



UNDER-VACUUM PRESERVATION OF TISSUES TO BE TRANSFERRED TO PATHOLOGY LABS

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Problem & Aim

Problem

- Formalin (4% Formaldehyde) is used worldwide as a fixative and preserver.
- Formalin is toxic, allergenic and a carcinogen (IARC, 2006).
- Surgical specimens to be processed for histopathological diagnosis are routinely immersed in Formalin, then transferred to Pathology labs.

Aim

A safe formalin-free procedure for transferring tissues to Pathology Labs.

Background & Instrumentation

Background

Tissue specimens to be transferred from the surgical theatres to the pathology labs are usually immersed in Formalin, used as a preserver and fixative. This procedure prevents fresh tissue collection for tissue banking and involves the handling of Formalin (a toxic and carcinogenic agent) by nurses in the surgical theatre. Indeed, trying to reduce exposure to formalin is now mandatory.

Instrumentation

To overcome these problems, we adopted an under-vacuum processing for large specimens (while small biopsies are directly immersed in formalin in pre-filled containers).

A High Vacuum specially designed unit (Tissue-safe*, by Milestone, Bergamo, Italy - www.milestonemedsrl.com) was located in the surgical theatres in our Hospital.

Immediately after removal, specimens were put in a plastic bag and processed under-vacuum (the whole processing takes less than 1 min.), then kept in a refrigerating unit at 2° - 4° C until transferred to the Pathology Lab. In the grossing room, the specimens were processed as usual. To test the safety and efficacy of the procedure, we have been checking the preservation of histological features, of antigen detectability and of nucleic acid integrity in tissues kept under-vacuum for various times (from a few hours up to 5 days).

* International Patent Applied For

Procedure

- 1) Tissue specimens are put into plastic bags.
 - 2) Processed under-vacuum at the surgical theatres (High Vacuum specially designed unit by Milestone, Italy).
 - 3) Kept at 2° - 4° C until transfer to the Pathology Labs.
- A few hours later, tissues arrive "fresh" at the grossing room, to be routinely processed for fixation, paraffin embedding and histopathological diagnosis. Occasionally, specimens operated on Friday are kept at the surgical theatre over the week-end.

