A novel approach to rapid onsite slow Mohs processing using the LOGOS tissue processor.

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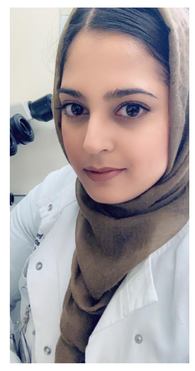

Introduction

Skin cancer is the most common type of cancer in England with 224, 000 instances identified in 2019 [1]. Rapid processing of histopathologic material is becoming increasingly desirable by clinicians treating skin cancer patients. Frozen section histopathology, in the form of conventional Mohs surgery histological assessments, makes cancer recognition challenging, particularly with Lentigo Maligna (LM) and Dermatofibrosarcoma protuberans (DFSP), as morphology is improved using formalin fixed paraffin embedded (FFPE) tissue-therefore slow Mohs is often implemented. The drawback is the requirement for a 12-24-hour delay period to enable adequate fixation before microscopic analysis of the H&E sections can be performed. The aim of this study was to determine an efficient and rapid processing method of producing FFPE sections for slow Mohs analysis of tumours. Consequently, patients can obtain their results quickly, ideally within 3 hours.

Method- Sample cohort

This study examined seventy-seven cases of skin malignancies. The tissues used were patient consented anonymised remaining samples, that were no longer required for diagnostic purposes.

Method- optimization and staining

The first sixty cases consisted of skin samples of various thickness, ranging from 2mm to 5mm. A total of seven different processing programs were tested on the first sixty cases to gain an insight into the quality of the processing. Seven different programs were tested because each program was suited to process a tissue of a specific (see Table 1 for summary of each program).

These programs were then modified, mainly by lengthening the fixation and impregnation stages of processing to find optimum protocols. Once the protocols were finalised, the last seventeen cases of known tumours were processed in accordance with their thickness.

To attain reliable results, program two (P2) trialled tissues measuring 2-3mm thick and program three (P3) processed tissues measuring 2-4mm in thickness. Program four (P4) was a universal program and therefore tested tissues measuring 2-5mm as some 2mm and 3mm tissues were quite fatty and therefore required slightly longer fixation periods. Program 5 (P5) processed tissue of 5mm thickness. This methodology allowed comparison of processing protocols amongst different tissue sizes to see which program was optimum for each tissue size. The best suited program was then selected for cases SM-21-61 to SM-21-77.

Following processing, the blocks were embedded producing paraffin blocks. Each block was sectioned to produce eight slides of 3µm thick sections. To limit the number of variables, the blocks were sectioned under consistent environmental conditions i.e., the water bath temperature was set at 37^{o}c, the same batch of slides were used to pick up sections.

One slide was stained using H&E, and the spare slides were kept for either repeat sections or further IHC work. H&E staining was performed by the Leica Autostainer, using commercially produced Harris' Haematoxylin and 0.8% aqueous Eosin. Both reagents were supplied by Leica Biosystems. Prior to loading the slides onto the Leica Autostainer, the slides were dried in a separate oven for 25 minutes at 60^{o}c.

Tissue Thickness	Program Name	Fixation Time (Hrs:Mins:Secs)	Wax Impregnation Time	Total Processing Time
Up to 2mm non fatty	P2	0:25:00	0:52:30	2:14:00
Up to 2mm non fatty version 2	P2V2	0:45:00	1:13:30	2:55:00
Up to 3mm non fatty	P3	0:30:00	1:03:30	3:05:00
Up to 3mm non fatty version 2	P3V2	0:55:00	1:27:30	3:54:00
Up to 4mm fatty/non fatty/universal	P4	0:50:00	1:30:30	4:17:00
Up to 5mm non fatty overnight	P5	2:00:00	5:50:30	16:27:00
Up to 5mm non fatty rapid	P5R	0:57:00	2:00:30	5:58:30

Table 1 Summary of processing times for each Logos program

Method-assessment criteria

To determine the quality of tissue processing, the FFPE sections were stained using H&E. The H&E sections were assessed microscopically via routine light microscopy by two of the authors GEO and MS, both UKNEQAS cellular pathology technique (CPT) current assessors. All H&E sections were scored by GEO and MS, in accordance with the UK NEQAS CPT assessment criteria to ensure consistency. Each assessor was required to assign a score out of 5 (see Table 2) to every slide resulting in a total score out of 10. The UK NEQAS assessment criteria lists possible artefacts and issues that can be encountered during each phase i.e. pre microtomy, microtomy, staining and post staining. If these are seen during H&E examination, the scores will be impacted.

