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Store glutaraldehyde fixed samples at -80°C: is it possible?

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The design and realization of experiments aboard the International Space Station (ISS) often clashes with greater difficulties than at ground level. Dimensions, weight, materials used, transport, extreme conditions are just some of the possible variables. During the planning of a research project, involving the study of microgravity effects on human bronchial mucosa on the ISS, we stumbled upon an "unusual" problem: is it possible, in order to minimize containment levels of the fixatives, to store biological samples fixed in 2.5% glutaraldehyde at -80 ° C without affecting transmission electron microscopy (TEM) analysis? Normal fixation procedures involve storage of fixed samples at 4°C. For reasons related to crew safety in fact, fixed samples should not be handled in the incubator (BIOLAB) and must be stored at a -80°C. Therefore, we had to verify that once brought back to room temperature, samples fixed at -80°C did not show morphological and ultrastructural alterations. Small bronchial tissue biopsies (about 0.2/0.3 mm3) were fixed in a glutaraldehyde solution at 2.5%, for 45'. After this passage the fixative solution was removed and samples were dried under the cabinet or replaced with DMSO 10% in Millonig's buffer. . In both cases samples were stored at -80°C for one week. At the end of the week we proceeded with the inclusion in EPON 812 resin for the subsequent analysis by TEM. Analysing both types of preparations through TEM we didn't observe any alteration or loss of morphology. The cellular structures were practically identical to the control samples fixed with the standard procedure and stored at 4°C. Even structures such as mitochondrial crests have retained their morphological integrity. In conclusion, we demonstrate preservation of samples fixed in glutaraldehyde for TEM at a temperature of -80 ° C is feasible and does not lead to the loss of tissue integrity. This will allow to store the samples of the experiments conducted under microgravity conditions, meeting the engineering requirements and safety for the astronauts on board.

References

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Key words

Electron Microscopy, Microgravity Environment, Space Medicine.