NCI Biospecimen Evidence-Based Practices	SNAP-FREEZING OF POST-SURGICAL TISSUE BIOSPECIMENS				
Author	Biorepositories and Biospecimen Research Branch			Revision # (Date)	1.0 (2/4/2025) from Original (1/25/2014)
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1.0 PURPOSE

The purpose of this document is to provide evidence-based guidance for the proper snap-freezing of human tissue biospecimens. This guidance is intended to support the development and execution of evidence-based Standard Operating Procedures (SOPs) for human biospecimen collection, processing, and storage operations.

2.0 SCOPE

This evidence-based best practice document is applicable to all human tissues that are to be preserved by snap-freezing. Biospecimens preserved under these procedural guidelines are suitable for downstream analysis of DNA, RNA, protein, and morphology endpoints. Additional analytical endpoints, including but not limited to cell viability, cell sorting, drug sensitivity testing, or use as donor specimens for xenografts or primary tissue culture, do not fall within the scope of this document.

3.0 DEFINITIONS

3.1 **Organ** – the complete or partial organ that is removed from the patient for dissection

3.2 **Module** – the portion(s) of the organ that is/are specifically removed for the creation of segments or aliquots

3.3 **Segment** – the component(s) that is/are dissected from the module that will be used to create the aliquot(s) for final labeling and submission

3.4 **Aliquot** – the final tissue component(s) that is/are dissected directly from the organ or the segment according to protocol

3.5 **Surgical Warm Ischemia Time** – the length of time a biospecimen is retained at physiological temperature, commencing with instrument-obstructed blood flow and terminating upon removal from the patient

3.6 **Surgical Cold Ischemia Time** –the length of time elapsed between the time of removal of the tissue from the patient and the time the tissue is preserved by freezing, placement in formalin, or other stabilization method

3.7 **Post-Mortem Interval (PMI)**–the length of time elapsed between the time of non-beating heart death and the time the tissue is preserved by freezing, placement in formalin, or other stabilization method

4.0 ENVIRONMENTAL HEALTH & SAFETY

4.1 Universal Precautions (CDC-1978) are used for all phases of organ/tissue dissection and handling. Reference 9.1.1.

5.0 RECOMMENDED MATERIALS/EQUIPMENT

5.1 Plastic-backed absorbent bench paper

5.2 New disposable dissecting equipment for each organ

5.3 Liquid Nitrogen (LN2)

5.4 Dewar flask

5.5 Cryogenic specimen storage container (cryovial, cryostraw, cryosette®, cryomold, or equivalent storage container designed for temperatures at or below -190°C), LN2 storage container or, in the event of immediate shipment, LN2 dry shipper

5.6 Should LN2 be unavailable, alternative freezing media may include: isopentane pre-cooled with LN2; isopentane cooled with dry ice; dry ice alone; -80°C freezer. When utilizing dry ice or -80°C for freezing and storing at -80°C, suitable cryogenic specimen storage containers designed for temperatures at or below -80°C will be acceptable, and shipment may be performed on dry ice.

6.0 PROCEDURAL GUIDELINES

6.1 Recording of biospecimen preacquisition data

6.1.1 Whenever possible, extensive data should be recorded relating to preacquisition conditions that may affect the integrity of the biospecimen. Such data may include patient information (including age, gender, diagnosis and treatment) as well as details relating to surgery and

biospecimen acquisition (including the use of anesthesia, warm ischemia time, and surgical procedure and duration).

6.2 Preparation of freezing containers and bench space

6.2.1 Pre-labeled cryogenic specimen storage containers for each organ being dissected should be identified and arranged before the organ is available for dissection.

6.2.2 Specimen containers should be appropriately labeled and organized, and tissues of different anatomic sites as well as tumor and normal tissues should be segregated to the extent possible.

6.2.3 Clean disposable scalpels and forceps should be used when cutting different tissue types of the same patient and specimens from different patients. Contact with absorbent materials, which may contaminate dissected research tissues or where capillary action may draw fluid from tissue samples, should be avoided.

6.3 Post-collection storage of tissue specimens on wet ice

6.3.1 Specimens may be placed in a sterile closed container on wet ice until dissection (See 8.1).

6.4 Minimizing cold ischemia time

6.4.1 Dissection should be accomplished soon after the specimen is released by the supervising physician. Cold ischemia time should be minimized as much as possible, optimally less than 20 min but no more than 1 hour (See 8.2). Cold ischemia time should be documented for every module or segment and for each subsequent aliquot.

