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

Luisa Ferrari,[1,2] Jasna Metovic,[2] Daniela Pulcinelli,[3] Antonio Lombardi,[3] Daniela Rullo,[4] Franco Papola,[3] Luca Ventura[4,5]

[1]Division of Pathology, Cardinal Massaja Hospital, Asti; [2]Department of Oncology, Pathology Unit, University of Turin; [3]Regional Centre of Immunohematology and Tissue Typing, San Salvatore Hospital, L'Aquila; [4]Division of Pathology, San Salvatore Hospital, L'Aquila; [5]Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, Italy

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/µL and absorbance ratio at 260/280 nm were calculated from 4 µL 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.

Correspondence: Luca Ventura, Division of Pathology, San Salvatore Hospital, Coppito, 67100, L'Aquila, Italy.
Tel.: 39.862.368766; Fax: 39.862.368718.
E-mail: lventura@asl1abruzzo.it

Key words: Pathology museums; paleopathology; wet specimens; storage fluids; ancient DNA.

Contributions: LF conceptualized analysis and investigation and wrote the manuscript. JM reviewed and edited the manuscript. DP, AL, DR prepared the material and performed analysis. FP provided critical feedback. LV conceptualized the investigation, collected and validated data, wrote and reviewed the paper.

Conflict of interest: The authors declare no conflict of interest.

Funding: None.

Ethics approval and consent: Not applicable

Consent for publication: Not applicable.

Availability of data and material: Not applicable.

Received for publication: 29 August 2022.
Revision received: 20 September 2022.
Accepted for publication: 20 September 2022.

Publisher's note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article or claim that may be made by its manufacturer is not guaranteed or endorsed by the publisher.

Licensee PAGEPress, Italy
Journal of Biological Research 2022; 95:10820
doi:10.4081/jbr.2022.10820

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/µL; 7 ng/µL) 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 depend 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.

References

1. Ferrari L, Coda R, Fulcheri E, Bussolati G. The role of the pathological anatomy museum: Past glory, present crisis, and future prospects. Pathologica 2001;93:196-200.
2. Ferrari L. From body preservation to pathology museums in Italy: Conservation and modern value of a historical and biological archive. Med Secoli 2021;33:441-82.
3. Fulcheri E, Micalizio S, Ferrari L. Valore museale delle soluzioni di dimora nelle preparazioni anatomiche umane. Museol Sci 2008;3:88-92.
4. Schäffer S, Zachos FE, Koblmueller S. Opening the treasure chest: A DNA-barcoding primer set for most higher taxa of Central European birds and mammals from museum collections. PLOS One 2017;12:e0174449.
5. Strauben N, Lyra ML, Paijmans JLA, et al. Successful application of ancient DNA extraction and library construction protocols to museums wet collection specimens. Mol Ecol Resour 2021;21:2299-315.
6. Ferrari L, Metovic J, Rullo D, et al. Histopathology and DNA evaluation of wet specimens from the Pathology Collection of Turin. Virchows Archiv 2019;475:S35.
7. Hühns M, Röpenack P, Erbersdobler A. Molecular and immunohistochemical characterization of historical long-term preserved fixed tissues from different human organs. PLOS One 2015;10:e0135297.
8. Hühns M, Erbersdobler A, Obliers A, Röpenack P. Identification of HPV types and *Mycobacterium tuberculosis* complex in historical long-term preserved formalin fixed tissues in different human organs. PLOS One 2017;12:e0170353.
9. Hargrave CW, Sutherland KG, Marsh-Matthews E, Matthews WJ. Multiple interacting factors affect pH in museum storage solutions. Collection Forum 2005;19:23-31.