

Stability of Cancer Biomarkers Under Ultra-Low Temperature Storage

Summary

The use of tumor markers to diagnose, assess prognosis, and select a suitable cancer treatment has become possible due to the development of inexpensive and user-friendly molecular analysis tools such as polymerase chain reaction (PCR). Maintaining the integrity of biomarkers during processing and storage is therefore of paramount importance for accurate diagnosis and prognosis of many diseases, including cancers. To safely store samples for research or clinical uses, freezing procedures are used to arrest the degradation of biomarkers. However, the stability of these molecules depends upon long-term storage conditions. This paper aims to demonstrate the necessity of ultra-low temperature storage in maintaining the stability of important cancer biomarkers in fluid, cell, and tissue biospecimens.



Background

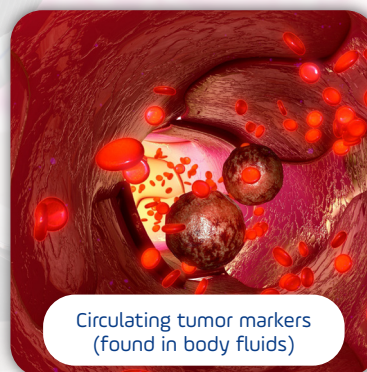
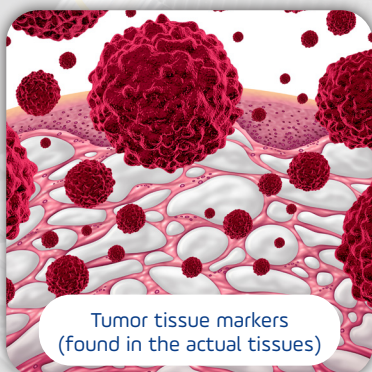
Analyzing frozen tissue and body fluid samples has become standard practice for suspected cancers following recent breakthroughs in practical applications of molecular diagnostics which detect cancer-associated biological molecules, often known as biomarkers. The potential use of molecular tools was first recognized in oncohaematology following the realization that specific chromosomal translocations could help diagnose various leukemias and lymphomas¹. Furthermore, the development of immunohistochemistry-based technologies for detecting specific antigens in tissue samples altered the approach to the most common oncological diseases, such as breast cancer, by customizing endocrine therapy to a laboratory test².

An accurate assessment of biomarkers in a biospecimen is essential for making an accurate disease diagnosis. Therefore, the key biological features of the biospecimen must be maintained during its processing and storage as biological specimens contain molecules such as lipases, carbohydrases, proteases, and nucleases³ which can degrade over time and at certain temperatures. As the activity of these molecules is dependent on the specific protein dynamics and ambient temperature, reducing protein activity by decreasing the temperature is a mechanism used to stabilize the biospecimens³.

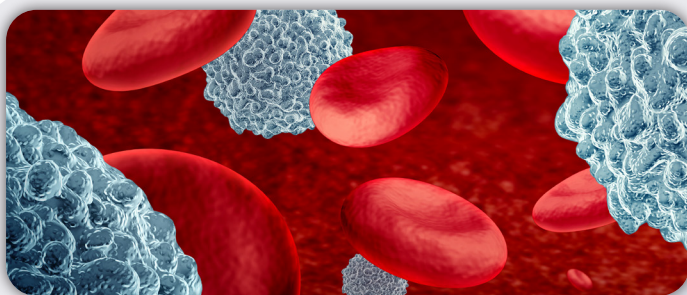
Biomarkers in biospecimens

In oncology, biomarker testing identifies genes, proteins, and other substances that can reveal information about a cancer since each type has a distinct pattern of biomarkers⁴. The data can then be used to diagnose or classify the cancer, estimate the prognosis, or select an appropriate treatment.