6.4.2 For tissue specimens collected postmortem, PMI should be minimized as much as possible, optimally less than 2 hours, but no more than 6 hours (See 8.3). This time should be documented for every patient and attached to the module and its aliquots.

6.5 Dissection notes

6.5.1 Dissection should be performed one organ at a time. Final aliquots should be no thicker than 0.4 cm and placed into the proper cryogenic specimen storage containers. If morphological analysis is anticipated then specimens can be surrounded by OCT medium prior to freezing; however, the use of OCT is not optimal for some specific molecular analysis methods (See 8.4).

6.6 Freezing of tissues

6.6.1 Optimally, the tightly sealed cryogenic specimen storage container should be frozen in LN2 vapor. This can be achieved by suspending a stainless steel beaker inside a bench-top Dewar flask pre-filled with LN2 (See Figure 7.1). The specimen storage container should then be placed inside the steel beaker for 2 minutes or less depending on the size of the specimen (See 8.5). Common alternatives to freezing in LN2 vapor may include freezing by immersion in LN2 or immersion in isopentane pre-cooled to -80°C or below (See 8.6).

6.6.2 If LN2 is unavailable at the physical site where specimens are collected and preserved, alternative freezing methods may used, and include immersion in isopentane pre-cooled with dry ice, placement on dry ice, or placement in a -80°C freezer. Freezing specimens directly on dry ice should be avoided if they are to be used for morphological analysis (See 8.7).

6.7 Transfer and storage of frozen biospecimens

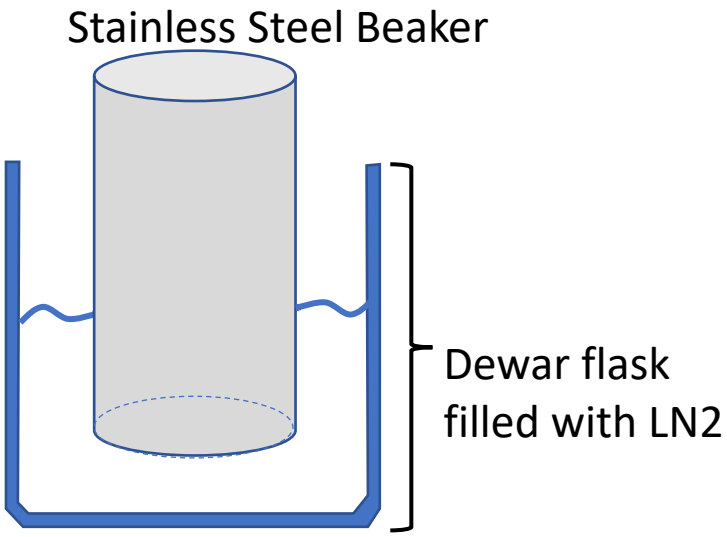
6.7.1 After freezing, the cryogenic specimen storage container should be transferred for storage in a LN2 vapor freezer. Should LN2 be unavailable, specimen storage containers may be stored at -70°C or colder (See 8.8).

6.7.2 Alternatively, the frozen specimens may be placed directly into a LN2 dry shipper for immediate transport (See 8.8). Specimen containers frozen in LN2 and destined for storage in LN2 should be held in LN2 vapor before and during transfer to repository/long-term storage. Should LN2 be unavailable, specimen storage containers may be shipped on dry ice.

Specimen containers destined for storage at -80°C should be held on dry ice before and during transport.

7.0 FIGURES

7.1 Experimental set-up for snap-freezing in LN2 vapor



8.0 SUMMARIES OF LITERATURE EVIDENCE

8.1 Incubation of specimens on wet ice as opposed to room temperature reportedly delays the onset of ischemia-induced effects for RNA analyses [1-3], although data conflict as to whether incubation on wet ice [4] or at room temperature in the absence of buffer [5] is the optimal ischemic condition for protein preservation.

