Mohs and the benefits of new embedding and staining systems

Guy Orchard reports on preliminary work to assess the benefits of the latest equipment designed to facilitate frozen section preparation and staining in a laboratory supporting a Mohs micrographic surgery service.

Mohs practice in the UK has been expanding steadily over the past decade. The incidence of skin cancer, in particular non-melanoma skin cancer (NMSC), has been steadily increasing. The most significant tumour type contributing to this increase is basal cell carcinoma (BCC). According to statistics from the British Skin Foundation website, BCC accounts for >80% of all the skin cancers recorded in the UK. It remains the most common skin cancer with large numbers of the Caucasian population likely to develop a BCC at some point in their lives. These tumours can occur anywhere on the body, but are more commonly seen in sun-exposed sites such as the face, head, neck and ears. It is also the case that BCCs can occur at the sites of former burned tissue, scars or ulcers that have damaged the integrity of the skin.

As a junior biomedical scientist, I recall seeing histology request forms of patients with BCCs and for the large part they were all patients in their 60s or over. Perceptually, I classified this particular common tumour as a cancer of older age. However, as time has gone by, we have seen the incidence of NMSC rise, primarily due to increased levels of exposure to the main causative agent, sunlight, more specifically the ultraviolet (UV) spectrum of light involving the wavelengths of UVA and UVB.

I manage a large Mohs laboratory, one of the largest of its type in the UK, and I see BCCs on patients in their early 30s and 40s. Admittedly, the large majority of these younger patients are strict 'sun worshippers' and also partial to the use of sun beds to top up their tans. The imperative observation is clear, in that the number of BCCs that pathology laboratories will be dealing with will continue to increase for the foreseeable future. This is in spite of a much clearer understanding of how we all should be protecting ourselves from the long-term effects of sun damage to our skin.

Mohs procedures in the UK are mostly confined to the surgical removal of BCCs and, to a lesser extent, squamous cell carcinoma (SCC) and lentigo maligna melanoma (LMM) from facial sites, using frozen section analysis. In the USA, Mohs practice is largely a private practice concern, so Mohs can be performed on almost any anatomical site. Classically, Mohs procedures offer the benefit of minimal excision margin clearance and therefore improved preservation of the surrounding uninvolved tissue. In addition, complete tumour clearance following Mohs procedures is assured, with accuracy values of over 95%, which remains unmatched by any other patient management approach in skin cancer treatment.

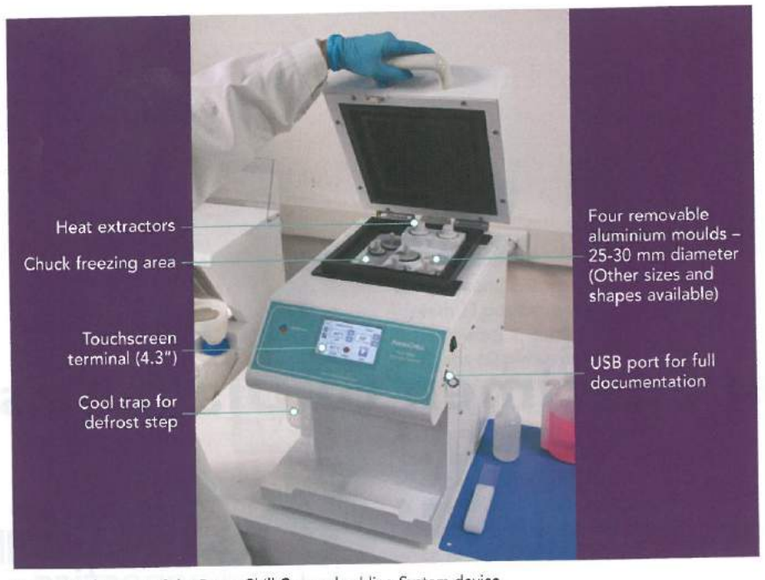

Fig 1. Key features of the PrestoChill Cryoembedding System device.

Equipment for frozen section analysis

Traditionally, we have regarded the cryostat as the most significant piece of equipment involved in the process of frozen section analysis. Indeed, there have been some great improvements in cryostat design in recent years. However, this is only part of the story as far as improving speed, efficiency and quality of frozen section assessments.

