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New embedding and staining systems PrestoCHILL and Presto stainer for application in the advancement of Mohs micrographic surgery

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ABSTRACT

Background: Mohs micrographic surgery (MMS) involves evaluation of frozen tissue sections to determine complete circumferential and deep tissue margin clearance of skin tumours. PrestoCHILL and Presto stainer devices are two new innovative tools which bring benefits of automation, speed and efficiency to the preparation of frozen section analysis in MMS. The devices were assessed at Viapath's Tissue Science Mohs laboratory at Guy's Cancer Centre.

Material and Methods: A total of 279 samples from 10 anatomically different facial sites. These included nose (95), lip (24), forehead (47), cheek (25), eyelids (34), temple (9), chin (15), ear (17), scalp (6) and neck (7). These were analysed using both devices simultaneously.

Results: The PrestoCHILL device was measured for accuracy of tissue orientation by determining how many of the cases examined microscopically had complete margin and full epidermis preservation. The precision and reproducibility of the Presto stainer was evaluated by the consistency of achieving ideal standards of staining quality as defined by the department's internal quality control check, on stained sections examined and evaluated microscopically. The mean (standard deviation) score for accuracy for the PrestoCHILL across all tissue facial sites was 93.5 (11)%; the mean (standard deviation) score for precision/reproducibility of the Presto stainer was 96.5 (11)% (both $p < 0.05$).

Conclusion: The devices combined offer an assured accuracy and precision performance, which is reproducible across all facial tissue types examined. The devices represent a key step forward in the introduction of improved automated embedding and staining procedures within MMS.

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KEYWORDS
Mohs; PrestoCHILL; Presto stainer; frozen sections

Introduction

Mohs micrographic surgery (MMS) is a rapidly expanding part of most modern day histopathology services. As a technique it is practiced throughout most of the western world as a treatment used for the removal of common skin cancers, mainly, although not exclusively from facial sites. The incidence of skin cancer and in particular, non–melanoma skin cancer (NMSC), has been steadily increasing. The most significant tumour type contributing to this increase is the basal cell carcinoma (BCC). According to the statistics from the British Skin Foundation website [1], BCC accounts for (>80%) of all the skin cancers recorded within the UK and remains the most common skin cancer. These tumours can occur anywhere on the body, but are more commonly seen in sun exposed sites, such as the face, scalp, neck and ears. MMS procedures in the UK are mostly confined to the surgical removal of BCCs and to a lesser extent squamous cell carcinomas as well as other rarer tumours. Most significantly as a surgical procedure MMS has the benefit of potentially minimising the amount of skin removed 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 [2–4].

Traditionally laboratory Mohs procedures are dependent on both speed and efficiency for provision of good quality, well-stained slides [5]. The cryostat plays a key role in this overall procedure, although the equally important and sometimes quite difficult process of tissue orientation, which is so crucial to the success of the Mohs techniques, should not be underestimated. Failure to orientate the tissue correctly can cause a host of problems in interpretation as the technique relies on being able to demonstrate the complete circumferential and deep tissue margins. Similarly staining procedures that are fast and efficient can save considerable time in the overall working day, in most busy Mohs clinics. This in turn can benefit patient care due to reduced waiting times. Here, we report on the evaluation of two new and innovative pieces of equipment for use in frozen section preparation and staining.

