

## Freezing of Tissue Specimens

Updated: 7<sup>th</sup> December 2011

(The document is being revised and additional references will be added subsequently)

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**Reference:** Mager SR, Oomen MHA, Morente MM, Ratcliffe C, Know K, Kerr DJ, Pezzella F, Riegman PHJ, "Standard Operating Procedure for the collection of fresh frozen tissue samples," *European Journal of Cancer***43**:828-834 (2007)

**Notes:** This article describes the guidelines for collection, processing and storage of fresh-frozen tissue samples established at the European Human Frozen Tumor Bank (TuBaFrost). The article describes the workflow for the tissue collection process, responsibilities and roles of various personnel involved and quality control aspects.

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**Reference:** Gal AA, "The Centennial anniversary of the frozen section technique at the Mayo Clinic," *Archives of Pathology and Laboratory Medicine***129**:1532-1534 (2005)

**Notes:** In celebration of 100 year anniversary of the landmark publication by Dr. Louis Wilson that described the tissue freezing technique developed by him at the Mayo Clinic, this article recounts the historical developments that lead to the modern frozen tissue.

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**Reference:** Steu S, Baucamp M, von Dach G, Bawohl M, Dettwiler S, Storz M, Moch H, Schraml P, "A procedure for tissue freezing and processing applicable to both intra-operative frozen section diagnosis and tissue banking in surgical pathology," *Virchows Archives***452**: 305-312 (2008)

**Notes:** Three different methods of freezing tissue samples were compared with the help of immunohistochemistry (IHC), DNA and RNA integrity analysis, PCR and Immunoblotting. The freezing protocols included tissue freezing with cold CO<sub>2</sub> flowing over tissue samples, snap freezing by direct immersion in LN<sub>2</sub> and OCT embedding and freezing in pre-cooled isopentane at -80C. Morphological artifacts were observed in samples frozen using CO<sub>2</sub> snow while all assay yielded satisfactory results for both LN<sub>2</sub> cooled and isopentane cooled samples.

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**Reference:** Bratthauer GL, "Preparation of frozen sections for analysis," *Immunocytochemical Methods and Protocols, Methods in Molecular Biology Eds. Oliver C and Jamur MC***588**: 67-73 (2010)

**Notes:** This chapter describes detailed protocol for freezing of tissue samples, frozen tissue sectioning and slide preparation for staining.

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**Reference:** Ardévol A, Cañas X, Remesar X, Alemany M, "Cooling rates of tissue samples during freezing with liquid nitrogen," *Journal of Biochemical and Biophysical Methods* **27**:77-86 (1993)

**Notes:** The article describes a very interesting aspect of tissue freezing, the thermal history experienced by the tissue which is closely depended on the specific heat and thermal conductivity of the tissue material. Observations show that fatty tissue like the adipose tissue or fat muscle have very low conductivity and thus take long to freeze compared to other tissues like liver. It is important to understand how these or other parameters like sample size etc. affect the freezing outcome. Hence if a protocol suggests freezing for a specific time period, all tissue types may not yield intended results.

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**Reference:** Dankwa EK, Davies JD, "Frozen section diagnosis: an audit," *Journal of Clinical Pathology* **38**:1235-1240 (1985)

**Notes:** This article may be of special interest to clinicians and pathologists as it describes potential sources of errors during diagnosis using frozen sections as well as avoidable and non-avoidable errors.

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**Reference:** Loken SD, Demetrick DJ, "A novel method for freezing and storing research tissue bank specimens," *Human Pathology* **36**:977-980 (2005)

**Notes:** An interesting alternative to the standard freezing methods is the use of pharmaceutical capsule to contain the specimen during freezing. This method combines the advantages of OCT specimen preservation with convenience of cryovial storage. The caveat is that the method can be applied only to small tissue samples.

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**Reference:** Serth J, Kuczyk MA, Paeslack U, Lichtinghagen R, Jonas U, "Quantitation of DNA extracted after micropreparation of cells from frozen and formalin-fixed tissue sections," *American Journal of Pathology* **156(4)**:1189-1196 (2000)

**Notes:** This article illustrates amicrodissection technique for, and quantitation of extracted DNA on histologically characterized cells from frozen as well as FFPE tissue sections. The results show that hematoxylin staining interferes with the extraction of DNA.

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**Reference:** Vega CJ, "Laser microdissection sample preparation for RNA analyses," *Methods in Molecular Biology, Eds. Mor G and Alvero AB* **414**:241-252 (2008)

**Notes:** The chapter describes detailed protocol for laser microdissection of frozen and FFPE samples for extraction of RNA.

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**Reference:** Studer D, Graber W, Al-Amoudi A, Egli P, "A new approach for cryofixation by high-pressure freezing," *Journal of Microscopy* **203(3)**:285-294 (2001)

**Notes:** A different approach to freezing biological samples involves using high pressures for upto 2000 bar to essentially vitrify the samples. Mainly targeted towards electron microscopy, this method yields excellent structural details in the cryofixed samples processed by freeze substitution.

