



Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Vacuum-based preservation of surgical specimens: An environmentally-safe step towards a formalin-free hospital

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ARTICLE INFO

Article history:

Received 2 March 2010

Received in revised form 7 April 2010

Accepted 13 April 2010

Available online xxx

Keywords:

Formalin

Tissue specimens

Histopathology

Under-vacuum sealing

Pathology

ABSTRACT

Formalin as a fixative has no practical substitutes, but is toxic and potentially carcinogenic, so caution of its use in hospitals and elsewhere is mandatory. In our hospital, preservation of surgical specimens into formalin to be transferred to pathology labs was replaced by under-vacuum sealing (UVS) tissues into plastic bags and preservation at 4 °C until transfer. Data analysis showed UVS processing to be superior in terms of staff satisfaction and of gross anatomic preservation; no problems in terms of technical feasibility or histopathologic preservation were encountered. Formalin was confined to pathology labs while its use on hospital premises was vastly reduced.

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1. Introduction

Formalin, a 4% solution of formaldehyde in water, is a world-wide and extensively used fixative for histopathological specimens. Since its discovery at the end of 19th Century (Fox et al., 1985), this aldehyde has been universally appreciated as a simple reagent that is a good antiseptic, penetrates tissues quickly (diffusion rate of 1 cm in 24 h) and is easy to handle. In tissues that are formalin-fixed, morphology is well preserved, as is tissue antigenicity, and immunohistochemical procedures of diagnostic interest have routinely been adapted to formalin-fixed tissues (Dabbs, 2008).

In addition to multiple industrial applications, the medical use of formalin as a tissue preserver and fixative is extensive, especially in pathology laboratories. In fact, the amount used in public hospitals in the Piedmont region (Italy) alone for the preparation of approximately 300,000 histological exams is in the range of 100,000 liters per year.

Tissues preserved in formalin are even sent by post, in the number of several thousands per year.

Despite its advantages, formaldehyde has some drawbacks that demand caution: it is allergenic to the skin and produces irritating vapors, causing asthma. The International Agency for Cancer Research

(IARC, 2006) has declared formaldehyde to be a Class 1 carcinogenic agent, and statistical evidence has been presented on a possible link between formaldehyde exposure and lymphohematopoietic malignancies (Beane Freeman et al., 2009).

Several attempts have been made to find a substitute for formalin, but so far all of the proposed alternatives have failed, being either ineffective or unreliable (Titford and Horenstein, 2005). A more practical approach would be to limit the use of formalin to pathology laboratories, where this toxic agent is carefully handled with hoods and gloves in safe environmental conditions, and to avoid its use in other less-protected areas of the hospital, such as in surgical theaters, where removed tissues are commonly placed in boxes full of formalin until transfer to the pathology labs. In fact, despite the advantages linked to this procedure (fixation and anti-sepsis begin immediately for the removed tissues and organs, and dehydration is avoided) several disadvantages are also recognized (see Table 1).

To overcome these problems, we proposed an alternative procedure, which is the under-vacuum sealing of tissues (UVS) in plastic bags inside the surgical theatre immediately after removal, and to keep them cooled at 4 °C until transfer to the pathology labs, where they are routinely processed.

Safety and advantages linked to this UVS procedure have already been reported elsewhere (Bussolati et al., 2008). This processing was tested for more than two years in a single surgical theater, and it is now being extended to the whole hospital.

The present study compares the compliance of personnel and the feasibility of this new procedure in a large regional hospital to the

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Table 1

A) Tissues immersed in formalin (large specimens)	
Disadvantages	
<ul style="list-style-type: none"> • Degradation continues in deep areas • Tissue banking is hampered • Formalin-containing vessels are heavy to carry • Spilling of formalin may occur • Fumes dispersed while grossing • Nurses refuse to handle this carcinogen inside the surgical theatre (without hoods) • Tissue forgotten by the surgeon because it is “already safe in formalin” 	
B) Tissues preserved under vacuum	
Merits	
<ul style="list-style-type: none"> • No more formalin in surgical theatre (except for small specimens, where pre-filled tubes are employed) • No spilling • No fumes • No drying of tissues • Colour, form and consistency are preserved • Lack of insulating air around tissues allows fast cooling • Tissues (bags) light and easy to carry • Structures (RNA, antigens) preserved for days • Banking (selective) and gene-expression profiling allowed • Fixation times can be standardized • Microbiological and viral analysis feasible 	

traditional process of immersing surgical specimens in formalin. The survey was conducted with questionnaires and interviews specifically dealing with the various steps of the processing that were given to all the staff (nurses, technicians and pathologists) involved.