Tumor markers are divided into two groups:



1. Tumor tissue (or cell) markers

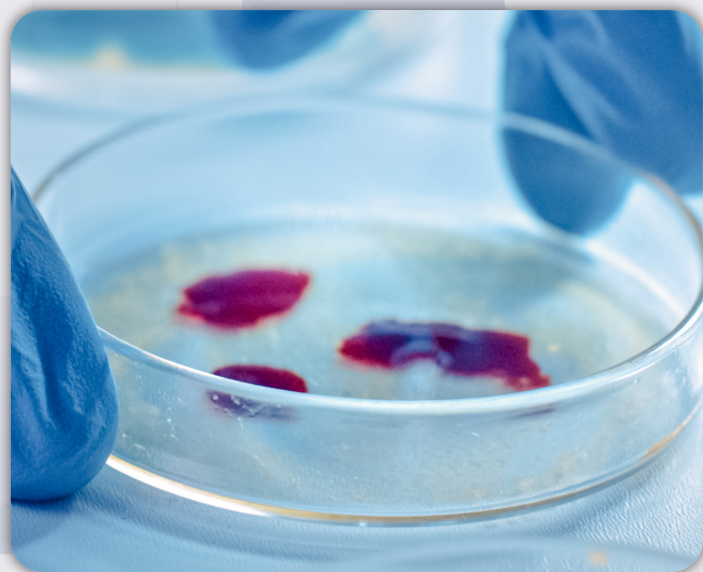


Stability of tissue nucleic acids under ultra-low temperature storage:

Ultra-low temperature frozen tissue is used for cancer biomarker testing because it provides high performance and high-quality nucleic acids (DNA and RNA) and proteins⁵. The suitability of tissue biospecimens for biomarker testing is highly dependent on how the biospecimens are processed and stored⁶. The effect of ultra-low temperature storage conditions on the stability of tissue nucleic acids has been described in several studies. Andreasson *et al.*⁷, for example, investigated the impact of long-term storage of endocrine tissues at -80°C on the RNA quality. According to their findings, storage at -80°C for up to three decades had no negative impact on the quality of RNA extracted from the stored tissues. Some studies, however, have reported susceptibility of RNA to ultra-low temperature storage. For instance, Jewell *et al.*⁸ analyzed the stability of DNA and RNA isolated from human tissues, including breast, colon, liver, lung, ovary, endometrium, and cervix, and frozen at -80°C for up to one year. Results from the study indicated that storage at -80°C was able to maintain the DNA and RNA quality in 80% and 60% of the tissues, respectively, suggesting that tissue DNA could be more stable than RNA. Additional research studies found that specimens maintained at temperatures between -70°C and -80°C for five years also had lower RNA quality⁹. However, Riddick *et al.*¹⁰ demonstrated that snap freezing prostatic tissue in isopentane supercooled in liquid nitrogen for between 10-20 seconds before storing at -80°C ensured the preservation of RNA and DNA.

Stability of tissue proteins under ultra-low temperature storage:

Studies comparing the proteome profiles of matched Formalin-Fixed Paraffin-Embedded (FFPE) and frozen tissues using mass spectrometry (MS) analysis have revealed that the number of proteins detected from FFPE is often lower than that of matched frozen tissue^{11,12,13}. There is also evidence that by keeping frozen tissue at or below -70°C , the proteome can be preserved for years¹⁴. McLeay *et al.*¹⁵ demonstrated that breast cancer specimens' quantifiable epidermal growth factor receptor activity could be maintained at temperatures below -70°C . In another study, Toppia *et al.*¹⁶ evaluated the effects of ultra-low temperature storage at -70°C and the addition of sodium molybdate on estrogen and progesterone receptors of ovarian and uterine specimens after eight weeks of storage. The study found that while estrogen and progesterone receptor concentrations were unaffected after the eight weeks, adding sodium molybdate to fresh and frozen tissue specimens significantly boosted estrogen and progesterone receptor concentrations.



