INTERVIEW

STANDARDIZE AND SECURE THE FREEZING OF TISSUES WITH FlashFREEZE

THE EXPERIENCE OF HOSPITAL CHU D'AMIENS - PICARDIE

Interview with Isabelle Torchy, Helath Executive and Eléonore Capliez, medical laboratory technician and former intern, at the Anatomo-CytoPathology Laboratory of Amiens-Picardie

Isabelle Torchy (left), Eléonore Capliez (right), and Cathy Jacquart, a laboratory technician using FlashFREEZE

Q: Can you introduce us to your laboratory?

Isabelle: We are part of the Center Hospitalier Universitaire d'Amiens which includes 2 sites, the North site where our laboratory is located, and the South site where we have an area dedicated to extemporaneous examinations and the reception of fresh samples.
Our laboratory currently processes 400 blocks per day and has 16 technicians and 6 medical pathologists. We support all types of histological and cytological sampling and carry out standard and special colorations, IHC, IF, enzymology and FISH.
As part of our tumor library activity, we freeze tissue samples from cancer patients for diagnostic or research purposes. These fresh samples must be frozen in less than 30 minutes to avoid nucleic acid degradation and then stored in freezers at -80°C.
We have embarked on an accreditation process in accordance with ISO 15 189, with the aim of automating, standardizing and tracing as far as possible each phase of sample processing. Our approach also involves minimizing the chemical, biological and musculoskeletal risks to which technicians are subjected.

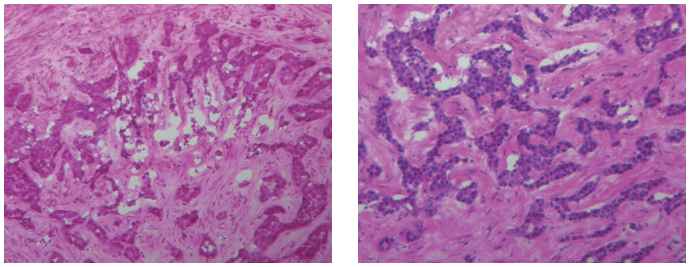
Q: What led you to become interested in FlashFREEZE?

Isabelle: In 18 months, the North and South sites of CHU d'Amiens will be grouped in a new building on the South site. The Anatomo-CytoPathology Laboratory will be located just above the operating rooms. The use of liquid nitrogen has been compromised for logistical reasons, as it would have required a dedicated elevator to transport liquid nitrogen to our facility and could not be stored near care facilities and operating rooms. Indeed, liquid nitrogen presents several dangers: anoxia in case of excessive evaporation; it can take the place of ambient oxygen; there is also a risk of cryogenic burn when handled; finally a risk of explosion if it is trapped inside a container.
This led us to seek a less dangerous and restrictive solution that would allow us to freeze our samples in an ultra-fast manner without compromising tissue preservation.
By talking with Paul Gomes, sales manager for MM FRANCE, I learned about the existence of the FlashFREEZE system which made it possible to **freeze our samples at -80°C, very quickly and in complete safety, while also standardizing the freezing process**. This was not possible with liquid nitrogen.
This new machine became even more interesting when our head of service, Pr. Denis Chatelain, wanted to create a partnership with the Biobank of Picardie, certified ISO 9001, to secure the preservation of our tumor library and enhance our samples. Therefore, we carried out a validation of the method of freezing fresh tissue fragments with FlashFREEZE according to ISO 15 189 and I entrusted this work to Eléonore Capliez . Eléonore got an internship in our laboratory and did her dissertation on this subject.

Q: What was this method validation and what results did you obtain?

Eléonore: **We carried out a comparative study between freezing samples using liquid nitrogen and freezing at -80°C with the FlashFREEZE unit and the Flashsolv liquid**.
The aim was to compare the performance of these two freezing techniques, in terms of tissue preservation, by comparing the tissue morphology and the immunohistochemical and molecular properties of frozen tissues.
The tissues frozen simultaneously by the two techniques were cut with cryostat and stained with Hematoxylin Phloxine (HP). The tissue fragments were then thawed, included in paraffin and stained with Hematoxylin Phloxine Saffron (HPS). Special colorations, Trichrome, Perls and PAS, were also made. The antigenic reactivities of the tissues were tested using immunohistochemical techniques with different antibodies. Finally, the preservation of nucleic acids in frozen tissues by these two techniques was evaluated at the Molecular Biology Laboratory of Amiens CHU. The quantity and quality of DNA and RNA extracted from frozen tissues were compared. Their quality was assessed by identification of fusion transcripts.
We performed this method validation for many healthy and tumor tissues, including tissues from placenta, uterus, ovary, colon, skin,

and adrenal glands. For each tissue type, we performed at least 30 mirror tests. **Of our 30 cryostat-cut and HPS-stained samples, 12 had better preserved morphology with FlashFREEZE, 3 had a morphology better preserved with liquid nitrogen and 15 had an equivalent morphological conservation between the two freezing methods.**