In June 2016, Menarini approached Viapath, St John's Institute of Dermatology to undertake an evaluation of some new pieces of equipment, the PrestoChill Cryoembedding System and the Presto automated processor/stainer. These two new pieces of equipment are distributed by Menarini and are highly innovative in design and most importantly have efficiencies of speed over conventional manual methods of frozen tissue section production and staining.

Manufactured for use in all forms of frozen section work, the PrestoChill Cryoembedding System and the Presto automated processor/stainer are designed to be used in a combined approach to the histological assessment of frozen section production and subsequent haematoxylin and eosin (H&E) staining. This includes their application in urgent frozen section production for operative assessment in diagnosis and margin clearance. In addition, they can be used in a host of special histological procedures requiring more precise tissue analysis. These procedures often encompass issues of increased complexity, either in terms of issues relating to the composition and nature of the tissues or the need for accuracy of tissue orientation during the freezing process. In the case of Mohs procedures, both of these complex issues are common challenges faced by histologists worldwide.

Traditionally, there has been a host of methods employed to improve efficiency and accuracy of tissue embedding for cryotomy. In conventional cryostats, tissue is embedded for frozen section by placing it face-up on a chuck in embedding medium. The tissue chuck is then placed on a metal cooling plate, often with a heat extractor applied to the top of the tissue to extract heat more effectively and flatten the surface of the tissue to create a flat plane. This method is fraught with problems, including tissue distortion and, most significantly, uneven embedding at the chuck face, leading to inadequate and inconsistent tissue orientation. These issues become more apparent with smaller and more friable pieces of tissue.

The additional pressure of producing adequate sections fast to meet the surgeon's turnaround times for reporting has led to increased interest in more effective alternative freezing methods. In this regard, the use of liquid nitrogen has gained some popularity. This has the benefit of reducing freezing artefact as the process is faster. However, it is often less precise in terms of accuracy of tissue orientation.

Fig 2. Tissue on the embedding platform (either spatula or glass disc) to ensure optimal orientation prior to loading into the PrestoChill Cryoembedding System device.

A wide range of devices currently produced has concentrated on the flattening of tissue at the surface of the chuck prior to tissue sectioning in an attempt to improve accuracy. However, this is only part of the overall requirement, as ideally the tissue needs also to be embedded in the right plane across its entire surface. Simply applying a flattening force across the tissue surface during the freezing is not always the answer, as tissue composition often affects the ability to lay such tissue flat without curling or folding. The issue is a complex one.

The new generation of embedding devices will need to combine the benefits of rapid freezing with concepts of tissue flattening and good orientation. Orientation of tissue will also depend on good visualisation as well as manipulation of the tissue during the freezing process. Traditionally, this has relied largely on the skill of the practitioner but the new methodologies are introducing improvements in this area, too. So, how can a modern piece of equipment manage these variables and perhaps improve efficiency and speed?

Comparative assessment

A total of 250 blocks were embedded on the PrestoChill and stained on the Presto benchtop processor/stainer. All the tissues selected were tested against conventionally frozen tissue to compare and contrast quality, speed, efficiency and accuracy. Both devices presented a high degree of innovative design and embraced the ideals of a fully automated solution for frozen section preparation and staining.

The key features of the PrestoChill device are stated as offering:

- excellent cryoembedding of small fragments including delicate tissues from Mohs surgery
- no freezing artefacts
- rapid freezing time of only 60 seconds
- no liquid nitrogen, CO_2 or isopentane
- perfectly 'flat plane' surfaces

The specifications of the device that enable these features to be delivered include:

- standardised freezing at –40°C for all types of tissue
- reduced trimming time at the cryostat due to improved planarity of the specimen
- employs 'face down' embedding techniques to enable perfectly 'flat plane' surfaces to be achieved
- eliminates retraction and compression artefacts in tissue, principally due to improved speed of freezing
- HEPA cap filter to improve operator safety

Fig 3. The freezing embedding chambers/wells of the PrestoChill Cryoembedding System. Two wells are 25 mm in diameter and two are 35 mm in diameter.

