

RESEARCH ARTICLE

Laboratory Attitudes and Practices in the Conservation of Biological Tissues for Research Purposes in Cote d'Ivoire

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Abstract:

Background: Tissue banks are indispensable structures for biological and medical research. These banks must provide high quality biological materials based on optimized procedures. But at present, there is no consensus on the best way to preserve biological tissues. Since 2009, the Institute Pasteur of Côte d'Ivoire has had a biobank which today houses the ECOWAS regional biobank. This biobank wishes to set up a biological tissue bank for research. In Côte d'Ivoire, several public and private structures handle biological tissues. However, we do not know which ones do the conservation of biological tissues and their related procedures. It is therefore essential to better understand the related procedures for the conservation of biological tissues in laboratories in order to develop a standard procedure that can be used in Côte d'Ivoire. **Methods:** This is a descriptive study carried out in biological analysis laboratories in the south of Côte d'Ivoire. **Results:** A total of 66 laboratories agreed to respond to the proposed questionnaire. Among them, only 21.21% conserved biological tissues of which 42.86% were human tissues, 35.71% animal tissues and 21.43% plant tissues. Human tissues were stored in formalin at room temperature for a period of 2 weeks to 6 months. Animal biopsies and surgical specimens were stored in formalin and at -80°C for an indefinite period of time. Plant tissues were stored at room temperature and +4°C for an indefinite period of time. **Conclusion:** The quality of conservation of biological tissues in Côte d'Ivoire remains to be improved.

Keywords: Côte d'Ivoire, Biological tissue conservation, Laboratories

Introduction

Tissue banks are indispensable structures for biomedical research that treat cellular, tissue and molecular pathologies [1-4]. Research studies aimed at improving the prevention, diagnosis and treatment of cellular and tissue diseases depend on a number of key resources, including biological tissue banks. These banks must provide high-quality biological materials based on optimized procedures for the reception, preservation and documentation of specimens. Several organizations have developed guidelines that can be used in the practice of biological tissue preservation. Despite the availability of such guidelines there is no consensus on how best to conserve biological tissues over the long term [5].

In 2009, the Institute Pasteur de Côte d'Ivoire (IPCI), a biological and medical research center, established a biobank that has been housing the regional biobank of the countries of the Economic Community of West African States (ECOWAS) since 2018. Samples of human, animal, plant, environmental and microbial origin are stored in this biobank. These samples come from several countries in the subregion, notably Benin, Togo, Burkina Faso, Mali and Nigeria, including Côte d'Ivoire. The IPCI biobank contributes to the epidemiological surveillance of influenza and measles in Côte d'Ivoire. It also participated in the USAID PREDICT 2 project whose overall objective was to strengthen the capacity of the human and animal disease surveillance system in high risk areas.

IPCI wishes to set up a human, animal and plant tissue bank according to the concept " One Health" within its research biobank. The successful operation of a biobank requires comprehensive interactions between the different actors involved in biobanking including the public, patients, health care providers, government and donors [6, 7]. There are several laboratories across the country that handle biological tissues for research purposes, but we do not know which ones store them and their related procedures. Therefore, appropriate assessment of laboratory attitudes and practices in preservation can ensure continued development and innovation in biomedical research. It is therefore essential to better understand the related procedures for the conservation of biological tissues in laboratories in order to develop a standard procedure that can be used in Côte d'Ivoire. The main objective of this study was to assess laboratory attitudes and practices in the conservation of biological tissues for research purposes in Côte d'Ivoire.

Methods

This is a descriptive study that was conducted from May to August 2019 in laboratories located in the South of Côte d'Ivoire. Its objective was to evaluate the attitudes and practices of laboratories in the conservation of biological tissues for research purposes. The study covered human, animal and plant health laboratories. Laboratories were selected using a purposive sampling technique from a pre-established list. This list consisted of 450 functional laboratories in southern Côte d'Ivoire. It was obtained from the Directorate of Health Establishments of the Ministry of Health and Public Hygiene, the Ministry of Agriculture and Rural Development and the Directorate of Veterinary Services of

the Ministry of Animal and Fishery Resources. From this list, the laboratories have been grouped into three classes:

- Human health laboratories: 360
- Animal health laboratories: 53
- Plant health laboratories: 37

Then the laboratories of each class constituted were chosen taking into account the level of the technical platforms with sophisticated equipment used for diagnosis and scientific research and also the private or public character of the laboratories. A total of 66 laboratories were selected: 45 human health laboratories, 15 animal health laboratories and 6 plant health laboratories.