Score	Rating
5	Excellent
4	Good
3	Pass
2	Borderline Fail
1	Fail
0	Non submission

Table 2 Scoring based on UK NEQAS CPT H&E assessment criteria

Results

Table 3 shows the breakdown of scores for each case.

Cases processed using program 2 achieved an average score of seven out of a possible ten. This score fluctuates between a 'pass' and 'good'. Tissues processed using program 3 achieved an average score of 6/10 which indicates a borderline pass. Program 4 achieved an average score of 6/10, again representative of a borderline pass. Program 5O was an overnight processing protocol for tissues with a thickness of 5mm and gained an average score of 6/10, which was also a borderline pass. Finally, program 5R was a rapid program used to process tissues with a thickness of 5mm and scored an average of 6/10, indicative of a borderline pass.

Upon examining individual cases processed by each program, 89% of cases processed using program 2 achieved a score between 6-9/10, suggestive of a pass with good nuclear and tissue constituent staining allowing good visualisation of cell components within the tissue sections. 91% of cases processed using program 3 attained a score of 6/10 and above (maximum 8/10). 70% of cases processed using program 4 scored either 6/10 or 7/10. Both demonstrating adequate/good nuclear and cytoplasmic staining. Tissues measuring 5mm in thickness were processed using an overnight (P5O) or rapid (P5R) protocol. Of those processed using the overnight program, 79% of cases scored between 6/10-8/10 (pass). 33% of cases processed using P5R passed with scores of 7/10, whilst the remainder of cases demonstrated staining quality in line with a borderline fail (Figures 1a-1g).

Table 3 Breakdown of scores for each case

Case	Score 1 (/5)	Score 2 (/5)	Total (/10)
SM-21-1	4	4	8
SM-21-2	3	4	7
SM-21-3	4	3	7
SM-21-4	4	4	8
SM-21-5	4	4	8
SM-21-6	4	4	8
SM-21-7	3	3	6
SM-21-8	4	3	7
SM-21-9	3	4	7
SM-21-10	3	4	7
SM-21-11	4	4	8
SM-21-12	4	4	8
SM-21-13	3	3	6
SM-21-14	3	3	6
SM-21-15	4	4	8
SM-21-16	4	3	7
SM-21-17	3	3	6
SM-21-18	4	3	7
SM-21-19	3	4	7
SM-21-20	4	4	8
SM-21-21	2	3	5
SM-21-22	3	4	7
SM-21-23	4	3	7
SM-21-24	3	2	5
SM-21-25	3	4	7
SM-21-26	3	4	7
SM-21-27	4	3	7
SM-21-28	4	4	8
SM-21-29	4	4	8
SM-21-30	5	4	9
SM-21-31	3	3	6
SM-21-32	3	4	7
SM-21-33	2	1	3
SM-21-34	4	4	8
SM-21-35	3	4	7
SM-21-36	3	2	5
SM-21-37	3	3	6
SM-21-38	3	3	6
SM-21-39	2	2	4
SM-21-40	3	3	6
SM-21-41	2	2	4
SM-21-42	2	2	4
SM-21-43	3	4	7
SM-21-44	4	3	7
SM-21-45	4	3	7
SM-21-46	3	4	7
SM-21-47	3	3	6
SM-21-48	3	4	7
SM-21-49	4	3	7
SM-21-50	3	4	7
SM-21-51	3	4	7
SM-21-52	2	1	3
SM-21-53	2	1	3
SM-21-54	1	2	3
SM-21-55	3	2	5
SM-21-56	2	3	5
SM-21-57	2	2	4
SM-21-58	2	2	4
SM-21-59	3	3	6
SM-21-60	3	3	6
SM-21-61	3	3	6
SM-21-62	3	3	6
SM-21-63	3	3	6
SM-21-64	3	3	6
SM-21-65	3	3	6
SM-21-66	2	2	4
SM-21-67	3	2	5
SM-21-68	2	2	4
SM-21-69	2	2	4
SM-21-70	1	1	2
SM-21-71	2	2	4
SM-21-72	1	1	2
SM-21-73	1	1	2
SM-21-74	1	1	2
SM-21-75	3	3	6
SM-21-76	3	3	6
SM-21-77	3	2	5