2. Material and methods

The present study was conducted in the S. Giovanni “Molinette” hospital of Turin (Italy), a teaching hospital with 1162 beds, 54,560 yearly admissions, and over 40,000 histopathological exams in the year 2008. The hospital was originally built in 1938 as a pavilion hospital. As a result, the main pathology laboratories are separated and located in a different building from the surgical theaters. The study involved four surgical theaters, all located in different areas, that produce over 90% of the surgical biopsies.

Biopsies were subdivided into two classes: “small”, i.e., less than 2 cm. in diameter, and “large”, or >2 cm in size. The latter corresponded to roughly 25% of the total number of specimens. The rationale for such subdivision is related to the well-known fact (Hewitt et al., 2008) that the penetration rate of formalin into tissues is in the range of 1 cm in 24 h, thus theoretically assuring fixation of “small” biopsies in acceptable times. These “small” biopsies are routinely collected in 50-ml containers pre-filled with buffered formalin (Diapath s.r.l., Martinengo, Bergamo, Italy).

The present study is concerned with “large” biopsies which, until now, were transferred from the operating theatre to the pathology laboratories in large plastic containers (ranging in size from 1000 to 5000 ml) filled with formalin. The volume of formalin varied according to the size of the specimen, but is recommended to be 20 times the weight of the specimen. It is customary in our hospital that surgical specimens are collected once a day, in the early afternoon, to be transferred to the pathology labs. Thus, in cases operated on Friday afternoon, or before long weekends, the specimens are transferred to pathology only on Monday.

For UVS processing, specimens were sealed into plastic bags immediately after removal in the vacuum apparatus (Tissue-safe® , Milestone, Bergamo, Italy). The process lasted a few seconds. The bags were labeled with identification data. The specimens were then kept in a refrigerator at 4 °C inside the premises of the surgical theater until they were transferred to pathology. Once the sealed bags, which were kept in a chilled plastic box, arrived at the pathology labs, the tissue was removed and routinely processed. This included grossing and then

fixation in buffered formalin (Diapath) under hoods and for a controlled time, followed by embedding in paraffin.

2.1. Evaluation of staff satisfaction, technical feasibility and the quality of tissue preservation

A series of questionnaires were distributed in sequence to the hospital staff, collected and statistically analyzed. Overall, the study was conducted over a time period of six months (Oct. 2008–April 2009).

2.1.1. Staff satisfaction

The first questionnaire, distributed on October 2008 to all the personnel of the surgical theaters and to the technical staff of the pathology laboratories ($N = 60$ and 58 , respectively) enquired about the satisfaction or dissatisfaction experienced with the traditional process of tissue handling (following categorical outcomes: very satisfied, satisfied, not satisfied) and with related problems or difficulties. One month after the introduction of the UVS processing, the same questionnaire was distributed to the same 58 technicians of the pathology laboratories as before and to 28 personnel of the surgical theatres now equipped with the Tissue Safe apparatus. Overall, after correcting for the missing values, the sample included 177 observations.

2.1.2. Technical feasibility

A questionnaire analyzed the technical feasibility of the different sequential steps involved in the transfer, examining either the traditional procedure employing formalin or the new UVS processing. The form accompanied single tissue specimens. Requests to fill out the forms were stopped after collecting 323 forms from senders (staff of the surgical theaters).