All laboratories volunteered to participate in the study were included in the study.

Data collection

Data collection was conducted by a previously trained PhD student. A pre-tested and validated questionnaire was used as a tool to collect data on laboratory attitudes and practices in the conservation of biological tissues. The questionnaire took approximately 30 minutes to complete.

Statistical analysis

Data entry and analysis was carried out using EpiData 3.1 and Excel respectively.

Ethical conditions

Arrangements have been made to ensure that this study is conducted under ethical conditions. Indeed, the investigation only began after obtaining the authorization of the heads of the selected laboratories. In addition, the information was collected with the consent of the participants and in absolute confidentiality, and made anonymous and coded.

Results

There were 66 laboratories participating in this study. The public laboratories were the most numerous at 84.84%. Of these, 53.03% were research laboratories. Of the 10 private laboratories surveyed, 7 were research laboratories. Of the 66 laboratories surveyed, only 14 keep biological tissues. Of these, laboratories that store human tissue were the most represented at 42.86% (See Table 1).

Table 1: Profile of surveyed laboratories

Characteristics	Staff size	Percentage (%)
Laboratory Status		
Publics	56	84.84
Private	10	15.16
Research Laboratories		
Yes	42	63.64
No	24	36.36
Human tissue conservation laboratories	6	-
Animal tissue conservation laboratories	5	-
Plant tissue conservation laboratories	3	-

Human tissue conservation laboratories

These laboratories keep tissue samples (i) to validate the diagnosis of patients suffering from tumors and cancers, (ii) to monitor the evolution of tumors and cancers, and (iii) for biomarker research. Of the six human tissue conservation laboratories listed, two laboratories conserved tissue only for diagnosis, one conserved tissue only for research, two conserved tissue for diagnosis and research and one conserved tissue only for diagnosis, research and therapy. All but two of these laboratories preserved several types of human biopsies and surgical specimens (one that preserved only ocular tissue and one that preserved only placental tissue). All of these laboratories stored human tissue in formalin at room temperature for between 2 weeks and 6 months. They also stored tissues in kerosene blocks after formalin fixation for an indefinite period of time. Except for one laboratory that stored tissues in formalin for an indefinite period of time. The biological tests performed on these tissues were histology and immunohistochemistry. Four laboratories performed only histological tests and the other two laboratories performed histological and immunohistochemical tests. All of the human tissue conservation laboratories stored their tissues in their own laboratories.

Animal tissue conservation laboratories

Animal tissue sample preservation laboratories are used (i) to validate the diagnosis of patients suffering from tumors and cancers, (ii) to monitor the evolution of tumors and cancers, and (iii) for biomarker research. Of the 5 animal tissue conservation laboratories listed, 4 laboratories only conserved tissues for research and one conserved them for diagnosis and research. There were 3 laboratories that preserved several types of animal biopsies and surgical specimens. The other two laboratories kept animal and insect surgical specimens.

respectively. Insect samples were kept at -80°C for an indefinite period of time, while animal biopsies and surgical specimens were kept in formalin and at -80°C for an indefinite period of time. Biological tests performed on these tissues were histology, ELISA, PCR and immunohistochemistry. One laboratory performed only PCR tests, 2 laboratories performed PCR and ELISA tests and 2 laboratories performed PCR and histology tests. All animal tissue preservation laboratories stored their tissues in their own laboratory.