- the mechanism of freezing is environmentally friendly and employs advanced technology involving helium gas as a refrigerant in a sealed stainless steel chamber; therefore, there is no standard compressor, which is often the component in traditional freezing devices that can go wrong, and no CFCs are released.
- the device has a fully automated defrost feature
- an easy-access USB port ensures that upgraded software can be installed and it also allows the downloading of event logs for full documentation and improved equipment management – this is a growing requirement under the new UKAS ISO 15189 regulations
- by modern freezing equipment standards the device is small in size, with a small footprint and is therefore more applicably suited in a Lean working environment.

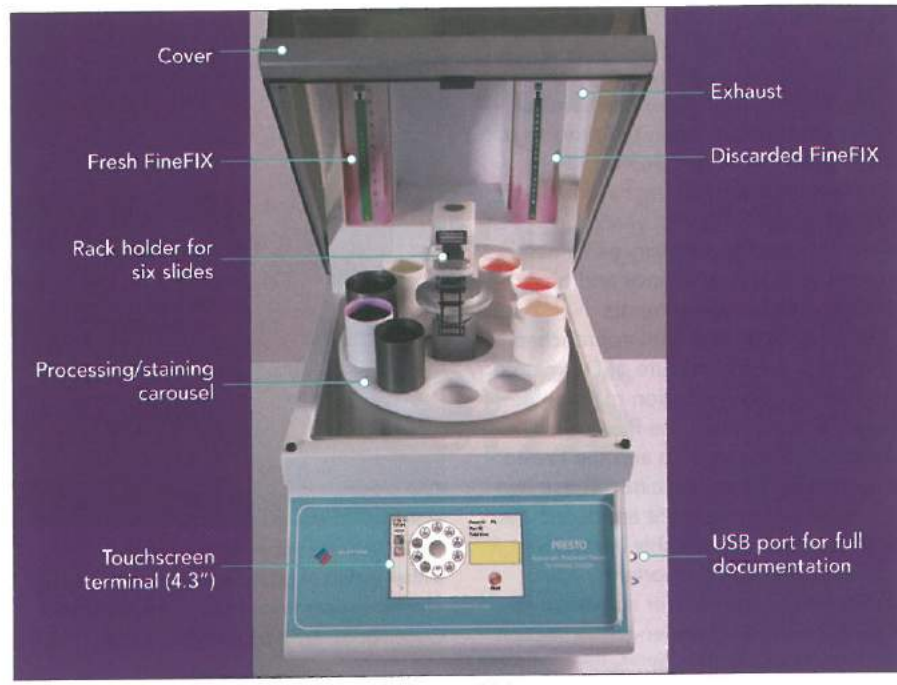

Fig 4. Key features of the Presto automated processor/stainer.

The key components and features of the device are illustrated in Figure 1. An automatic defrost cycle reduces the formation of ice and is facilitated by a heater embedded in the freezing platform, along with a vacuum pump to extract water vapour from the embedding chambers. Any vapours are condensed and collected in a cold-trap placed in the front of the unit for easy handling. The HEPA filter feature is a nice health and safety extra of this device. The integrated software can enable defrost cycles to take place when the machine is in 'off' mode, yet it can be automatically restarted swiftly and effectively, ensuring the device is available for use as required.

Like the equipment itself, the consumables are also innovative. The use of an optimising cryoembedding compound, called MCC, in conjunction with PrestoChill paper on which to rest the tissues before freezing, are new developments (Fig 2). The speed of embedding was confirmed to be no more than 60 seconds with all the tissues tested. There are also four embedding wells which increase the speed at which multiple tissue slices can be assessed.

We assessed fresh de-bulk tissue directly on the PrestoChill device and compared these with our conventional, manually operated procedures. All blocks exhibited optimal 'flat plane' surfaces, making sectioning in the cryostat swift and effective. Uneven or irregular tissues composed of multiple components, such as fatty tissue and cartilage, posed some difficulties in orientation; however, trials to assess a slight modification to the visualisation and manipulation of the tissue prior to freezing in the PrestoChill device have shown that complex tissues can be embedded correctly and effectively.