Tissue safe

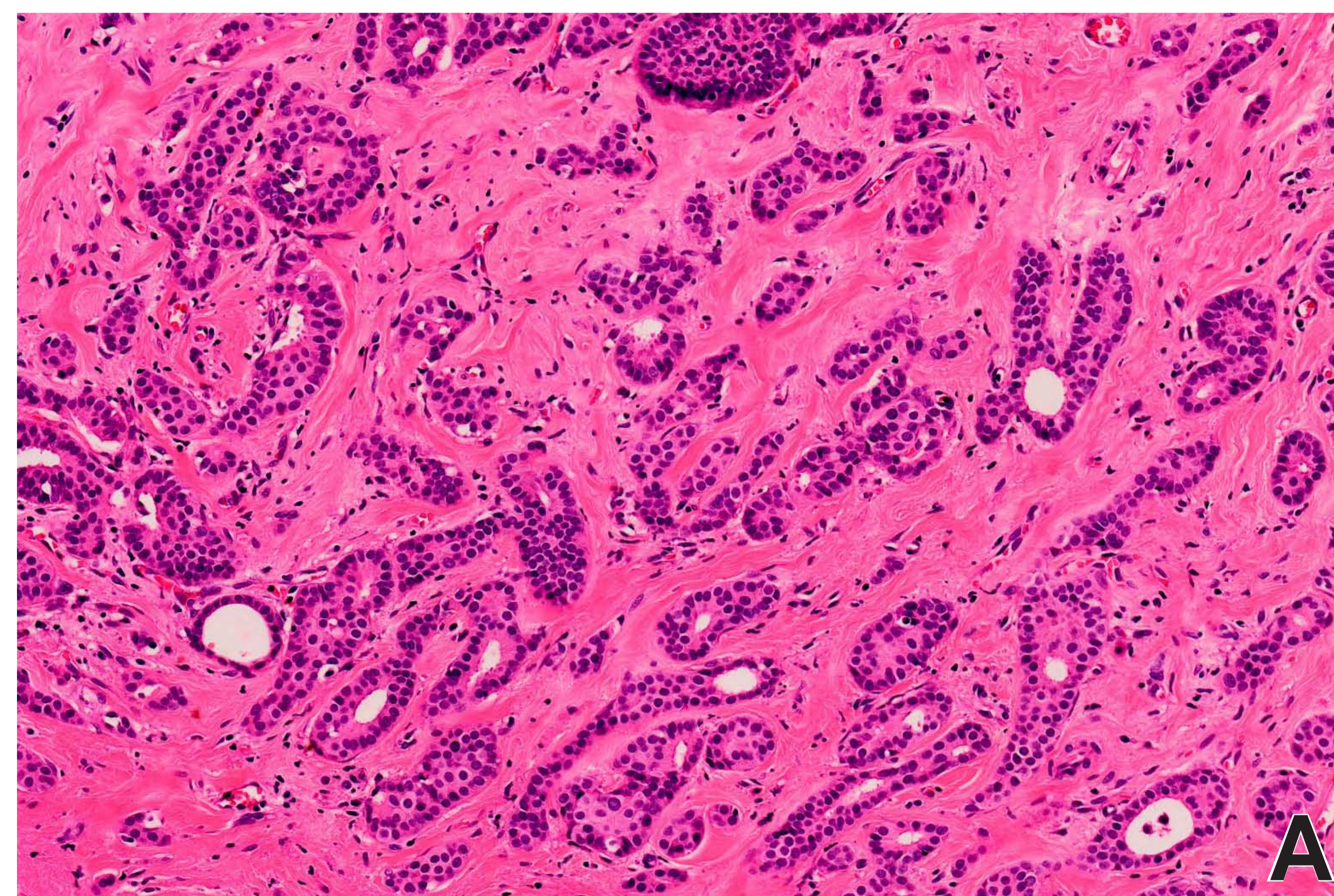


High Vacuum unit located at the surgical theatre in our hospital.