2.1.3. Quality of tissue preservation

Questionnaires enquiring about tissue preservation at either the gross or microscopic level were collected from 46 members of pathology staff (24 medically qualified, 22 biologists or technicians). The questionnaires regarded the quality (form, colour and consistency) of the gross anatomic preservation of different organs and tissue specimens (esophagus/stomach, colon, kidneys/prostate, breast, thyroid, liver/spleen), and qualified each as weak, satisfactory or good. They surveyed the preservation of tissues processed either with formalin or UVS.

A final questionnaire, distributed to the same staff, was related to the quality of the histo-pathological and immuno-histochemical preservation of surgical biopsies processed with the new UVS procedure.

3. Estimation method

A general linear regression model was used to test whether there exists a positive and significant correlation between the tissue handling procedure and the hospital staff satisfaction or the tissue quality conservation indicators (detailed description of the method can be found in the [Appendix A](#)).

4. Results

4.1. Hospital staff satisfaction

In order to test if the operator acceptance of the tissue handling procedure was positively correlated with the alternative procedure in which tissues are sealed under-vacuum in plastic bags, this study used a cross-sectional survey design in which hospital staff (nurses, pathology laboratory technicians, biologists, and physicians) from the San Giovanni Battista University Hospital (Turin, Italy) completed a questionnaire (reported in the [Appendix A](#)). The sample included 177

respondents. We create a binary variable that takes the value one if respondents work with the under-vacuum procedure and zero otherwise (that is, if they practice the fixation of tissues with formalin). Other than the tissue handling procedure, the following factors that may influence respondent satisfaction were measured using self-report questionnaires and included as explanatory variables: demographic variables (age, sex), professional activity indicators (whether respondents are nurses or physicians, pathology laboratory technicians and biologists), symptom perception (whether the respondents from the hospital staff perceive symptoms such as cough, chest pain, shortness of breath, and wheezing deriving from the use of tissue conservation procedure), difficulties (whether respondents encounter difficulties in using the tissue conservation procedure), and time of experience with the conservation procedure. The sample was divided into two categories: the first sub-sample includes personnel who were experienced with the traditional processing with formalin, and the second sub-sample includes personnel who were experienced with the new preservation method.

The most important statistic concerns the indicator of satisfaction, which increased with the UVS procedure (statistics reported in [Supplementary Table 1](#) in the [Appendix A](#), which provides descriptive statistics including means, standard deviations (SDs) and percentages for all relevant sample variables, as well as responses to the questionnaire concerning staff satisfaction). Briefly, 67% of respondents reported that they were very satisfied with the UVS based preservation mechanism, whereas for those who were using formalin, only 39% report satisfaction with the method of preservation. For the UVS procedure, 24% answered that they are satisfied, versus 41% of the sub-sample who used formalin. Finally, 8% of the sub-sample who preserve tissue with the UVS processing answered that they were dissatisfied with the preservation method, versus 39% of those who use the formalin fixation method. It is worth noting that respiratory symptoms such as cough, chest pain, shortness of breath, and wheezing increased with formalin use (34% versus 4% for UVS). Among personnel who used the UVS processing, 10% reported that they encountered difficulties with the preservation procedure, versus 39% of personnel who operated with formalin.

[Supplementary Table 1c](#) shows coefficients for hospital staff satisfaction calculated with the conservation procedure equation estimated using the ordered probit specification. From our empirical results, it arises that suffering from respiratory symptoms and having difficulties in using the tissue conservation procedure both have a strong negative impact on the satisfaction with the conservation procedure employed. The most interesting results concern the under-vacuum procedure: this technique in fact has a double effect on hospital staff satisfaction: a direct effect and an indirect one. The under-vacuum procedure directly improves operators' satisfaction and decreases the probability of suffering from respiratory symptoms, which, in turn, decreases the negative influence on operators' satisfaction.

4.2. Technical feasibility of the procedure

Our data indicate that no technical inconveniences were encountered when using the new UVS procedure (data available on request).