8.2 Cold ischemia has elicited quick and selective alterations in RNA transcript levels [6-8] and protein expression [4] after as little as 15-20 min at room temperature. Importantly, studies investigating similar timepoints extend the window of stability citing significant and selective changes after 30 min [5, 9], 60 min [10], 120 min [11], 6 h [1], or no change after 2 h [12, 13] or 5 h [14]. Other analytical endpoints appear to be more robust, as a cold ischemia time of 60 min or less did not affect yields of DNA [9], RNA [15, 16] or protein [9], PCR amplification of DNA targets [9], or DNA quality [9, 14] and evidence of protein degradation was

first reported after 24 h at room temperature [11]. Similarly, cellular analysis by flow cytometry was not significantly affected by ischemia time of 4 h or more [17]. While reports conflict whether RNA quality is adversely affected by progressive ischemia, the earliest reported onset of significant RNA degradation as determined by RIN was after 45 min at room temperature in thyroid and colon [18]. Additional variables confounding investigation of ischemia-induced effects on RNA quality include: (a) tissue-specific differences in the timing and magnitude of effect as significant RNA degradation has also been reported after 12 h in liver and 18 h in an ovarian carcinoma specimen [2], (b) increased variability among RNA integrity numbers (RIN) following 30 to 120 min of ischemia [8, 19], (c) tissue composition-dependent differences in RIN, with lower RINs reported for specimens rich in connective tissue [20], (d) manual versus automated extraction methods [21], (e) and analysis [22, 23].

8.3 PMI does not significantly alter DNA [24], RNA [24-28], or protein [24, 28] yields with a few notable tissue-specific exceptions in frontal cortex [24, 29] and spleen [30]. Although RNA degradation has been reported in autopsy specimens [26, 31], reports conflict as to whether there is no clear relationship between the degree of degradation and PMI [25, 28, 30-35] or whether a weak but significant negative correlation is present [29]. The majority of DNA and RNA [25, 26, 28, 30, 31, 34, 36, 37] and protein expression analyses [28], as well as protein methylation activity [38], do not appear to be affected by a PMI of 1-5 days or less.

8.4 DNA and RNA yields, RNA quality, PCR and RT-PCR analyses, and immunohistochemistry and Western blot analyses generated equivalent results in OCT-embedded specimens immersed in isopentane pre-cooled with LN2 and case-matched controls preserved by immersion in LN2, suggesting these molecular and immunoassays are not adversely affected by the presence of OCT or isopentane [39]. Although OCT-embedding has been reported to interfere with subsequent PCR analysis of amplicons longer than 280 bp [40], a more recent study employing a column-based extraction method observed no deleterious effects of OCT on PCR analysis of amplicons ranging in length from 267-927 bp when compared to untreated controls snap frozen in LN2 [39]. Receptor binding capability as determined by radioligand-binding and ligand

titration also produced equivalent results between OCT-embedded and unembedded controls [41], while interference due to OCT has been reported for the dextran-charcoal and Lowry Protein assays [42]. While OCT has also been shown to interfere with mass spectrometry-based proteomic analyses in an animal model [43], a recent study using a human cell line demonstrated that OCT compound can be successfully removed by ether-methanol precipitation or filter-aided sample preparation [44].

8.5 Superior morphology was observed when specimens were frozen in LN2 vapor using a double-walled vessel either alone [45] or in media [46] compared to those directly immersed in LN2 [45, 46] or on dry ice with a cooling device [45]. While cellular dehydration and extra- and intracellular ice crystal formation and their resultant artifacts occurred more prominently at cooling rates slower than LN2 (an estimated 2000°C/min) [47], direct immersion in LN2 can result in the Leidenfrost effect, the creation of na insulating vapor layer upon contact with a substance hotter than the liquid's boiling point [48].

8.6 Specimens frozen by immersion in LN2 or isopentane pre-cooled to -80°C produced comparable DNA, RNA and protein yields; RNA purity and integrity; and RNA and protein expression levels [39]. However, morphology was modestly superior among specimens immersed in isopentane pre-cooled to -80°C than those immersed in LN2 [39], but morphology was equivalent among specimens frozen in isopentane pre-cooled with either LN2, dry ice, or a -100°C freezer [49].