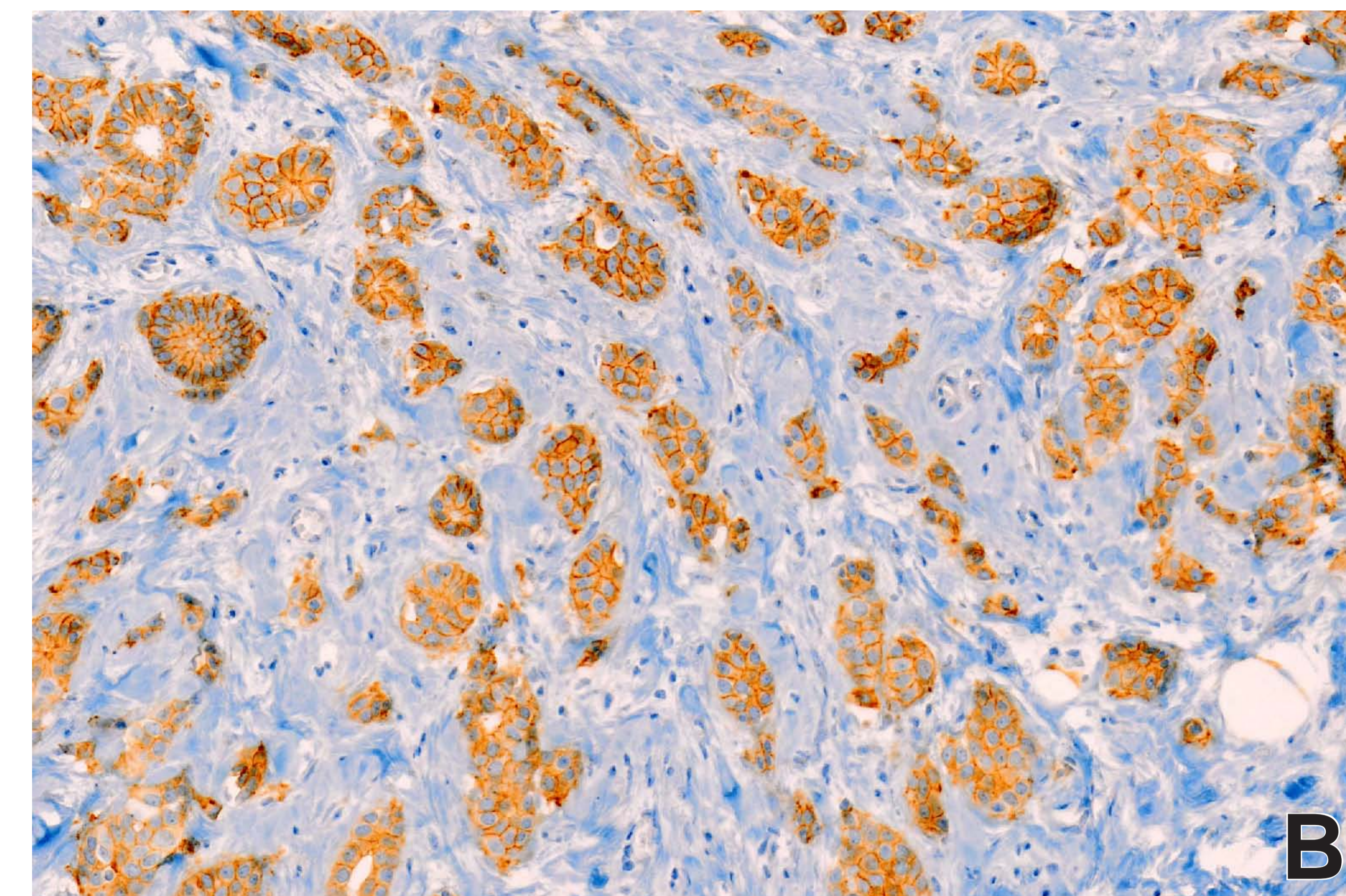
Results

The experience accrued on over 1000 cases (mainly of breast, thyroid and colon lesions) processed under-vacuum and sent to the Pathology labs for grossing allows to conclude that this processing is well appreciated by both nurses and laboratory personnel. Fresh under-vacuum tissues kept in the refrigerating unit (2°-4° C) up to 2-3 days still retain an excellent morphology, as well as preservation of immunohistochemically detectable antigens and of nucleic acids. Tissues banking, cell cultures and electron microscopy were still feasible in such material. This procedure offers advantages in the processing of breast biopsies.

