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pH evaluation of storage fluids and ancient DNA extraction from wet specimens in pathology museums

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Pathology museums host ancient samples obtained during autopsies and generally used for educational purposes in the past. Such collections consist of dry and wet specimens showing diseases that no longer exist or with their natural course unmodified by modern therapies.^{1,2} In wet specimens, the preservation of macroscopic features due to the storage fluid has a great historical and paleopathological interest. Unfortunately, both original fixatives and storage fluids strongly influence tissue antigens and nucleic acids preservation.³ In recent years, specific attention is being paid to the study of wet preparations by modern investigation techniques. Ancient DNA (aDNA) has been investigated in specimens from natural history museums, but the experience with human material is still limited.^{4,5} In such cases, time elapsed between death and fixation, as well as the chemical composition of fixation and storage fluids may irreversibly damage the DNA, thus routine techniques may result ineffective. We suggest a simple and effective approach to aDNA collection and extraction from fluid preserved specimens.

Ten wet specimens dating back to 19th-20th century were selected from the Pathology Collection of Turin and submitted to histopathologic re-evaluation.⁶ Most of them were in their original jars with labels describing year, necropsy number and diagnosis. As the chemical composition of storage fluids was unknown, pH value was assessed using a consumer-type pH tester, calibrated before each use. Four cases representative of different classes of pH value underwent DNA analysis by conservative sampling. In two of them autopsies had been performed in 1896 and in 1899, whereas no date was indicated in the other two cases. Samples were submitted to routine histology, histochemistry and immunohistochemistry. Additional tiny samples underwent DNA extraction and analysis by spectrophotometry and electrophoretic runs in agarose gel. In order to verify DNA integrity, Short Tandem Repeat (STR) analysis usually employed for personal identification was performed.

Tiny fragments of tissue were frozen at -20°C to obtain sixty 10 µm-thick sections, collected in microtubes containing 1 mL of digestion solution (75 mM NaCl, 10 mM tris, 0.5 mM EDTA, pH 8.0) and 100 µL of proteinase K solution (18 mg/mL). The samples were incubated at 56°C for 48 h and 50 µL fresh of proteinase K solution were added for 72 h. Subsequently, 400 µL of solution were

extracted with magnetic beads using a Roche MAGNA PURE COMPACT instrument.

DNA quantity and quality were evaluated using the full absorption spectrum (220/340 nm) obtained by the Nanophotometer P 300 spectrophotometer. DNA concentration in ng/ μ L and absorbance ratio at 260/280 nm were calculated from 4 μ L samples. The quality of DNA was also observed by electrophoretic run in 1.3% of agarose gel. In order to verify DNA integrity, short tandem repeat (STR) analysis was performed using the PowerPlex 16 HS system (PROMEGA) employed for personal identification.

The cases were originally diagnosed as lymphosarcoma (two), uterine myosarcoma, esophageal, gastric, and rectal cancers, pancreatic tumor, lung cancer, and pleural sarcomas (two). The pH values of storage fluids ranged between 1.46 and 4.65. The pH value of the four specimens submitted to aDNA extraction and analysis was 2.56, 3.15, 4.45, and 4.65. The revised diagnoses of the selected cases were lung carcinoma, uterine leiomyosarcoma (dating back 1899), lung metastases from squamous carcinoma of unknown primary (dating back 1896), and lung metastases from uterine leiomyosarcoma. As for aDNA extraction, the first two samples gave negative results on both spectrophotometric analysis and electrophoretic run. The other two showed a low quantity of DNA (6 ng/ μ L; 7 ng/ μ L) of moderate quality, with an absorbance ratio of 1.53 and 1.50 at the spectrophotometric analysis. The electrophoretic analysis showed a light band of DNA with molecular weight around 1000 bp in both samples. STR analysis displayed DNA fragmentation, evidenced by ladderization of the electropherograms result.

Pathology collections are actual biological archives and a great resource for research. Museum wet specimens may represent a valid source of aDNA to investigate genetic molecular features of ancient diseases.^{7,8} Long-term quality of fluid-preserved specimens depends on multiple factors, such as effective initial fixation and pH level of storage solutions.⁹ Rapid and thorough fixation prevents protein autolysis, coagulates cell contents, and sterilizes specimens. Acidic conditions of storage solutions cause decalcification, whereas alkaline conditions cause clearing of soft tissues and proteins.

The issue of pH levels in preserving solutions has been recognized as an important factor affecting specific biomolecules such as DNA. It is well known that DNA is better preserved in a slightly alkaline medium. The quantity and quality of aDNA

from wet specimens strongly depends on the pH value of storage fluids. Various technologies are available, but the most accurate pH measurements can be obtained with a pH meter. Whatever solution was employed for fixation and subsequent storage, its pH value predicts the quantity and quality of aDNA that can be obtained from the sample. We established that aDNA extraction may be acceptable also in specimens preserved in moderately acid solutions.

In conclusion, our preliminary results showed that pH value measurement in storage fluids may represent a good screening method for the evaluation of aDNA preservation. This simple and effective method may help to select cases to be submitted to DNA collection and subsequent extraction.

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