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LETTER TO THE EDITOR

Tissue transfer to pathology labs: under vacuum is the safe alternative to formalin

Gianni Bussolati · Luigi Chiusa · Antonio Cimino · Giuseppe D'Armento

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The time-honoured use of formalin, both as a preserver and fixative for histological processing is encountering increasing criticisms because of toxicity and environmental concerns. Moreover, the declaration recently issued by the International Agency for Research on Cancer [1] which classified formaldehyde as a class 1 carcinogen has definitely increased the request by health authorities, technicians and practicing pathologists to reduce exposure. Such requests contrast with the considerable advantages offered by this safe, chip, reliable fixative. Although it should be acknowledged that in modern pathology laboratories, visitors are not any more met by the permeating flavour of a stingy scent because formalin processing is mostly carried out under aspiration hoods, a critical passage is still represented by the transfer of tissues from the surgical theatre to the pathology lab. Apart from the small biopsies, which are directly collected into pre-filled containers and cause no concern, problems are encountered with the immersion of large specimens and organs into large boxes to be filled with formalin. A list of such problems follows:

1. Plastic containers are large and relatively heavy, while on occasion spilling may occur.
2. Immersion into formalin prevents the collection of fresh material for tissue banking. Fixation starts, but only at the periphery. Discoloration occurs, while a delay in the transfer to pathology labs is somehow justified by the fact that 'the tissue is already in formalin'.
3. Nurses at the surgical theatre are becoming increasingly concerned for toxicity and cancerogenicity issues because the fluid has to be managed freely and not under hood.
4. When the container arrives at the pathology lab, its opening, extraction of the specimen and reduction of the latter constitutes a major cause of diffusion of formaldehyde fumes.

To circumvent such problems, we adopted a transfer under vacuum. We selected a semi-professional machine, of a relatively limited cost and size (Mod. VAC 10, by Milestone, Bergamo, Italy; see http://www.milestonemedsrl.com) (Fig. 1) and capable of handling large bags. After testing the use of such machine on different tissue specimens and organs (colon, gallbladder, spleen and kidney) and checking the safety of the processing from the point of view of histological preservation, we trans-

Fig. 1 The under vacuum machine used in our hospital

G. Bussolati (✉) · L. Chiusa · A. Cimino · G. D'Armento
Department of Biomedical Sciences and Human Oncology,
University of Turin,
Via Santena n. 7,
10126 Torino, Italy
e-mail: gianni.bussolati@unito.it

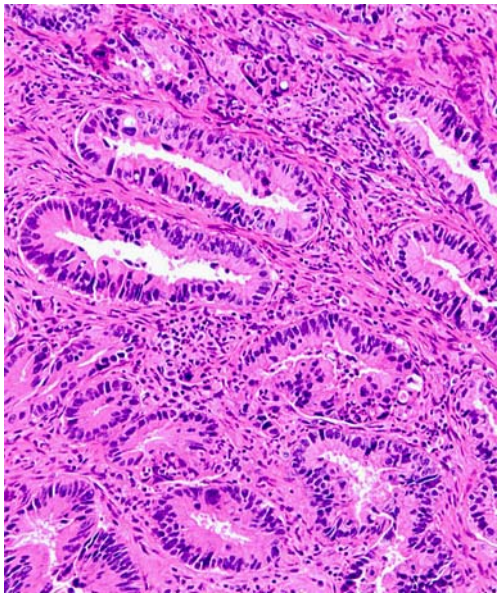

Fig. 2 Adenocarcinoma of the colon. The specimen was kept under vacuum at 4°C for 48 h, then routinely processed with formalin fixation and paraffin embedding. The structure is preserved and diagnosis is feasible. H&E. 150×

ferred the machine to a surgical theatre of our Hospital, where it is now in use by nurses for one year. Large surgical specimens (from thyroid, breast, colon and other organs) immediately after removal are put in plastic bags with or without an identification label (which can alternatively be attached on the surface of the bag). Vacuum and sealing processes takes approximately 15 s. The specimen is then kept in a refrigerator for up to a few hours, although preferentially soon transferred to the pathology lab inside a container (we routinely use a pick-nick box with chilling devices inside). On rare occasions, because of urgency operations during the weekend, the under-vacuum tissues are kept in the refrigerator for 1–2 days and arrived in the laboratory after a long delay.

Upon arrival of the specimen to the pathology lab, reduction takes place so that selected block are inserted into cassettes for routinary fixation in formalin and paraffin embedding, or frozen for tissue banking. The rest of the specimen is kept under formalin in sealed boxes as a reserve.

As a review of the results of the above processing, the experiences gained after 1 year can be summarized as follows:

1. No drawbacks or problems were encountered in approximately 2,000 cases so far processed, as far as the morphological preservation or the immunohistochemical reactivity are concerned. Preservation was less optimal in cases when the under-vacuum specimen had been stored at the surgical theatre for long time, but even this did not prevent histopathological processing and reading (Fig. 2).
2. Under-vacuum processing is very well accepted by the personnel of the surgical theatre, who find it safe and easy. Previously, the consumption of formalin in that place was 15 l per week, and it is now reduced to null.
3. Under-vacuum processing routinely provides material for tissue banking. The quality of RNA preservation is of course related to the time of processing, but even tissues kept in the fridge under vacuum for long time provided nucleic acids of acceptable quality (Fig. 3a,b). This finding is in agreement with reports on the stability of RNA in non-fixed surgical specimens kept on ice [2].

In conclusion, tissue transfer in under-vacuum conditions meets the request of health authorities and involved personnel to reduce exposure to formaldehyde. Absence of air decreases autolytic processes, favours the cooling of specimens (because of the absence of insulating air) and

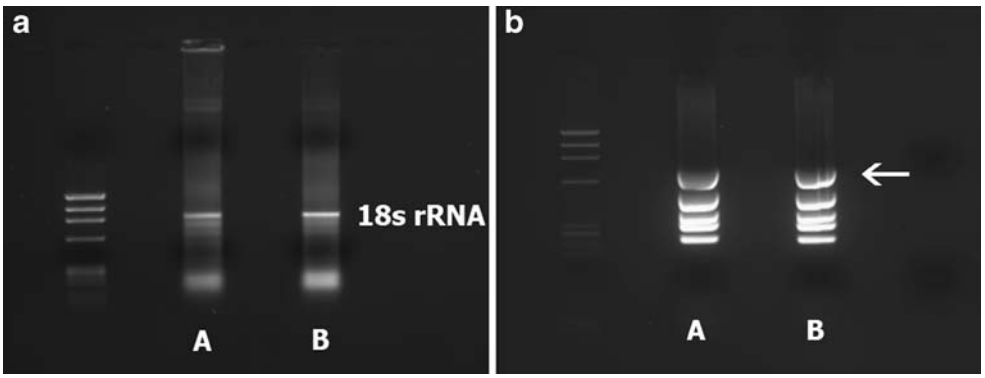

Fig. 3 Colon mucosa either (*A*) frozen immediately after removal or (*B*) preserved under vacuum at 4°C for 48 h. The extracted RNA is of acceptable and similar quality. **a** Shows 1% denaturing agarose gel of total RNA running: the 18s band is visible and no degradation is appreciable. **b** Represents RT-PCR products of cytokeratine 20 mRNA of different bp number. The upper band (*arrow*) is related to a 716-bp product

gives rise to a relatively small and light bag, easy to carry as compared to formalin-filled containers.

The goal of a formalin-free Hospital is still far away, but the present proposal represents a step forward.

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