Breast Cancer

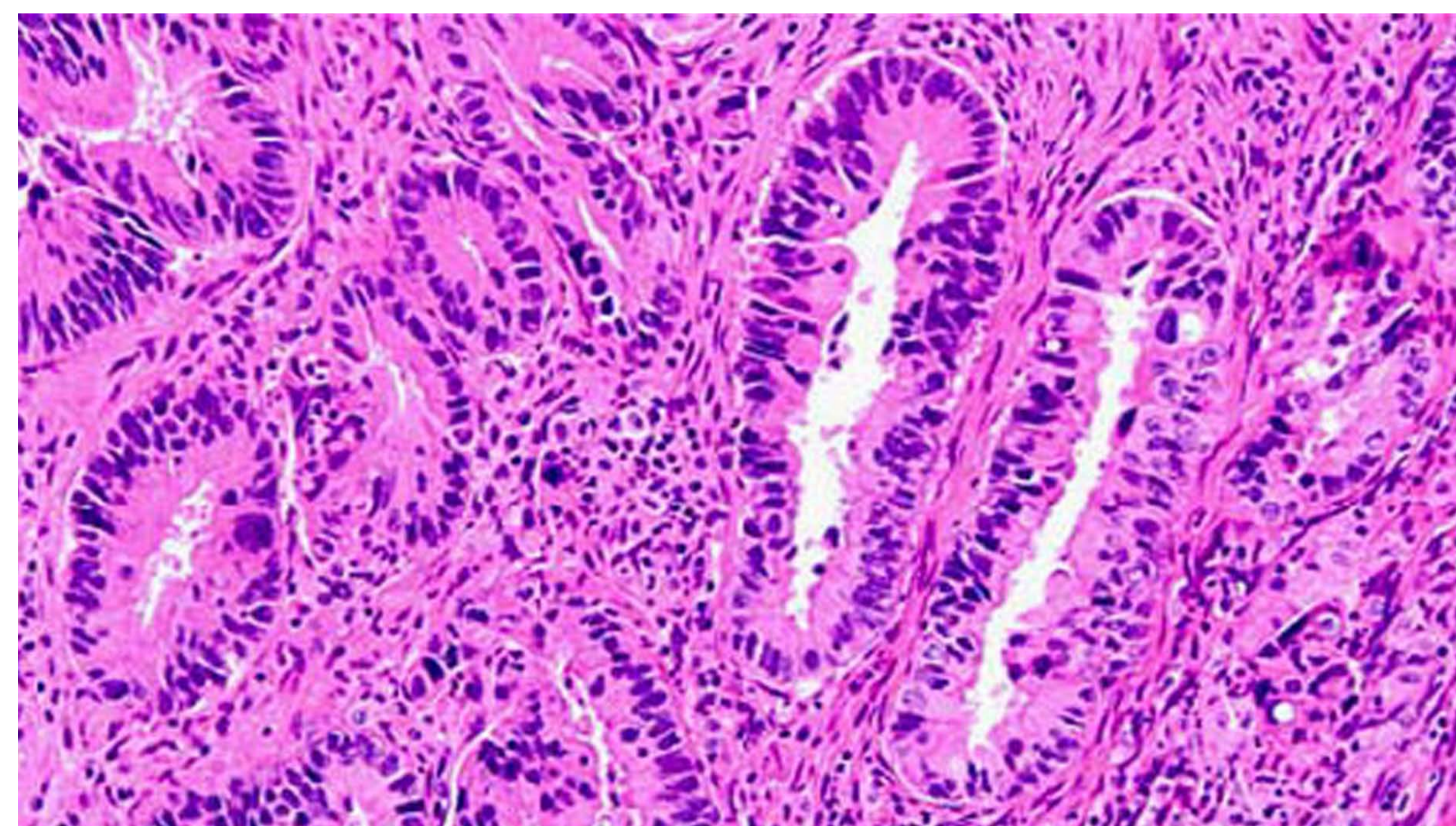


H&E of an infiltrating ductal carcinoma on the breast. The specimen was kept under-vacuum at 4°C for 72 h, then routinely processed with formalin fixation and paraffin embedding.

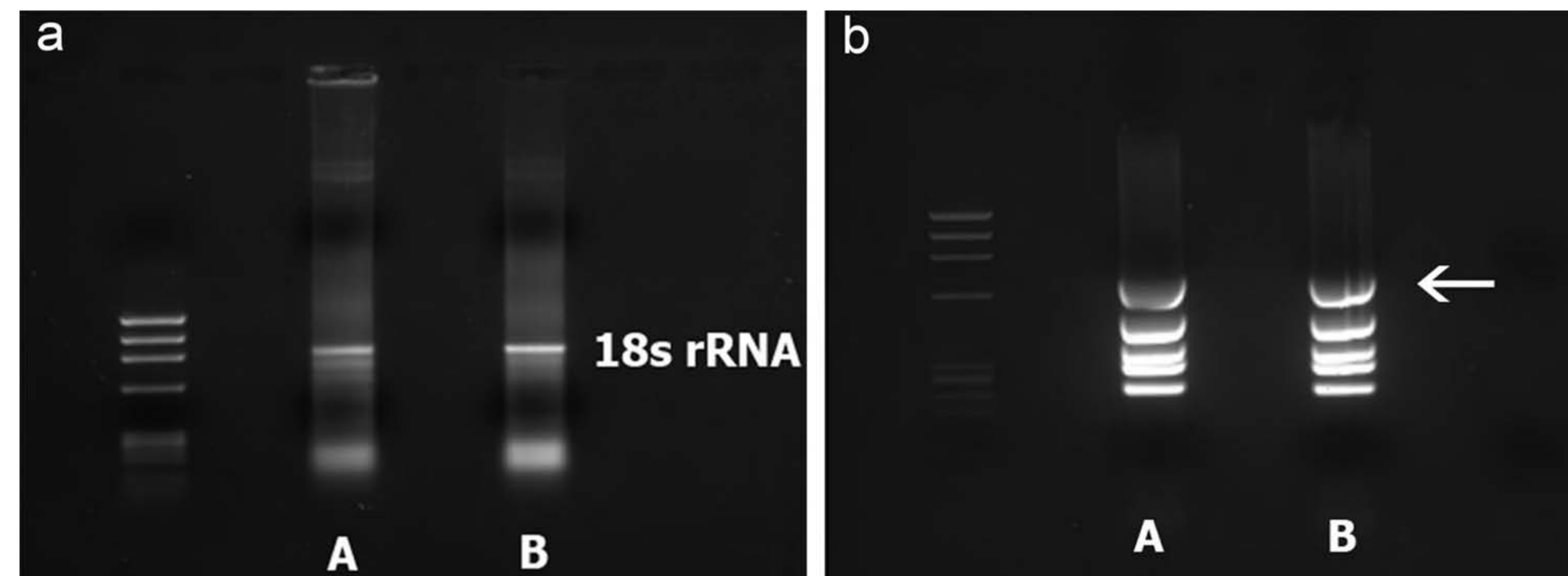


Same case of breast cancer. Immunohistochemical staining for HER2 protein shows a continuous membrane decoration in >30% cancer cells (3+score).

