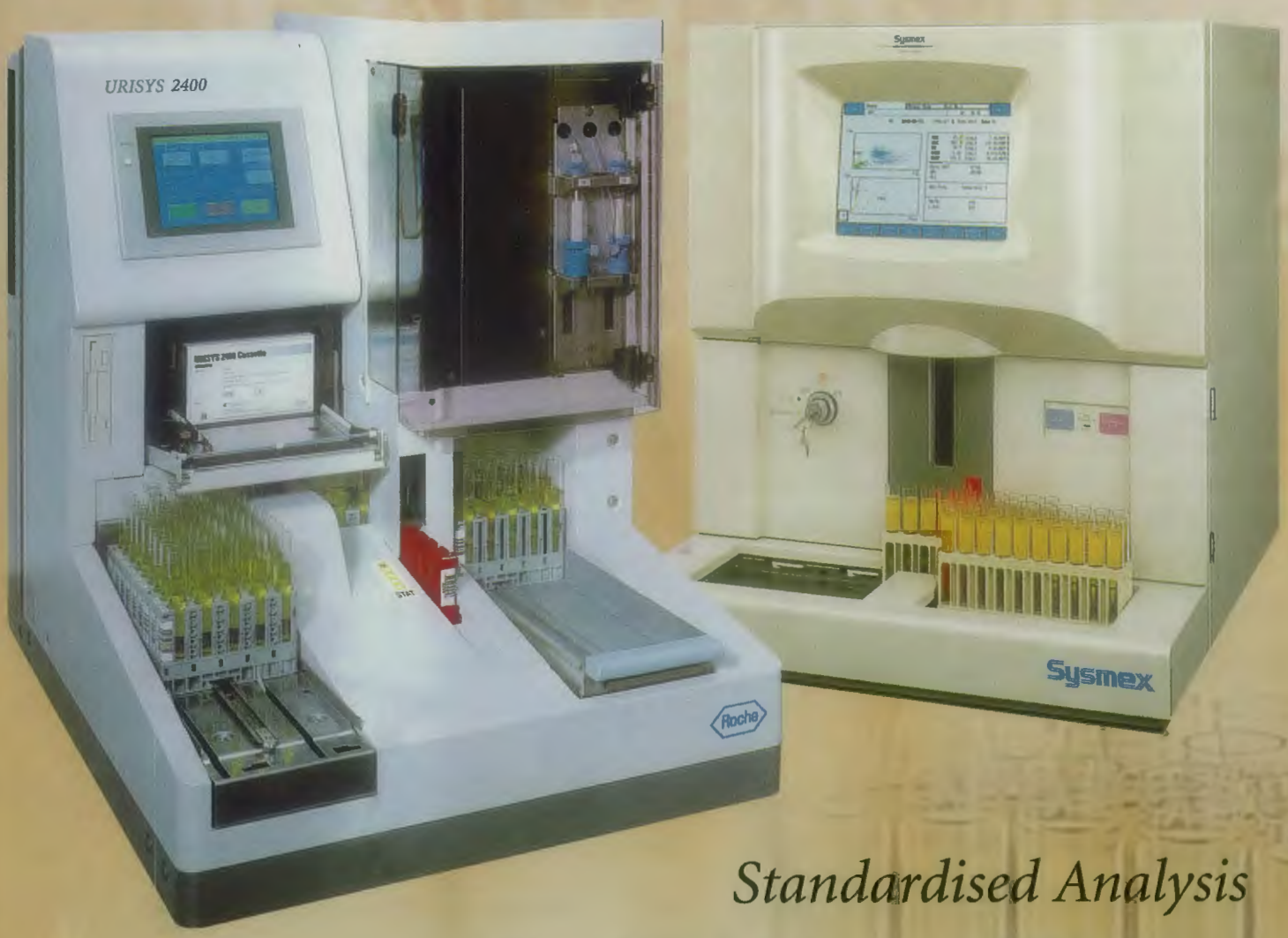

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SOUTH ISLAND SEMINAR 2002

This years South Island Seminar (SIS) was held at Tekapo on the 16th of March. There was what can only be described as an overwhelming response to the event with 166 registrations. There was a wide scope of presentations, which covered almost all laboratory disciplines. The presentations were all of a high standard and showed the hard work that had gone into them. The relaxed nature of the audience seemed to bring the best out of our presenters. The Tekapo Community Hall bought us all back to grass roots (in all senses!), but added to the event with its isolation and fantastic setting nestled tightly under the Southern Alps. The presentations from the day are all summarised below.