The device was easy to maintain and operate. The overall quality of freezing was much faster than conventional procedures, with additional improvements of flat, easy-to-trim block surfaces and reduced embedding medium usage. The ability to embed more than one block at a time also provides efficiencies of speed.

The PrestoChill has four embedding well chambers (Fig 3). There are two routine embedding well sizes (25 mm and 35 mm in diameter) and these cover the majority of tissue samples examined in an average Mohs laboratory. An interesting option is that wells of deeper depths can be used and easily inserted to replace the normal wells provided with the machine, which is beneficial when embedding denser or thicker tissue pieces. The inclusion of a large-diameter well would benefit the design further, as this would cover the full repertoire of tissue pieces dealt with in a Mohs laboratory.

Tissue pieces that, when sectioned, cover most of a glass slide with a single section are encountered occasionally and so a well with a wider diameter to enable such tissues to be embedded would be beneficial. If this well could be interchanged with the conventional well size diameters when not in use then that would be an additional advantage.

The device performed effectively on all the tissues embedded and results were of a consistently high standard. The benefits of speed make this automated platform an attractive development in tissue cryotomy.

Fig 5. Presto automated processor/stainer slide rack showing a six-slide capacity.

Innovative staining

Once the tissues have been embedded and sectioned, the use of the Presto processor/stainer device provides additional innovative design features (Fig 4), which include:

- four-minute fixation, H&E staining, dehydration and clearing time
- staining a maximum of six slides per rack, enabling high productivity
- fully automated platform with flexible modifications of staining protocols for more tailored needs
- open staining reagent platform
- downloadable documentation through a USB port for logging/tracking events
- inbuilt exhaust system
- standardised results
- less operator variation with quality results.

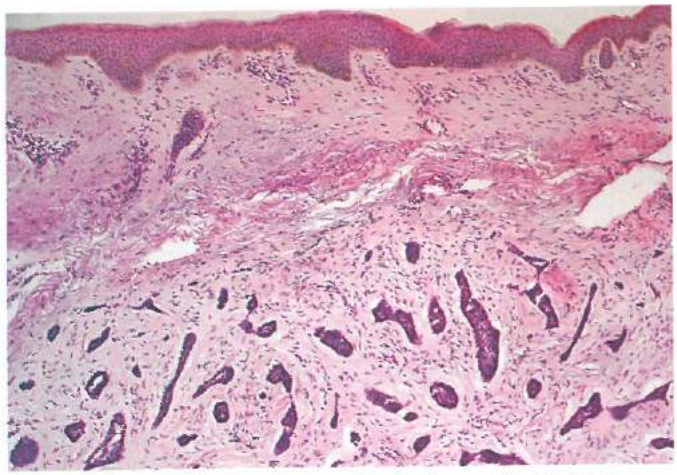

Fig 6. Morphoeic basal cell carcinoma pattern in tissue embedded on the PrestoChill Cryoembedding System and stained on the Presto automated processor/stainer devices (H&E stain, original magnification x20).

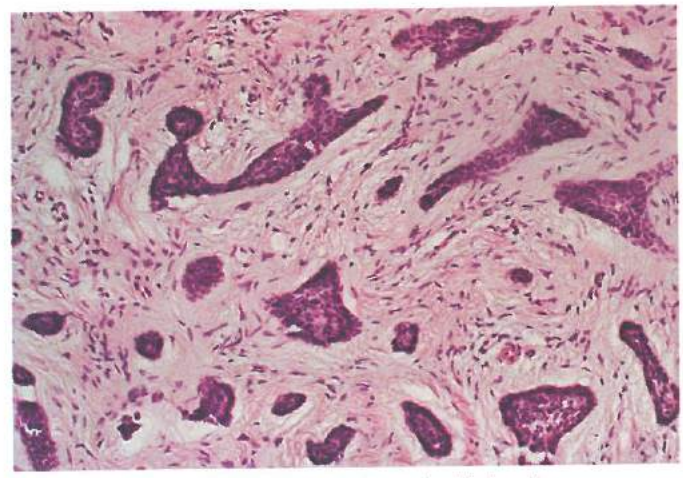

Fig 7. Islands of basal cell carcinoma in tissue embedded on the PrestoChill Cryoembedding System and stained on the Presto automated processor/stainer devices (H&E stain, original magnification x40).