4.3. Tissue preservation quality

We evaluated the gross anatomic preservation of different organs and tissue specimens, including esophagus/stomach (1), colon (2), kidneys/prostate (3), breast (4), lung (5), endocrine/thyroid (6), and liver/spleen (7), each to be qualified as 1 = weak, 2 = satisfactory or 3 = good (see [Supplementary Tables 2a and 2b](#) in the [Appendix A](#), which show a simple descriptive analysis that presents sample means and standard deviations for the questionnaire). In order to make comparisons between the formalin and UVS procedure, the pathology

samples were divided into two categories based on the method of tissue conservation, either formalin fixation or UVS preservation.

It is worth noting that the quality of gross anatomic preservation increases with the UVS procedure. Those samples present better colour, form and consistency for all different organs and tissue specimens. The average colour, form and consistency of the esophagus/stomach, colon, kidneys/prostate, thyroid, and liver/spleen sealed under-vacuum in plastic bags were in the good range, versus the satisfactory range for tissue fixed with formalin. These gross anatomical parameters are pertinent for pathological evaluation and diagnosis, especially in hollow organs such as stomach and colon, where formalin-induced discoloration, hardening and retraction of the mucosa may hamper the proper recognition of ulcerative and infiltrative foci, or the evaluation of the correct distance of the lesion from resection margins. The evaluation was rather subjective, hence we opted for a 3 scale evaluation into weak, satisfactory or good quality. The final data (as extensively reported in [Supplementary Tables 2a and b](#) in the [Appendix A](#)) stress the importance of sealing and cooling conditions for a good preservation of both structure and consistency in esophagus, stomach and colon. In solid organs as kidney, liver and prostate the improvement of gross anatomical features, linked to the use of UVS as compared to formalin preservation, was lower, though still significant.

Thus, we can conclude from our empirical results that the UVS procedure is more effective than formalin for preserving the quality of tissue. The age and sex of the involved personnel had no influence on the results. In each estimation model, we have tested for multicollinearity by using the Variance Inflation Factor (VIF) and Tolerance ($1/VIF$) ([Wooldridge, 2002](#)). We found that VIF for all the independent variables in both the equation models were quite low. Therefore, we can safely assume that there are no problems of multicollinearity.

Finally, data of the questionnaire collected from members of the pathology staff concerning the quality of histo-pathological and immuno-histochemical preservation of tissues processed with the UVS procedure were categorized as either no damage or damaged. No damage affecting histopathological reporting, including tumor classification, grading and staging, was ever noticed in the period of use of UVS processing. We therefore concluded that the procedure was safe and reliable. Moreover, a neat improvement in the quality of histological features was noticed in solid organs such as kidney, liver, prostate and breast when kept for several hours, or even over the week-end, in UVS at 4 °C instead of formalin. In fact, in the latter conditions at variance with the former, areas deeper than 1 cm. (the penetration front of formalin) underwent autolysis resulting in poor structural preservation.

In our Institution the number of immunohistochemical and FISH (fluorescent *in situ* hybridization) procedures performed for diagnostic purposes amounted in year 2009 to 40.995 and 554, respectively, but no reports of damage affecting results was ever related to UVS processing. However, for a more objective evaluation, we checked the immunohistochemical values reported for breast cancer prognostication in 375 consecutive cases diagnosed between 06/2005 and 06/2007, before adoption of UVS processing, and the same number of cases in the two years after. Percentages of positivity for ER, PgR and Ki67 (>21%) were respectively 83.2, 84 and 40.8% before, and 86.9, 81.06 and 39.7%, after (non significant difference). In addition, we observed that UVS processing of breast cancer specimens facilitates gene expression profiling, since in 40 consecutive breast cancer specimens collected at times ranging from 1 to 72 h after removal, the quality of RNA was optimal in all cases (RIN value >7) ([Sapino et al., 2009](#)).

5. Conclusion

Formalin, a buffered solution of formaldehyde, is extensively used for histopathological preservation, and its substitution with alternative fixatives cannot be foreseen at present ([Dabbs, 2008](#); [Hewitt et al., 2008](#)). Concerns of carcinogenic activity of this mutagenic aldehyde

have been raised (IARC, 2006), and evidence has recently been presented on its possible association with leukemia (Beane Freeman et al., 2009), an observation that might fit with data reporting an excess of deaths due to cancer of the lymphatic and hematopoietic systems among British pathologists (Hall et al., 1991). Still, the major concern is linked to the production of toxic, irritating and allergenic vapors.

