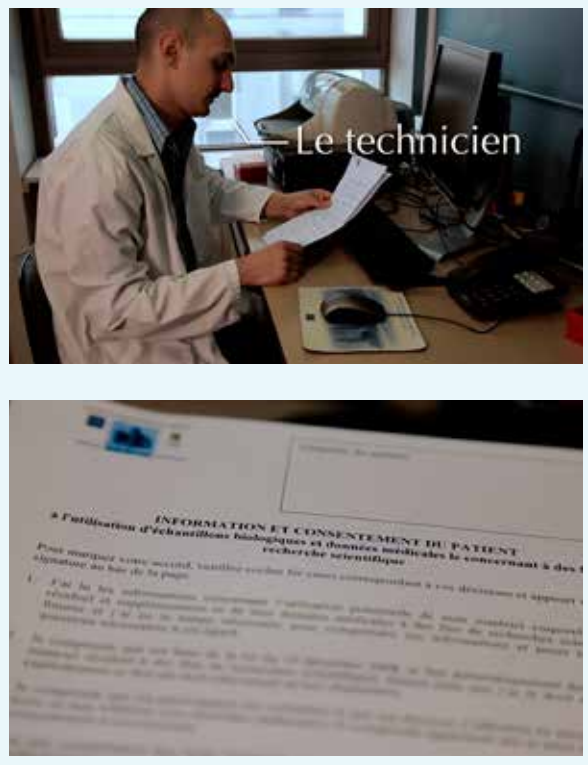


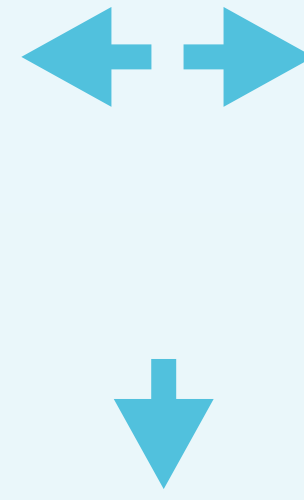
# IMPROVING TRANSLATIONAL RESEARCH SAMPLE COLLECTION IN INTERNATIONAL TRIALS

Use Standard Operating Procedures to document the methodology you use. Be able to trace who has done what, when and how!



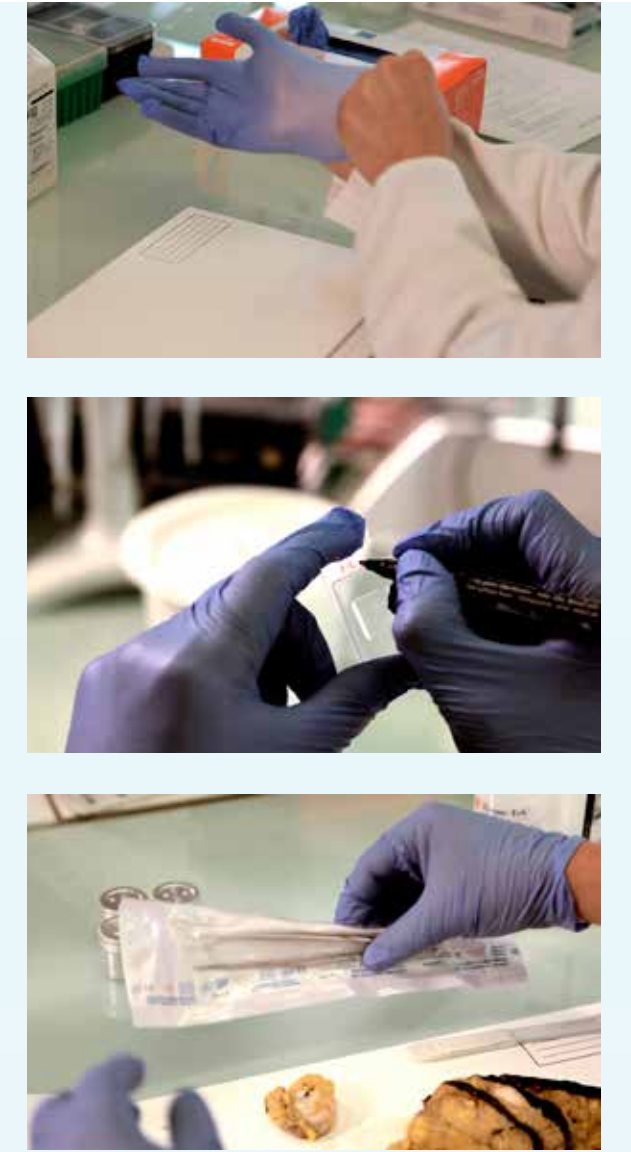
## Important communications:

- Before any sampling takes place it is important to notify the staff involved in the sampling. These can be the surgeons, the nursing staff or radiology staff depending on the type and origin of the tissue you would like to collect.
- It is important that the staff involved have made sure that the patient has consented for tissue collection for research purposes.



## Important recommendations:

- Always wear gloves
- Use sterile instruments that are DNA-ase and RNA-ase free.
- Don't use the same instrument to sample normal and tumour tissue respectively.
- Don't sample macroscopically evident zones of necrosis.
- If you take normal tissue, please take it as far as possible from the tumour; avoid sampling only fat as normal tissue (unless that is intended).
- Don't ever take a tissue sample for research without checking the informed consent and consulting with the pathologist.

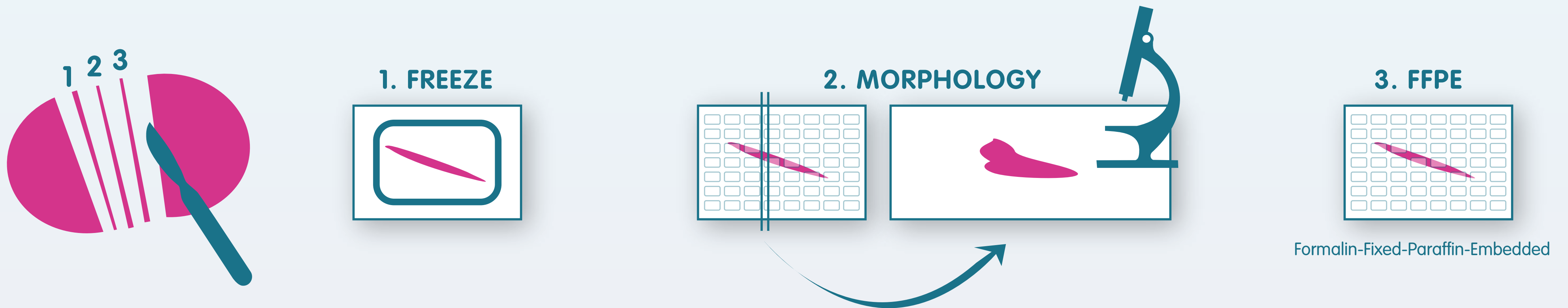


## Once the tissue is sampled:

- Immediately send the tissue to the laboratory. It is crucial that the cold ischemia time be as short as possible, preferably <30min from the moment of resection.
- The time lapse between sampling and freezing of the tissue should be recorded in real time.
- The vial should be clearly labelled using patient unique identifiers and using ink that is resistant to humidity and cold.
- It is crucial to ensure that the sample is neither squeezed nor fragmented after sampling. The tissue should be manipulated gently, if at all.

## Use the mirror-image principle to cut the samples!

- This means that one cut section is used for morphology evaluation, and that adherent sections on both sides of the slide used for morphology evaluation are frozen and respectively fixed and paraffin-embedded.
- Mirror-images are important to avoid heterogeneity between a frozen sample and a corresponding fixed sample from the same tumour.



