

Evaluation of tissue preservation using a vacuum-based refrigeration system for specimen transfer from theatre to laboratory

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Abstract

Specimen preservation is essential to allow adequate morphological appraisal and successful application of immunohistochemistry. Traditionally immersion in formalin is ideal for morphology and immunohistochemistry but does not permit collection of fresh frozen tissue, the ideal for research purposes and Biobanking. We evaluated a novel system for handling fresh specimens (TissueSAFE™) which employs vacuum sealing and storage at 4°C for transfer to the pathology laboratory.

Tissue slices from colectomy specimens were vacuum sealed using TissueSAFE™ and stored at 4°C prior to sampling mucosal, nodal and tumour tissue at subsequent time points (up to 72 hours) for snap freezing. In addition, composite paraffin blocks were generated at the same time points following an additional 24hrs in formalin. H&E staining and immunostaining using a range of antibodies was applied to the paraffin blocks and DNA was extracted from the frozen tissue samples for comparison between times in TissueSAFE™.

There was no appreciable difference in morphology or immunohistochemical staining between any of the samples for any of the antibodies assessed. Extracted DNA quantities and quality were preserved with no depreciation with time in TissueSAFE™. In conclusion, TissueSAFE™ preserves tissue for morphological examination, immunohistochemistry and DNA extraction for up to at least 72 hours.



Figure 1. TissueSAFE™ vacuum-based refrigeration system for specimen transfer.

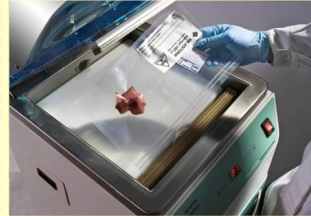


Figure 2. Vacuum sealing of fresh tissue specimen prior to transfer at 4°C in a refrigerated container to the pathology laboratory.

Background

Preservation of surgical specimens submitted for histopathological examination is essential to allow adequate morphological appraisal and successful application of immunohistochemistry and other laboratory tissue investigations. For decades, the traditional approach to handling surgical samples removed in theatre has been immersion in formalin at room temperature prior to transport to the laboratory. Although this is ideal for morphology and immunohistochemistry, it does not permit collection of fresh frozen tissue, which provides optimal material for research purposes and Biobanking. Furthermore, fresh samples are increasingly required for nucleic acid extraction for a variety of downstream applications relevant to both research and diagnostics. In addition, variable time intervals both from removal of specimen from the patient to first immersion in formalin and to subsequent specimen dissection and tissue sampling by the pathologist can lead to problems with fixation which can in turn affect the results of immunohistochemistry or molecular investigations. Uncertainty over the effects of such time intervals on the preservation of tissue and potentially the results of subsequent investigations necessitates further investigation. We have evaluated a novel system for handling fresh specimens in theatre (TissueSAFE™) which employs vacuum sealing and storage at 4°C for transfer to the pathology laboratory (Figures 1 and 2). In addition to eliminating the use of formalin from theatres, this system potentially offers more control over the adequacy and duration of formalin fixation. Before gaining acceptance in laboratory practice, the tissue effects of this novel system of specimen handling must be scrutinised. We compared tissue samples handled routinely, and those handled using TissueSAFE™ methodology, with respect to morphology, immunohistochemistry and DNA extraction, at various timepoints after removal from the patient.

Materials & Methods

Specimen Handling

Four colectomy specimens (two neoplastic and two non-neoplastic) were transferred from theatre to laboratory on ice immediately after removal from the patient. After routine diagnostic dissection and examination, large tissue slices from each specimen were vacuum sealed using TissueSAFE™ and stored at 4°C prior to sampling mucosal, nodal and (for neoplastic specimens) tumour tissue at subsequent time points, specifically 0, 4, 24, 48 and 72 hours. At each time point small (4mm diameter) tissue samples were taken for snap freezing in liquid nitrogen. In addition, composite paraffin blocks of normal mucosal, lymph node and (for neoplastic specimens) tumour tissue were generated at the same time points. These composite blocks were placed in formalin for 24hrs prior to routine processing. Control composite paraffin blocks were prepared from tissue which had been placed directly into formalin at the time of specimen transfer to the laboratory. For one specimen a set of frozen control tissue samples was taken after tissue exposure to room temperature for 4 hours.

H&E staining and immunostaining using a range of nuclear and membrane-directed antibodies (Table 3, AE1/3, MIB1, CDX2, PMS2) were applied to all the paraffin blocks and DNA was extracted from all the frozen tissue samples for comparison between times in TissueSAFE™.