Plant tissue conservation laboratories

Of the three plant tissue conservation laboratories, two were only conserving tissue for research, and one was conserving tissue for diagnosis and research. Meristems (secretory tissues), nodes and internodes were the plant tissues conserved by these laboratories. These plant tissues are stored at room temperature or at $+4^{\circ}\text{C}$ for an indefinite period of time. The biological tests performed on these tissues were PCR and germination tests. All the plant tissue conservation laboratories stored their tissues in their own laboratories.

Discussion

This study gave us an overview of the conservation of human, animal and plant tissues in Côte d'Ivoire. Most of the human and animal tissue conservation laboratories surveyed store their samples in formalin for a period ranging from one week to several years. Formaldehyde, although it is the cornerstone of tissue fixation [8], is recognized by WHO as a Class I carcinogen [9]. Also, several studies have shown the toxicity of formalin and its effect on cell death, denaturation of nucleic acids and proteins [10, 11] and loss of enzyme activity [12]. Some of the human and animal tissue conservation laboratories investigated preserve their samples at -20°C to -30°C and at -80°C for several years for molecular biology testing. One study showed that liver samples stored between -20°C and -30°C were visibly degraded after 7 years of storage. As RNA is considered to be the most vulnerable molecular component of unfixed tissues, some studies have shown that the quantity and quality of RNA in tissues was not affected for storage at -70°C for 15 years [13]. However, other studies have shown a significant decrease in the integrity of RNA in tissues stored at -70°C or -80°C for 5 years or more [14, 15]. However, the laboratories surveyed are faced with freeze-thaw cycles due to untimely power outages. This can lead to a reduction in RNA integrity in tissue samples stored at -80°C . Several other studies have shown that repeated freeze-thaw cycles result in significant decreases in RNA integrity, particularly in autopsy brain tissue [16-18]. Unlike RNA, the yield and integrity of DNA remains unchanged in long-term storage at -80°C [15].

Plant tissue conservation laboratories, on the other hand, preserve their samples at room temperature and $+4^{\circ}\text{C}$ for several years for genetic studies without the use of conservation media, although such media do exist. Indeed, studies have shown that the conservation of plant tissues in silica gel (silica-gel) allows the obtaining of good quality DNAs compared to tissues conserved at room temperature [19].

Conclusion

This study was carried out in order to know the state of the art of biological tissue conservation in Côte d'Ivoire for research purposes, through a personalized questionnaire intended for biological and medical laboratories. The results showed a notable lack of conservation equipment and organization. Most of the laboratories surveyed do not follow the international standard procedure for the conservation of biological tissues. It therefore seems necessary to develop a standard procedure for the conservation of biological tissues that can be used in Côte d'Ivoire. This procedure could improve the quality of tissue conservation in order to enhance the value of biomedical research in Côte d'Ivoire and even in Africa.

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References

1. Herpel E, Koleganova N, Schirmacher P. [Tissue bank of the National Centre for Tumour Disease. An innovative platform for translational tumour]. *Pathologie*. 2008;29 Suppl 2:204-209. doi:10.1007/s00292-008-1067-2
2. Riegman PHJ, Dinjens WNM, Oosterhuis JW. Biobanking for Interdisciplinary Clinical Research. *Pathobiology*. 2007;74(4):239-244. doi:10.1159/000104451
3. Riegman PHJ, Morente MM, Betsou F, de Blasio P, Geary P. Biobanking for better healthcare. *Molecular Oncology*. 2008;2(3):213-222. doi:10.1016/j.molonc.2008.07.004
4. Stege A, Hummel M. [Experience with establishment and operation of a biobank]. *Pathologie*. 2008;29 Suppl 2:214-217. doi:10.1007/s00292-008-1043-x
5. Barr M, Souan L, MacGabhann P, et al. The Establishment of an ISO Compliant Cancer Biobank for Jordan and its Neighboring Countries Through Knowledge Transfer and Training. *Biopreservation and Biobanking*. 2014;12(1):3-12. doi:10.1089/bio.2013.0072
6. Husedzinovic A, Ose D, Schickhardt C, Frohling S, Winkler EC. Stakeholders' perspectives on biobank-based genomic research: systemic review of the literature. *Eur J Human Genet*. 2015;23(12):1607-14. <https://doi.org/10.1038/ejhg.2015.27>
7. Manolio TA, Abramowicz M, Al-Mulla F, Anderson W, Balling R, Berger AC, Bleyl S, Chakravarti A, Chantratia W, Chisholm RL, et al. Global implementation of genomic medicine: we are not alone. *Sci Transl Med*. 2015;7:290. <https://doi.org/10.1126/scitranslmed.aab0194>
8. Le Guellec S, Lacroix-Triki M, Delord J-P, Ben Allal C, Rochaix P. Les nouveaux fixateurs tissulaires. *Revue Francophone des Laboratoires*. 2009;2009(408):25-32. doi:10.1016/S1773-035X(09)70047-4