Once the processing protocols were optimised, phase 2 of this study incorporated processing tissues of known malignancy and selecting the best program to use for each of these cases in accordance with their thickness. Figure 2 shows the H&E scores achieved once the H&E slides were examined.

41% of cases achieved a borderline pass, showing adequate cellular detail and a sufficient quality of H&E staining, the rest of the cases showed suboptimal staining with multiple artefacts and poor quality of cellular detail (Figures 3(a&b), 4, 5 and 6).

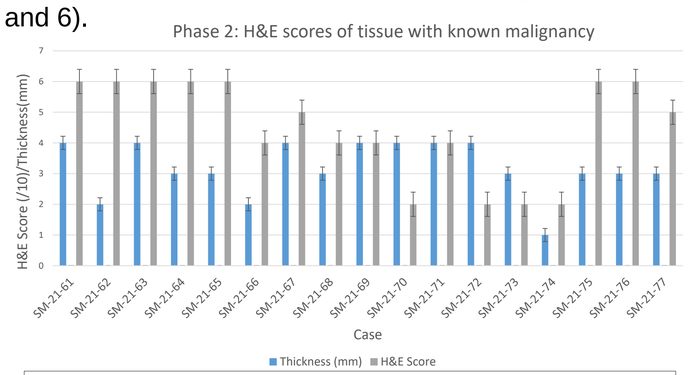

Figure 7 Chart of H&E scores for cases of known malignancy

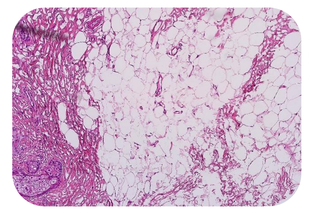

Figure 1a. SM-21-7 (x10 magnification) H&E showing distorted adipocytes

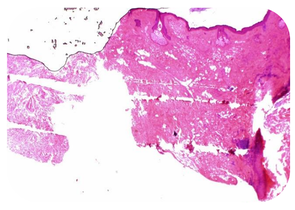

Figure 1b. SM-21-9 (x2 magnification) H&E showing knife marks due to poor processing.

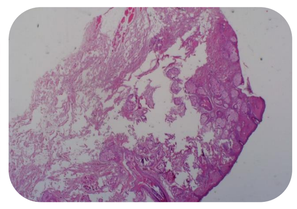

Figure 1c. SM-21-13 (x2 magnification) H&E shows poorly processed tissue with holes.

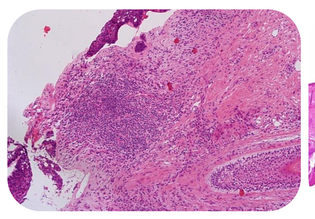

Figure 1d. SM-21-18 (x20 magnification) H&E section shows BCC and squamous cells.

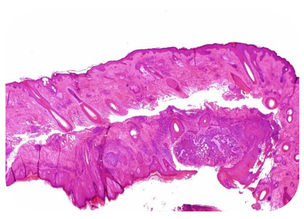

Figure 1e. SM-21-24 (x2 magnification) H&E section shows folds and creases as well as tearing within the section.