DNA extraction

DNA was manually extracted from the snap frozen biopsy material using Qiagen Blood & Tissue Kit (Qiagen, West Sussex UK). Biopsy material was placed into a 190µl digestion/Proteinase mixture and left to incubate overnight at 55°C. Following the incubation period DNA was isolated in accordance with the manufacturer's protocol, with DNA being eluted into a final volume of 50µl.

DNA quantification

1.5µl of DNA was quantified by spectrophotometry using the Nanodrop ND-1000 (Thermo-Scientific, Wilmington USA). Optical density was measured at 260nm and concentration levels given in ng/µl.

DNA quality assessment

For quality assessment, DNA was amplified using a specimen control size ladder from Invivoscribe (Invivoscribe Technologies, France) based on the BIOMED-2 protocol (Tables 1 and 2). The reaction mix contained 2.5µl specimen ladder, 0.125µl AmpErase (Qiagen) and 2µl DNA for a final volume of 27.125µl per sample. A negative control was included in each group, in which water was used in place of DNA. PCR was performed on the Applied Biosystems GeneAmp 2700 thermocycler (Applied Biosystems, Warrington UK) using the cycling conditions given in Table 2. 7µl of amplified DNA was run out on a 2% TBE agarose gel for visual examination of DNA quality.

Table 1. Primers used in PCR with the gene targeted for sequencing DNA quality

Target	Forward	Reverse
1. <i>Thymidylate synthase</i> gene (chr5:2)	5' ACCCGAATCTTCCAAATCC	5' TTTGAGAAAGGCTCTTCTG
2. <i>β-actin</i> gene (chr4:2)	5' TTTTACTACTTCCAGCCCA	5' AATGCTGTCCTTCTTCTG
3. <i>Prothymosin</i> subunit 1 gene (chr11:1)	5' TCCATATGTTTCTTCTTCTG	5' CCGATCTCTCTTCTTCTG
4. <i>β-actin</i> gene (chr4:2)	5' CCGAATCTTCCAAATCC	5' TTTGAGAAAGGCTCTTCTG
5. <i>β-actin</i> gene (chr4:2)	5' CCGAATCTTCCAAATCC	5' TTTGAGAAAGGCTCTTCTG

Table 2. Cycling conditions used for Invivoscribe PCR

1 hold	3 Temp. 35 cycles	2 holds
94°C 10 min.	94°C 45 sec. 60°C 45 sec. 72°C 90 sec. 72°C 7 min. 15°C ∞	

Table 3. Antibodies, sources and conditions used for immunohistochemistry

ANTIBODY	CLONE	SUPPLIER	RETRIEVAL	DILUTION
AE1/3	AE1/3	DAKO, CAMBRIDGE, U.K.	ER2 20°	1:80
ki-67	MIB-1	DAKO, CAMBRIDGE, U.K.	ER1 30°	1:200
CDX2	AMT28	LEICA, LEICA MICROSYSTEMS, U.K.	ER2 20°	1:50
PMS2		BD PHARMINGEN, OXFORD, U.K.	ER2 40°	1:100

Table 3. Antibodies, sources and conditions used for immunohistochemistry.

Results

Morphology and Immunohistochemistry

There was no appreciable difference in morphology or immunohistochemical staining between the samples taken at any of the timepoints for any of the four antibodies assessed. Two consultant pathologists blinded with respect to specimen times in TissueSAFE™ were unable to discriminate between samples. Figure 3 shows an example of a H&E-stained section of a composite block taken after 72 hours in TissueSAFE™ (followed by 24 hours in formalin). Figure 4 represents high power images from the same tumour as Figure 3, demonstrating no appreciable difference in tumour morphology between the TissueSAFE™ 72 hour sample and the control block immersed in formalin from time of receipt in the laboratory (96 hours total in formalin). Figure 5 (a-e) shows excellent immunostaining for relevant tissue components on a composite block after 72 hours in TissueSAFE™.