Metastasis of a neuroendocrine tumor, X10 magnification.
Better differentiation of nuclei and cytoplasm with FlashFREEZE (right photo) than with liquid nitrogen (left photo).

These results can be explained by the fact that **freezing with FlashFREEZE is milder than freezing with liquid nitrogen and avoids cell alteration when changing temperature**. Infact, with liquid nitrogen, the cells are frozen at -196°C and then return to -80°C when stored in the cryotubes; this thermal shock can cause their alteration. **With FlashFREEZE, the cells are frozen and stored at the same temperature, which certainly explains why we obtained more qualitative results than with liquid nitrogen**.
For microtome cutting, the morphological preservation is equivalent between the two freezing methods, both for HPS staining and for special staining.
Immunohistochemistry results demonstrate that cytoplasmic and nuclear labeling are equivalent with both freezing methods.
Regarding molecular biology tests, we found that FlashFREEZE did not alter the samples more than liquid nitrogen. All the DNA obtained had a quality of 600 bp, which means that the samples are well preserved by the two freezing techniques. RNA is also preserved in the same way by both freezing techniques.
In terms of usability, we were able to freeze up to 25 cryotubes (1.2 mL) in maximum 120 seconds at –80°C. **FlashFREEZE allows to freeze cryotubes up to 50 mL and keeps samples at -80°C while waiting to transfer them to a -80°C freezer**.

In conclusion, we have validated the freezing method with the FlashFREEZE unit. This system allows an equivalent or higher quality of tissue preservation compared to liquid nitrogen freezing. Moreover, FlashFREEZE provides the following benefits for the laboratory:

- **User safety and a simplified freezing method, by replacing hazardous liquid nitrogen with a simple-to-use device** and using a non-CMR coolant, the Flashsolv.
- **Time-savig freezing procedures**
- **Standardization of the freezing tissues method**

Q: Have you studied slow freezing with FlashFREEZE?

Isabelle: We will soon start this study on kidney biopsies, skin samples, and muscles that require a mild freezing, process less rigid than with liquid nitrogen in order to better conserve cells and limit artifacts on the blade. Unfortunately, this technique cannot be standardized and uses isopentane, a C.M.R agent (carcinogenic, mutagenic and reprotoxic). The FlashFREEZE and the Flashsolv, which is based on ethanol and is not C.M.R., would solve these problems.

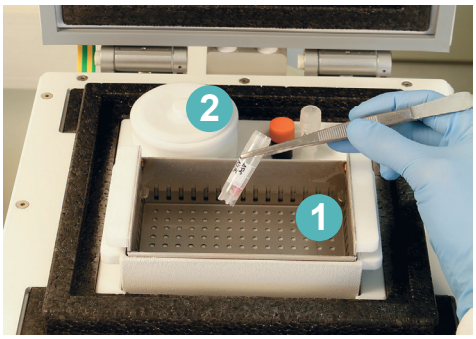
Q: What improvements would you like to make to FlashFREEZE?

Isabelle and Eléonore: We would like it to be possible for quick freezing to trace the patient ID, the date and time of freezing, the batch and the expiry date of the Flashsolv.

Q: In fact, this traceability already exists for slow freezing but indeed, it should also be available for rapid freezing. We will talk to the manufacturer Milestone who is always very reactive. Congratulations to Ms Capliez for this beautiful validation work and congratulations Mrs Torchy for your guidance and your willingness to make your team grow!

This interview has been conducted by E. Michel and V. Lafargue of MM France company - exclusive Milestone distributor in France.

FlashFREEZE Unit:
30x54x45 cm (LxPxH)
25 kg



1 - Freezer tank may contain up to 25 cryotubes
2 - Container for storing the cryotubes at -80°C while waiting for their transfer to the freezer

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