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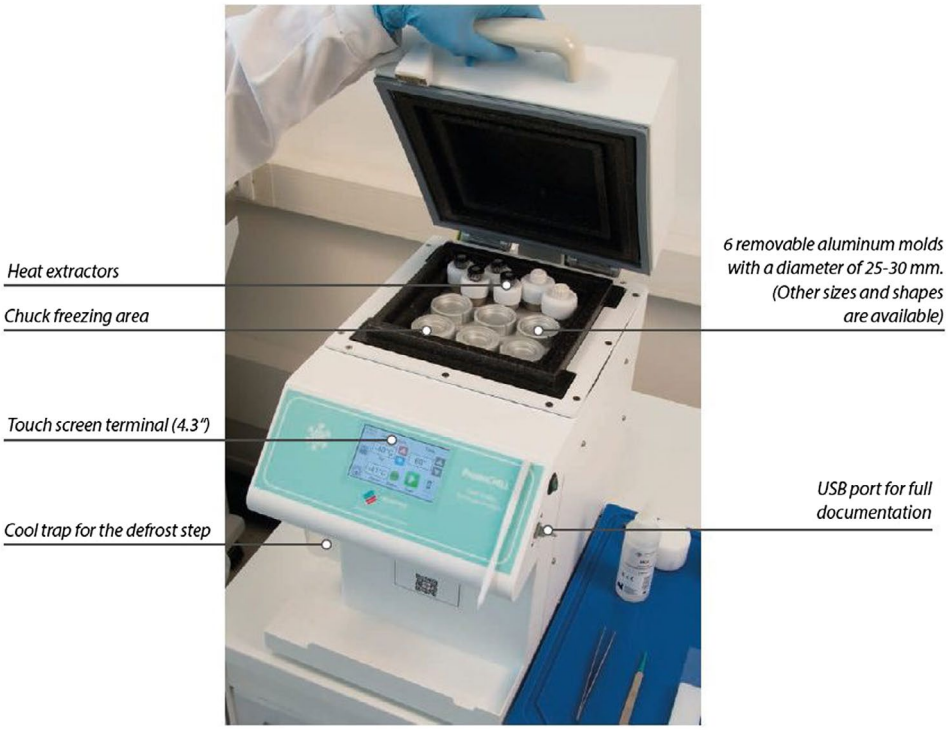

Figure 1. Showing the key features of the PrestoCHILL cryoembedding system device (provided by A. Menarini diagnostics).

Figure 2. Showing on tissue a glass disc to ensure optimal orientation prior to loading into the PrestoCHILL Cryoembedder System device.

Material and methods

A total of 279 frozen tissue samples from 10 anatomically different facial sites were analysed. These included nose (95), lip (24), forehead (47), cheek (25), eyelids (34), temple (9), chin (15), ear (17), scalp (6) and neck (7).

All the staff using the PrestoCHILL and Presto Stainer devices was given training guidance on how to use them (Figures 1–5). The devices were assessed for accuracy of embedding and precision/reproducibility of staining. In terms of accuracy, this was accessed by examining microscopically if full margins and complete epidermis were demonstrated on all the block samples tested by the first and second authors (GEO and MS). The precision and reproducibility of the Presto stainer was evaluated by the consistency of achieving ideal standards of staining quality as defined by the department's internal quality control checking procedures (Chart 1), performed on stained sections examined and evaluated microscopically by the first and second authors (GEO, & MS). Any stained slides that did not meet the desired staining outcome were deemed as fails. The data were then compared using the chi-squared test on Microsoft Excel and t-test and correlation on Minitab 17 (Coventry, UK). In addition, comments were made on the speed of tissue orientation on the PrestoCHILL compared to the standard procedures employing cryosprays. The PrestoCHILL Cryoembedding System and the Presto automated processor/stainer (manufactured by Milestone Srl – via Fatebenefratelli 1–5, 24,010 Sorisole (BG) Italy and distributed throughout Europe by Menarini Diagnostics, 405 Wharfedale Road, Winnersh, Wokingham RG41 5RA) [6,7]. These devices are designed to be used in a combined unified approach to the histological assessment of frozen section production and subsequent HE (haematoxylin and eosin) staining. The cryosprays

Figure 3. The freezing embedding chambers/wells of the PrestoCHILL Cryoembedding System. Note two wells are 25 mm in diameter and two are 35 mm in diameter.

were manufactured by (Leica Microsystems (UK) Ltd, Milton Keynes, MK14 6FG, UK) and Cryoembedder (cryoEMBEDDER® System, SLC, 84,121, USA) [8] devices routinely used within the department. The speed of staining of the Presto Stainer was also compared with the department's Linistat Linear Stainer (Thermo Fisher Scientific, 168 Third Avenue, Waltham, MA USA 02451) [9].

Results

The accuracy of the PrestoCHILL device is shown in (Table 1). Statistical analysis of data from all the 10 tissue sites accessed demonstrated that the mean (standard deviation) score for accuracy regarding all tissue facial sites for the PrestoCHILL was 93.5 (11)%.