8.7 Specimens frozen by placement in a -70°C freezer or immersion in LN2 produced comparable RNA quality and were successfully used in the construction of a cDNA library [27]; and specimens frozen in a -20°C or -70°C freezer displayed epidermal growth factor receptor (EGFR) activity that was comparable to specimens immersed in LN2 [50]. However, freezing specimens on dry ice using a cooling device [45] or by the carbon dioxide quick freeze method [39] compromised morphology in comparison to LN2 due to the formation of macro- and microscopic cracks. Similarly, microscopic and ultrastructural damage due to ice crystal formation were more prevalent among specimens embedded in OCT and frozen in a -20°C cryostat compared to those immersed in isopentane pre-

cooled to -60°C [51, 52] or -112°C [53], although effects may be influenced by tissue type [51].

8.8 Potential effects of storage temperature on DNA and RNA analyses have not been investigated. Although EGF-R activity was reduced by the initial freeze, short term storage for up to 21 days at -20°C, -70°C, or LN2 produced equivalent results [54]. Specimens stored in a LN vapor freezer or at -80°C displayed similar gross morphology and equivalent levels of rubidium, iron, and zinc [55].

8.9 Potential effects of the temperature and conditions of shipment on DNA, RNA, and morphological analyses have not been investigated. Shipment of breast cancer specimens on dry ice, as opposed to in a LN2 dry shipper, resulted in a reduction in ER binding and a subsequently lower incidence of ER-positive cases [56].

9.0 REFERENCES

9.1 Laboratory Guidelines

9.1.1 Universal Precautions (CDC, 1987): http://www.cdc.gov/mmwr/preview/mmwrhtml/00000039.htm; Infection Control Tools and Resources, CDC: https://www.cdc.gov/infection-control/php/tools/index.html.

9.1.2 CLSI IL-28A: Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline—Second Edition S Hewitt, personal communications, draft CLSI IL-28a.

9.1.3 CLSI MM13-A: Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline. 2008. Vol25, No 31.

9.1.4 Mager SR, Oomen MH, Morente MM, Ratcliffe C, Knox K, Kerr DJ, Pezzella F, Riegman PH: Standard operating procedure for the collection of fresh frozen tissue samples. *Eur J Cancer* 2007, 43(5):828-834.

9.1.5 Morente MM, Mager R, Alonso S, Pezzella F, Spatz A, Knox K, Kerr D, Dinjens WN, Oosterhuis JW, Lam KH *et al*: TuBaFrost 2: Standardising

tissue collection and quality control procedures for a European virtual frozen tissue bank network. *Eur J Cancer* 2006, 42(16):2684-2691.

9.2 Literature References

1. Micke, P., et al., *Biobanking of fresh frozen tissue: RNA is stable in nonfixed surgical specimens.* Lab Invest, 2006. 86(2): p. 202-11.
PubMed Abstract NCI Biospecimen Research Database Curation

2. van Maldegem, F., et al., *Effects of processing delay, formalin fixation, and immunohistochemistry on RNA Recovery From Formalin-fixed Paraffin-embedded Tissue Sections.* Diagn Mol Pathol, 2008. 17(1): p. 51-8.
PubMed Abstract NCI Biospecimen Research Database Curation

3. Hůlková, M. and J. Zeman, *Placental tissue as model for pilot study focused on RNA analysis from human foetal tissue.* Prague Med Rep, 2011. 112(2): p. 93-101.
PubMed Abstract NCI Biospecimen Research Database Curation

4. Espina, V., et al., *A portrait of tissue phosphoprotein stability in the clinical tissue procurement process.* Mol Cell Proteomics, 2008. 7(10): p. 1998-2018.
PubMed Abstract NCI Biospecimen Research Database Curation

5. Walker, L.A., et al., *Tissue procurement strategies affect the protein biochemistry of human heart samples.* J Muscle Res Cell Motil, 2011. 31(5-6): p. 309-14.
PubMed Abstract NCI Biospecimen Research Database Curation

6. Huang, J., et al., *Effects of ischemia on gene expression.* J Surg Res, 2001. 99(2): p. 222-7.
PubMed Abstract NCI Biospecimen Research Database Curation

7. Spruessel, A., et al., *Tissue ischemia time affects gene and protein expression patterns within minutes following surgical tumor excision.*

Biotechniques, 2004. 36(6): p. 1030-7.
PubMed Abstract NCI Biospecimen Research Database Curation