9. Hofman P. Quels fixateurs ? Pour quelles indications ? Principaux critères d'évaluation des méthodes alternatives à la fixation par le formol. *Revue Francophone des Laboratoires*. 2009;2009(408):45-48. doi:10.1016/S1773-035X(09)70050-4
10. Srinivasan M, Sedmak D, Jewell S. Effect of Fixatives and Tissue Processing on the Content and Integrity of Nucleic Acids. *The American Journal of Pathology*. 2002;161(6):1961-1971. doi:10.1016/S0002-9440(10)64472-0
11. Masuda N, Ohnishi T, Kawamoto S, Monden M, Okubo K. Analysis of chemical modification of RNA from formalin-fixed samples and optimization of molecular biology applications for such samples. *Nucleic Acids Research*. 1999;27(22):4436-4443. doi:10.1093/nar/27.22.4436
12. Lou JJ, Mirsadraei L, Sanchez DE, et al. A review of room temperature storage of biospecimen tissue and nucleic acids for anatomic pathology laboratories and biorepositories. *Clinical Biochemistry*. 2014;47(4-5):267-273. doi:10.1016/j.clinbiochem.2013.12.011
13. Yasojima K, McGeer EG, McGeer PL. High stability of mRNAs postmortem and protocols for their assessment by RT-PCR. *Brain Res Brain Res Protoc*. 2001;8(3):212-218. doi:10.1016/S1385-299X(01)00119-2
14. Leonard S, Logel J, Luthman D, Casanova M, Kirch D, Freedman R. Biological stability of mRNA isolated from human postmortem brain collections. *Biol Psychiatry*. 1993;33(6):456-466. doi:10.1016/0006-3223(93)90174-c
15. Chu T-Y, Hwang K-S, Yu M-H, Lee H-S, Lai H-C, Liu J-Y. A research-based tumor tissue bank of gynecologic oncology: characteristics of nucleic acids extracted from normal and tumor tissues from different sites. *Int J Gynecol Cancer*. 2002;12(2):171-176. doi:10.1046/j.1525-1438.2002.01085.x
16. Ma Y, Dai H, Kong X. Impact of warm ischemia on gene expression analysis in surgically removed biosamples. *Analytical Biochemistry*. 2012;423(2):229-235. doi:10.1016/j.ab.2012.02.003
17. Sherwood KR, Head MW, Walker R, Smith C, Ironside JW, Fazakerley JK. RNA integrity in post mortem human variant Creutzfeldt-Jakob disease (vCJD) and control brain tissue: RNA integrity in human vCJD brain tissue. *Neuropathology and Applied Neurobiology*. 2011;37(6):633-642. doi:10.1111/j.1365-2990.2011.01162.x
18. Atz M, Walsh D, Cartagena P, et al. Methodological considerations for gene expression profiling of human brain. *Journal of Neuroscience Methods*. 2007;163(2):295-309. doi:10.1016/j.jneumeth.2007.03.022
19. Tissus végétaux préservés en vue d'études génétiques. Muséum national d'Histoire naturelle. <https://www.mnhn.fr/fr/collections/ensembles-collections/botanique/tissus-vegetaux-preserves-vue-etudes-genetiques>. Accessed December 19, 2019.