The results seemed to show no specific tissue types which produced significantly lower scores than any other. Although it was evident that accuracy scores for the ear sites and eyelid areas were less than other sites assessed. This was due to the uneven nature of the cartilaginous connective material present within the ear tissue and the presence of conjunctival tissue in some cases of eyelid blocks, making orientation difficult.

There were minor differences recorded for staining precision/reproducibility results achieved with the Presto stainer across all the tissue types accessed. The mean (standard deviation) score for precision/reproducibility of the Presto stainer was 96.5 (11)%.

The precision of the Presto stainer device are shown in (Table 1).

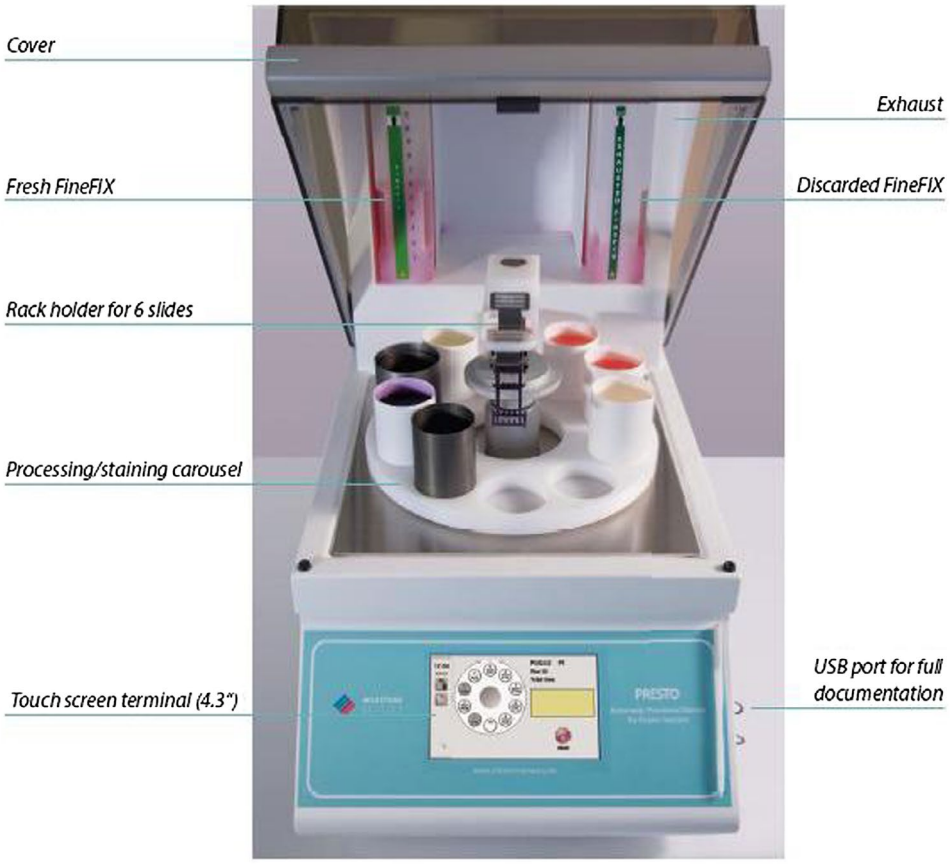

Figure 4. Presto automated processor/stainer, showing key features of the Presto automated processor/stainer (provided by A. Menarini diagnostics).

Figure 5. Presto automated processor/stainer slide rack showing a six slide capacity (provided by A. Menarini diagnostics).

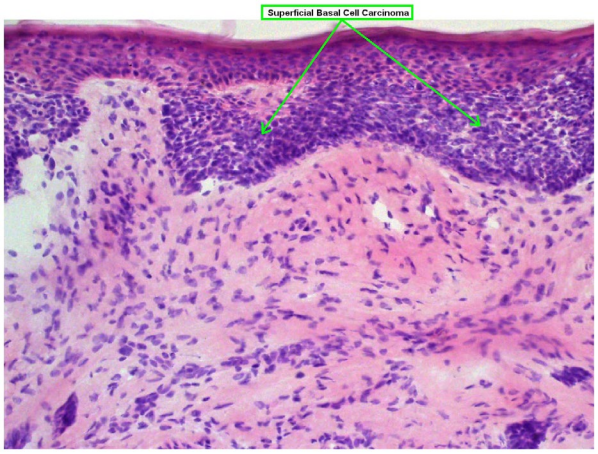

Figure 6. Superficial basal cell carcinoma in tissue embedded on the PrestoCHILL Cryoembedding System and stained on the Presto automated processor/stainer devices (H&E stained original magnification × 20).