2. Circulating tumor markers

Tumors release a certain amount of their fragments into the peritumoral area. These fragments can be individual malignant cells, clusters, proteins, or nucleic acids. For instance, circulating free DNA (cfDNA) in blood plasma refers to various freely circulating DNA in the bloodstream, such as circulating tumor DNA (ctDNA)¹⁷. As a result, these molecules can be found in multiple body fluids, including serum, saliva, and urine, and can be used as tumor indicators¹⁷. Therefore, plasma storage conditions are significant preanalytical factors influencing circulating tumor marker analysis outcomes. Chan *et al.*¹⁸ recommend that for proper cfDNA preservation, once the plasma is separated it should be stored at -80°C , and that repeated freeze-thaw cycles should be avoided. Other serum components, such as matrix metalloproteinase-7 (MMP-7), remained stable after five years of storage at -20°C and could remain stable for at least 100 years at -75°C , according to the Arrhenius equation¹⁹. The stability of purified and non-purified forms of nucleic acid extracted from JB-1 tumor cells and fine-needle aspirates of solid tumor tissue has also been demonstrated at storage temperatures of -80°C for up to one year²⁰.

Woodrum *et al.*²¹ studied the stability of free prostate-specific antigen (PSA) in serum under different storage conditions and found that free PSA levels dropped dramatically at higher temperatures. For each month in storage, temperatures of -20°C resulted in a 0.9% loss, whereas storage at -70°C resulted in a 0.4% loss. In another study, Schiele *et al.*²² investigated the stability of apolipoprotein concentration in human serum at various storage temperatures (-20°C and -80°C) for up to four years. They discovered that storage for three months at temperatures between -20°C and -80°C or for four years at -80°C had no significant effect on apolipoprotein concentrations. Qvist *et al.*²³ evaluated the impact of different storage temperatures (-20°C , -80°C , and -150°C) on the stability of C-telopeptides of type I collagen (CTX) in blood and serum samples. CTX is one of the biochemical markers of bone turnover used in diagnosing and prognosis metastatic bone cancer²⁴. After three years of storage at -20°C , -80°C , or -150°C , no significant drop of CTX levels could be identified in either serum or plasma samples, indicating that CTX was stable when frozen at temperatures below -20°C , proving that the use of medical freezers or Ultra-Low Freezers that reach this temperature for the purpose of the storage of these samples is possible.

Reliable Ultra-Low Freezers for modern cancer research

Ultra-Low Freezers are essential for the storage of samples in cancer research and are especially important in maintaining the stability and quality of biomarkers. Hence, when selecting ultra-low freezers to purchase and use, the performance and the reliability of these units should be carefully considered. The flexibility in offering varying set points can help medical professionals choose the right storage temperature depending on the type of biomarkers being frozen and the length of the storage required. Moreover, the Ultra-Low Freezers must maintain a uniform temperature inside their cabinets to best maintain the samples, while superior door opening recovery (DoR) and hold over times will help ensure their safety even during adverse events. Certifications such as the EU MDR ensure compliance with regulations that govern the production of quality medical devices. These, along with 24/7 real-time monitoring systems can ensure that specimens are always stored in the most ideal conditions. Furthermore, energy-efficient operations and green refrigerants will ensure that laboratories will be able to achieve considerable sustainability levels.

Conclusion

Specimens stored at ultra-low temperatures are generally preferred in cancer biomarker testing because most biomarkers, such as nucleic acids and protein molecules, are prone to degradation due to fixation procedures. On the other hand, biospecimens also contain degradative molecules like proteases, lipases, and nucleases, whose activities are greatly influenced by temperature. As a result, appropriate storage temperatures must be chosen to preserve the biomarkers. Various studies have demonstrated that if biospecimens are used mainly for DNA and protein analysis, long-term storage at temperatures between -70°C or -80°C can be effective. It is for this reason that reliable Ultra-Low Freezers should be employed to store these delicate samples: by providing temperature uniformity and sample safety with these products, researchers can use them to store their biologicals for extended periods of time.

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