Indeed, a positive relationship between formalin and respiratory symptoms has been reported not only in workers in match factories (Vaughan and Black, 1939), but also in hospital staff members professionally exposed to this substance (Hendrick and Lane, 1975). Accordingly, our results support a positive and significant relationship between formalin and the probability of reporting distressing respiratory symptoms.

The ultimate goal of our approach was to reduce the use of formalin outside protected areas (i.e., fume hoods in pathology labs and pre-filled containers for small biopsies). Thus, we focused our attention to the practice of immersing surgical biopsies in large containers filled with formalin inside the surgical theater and their transfer to the pathology labs in due time. This process is endowed with several inconveniences (see Table 1a). An alternative process, whereby specimens are sealed under-vacuum into plastic bags and kept at 4 °C until transfer for grossing in the pathology labs was originally proposed by our group (Bussolati et al., 2008) and has been tested for over two years. In our experience, this process offers merits (see Table 1b) in terms of simplicity, feasibility and preservation of the original characteristics of tissues.

The extension of UVS processing to the whole hospital (a large, regional hospital) required a series of validation tests, to be checked with questionnaires analytically and statistically analyzed.

The results unanimously indicate a high degree of satisfaction for the new procedure (as compared to the traditional use of formalin) by both nurses from the surgical theater and technicians of the pathology labs. Not only did the UVS procedure avoid exposure to the distressing vapors of formalin, but it was also found to be easy and practical.

Further series of questionnaires specifically dealt with the feasibility and possible intricacies linked to the use of the UVS machine for different tissues and organs to be transferred from the surgical theater to the pathology labs. No specific difficulties were noted, and the evaluation of gross anatomic features was improved with the UVS procedure (as opposed to fixation in formalin) in terms of preservation of form, colour and consistency of the specimens. Finally, an inquiry among 46 members of pathology staff (24 medically qualified, 22 biologists or technicians) after several months of use of the UVS procedure did not reveal any difficulties in the diagnostic process linked to the use of the new procedure.

The conclusion of the present survey on the feasibility, compliance and quality assurance of a new procedure for transferring surgical specimens is positive. The UVS procedure was met with favor by the staff and did not present specific problems of practical or diagnostic interest. As a result, the new procedure has been adopted as the standard in our hospital.

Additional bonuses are linked to the possibility of standardizing fixation times and of implementing tissue banking. In fact, we can now determine the starting time of fixation in formalin, thus avoiding over-fixation, which can cause detrimental effects on immunophenotyping of the specimen, an issue that is presently regarded as mandatory for breast cancer processing (Goldstein et al., 2003; Wolff et al., 2007). An additional bonus of the novel U–V procedure is the preservation of RNA, which is enhanced by the storage at 4 °C (van

Maldegem et al., 2008), thus permitting tissue banking and gene expression profiling.

In conclusion, the present study shows a pathway towards a progressive reduction of the exposure of nurses, pathologist and technical personnel to formaldehyde vapors. The use of formalin has been restricted to dedicated areas in the pathology laboratory, and transfer of large boxes filled with fixative throughout the hospital was cancelled. In addition, the simple UVS processing offered advantages in terms of staff satisfaction, tissue preservation and cost. Complete elimination of formalin is still out of reach, but its substantial reduction from hospital premises is attainable and meets requests of environmental safety.

Acknowledgements

This work was supported by grants from Ricerca Sanitaria Finalizzata 2008 Regione Piemonte, European Commission Project IMPACTS – contract no: 037211 and Ministero per lo Sviluppo Economico (MISE, Rome) within the frame of the MISE-ICE-CRUI Project.

The active collaboration of the staff personnel of nurses and technicians of surgical theatres and pathology laboratories is gratefully acknowledged.

We would like to thank Dr. C. Marchiò and Mrs A. Davello for careful review and formatting of the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.scitotenv.2010.04.022](https://doi.org/10.1016/j.scitotenv.2010.04.022).

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