For both accuracy and precision/reproducibility, the value for p was < 0.05 by chi-squared test. Although, this varied with type of tissues.

In terms of the information of the speed of procedures, there were some differences. The average speed for embedding on the PrestoCHILL was similar to that of the cryoEMBEDDER and cryospray technique with average time of 4 minutes. With regards the speed of staining, the Presto stainer was three times as fast as the conventional Linistat Linear Stainer, with average times of 5 and 15 minutes, respectively. An important point to note is that the Presto stainer is essentially a batch stainer that can stain six slides at a time but within 5 min with each batch.

Table 1. Summary of total number of tissue types evaluated with accuracy and precision/reproducibility outcomes.

Tissue type	Number of blocks	Accuracy of PrestoCHILL (%)	Precision of prestostainer
Nose	95	94	97
Lip	24	93	98
Forehead	47	95	97
Cheek	25	94	96
Eye socket area	34	85	94
Temple	9	95	96
Chin	15	96	96
Ear	17	93	97
Scalp	6	94	96
Neck	7	96	98
Total	279	93.5	96.5

In terms of evaluation of assessors' comments, the devices were both found to be easy to use, with improvements noted over the more manual procedures and with clear benefits in terms of speed of procedures for staining compared to the existing employed methods. Certain tissues types notably cartilaginous containing tissue and to a lesser extent conjunctival tissue did cause some difficulties with correct orientation, since these tissues do require 'pushing down' when embedding. The use of 1 mm thick glass discs to lay the tissue pieces down flat to aid orientation before placing in the PrestoCHILL device improved visualisation of smaller tissues. This process mimics the manual use of a glass slide, to do the same thing when using the routine cryoembedder procedure. The PrestoCHILL device embedding wells were not large enough to deal with the largest tissue pieces presented, in such instances the tissue needed to be cut into two to enable adequate embedding. This was a rare finding. Both accuracy precision/reproducibility were strongly correlated ($r_p = 0.84$, $p < 0.001$).

Discussion

These results indicate a significant improvement in terms of accuracy and precision of employing the PrestoCHILL and Presto stainer devices over more conventionally used Mohs histological equipment. The accuracy and precision/reproducibility scores were statistically significant and indicated no deviation from data from existing employed techniques. The issue of correct tissue orientation during embedding is not simply about applying a flattening force across the tissue surface during the freezing steps as tissue composition often affects the ability to lay such tissue down flat without curling or folding occurring. The new generation of embedding devices will need to combine 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 enabling improvements in this area too.

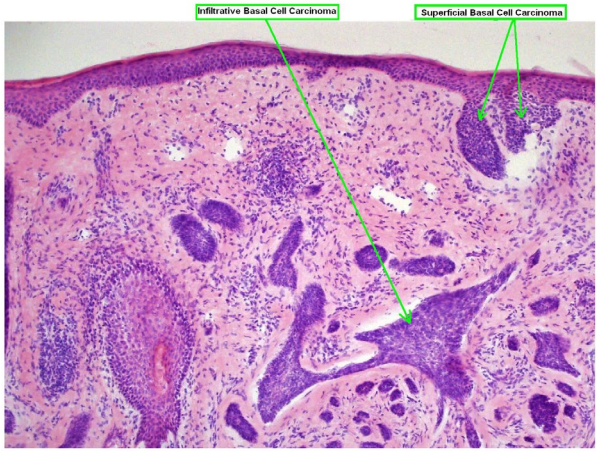

Figure 7. HE of superficial basal cell carcinoma tumour cells budding down from the epidermis more infiltrative basal cell carcinoma tumour nests deeper in the dermis. The tissue is embedded on the PrestoChill Cryoembedding System and stained on the Presto automated processor/stainer devices (original magnification × 10).