Something FISHY with Cytogenetics
Judy Moodie Healthlab Otago

Judy gave us an insight into the wide world of cytogenetics. We now all now know how cells are cultured so the metaphases can be observed and chromosome analysis done. This is a very labour intensive field and obviously takes a lot of very specialized skills. FISH techniques were explained and a case study was presented showing the importance of accurate diagnosis.

Paroxysmal Nocturnal Haemoglobinuria
Peter Hugo Healthlab Otago

Peter reviewed an article published by Dr Peter Hillman from the *British Journal of Haematology* 2000 **108** 470 - 479. He summarised the pathology of the disease, its diagnosis and present understandings of the molecular deficiencies that cause the characteristic symptoms.

Life in the Mortuary
Di Woodford Healthlab Otago

Di shocked us all with her dramitic insight into the life and times of the Mortuary technician. The eloquent way that organs are removed and cuts are strategically made impressed everyone. I suppose it is a little sobering to suggest that we all will end up in a fridge here!

A Story with a Ring to it
Frances Cadman Medlab South Christchurch

Frances explained to us the epidemiology of the protozoan malaria, which infects humans. The life cycle from the female anopheles mosquito as the vector to the human erythrocytes as the host for the growing trophozoites was all described. The identifying characteristics of each malarial species were outlined and case studies shown. And as Frances outlined malaria is not endemic to the West Coast despite what some GPs think!

Same virus - different disease
Lisa Tweedie Canterbury Health Laboratories

Lisa presented an overview of Varicella-zoster virus infections. The pathogenesis and clinical presentation of Chickenpox (the acute form of the viral infection) was described and compared with Shingles (the reactivated form of the same virus). Latency and reactivation, potential complications and methods of detection by culture, PCR and serology were also described.

Spiralling out of Control
Alvin Chua Canterbury Health Laboratories

Alvin captured our attention with an interesting case study.

A 43 yr old male had been unwell for several days with headache, abdominal pain, rash, red eyes and yellow skin, also fever and chills. Tests results showed abnormal LFT's and CRP, the blood culture and initial leptospira tests were negative. The CSF showed low numbers of wbc and elevated CSF glucose but no bacterial growth.

The patient showed signs of possible leptospira toxic shock. Samples for PCR were collected (urine, blood and CSF). Leptospiral PCR tests were positive. Further serology testing at 11 days showed positive results.

An interesting case study was presented which highlighted the usefulness of molecular diagnostic techniques in clinical testing and the need to use all available information (clinical details, other test results, patient history) to make the appropriate decisions for test selection.

It's a Dirty World we live in
Rob Siebers Wellington School of Medicine and Health Sciences

Rob gave us a summary of the asthma research that he has been involved with. A population of primary school children from both a small town and surrounding rural farming areas were tested for allergic disease. The findings that rural children had higher rates of allergic symptoms and disease seemed to raise the eyebrows of the audience. This was a most interesting topic and certainly generated a lively response. Those dam dust mites certainly have a lot to answer for in New Zealand.

Epidemiology of Meningococcal Disease
Youssef Dabous Healthlab Otago

Youssef highlighted the incidence of meningococcal disease in New Zealand. He outlined the fact that we are in the 11th year of an epidemic which has claimed the lives of many young people. Preventative measures were outlined in typical Youssef manner which the audience found very amusing.

Treatment of Meningococcal Sepsis
Dr Jim Faed Director Dunedin NZBS

Jim was our guest presenter and started by outlining the disease state caused by overwhelming septicaemia. The importance of the control of Disseminated Intravascular Coagulation (DIC) is seen as a major factor in disease progression. An overview of the importance of Protein C consumption was outlined. Jim presented case studies from patients treated at Dunedin Hospital outlining the use of Protein C levels to determine the level of treatment used. Once Protein C levels dropped below 30% an aggressive plasma exchange programme was implemented. Problems of this treatment from the size of cannulae (often small children have the disease), to the availability of fresh plasma were all explained. This presentation was an excellent insight into the clinical - laboratory overlap. Just to show how much Jim is in demand he got beeped regarding a plasma exchange case during this presentation!