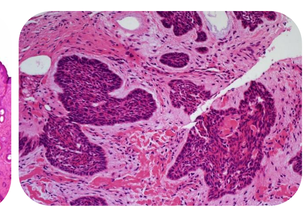

Figure 1f. SM-21-28 (x20 magnification) H&E shows BCC tumour deposits.)

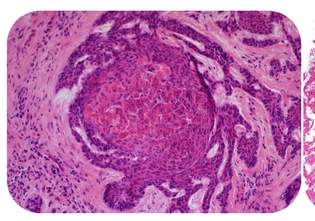

Figure 1g. SM-21-48 (x20 magnification) H&E showing amyloid deposits within a tumour nest of BCC.

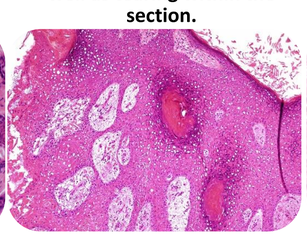

Figure 3a. shows H&E staining (x10 magnification) of a SCC SM-21-61 score: 6/10. Folding and holes visible due to poor processing.

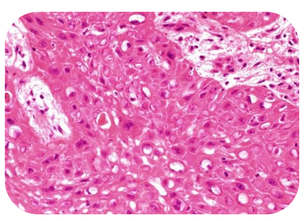

Figure 3b. shows H&E staining (x20 magnification) of islands of abnormal tumour cells in SM-21-61.

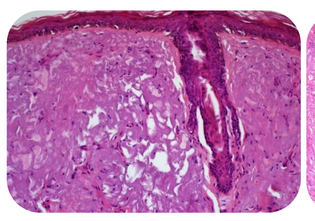

Figure 4. is a H&E stained LM case SM-21-62 (x10 magnification), score: 6/10. Section shows multiple holes with a stretched appearance to the epithelium, tumour cells are poorly preserved as well as cracks within the section.

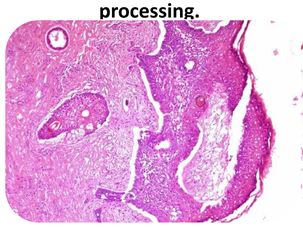

Figure 5. SM-21-77 demonstrates H&E staining of a nodular BCC case (x10 magnification), score: 5/10, processing artefact visible with stretching and tearing within the section.

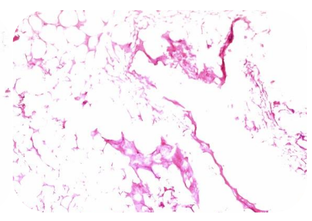

Figure 6. SM-21-71 (x10 magnification) of DFSP case, score: 4/10. Section is full of holes and tissue integrity has been compromised, adipocytes have lost their structure.

Summary Table

- This research project investigated the quality of H&E staining produced from rapid fixation of tissue samples with varying thickness levels. To achieve optimal processing, different protocols were tested. Consequently, tissues with thickness levels measuring 3mm and less were processed sufficiently to yield good quality H&E sections where tumour morphology was apparent and tissue components were well preserved achieving scores of 6/10 and above.
- Unfortunately, thicker tissue samples measuring 4mm-5mm did not produce good quality sections with a high level of distortion and suboptimal tissue component preservation making diagnosis extremely challenging.
- Larger studies are needed to substantiate the results, however these findings support the use of rapid on site slow Mohs processing for smaller tissues.
- This work represents an advance in biomedical science because it demonstrates that the majority of slow Mohs samples are of larger tissues where clear deep dermal visualisation is imperative to conclude 100% tumour clearance. Rapid paraffin processing techniques have the potential to significantly improve the process and subsequent cure rates for Mohs patients receiving Mohs treatment, as paraffin sections provide the Mohs surgeon with higher-quality of morphological detail than typical frozen sections.

Acknowledgements

This work was completed as part of the University of Greenwich Biomed online MSc course as the final project dissertation and achieved a distinction. Thank you to the co-authors and the University of Greenwich team for your support with this project.

References

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