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**Reference:** Ahmed MM, Gardiner KJ, "Preserving protein profiles in tissue samples: Differing outcome with and without heat stabilization," *Journal of Neuroscience Methods***196**:99-106 (2011)

**Notes:** To minimize the detrimental effects of tissue processing methods and delay in processing on the post translational modifications and phosphorylation states of proteins, a method of heat stabilization is proposed. It is hypothesized that heat stabilization causes rapid and effective inactivation of various enzymes responsible for protein modifications and degradation thereby preserving these biomarkers and their phosphorylated states more effectively when preserved using standard techniques.

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**Reference:** Turbett GR, Sellner LN, "The use of optimal cutting temperature compound can inhibit amplification by polymerase chain reaction," *Diagnostic Molecular Pathology***6(6)**:298-303 (1997)

**Notes:** Since OCT is used frequently for embedding of tissue samples, evaluation of the interaction between OCT and the biological components of the tissue is necessary. In this article the effects of OCT on the stability of nucleic acids, which are important biomarkers for diagnostic purposes, are studied. The experiments indicated that OCT caused degradation of DNA, but did not affect the quality of RNA, and the authors cautioned against use of OCT during routine storage of tissue samples.

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**Reference:** Callis G, "Preparation and snap freezing of murine tissues for research immunohistochemistry and routine hemotoxylin& eosin," *Histologic***37(1)**:4-7 (2004)

**Notes:** Preservation and use of murine tissues for histology has its own challenges and the author describes various methods for freezing adopted in their laboratory to achieve optimum results.

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**Reference:** Fail R, Della Speranza V, "A method to repair freeze artifact in skeletal muscle biopsies," *Histologic***36(1)**:10-11 (2003)

**Notes:** The article describes one easy way to correct some of the freezing artifacts by thawing and refreezing of samples which produced good results in their laboratory. Also an important point to note is the authors advocate for gum tragacanth as a freezing-embedding medium instead of OCT as it better supports and preserves orientation of the tissue sample during freezing.

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**Reference:** Perlmutter MA, Best CJM, Gillespie JW, Gathright Y, Gonzalez S, Velasco A, Kinehan WM, Emmert-Buck MR, Chuaqui RF, "Comparison of snap freezing versus ethanol fixation for gene expression profiling of tissue specimens," *Journal of Molecular Diagnostics***6(4)**:371-377 (2004)

**Notes:** Comparison of snap frozen sections with ethanol fixed paraffin embedded samples were made using molecular profiling assays. Based on the results, freezing is the recommended method for proper preservation of the genetic material which will be used for gene expression analysis compared to fixation techniques.

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**Reference:** Perlmutter MA, Best CJM, Gillespie JW, Gathright Y, Gonzalez S, Velasco A, Kinehan WM, Emmert-Buck MR, Chuaqui RF, "Comparison of snap freezing versus ethanol fixation for gene expression profiling of tissue specimens," *Journal of Molecular Diagnostics***6(4)**:371-377 (2004)

**Reference:** Gillespie JW, Best CJM, Bichsel VE, Cole KA, Greenhut SF, Hewitt SM, Ahram M, Gathright Y, Merino MJ, Strausberg RL, Epstein JI, Hamilton SR, Gannon G, Baibakova GV, Calvert VS, Flaig MJ, Chuaqui RF, Herring JC, Pfeifer J, Petricoin EF, Linehan WM, Duray PH, Bova GS, Emmert-Buck MR, "Evaluation of non-formalin tissue fixation for molecular profiling studies," *American Journal of Pathology***160(2)**:449-457 (2002)

**Notes:** Comparison of snap frozen sections with ethanol or formaldehyde fixed paraffin embedded samples were made using molecular profiling assays. Based on the results, freezing is the recommended method for proper preservation of the genetic material which will be used for gene expression analysis compared to fixation techniques.

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**Reference:** Ericsson C, Franzén B, Nistér M, "Frozen tissue biobanks. Tissue handling, cryopreservation, extraction, and use for proteomic analysis," *Acta Oncologica***45**:643-661 (2006)

**Notes:** A good review discussing various aspects of tissue handling, processing, freezing, extracting of proteome and proteomic analysis from the perspective of the frozen tissue banks.

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**Reference:** Bischof J, Christov K, Rubinsky B, "A morphological study of cooling rate response in normal and neoplastic human liver tissue: Cryosurgical implications," *Cryobiology***30**:482-492 (1993)

**Notes:** An excellent article that describes the relationship between the cooling rate that the tissue samples are exposed to and the resulting morphology with normal and neoplastic human liver tissue as model system. Although the article is primarily focused on cryosurgical applications, the overall discussion about the effects of freezing on tissue is relevant to the diagnostic pathology community as well.

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**Reference:** Strambini GB, Gabellieri E, "Proteins in frozen solutions: Evidence of ice-induced partial folding," *Biophysical Journal* **70**:971-976 (1996)

**Notes:**

Another article from a different field of research, which describes the effects freezing on the tertiary structure of isolated protein. Though the research involves use of isolated proteins, the information is relevant to the histology community because of potential relationship between freezing methods and antigenicity of biomarkers of interest.