The specifications of the device also include a smaller footprint than most modern automated or semi-automated staining platforms. The key specifications include:

- 4.3 inch touchscreen terminal
- one USB port
- processing/staining carousel
- compact overall dimensions (height 80 cm/31.5 inches [with the cover open 87.5 cm/34.4 inches], width 40 cm/15.7 inches, and depth 92 cm/36.2 inches)
- weight of 60 kg (132 lbs).

In addition, there are also innovative, environmentally friendly consumables associated with the device, most notably the proprietary FineFix (formalin-free fixative) solution.

The device can handle six slides (Fig 5) at a time and the fixation, dehydration and clearing steps were consistently achieved within one minute on all the tissue sections stained. The H&E staining itself is extremely fast and efficient, and elimination of lengthy washing steps enables the speed of staining to be achieved on average in three minutes.

Programme adjustments can be made easily to lengthen staining steps to account for operator choice and surgeon preferences for staining intensity and contrast. It also permits adjustment of staining times when dealing with thicker tissue sections. There exists several programme options on the device to achieve this, making adjustments easy and swift. You are also able to add your own programmes and protocols. There is also economy of usage as the FineFix can be used for up to 40 staining batch runs before being discarded, and the H&E staining solutions need to be changed at the end of the day, depending on usage.

The staining results (Figs 6 and 7) were comparable to the quality of those achieved using conventional tissue staining. Only minor staining artefacts were evident, some of which included tissue squames. Once again, this compared favourably with the results of the conventional staining methods employed. There was no evidence of inadequate dehydration or inadequate clearing of the tissue sections. The key benefit was a staining time approximately three to four times faster than existing semi-automated staining techniques, and time-saving improvements were evident in all steps of the staining procedure on the Presto processor/stainer device.

Exciting developments

The innovative, complementary devices described here represent very exciting developments in the field of fresh frozen section preparation and staining. They offer a comprehensive approach to tackling the whole range of technical challenges faced in producing good-quality, well-stained frozen sections in a rapid-response format. The careful attention to detail and design, with improved consideration of options to advance efficiency, mark both devices out as being of some merit. Clearly designed to be used together to ensure optimal time-saving performance, these devices are also highly automated, which brings benefits to any frozen section laboratory.

Within the UK, the fastest growing cellular pathology specialty in which such devices are likely to gain exposure has to be within the field of Mohs procedures, mainly because of the evident improvements in speed and efficiency. It should, however, be pointed out that this does not restrict application of the devices to other areas of frozen section work.

The clean and compact nature of both devices also embraces benefits in the Lean environment of laboratory design. Relatively speaking, the footprints of both devices are small compared to conventional staining and tissue embedding equipment. Maintenance requirements are also minor, and the reagent management process is simple, fast and efficient. The ability to download tracking logs also lends use of the devices to equipment management initiatives that are increasingly being required from UKAS ISO 15189 standards of equipment management and performance in all laboratories across the UK.

The benefits of an almost fully automated process improve standardisation of practice and ultimately will improve overall quality of performance. The options to adjust staining times swiftly and easily and thus enable a more tailored approach to staining gives the Presto processor/stainer some added advantages.

Finally, currently there are no clearly defined UK NEQAS standards of quality performance in Mohs procedures, or indeed in fresh frozen section production. This situation is under review and as current practice of performing frozen sections is highly variable, with application of a wide assortment of manual and semi-automated procedures, it follows that devices that can standardise these steps will be popular.

A brief glance over the past 20–30 years within the field of tissue and cellular science has shown us how automated platforms have been embraced and have standardised practices generally for the better, particularly fully automated platforms. Perhaps it is now time to review this in the light of our frozen section work, too. IP

Dr Guy Orchard, Consultant Grade Biomedical Scientist and Laboratory Manager, Viapath, St John's Institute of Dermatology, St Thomas' Hospital, London.