8. Bray, S.E., et al., *Gene expression in colorectal neoplasia: modifications induced by tissue ischaemic time and tissue handling protocol.* Histopathology, 2010. 56(2): p. 240-50.
PubMed Abstract NCI Biospecimen Research Database Curation

9. Johnsen, I.K., et al., *Evaluation of a standardized protocol for processing adrenal tumor samples: preparation for a European adrenal tumor bank.* Horm Metab Res, 2010. 42(2): p. 93-101.
PubMed Abstract NCI Biospecimen Research Database Curation

10. Jones, R.J., et al., *The impact of delay in cryo-fixation on biomarkers of Src tyrosine kinase activity in human breast and bladder cancers.* Cancer Chemother Pharmacol, 2008. 61(1): p. 23-32.
PubMed Abstract NCI Biospecimen Research Database Curation

11. De Cecco, L., et al., *Impact of biospecimens handling on biomarker research in breast cancer.* BMC Cancer, 2009. 9: p. 409.
PubMed Abstract NCI Biospecimen Research Database Curation

12. Blackhall, F.H., et al., *Stability and heterogeneity of expression profiles in lung cancer specimens harvested following surgical resection.* Neoplasia, 2004. 6(6): p. 761-7.
PubMed Abstract NCI Biospecimen Research Database Curation

13. Sehringer, B., et al., *Evaluation of different strategies for real-time RT-PCR expression analysis of corticotropin-releasing hormone and related proteins in human gestational tissues.* Anal Bioanal Chem, 2005. 383(5): p. 768-75.
PubMed Abstract NCI Biospecimen Research Database Curation

14. Jewell, S.D., et al., *Analysis of the molecular quality of human tissues: an experience from the Cooperative Human Tissue Network.* Am J Clin Pathol, 2002. 118(5): p. 733-41.
PubMed Abstract NCI Biospecimen Research Database Curation

15. Hatzis, C., et al., *Effects of tissue handling on RNA integrity and microarray measurements from resected breast cancers.* J Natl Cancer Inst, 2011. 103(24): p. 1871-83.
PubMed Abstract NCI Biospecimen Research Database Curation

16. Sewart, S., et al., *Molecular analysis of a collection of clinical specimens stored at 4 degrees C as an alternative to snap-freezing.* Int J Oncol, 2009. 35(2): p. 381-6.
PubMed Abstract NCI Biospecimen Research Database Curation

17. Bergers, E., et al., *The influence of fixation delay on mitotic activity and flow cytometric cell cycle variables.* Hum Pathol, 1997. 28(1): p. 95-100.
PubMed Abstract NCI Biospecimen Research Database Curation

18. Viana, C.R., et al., *The interference of cold ischemia time in the quality of total RNA from frozen tumor samples.* Cell Tissue Bank, 2012.
PubMed Abstract NCI Biospecimen Research Database Curation

19. Freidin, M.B., et al., *Impact of collection and storage of lung tumor tissue on whole genome expression profiling.* J Mol Diagn, 2012. 14(2): p. 140-8.
PubMed Abstract NCI Biospecimen Research Database Curation

20. Bao, W.G., et al., *Biobanking of Fresh-frozen Human Colon Tissues: Impact of Tissue Ex-vivo Ischemia Times and Storage Periods on RNA Quality.* Ann Surg Oncol, 2012.
PubMed Abstract NCI Biospecimen Research Database Curation

21. Bertilsson, H., et al., *RNA quality in fresh frozen prostate tissue from patients operated with radical prostatectomy.* Scand J Clin Lab Invest, 2010. 70(1): p. 45-53.
PubMed Abstract NCI Biospecimen Research Database Curation

22. Copois, V., et al., *Impact of RNA degradation on gene expression profiles: assessment of different methods to reliably determine RNA quality.* J Biotechnol, 2007. 127(4): p. 549-59.
PubMed Abstract NCI Biospecimen Research Database Curation

23. Strand, C., et al., *RNA quality in frozen breast cancer samples and the influence on gene expression analysis--a comparison of three evaluation methods using microcapillary electrophoresis traces.* BMC Mol Biol, 2007. 8: p. 38.
PubMed Abstract NCI Biospecimen Research Database Curation

24. Naber, D. and H.G. Dahnke, *Protein and nucleic acid content in the aging human brain.* Neuropathol Appl Neurobiol, 1979. 5(1): p. 17-24.
PubMed Abstract NCI Biospecimen Research Database Curation