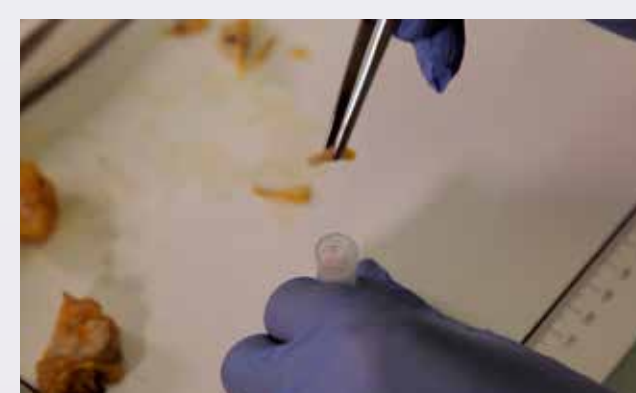
## There are 3 options to freeze your tissue:

### USING LIQUID N2:



- The tissue is rapidly frozen in liquid nitrogen, at temperatures of -160 till -190°C, before being transferred to the -80° freezer.
- The main disadvantage of this method is freezer-burn artefacts.
- + The main advantage is that it's easy and relatively rapid.

### USING RNA-LATER:



- The tissue is first immersed in RNA-later.
- The sample can then be stored at room temperature or even at 4° to 5°C in the fridge for several days before being transferred to the -20°C freezer for several weeks before being transferred to the -80°C freezer.
- Gradual accommodation of the tissue to lower temperatures is a preferred option to consider. Sample size should be limited to ensure RNA-later can penetrate.
- The main disadvantages of this method are the difficulties to evaluate the morphology, especially the lymphocyte infiltration.
- + Major advantages include excellent RNA-preservation, no need for dry ice or liquid nitrogen, and it's ease of use in general.

### USING OCT:



- The tissue is embedded in OCT as a cryoprotective measure, before being transferred to the -80°C freezer.
- It is important that all tissue is well embedded within the OCT, so that the tissue has no contact with air anymore.
- The tissue sample should be flat, and don't add too much OCT.
- The sample is now transferred to the -80°C freezer.
- A big disadvantage is that PCR-reactions can be inhibited.
- + The main advantage of this method is that it protects against freezer-burn artifacts, morphology is well preserved, and it protects against potential freeze-thaw cycles when transferring a sample from one storage site to another.

## Fixing the tissues in formalin:

- Always wear gloves.
- Limit the cold ischemia time as much as possible; thus, fix the cores or the samples in fixative as soon as possible!
- When using fixative, buffered formalin is most often used. Please use buffered formalin in order to fix the samples well.
- If you use other fixative than formalin, please validate this fixative before adapting it as routine! Finally, don't forget: time of fixation is best limited to 6 to 48h; and, underfixation can be as detrimental to epitopes as overfixation!

## Once the samples have been stored:

- Consider at least 2x/year a random quality control check of DNA, RNA and protein of at least 1% of your samples, both if the samples are used for research or diagnostic purposes.
- Also consider using dry ice when transferring samples from one repository to another in order avoid freeze-thaw cycles on the same sample.
- Don't forget to ensure that all equipment used is validated, whenever applicable.
- It is strongly suggested to have a continuous temperature monitoring of all crucial fridges and freezers, implementing an associated instant alarm notifying system.

For related information, please consult our other educational material at [www.BIGagainstbreastcancer.org](http://www.BIGagainstbreastcancer.org)

### DISCLAIMER

This educational material aims to illustrate "Good Laboratory Practice" in terms of handling samples in clinical cancer trials. To this end, focus has been put on the most frequently used methods, knowing that other methodologies and preservatives/fixatives may also be used in some laboratories. No judgment is made about such other methodologies and preservatives/fixatives. The Breast International Group (BIG) shall not be liable in any way with regard to the application of any content presented here.

### ACKNOWLEDGEMENTS

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What to do before sampling?

What to do during sample handling?

What to do after sample handling?