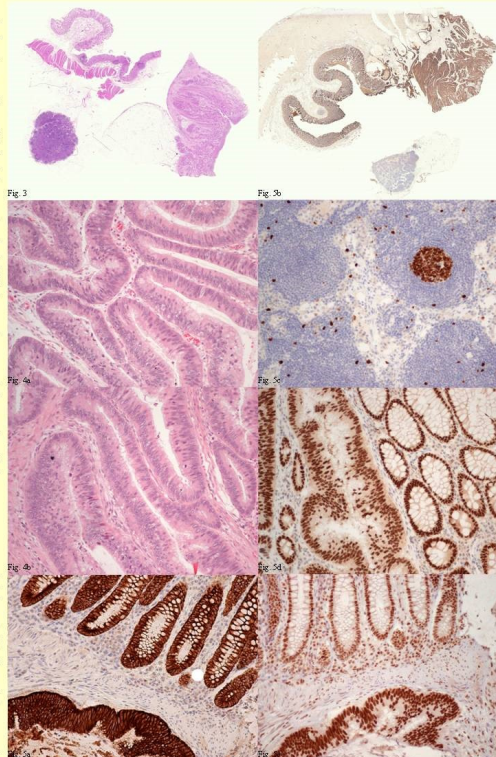


Figure 3. H&E stained section of a composite block after 72 hours in TissueSAFE™. Figure 4. High power images from tumour after 72 hours in TissueSAFE™ (4a) and formalin fixed control (4b) showing no morphological differences. Figure 5. Immunostaining of a composite block after 72 hours in TissueSAFE™ (a, b, AE1/3; c, MIB-1; d, CDX2; e, PMS2).

DNA

DNA was extracted from a total of 37 samples, taken from the four colectomy resection specimens at varying timepoints (Table 4). DNA quantification ranged considerably, from <100 to >1500ng/µl, but this was unrelated to time (Figure 6) and most likely reflected variation in tissue sample size. The five samples assessed after 72 hours in TissueSAFE™ all had >300ng/µl DNA. The control samples exposed to air for 4 hours both showed poor quantity DNA (10 and 60ng/µl). The A260/280 ratio for all samples was in the range between 1.75-1.92, indicating suitable quality DNA. The visual assessment of DNA quality from the Invivoscribe PCR showed that most samples amplified successfully, with no loss of DNA quality with time demonstrable (Figure 7).

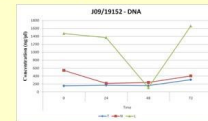


Figure 6. DNA quantification versus time in TissueSAFE™.

Discussion

This small study demonstrates the potential for a novel approach to specimen transfer from theatre to laboratory, specifically immediate vacuum sealing after removal from the patient and transfer to the laboratory at 4°C. Although this was not the procedure adhered to strictly in this study, some modifications were necessary to ensure normal specimen examination was not compromised. Large tissue slices, rather than whole specimens, were vacuum sealed, after preliminary dissection in the laboratory. As such, vacuum sealing was delayed (by an estimated 15-20 minutes). This delay would not occur were the entire specimen to be vacuum sealed immediately in theatre.

In the specimens evaluated in this study, up to 72 hours in TissueSAFE™ made no appreciable difference to morphological evaluation, immunohistochemistry (for the four antibodies assessed) or to DNA quantity or quality. In contrast, exposure to air at room temperature for four hours was sufficient to induce significant DNA degradation in control samples. The relevant importance of vacuum sealing versus refrigeration has not been assessed by this study. Along with extrapolation to RNA preservation studies, this would be of potential interest for future studies examining the potential utility of the TissueSAFE™ system.

Table 4. DNA extraction at various time points from four colectomy specimens.

Conc. (ng/µl)	J0919152					
	T	N	L	T	N	L
0	155.3	392.85	350.26	372.52	350.26	372.52
4	543.33	218.24	218.54	451.81	218.54	451.81
24	1472.46	1368.61	1368.61	1368.61	1368.61	1368.61

Conc. (ng/µl)	J0919152					
	T	N	L	T	N	L
0	145.53	211.53	430.38	1244.46	430.38	1244.46
4	576.83	3205.53	1556.65	836.83	1556.65	836.83

Conc. (ng/µl)	J0919152					
	T	N	L	T	N	L
0	420.43	244.35	432.15	372.52	350.26	372.52
4	850.46	785.33	597.26	785.33	597.26	785.33
24	1565.22	437.33	1567.88	437.33	1567.88	437.33

Conc. (ng/µl)	J0919152					
	T	N	L	T	N	L
0	172.83	83.86	123	804.78	83.86	123
4	121.4	19.46	10.23	18.16	19.46	10.23

Table 4. DNA extraction at various time points from four colectomy specimens.

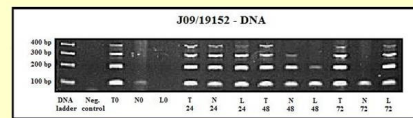


Figure 7. Visual assessment of DNA quality from the Invivoscribe PCR showed that most samples amplified successfully with no loss of DNA quality with time demonstrable.