25. Preece, P., et al., *An optimistic view for quantifying mRNA in post-mortem human brain.* Brain Res Mol Brain Res, 2003. 116(1-2): p. 7-16.
PubMed Abstract NCI Biospecimen Research Database Curation

26. Preece, P. and N.J. Cairns, *Quantifying mRNA in postmortem human brain: influence of gender, age at death, postmortem interval, brain pH, agonal state and inter-lobe mRNA variance.* Brain Res Mol Brain Res, 2003. 118(1-2): p. 60-71.
PubMed Abstract NCI Biospecimen Research Database Curation

27. Kobayashi, H., et al., *Stability of messenger RNA in postmortem human brains and construction of human brain cDNA libraries.* J Mol Neurosci, 1990. 2(1): p. 29-34.
PubMed Abstract NCI Biospecimen Research Database Curation

28. De Paepe, M.E., et al., *Postmortem RNA and protein stability in perinatal human lungs.* Diagn Mol Pathol, 2002. 11(3): p. 170-6.
PubMed Abstract NCI Biospecimen Research Database Curation

29. Birdsill, A.C., et al., *Postmortem interval effect on RNA and gene expression in human brain tissue.* Cell Tissue Bank, 2011. 12(4): p. 311-8.
PubMed Abstract NCI Biospecimen Research Database Curation

30. Heinrich, M., et al., *Successful RNA extraction from various human postmortem tissues.* Int J Legal Med, 2007. 121(2): p. 136-42.
PubMed Abstract NCI Biospecimen Research Database Curation

31. Johnson, S.A., D.G. Morgan, and C.E. Finch, *Extensive postmortem stability of RNA from rat and human brain.* J Neurosci Res, 1986. 16(1): p. 267-80.
PubMed Abstract NCI Biospecimen Research Database Curation

32. Cummings, T.J., et al., *Recovery and expression of messenger RNA from postmortem human brain tissue.* Mod Pathol, 2001. 14(11): p. 1157-61.
PubMed Abstract NCI Biospecimen Research Database Curation

33. Atz, M., et al., *Methodological considerations for gene expression profiling of human brain.* J Neurosci Methods, 2007. 163(2): p. 295-309.
PubMed Abstract NCI Biospecimen Research Database Curation

34. Schramm, M., et al., *Stability of RNA transcripts in post-mortem psychiatric brains.* J Neural Transm, 1999. 106(3-4): p. 329-35.
PubMed Abstract NCI Biospecimen Research Database Curation

35. Sherwood, K.R., et al., *RNA integrity in post mortem human variant Creutzfeldt-Jakob disease (vCJD) and control brain tissue.* Neuropathol Appl Neurobiol, 2011. 37(6): p. 633-42.
PubMed Abstract NCI Biospecimen Research Database Curation

36. Larsen, S., et al., *Northern and Southern blot analysis of human RNA and DNA in autopsy material.* APMIS, 1992. 100(6): p. 498-502.
PubMed Abstract NCI Biospecimen Research Database Curation

37. Gala, J.L., et al., *HIV-1 detection by nested PCR and viral culture in fresh or cryopreserved postmortem skin: potential implications for skin handling and allografting.* J Clin Pathol, 1997. 50(6): p. 481-4.
PubMed Abstract NCI Biospecimen Research Database Curation

38. Goggins, M., J.M. Scott, and D.G. Weir, *Regional differences in protein carboxymethylation in post-mortem human brain.* Clin Sci (Lond), 1998. 94(6): p. 677-85.
PubMed Abstract NCI Biospecimen Research Database Curation

39. Steu, S., et al., *A procedure for tissue freezing and processing applicable to both intra-operative frozen section diagnosis and tissue banking in surgical pathology.* Virchows Arch, 2008. 452(3): p. 305-12.
PubMed Abstract NCI Biospecimen Research Database Curation

40. Turbett, G.R. and L.N. Sellner, *The use of optimal cutting temperature compound can inhibit amplification by polymerase chain reaction.* Diagn Mol Pathol, 1997. 6(5): p. 298-303.
PubMed Abstract NCI Biospecimen Research Database Curation

