Pathos – a tragedy for conventional tissue processing

Tissue processing changed little over the course of the last century until the introduction of microwave equipment into histopathology. Now, the technology that revolutionised work in the kitchen appears set to do the same in one of the oldest disciplines in pathology, as Sue Wollington explains.

In Germany, in 1909, Arendt described the first automatic tissue processor thus: 'This apparatus received pieces of fresh tissue and after due time returned them infiltrated with paraffin – a notable technical achievement'. I wonder how he would have described the piece of apparatus about to be described? So, welcome to the 21st century and to Pathos, the revolutionary automated microwave high-throughput batch histoprocessor.

Method overcomes the last remaining objection to the use of microwave histoprocessors, namely the lack of automation. Most commercial microwave appliances designed for processing tissues have been visually and functionally related to the domestic microwave, apart from those manufactured by Milestone, an innovative Italian company that has for years been in the forefront of microwave technology. The many global patents have made its equipment second to none. As many readers will be aware, current government initiatives seek to improve tumourṇards, reduce costs and be more environmentally friendly. The Milestone family of histoprocessors has fulfilled these aims but did not include automation. Now, Pathos has changed all that. The implications are amazing: higher throughput, same-day processing for same-day diagnosis, less reagent usage and a greener and safer working environment.

Pathos process

So, how does Pathos achieve all this? Pathos technology is based on patented procedures pioneered by Milestone which reduce the steps in processing tissue to three or four, rather than the traditional 12 to 14. Pathos is a manageable size (800 x 1000 x 2000 mm), which will fit through most doors and easily into most laboratories. It has in-built filtration and agitation and requires one power socket; it has a safe working area with a safety shield and reagent drawer; and functionality is controlled by a Windows-based operating system with a colour touch-screen terminal loaded with Milestone's user-friendly HistoEasy software.

It is an open system that can utilise reagents normally held in the laboratory. These are the fixatives of choice, alcohol

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(Industrial methylated spirit (IMS)), isopropyl alcohol and histology-grade paraffin wax. Another option is to use Milestone’s JFC, a one-step solution that is useful for very fatty tissue (e.g., breast tissue) and further reduces the number of processing steps and the amount of reagent used.

Pathos has only six tanks in the reagent drawer, each with a 5 L capacity. One of these is generally spare or filled with a fluid for occasional use (e.g., formalin-free fixative such as FineFix) or a one-step dehydrating and clearing reagent (e.g., JFC). The machine can upload reagents automatically at the touch of a button from most types of container, reducing manual handling and saving operator time. Pathos documents all steps in each program and fulfills GLP/GM and ISO requirements.

The HistoEasy software also alerts the operator of the need to change reagents, either in relation to the number of cassettes processed or the number of cycles run. Reagent in two of the tanks is moved forward during changes and reused as rinsing reagents, thereby further reducing the amount of new reagent used. Wax is kept molten in a separate retort and remains uncontaminated, which means it only needs to be topped up, filtered or changed according to good housekeeping protocols. No flush cycle is required, not only saving on reagents but reducing the downtime normally associated with conventional histoprocessors.

Pathos achieves this in much the same way as do other members of the Milestone family of histoprocessors. The first optional step is rapid fixation by microprocessor-controlled microwave irradiation. Two quick rinses in alcohol follow, then rapid dehydration/clearing of tissue takes place under the same conditions, followed by ‘soft’ microwave evaporation under vacuum. This removes all traces of solvent without damaging the tissue. The final step is impregnation in paraffin wax. Operation is easy, just select the program on the touch screen, press start and walk away.

Pathos flexibility
Cassettes can be loaded in three layers, each holding 70 cassettes, making a total capacity per run of 210 cassettes. Sensors located in the wall of the processing retort permit less reagent to be used if only one or two layers of the rack are full. These data are stored by the reagent management system and contribute to the extended life of the reagents.

Another benefit of the Pathos processor is the ability to process different thicknesses of block at the same time. Tissue sizes can be mixed as long as processing time is set for the thickest sample size present. Smaller samples will not be harmed by over-processing and, once embedded, blocks are as easy (or easier) to cut than those subjected to conventional processing. This is due to the fact that the tissue has not spent long periods of time in harsh chemicals, making it over-hardened or brittle.

The ability of the processor to cope with tissue samples up to 5 mm thick is
a huge advantage. It is extremely difficult to cut unfixed tissue at a thickness of 2 mm; however, a special trimming board is also available that makes it easier to cut block thicknesses between 1.5 and 5 mm, helping to maintain a realistic standard block size. In addition, special cutMATE tools can be provided that are held like forceps to enable consistent thicknesses to be produced. Their use is a matter of personal choice and by no means a necessity in order to achieve perfect results. As with all microwave equipment, the use of all-plastic cassettes is essential; however, the user is not restricted to one particular supplier.

Although tissue thickness can be mixed, the best use of Pathos is achieved by processing smaller blocks first, as this permits a more even spread of work during the day and eliminates the need for costly out-of-hours working. Pathos also has a delayed start facility, which enables processing to be carried out at any time of the day or night.

As a guide, small biopsies can be processed from almost fresh in one hour, and tissue in standard-sized cassettes in three to four hours. Furthermore, it is possible to process tissue in super cassettes (eg fixed brain at just over 6 mm thick) in 4.5 hours by adapting one of the programs. Thin blocks and needle biopsies do not tend to curl, as is common with conventional processing, which makes embedding and sectioning easier.

Embedding has always been something of a bottleneck in the work of the histopathology laboratory. Staff often arrive early in the morning in order to complete this so that sectioning and staining is not delayed. Some would advocate automatic embedding but with Pathos this is unnecessary as there is no bottleneck in the first place. Blocks are ready to trim throughout the day or, more importantly, whenever staff are available to deal with them.

Pathos benefits
Pathos has benefits for everyone. For the person trimming, there is no need to trim blocks between 1.5 and 2.5 mm, and standard trimming procedures do not present a problem. If Fine Fix is used as the fixative then the working environment is greatly improved and the potential for molecular techniques to be carried out on the tissue is greatly enhanced.

In addition, there is the ability to process according to workload requirements and thus remove embedding bottlenecks and provide the facility to process a large number of cassettes on the same day. There is also considerable reduction in reagent usage, less downtime because there is no flush cycle, less manual handling and a better working environment because there is no need to use xylenes in the processing schedule.

For the pathologist, benefits include same-day diagnosis for timely patient care, a more even, less stressful workload and the ability to meet achievable cancer targets. For the administrator or budget-holder there are reduced reagent costs, a safer working environment and less pressure from clinicians.

The most significant benefit, however, is to the patient, who can have results the same day, reducing stress to them and their families from waiting to hear the results of tests that often have frightening consequences. It is all too easy to forget the patient while concentrating on the work and how much it costs; however, Pathos redresses the balance, providing benefits for biomedical scientists, clinicians and patients alike.