It's all in your head
Katrina Monaghan Canterbury Health Laboratories

Katrina presented a series of case studies to illustrate the use of CSF cell counts, biochemistry and patient history to rationalise and select appropriate PCR diagnostic tests in meningitis or encephalitis cases. The clinical history of the patients (e.g. rash, recent herpes infection,

recent activities etc) was used in conjunction with CSF results to direct the laboratory in the choice of PCR testing required. Katrina showed that in most cases this produced timely and productive results allowing for rapid, appropriate management of the patients

Establishing Quality Control Procedures for Collecting and Labelling of Laboratory Specimens
Stephen Silk Hutt Valley Hospital

Stephen talked about the need for documentation of problems associated with collecting and labelling of specimens. The statistics he had collected showed specimen problems that we are all too familiar with. The need for a system of documentation outlining the correct methods of collection is essential. Problems are always going to occur but as Stephen quotes, 'a specimen rejection policy must meet the needs of your customers as well as your conscience'.

Phlebotomy - "The new SIG on the block"
Ailsa Bunker Middlemore Hospital

Ailsa inspired us all with her enthusiasm for the phlebotomy SIG. As was outlined there is presently no nationally recognised qualification for phlebotomy. The framework has been put in place and now the aim is to get something in place in the near future. A dedicated team of laboratory people from all over New Zealand is meeting to nut this out. I am sure the team has the goods to deliver on this one.

CPD Why Why Why?
Graeme Broad Medlab Hamilton

Graeme took a serious and not so serious look at the CPD programme. He balanced his arguments by using clear and not so clear examples. He teased us with cans of speights and made all the audience laugh about our uncontrollable traits. And no Graeme, the Chiefs will not win the Super 12!

Current Trends in Point of Care Testing
Chris Bugden Canterbury Health Laboratories

Chris explained his involvement in the Point of Care (POC) testing in the Canterbury Health region. This is a field that is expanding but with it comes a logistical nightmare. Coordinating training logs and the control of equipment calibration and accuracy are typical problems. There have been problems in the past with POC equipment companies dealing direct with hospital wards without the laboratory even being involved. With Chris employed as the POC coordinator this should make these sort of practises a thing of the past. Watch this field it is only going to expand.

NZIMLS Discussion
Trevor Rollinson Secretary/treasurer NZIMLS

Trevor introduced Jan Deans and Ann Buchanan as the southern region delegates and Harold Neal outlined the implications of the new Health Advocacy Bill to us as medical laboratory workers.

The meeting was closed and the job of organizing the 2003 South Island seminar was passed on to Shona Johnstone and the able team from Nelson Hospital.

The Med-Bio Ltd award for the best first time presenter went to Youssef Dabous (pictured left)

The Abbott award for the best overall presentation went to Alvin Chua.

Thanks to all the sponsors for the day and night, Med-Bio Ltd, Abbott Diagnostics, Roche Diagnostics, Beckman Coulter, Global Science, Biolab and Medlab.

Then the fun began...!

Terry Taylor
SIS Convenor 2002
Healthlab Otago

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Winner of the Med-Bio Journal Award for the best original article in the last issue of the Journal (April 2002) goes to Rebecca Wilson from Southern Community Laboratories, Dunedin for her article: Is the TRACE spectrophotometric method specific enough to replace flame photometry for the analysis of lithium?
NZ J Med Lab Science 2002; 56: 9-11

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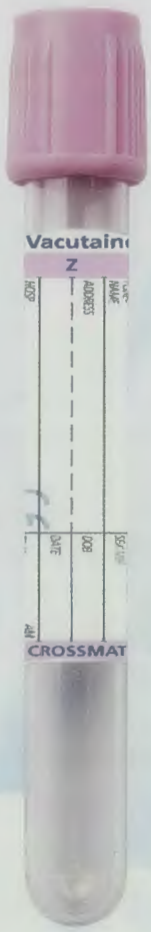
Draw volume:	7ml
Carton Size:	1000



- Special PINK cap colour for unique identification of all transfusion samples
- Special block style label with 'Crossmatch' clearly displayed and large 75mm label length

BD
8 Pacific Rise, Mt. Wellington, Auckland, New Zealand
Telephone: 64 9 574 2468 Facsimile: 64 9 574 2469

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