41. Pasic, R., B. Djulbegovic, and J.L. Wittliff, *Influence of O.C.T. embedding compound on determinations of estrogen and progestin receptors in breast cancer.* Clin Chem, 1989. 35(12): p. 2317-9.
PubMed Abstract NCI Biospecimen Research Database Curation

42. Muensch, H. and W.C. Maslow, *Interference of O.C.T. embedding compound with hormone receptor assays.* Am J Clin Pathol, 1984. 82(1): p. 89-92.
PubMed Abstract NCI Biospecimen Research Database Curation

43. Schwartz, S.A., M.L. Reyzer, and R.M. Caprioli, *Direct tissue analysis using matrix-assisted laser desorption/ionization mass spectrometry: practical aspects of sample preparation.* J Mass Spectrom, 2003. 38(7): p. 699-708.
PubMed Abstract

44. Weston, L.A. and A.B. Hummon, *Comparative LC-MS/MS analysis of optimal cutting temperature (OCT) compound removal for the study of mammalian proteomes.* Analyst, 2013. 138(21): p. 6380-4.
PubMed Abstract

45. Vonsattel, J.P., et al., *An improved approach to prepare human brains for research.* J Neuropathol Exp Neurol, 1995. 54(1): p. 42-56.
PubMed Abstract NCI Biospecimen Research Database Curation

46. Adam, M., et al., *The effect of liquid nitrogen submersion on cryopreserved human heart valves.* Cryobiology, 1990. 27(6): p. 605-14.
PubMed Abstract NCI Biospecimen Research Database Curation

47. Bischof, J., K. Christov, and B. Rubinsky, *A morphological study of cooling rate response in normal and neoplastic human liver tissue: cryosurgical implications.* Cryobiology, 1993. 30(5): p. 482-92.
PubMed Abstract NCI Biospecimen Research Database Curation

48. Leidenfrost, J.G., C. Embach, and C.S.E. Wares, *A Tract about Some Qualities of Common Water*. 1964: Carolyn S.E. Wares.

49. Wu, J.S., G.R. Hogan, and J.D. Morris, *Modified methods for preparation of cryostat sections of skeletal muscle.* Muscle Nerve, 1985. 8(8): p. 664-6.
PubMed Abstract NCI Biospecimen Research Database Curation

50. Crawford, D., et al., *New storage procedure for human tumor biopsies prior to estrogen receptor measurement.* Cancer Res, 1984. 44(6): p. 2348-51.
PubMed Abstract NCI Biospecimen Research Database Curation

51. Mellen, P. and G. Clark, *Isopentane frozen sections for intraoperative diagnosis.* J Histotechnol, 1991. 14(4): p. 285.
NCI Biospecimen Research Database Curation

52. Erickson, Q.L., et al., *Flash freezing of Mohs micrographic surgery tissue can minimize freeze artifact and speed slide preparation.* Dermatol Surg, 2011. 37(4): p. 503-9.
PubMed Abstract NCI Biospecimen Research Database Curation

53. McGinley, D.M., Z. Posalaky, and I.P. Posalaky, *The use of fresh-frozen tissue in diagnostic transmission electron microscopy.* Ultrastruct Pathol, 1984. 6(1): p. 89-98.
PubMed Abstract NCI Biospecimen Research Database Curation

54. McLeay, W.R., et al., *Epidermal growth factor receptor in breast cancer: storage conditions affecting measurement, and relationship to steroid receptors.* Breast Cancer Res Treat, 1992. 22(2): p. 141-51.
PubMed Abstract NCI Biospecimen Research Database Curation

55. Mackey, E.A., et al., *Quality assurance in analysis of cryogenically stored liver tissue specimens from the NIST National Biomonitoring Specimen Bank (NBSB).* Science of the Total Environment, 1999. 226(2-3): p. 165-176.
PubMed Abstract NCI Biospecimen Research Database Curation

56. Muschenheim, F., J.L. Furst, and H.A. Bates, *Increased incidence of positive tests for estrogen binding in mammary carcinoma specimens transported in liquid nitrogen.* Am J Clin Pathol, 1978. 70(5): p. 780-2.
PubMed Abstract NCI Biospecimen Research Database Curation

10.0 REVISION HISTORY

10.1 Revision 1.0 (2/4/2025): 508 Compliance

To achieve 508 compliance, formatting changes were applied and alternative text was added to the original version (1/25/2014).