Of merit in terms of design is the clean and compact nature of both devices which embrace the benefits of a 'Lean' working in the modern laboratory layout. The footprints of both devices are small compared to conventional staining and embedding equipment. In addition, the use of embedding medium MCC also has advantages over conventionally used OCT embedding medium in that the blocks produced from MCC were uniformly flat with no surface defects sometimes seen with OCT, such as air bubbles and tissue retraction features. This makes subsequent cryotomy easier to perform with MCC.

Maintenance requirements are also minor and the reagent management process is simple, fast and efficient. The ability to download tracking logs, also lends the devices to equipment management initiatives which are increasingly being required from UKAS ISO 15189 standards of equipment management and performance in all laboratories across the UK.

H&E Quality Control Log

Date	Acceptance criteria (tick as required)	Undesirable findings (tick as required)	Further comments/observations
	nuclei stained purple ☐	loss of the chromatin granularity ☐	
	clear demonstration of nuclear detail ☐	excessive cytoplasmic staining ☐	
	haematoxylin well differentiated ☐	excessive connective tissue staining ☐	
	connective tissue background remains ☐	excess differentiation - sections too red ☐	
	minimal cytoplasmic staining ☐	eosin intensity too weak- loss of selective demonstration of different components at low power ☐	
	correct colour and effectiveness of eosin demonstrates collagen cytoplasm, red blood cells, cellular granules, amyloid ☐	eosin intensity too strong colour and detail of the nuclear stain obscured, selectivity reduced ☐	
	appropriate section thickness ☐		
	Validated by:		

Figure 8. Staining quality parameters as defined by the department's internal quality control checking.

In terms of HE staining, the Presto stainer can complete the staining procedure within an average of 5 minutes. This is three times faster than the time achieved with the conventional Linistat Linear Stainer. The main reason for this improvement is due to the elimination of lengthy washing steps and also the use of some innovative environmentally friendly consumables associated with the device, most notably the FineFix (formalin free fixative) which is a proprietary solution. The device can handle six slides (Figure 5) at a time and the fixation, clearing and dehydration steps were consistently achieved within 1 minutes on all the tissue sections stained.

Both Presto devices are also ergonomically and IT friendly, as both have automated touch screens. With regard to the Presto stainer there is also an ability to set several different protocols with different time frames for each step, with the additional benefit of being able to dip or shake the slides during the staining procedure. The Presto stainer also has a built in filter which makes fume extraction an on-board facility with this device.

Another merit is also the fact that the Presto stainer uses relatively small volumes of water when running the device, compared to the Linistainer which has running water as a constant feature, while the machine is in use.

The staining results achieved were highly comparable to the quality of the conventional tissue staining results achieved (Figures 6 and 7). This again, compared favourably to conventional staining methods employed. There was no evidence of inadequate dehydration or inadequate clearing of the tissue sections seen. The key benefit was a staining time approximately three times faster than our existing semi-automated staining techniques.

These innovative complimentary devices are very exciting developments in the field of fresh frozen section preparation and staining. They represent a comprehensive approach to tackling the whole gambit of technical challenges faced in producing good quality well-stained frozen sections in a rapid response format.

The benefits of a nearly fully automated process, also improves standardisation of practice and ultimately will improve overall quality of performance. Since the current practice of performing frozen sections is highly variable with a wide assortment of manual and semi-automated procedures, it follows that devices that can consistently standardise these steps will be popular. History tells us a lot as a brief glance back over the last 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.

This work represents an advance in biomedical science because it demonstrates the improved efficiency and speed for the use of automated embedding and staining platforms in histological MMS investigations.

Summary table

What is known about this subject:
- Automated and semi-automated platforms for use within the field of cryotomy are few and far between.
- Innovative concepts and designs that enhance the speed and efficiency of working practice within the field of MMS are highly desirable and beneficial.

What this study adds:
- The introduction of newly designed equipment for embedding and staining of frozen section tissue
- Statistically-based evidence of improved efficiency, speed and quality of end results achieved for MMS-stained slide sections

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Disclosure statement

No potential conflict of interest was reported by the authors.

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