Colon Cancer

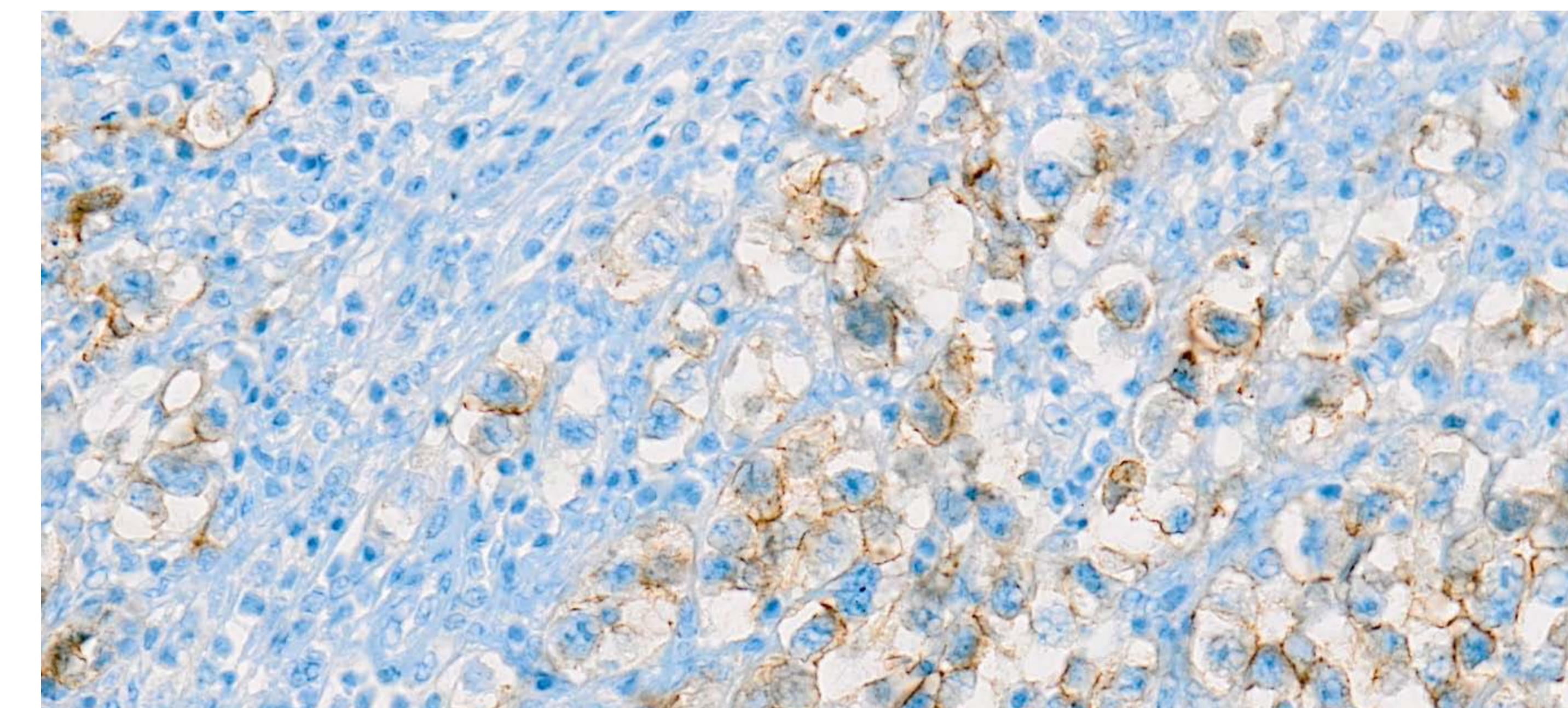


H&E of an adenocarcinoma of the colon. The specimen was kept under vacuum at 4°C for 48 h, then routinely processed with formalin fixation and paraffin embedding. The structure is preserved and diagnosis is feasible.



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.

Seminoma of the testicle



Specimen kept under-vacuum at 4°C in the surgical theatre over the week-end. Immunohistochemical staining for Placental Alkaline Phosphatase.

Conclusions

Tissues preserved under-vacuum: merits

- No more formalin in surgical theatre
- No spilling
- No fumes
- No drying of tissues
- Colors preserved
- Lack of insulating air around tissues allows fast cooling
- Tissues (bags) light and easy to carry
- Structure (RNA, Antigens) preserved up to days
- Banking (selective) allowed
- Cell cultures feasible.