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Long-term storage of liquid Biospecimens

The meaning of a long-term storage below the recrystallization temperature with focus on biomarkers

White Paper

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Content:

1. Background for long-term storage of liquid biological samples

There exists a variety of liquid biological samples that are cryopreserved in biobanks. These include plasma, serum, cerebrospinal fluid and urine. These samples contain not only cells, but also proteins, lipids and metabolites of all kinds that can be utilized as biomarkers. In order to promote research in the field of biomarkers and to safely use them diagnostically, specimens are required exhibiting the highest possible quality. This is essentially determined by the pre-analytical methods (sample preparation, freezing, storage) the sample is subjected to. The process steps mentioned cause 60-90% of diagnostic errors[1–3]. At present, the samples are mostly placed directly into freezers for storage at -20 to -80°C, where they cool down with very slow cooling rates of 1-2°C/min. This process irreversible impairs macromolecules which affects subsequent proteomic studies[4–8]. The actual nature of the sample cannot be reconstructed. Long-term storage at temperatures around -80°C and repeated heating of the samples also lead to denaturation of many proteins in the samples. Therefore the titer after thawing cannot be reliably determined[9–11]. Both serous biomarkers and diagnostically encouraging proteomic cancer biomarkers are very susceptible to freeze/thaw cycles and to long-term storage in frozen state at high temperatures[12,13]. Hence it is of great importance in the context of increasing and ensuring diagnostic quality to store the sample material in the best possible way.

2. The storage stability of plasma and serum

Plasma and serum represent a very easy-to-acquire specimen material. All tissues and organs have contact with them, so that macromolecules and therefore biomarkers gather in large numbers. A number of studies have shown that biomarkers in these specimen material show different reactions to the storage temperature. It was shown for instance that samples are subjected to further biochemical processes which result in protein modifications when stored at -20°C, such as oxidation and amino acid elimination. For the matrix metalloproteinase MMP-9 was shown that her activity decreased to 65% within two years when stored at -80°C due to degradation[9]. Proteins with coagulant effect show a significant degradation during storage for more than two years at -74°C[14]. These detrimental processes don't occur during storage at lower temperatures[15]. Just as important as the lowest possible storage temperature is avoidance of cyclical heating of the samples during handling. Just two freeze/thaw cycles cause in some proteins a significant degradation or a reduction in their activity. Therefore it is recommended to stabilize this kind of sample material with Glycerol and store it at temperatures markedly below -80°C[16,17].

3. The storage stability of urine and cerebrospinal fluid (CSF)

Urine can be obtained non-invasively and comprises a number of different proteins, which not only reflect the physiological state of the kidneys and the urogenital tract, but also of the blood[18]. Several disease specific biomarkers show a significant degradation during storage for 6-8 months at -20°C. These include for example Albumin and N-acetyl glucosaminidase[19]. For some biomarkers indicating acute kidney injury was shown that high storage temperatures lead to deviations between identical samples[20].

Due to direct contact of the cerebrospinal fluid with the brain and the spinal cord it is very well applicable for diagnosis and research of diseases of the central nervous system. For amyloid β (I-42), a biomarker of Alzheimer's disease, has been shown that repeated thawing by handling operations reduces the concentration significantly. Storage of amyloid β and τ at -20°C and at -80°C causes a reduced sample quality in just three months and therefore storage at much lower temperatures is recommended[12,21,22].

4. Conclusion

In fluid biospecimens there is a variety of biomarkers with great clinical importance. Furthermore, the samples contain a whole range of unknown biomarkers with importance for future diagnostic and therapeutic applications. In order to preserve the value of the samples and to justify their costly storage, samples should be stored at very low temperatures. Only in that manner it is possible to maintain the value of the biospecimens over a long period of time. For this purpose Hubel et al. published among others the following recommendations[12]:

- Liquid biospecimens should be stored in the gas phase of liquid nitrogen
- The samples should be frozen controlled at a constant freezing rate
- Fluctuations in the sample temperature (e.g. by opening of refrigerators) and freeze/thaw cycles should be minimized

All these requirements are met using the Askion C-line ® system (workbench WB220/230 and hermetic storage HS200)[1]. The entire system is designed to preserve specimens best possible using storage temperatures below -150°C with minimal fluctuations in temperature during specimen handling.

[1] For further information please visit www.askion.com

5. References

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