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Biological quality evaluation of samples stored in biobank of Pasteur Institute of Côte d'Ivoire : case of influenza in nasopharyngeal and meningococcal in cerebrospinal fluid (CSF) samples

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ABSTRACT

The preservation and use of biological samples for therapy, diagnosis and research have been common practice for a long time. To maintain the stability of biomolecules (DNA, RNA, proteins) contained in the samples stored at the biobank of Pasteur Institute of Côte d'Ivoire, we evaluated the quality of the conservation of influenza nasopharyngeal and meningococcal CSF samples. Two (2) samples were chosen for this evaluation, namely Influenza A nasopharyngeal samples and *Streptococcus pneumoniae* CSF samples. After a few years of storage, these same samples were used to perform a PCR test. The results of the PCR tests showed a discordance of 36.4% for the *Streptococcus pneumoniae* CSF samples compared to 15.4% for the nasophryngeal samples. This study validates the need to write procedures for sample conservation at the level of the analysis laboratories and the Biobank. Therefore, a reflection must be carried out to standardise conservation techniques and a sample conservation evaluation programme.
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Keywords: Preservation; Influenza A nasopharyngeal samples; *Streptococcus pneumoniae* CSF samples; Discordance.

INTRODUCTION

Biobanks are structures that house several biological samples. Thanks to these biobanks, the number of biological materials and databases has increased at a rapid pace in recent years (Zawati, 2013; Betsou, 2017). Whether longitudinal or disease-specific, large or small, these biobanks are invaluable resources for research and are essential for the advancement of knowledge in the field of health (Zawati, 2013). In this regard, biobanks must ensure that biological material is obtained in a manner that respects the rights of individuals and must meet technical requirements in order to be usable.

A biobank is considered to be a physical space or infrastructure that allows a number of biological samples to be stored under perfect

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conditions in an organised manner. Beyond their physical infrastructure, biobanks are made up of a database that allows for real-time and detailed knowledge of the status of each sample (Julio et al., 2012).

The biobanking process oriented to biological research deals with the collection, transportation, processing and storage of biological samples for molecular, cellular and biochemical diagnosis of diseases. In the West, studies show that thousands of high-quality biological specimens and samples collected by researchers from academia, research institutes, hospitals and commercial organisations are often stored in cold environments in several biobanks (Vaught et al., 2011; Clément et al., 2009; Massett et al., 2011; Whyte, 2009; Zhang et al., 2019; Betsou, 2017). Conservation best practice guidelines have been adapted to fit a resource-poor environment and have been instrumental in developing a quality management system, including standard operating procedures and a quality control regime (Soo et al., 2017).

In view of the high stakes involved in the storage and use of human biological samples in diagnosis, therapy and research, it is imperative to maintain the integrity of the samples stored in the biobank and to use them for future studies. Biological characteristics can be influenced by the variation in temperature that samples undergo. This can have an impact on the results of analyses of the stored biomolecules (proteins, DNA, RNA) (De Paoli, 2005).

In Africa, recent studies have reported the existence of biobanking that can meet the demand for high quality biospecimens for cancer diagnosis, pathogenesis and translational medical research (Yakubu and De Vries, 2020; Nnamuchi, 2016; Mwaka and Horn, 2019; de Menil et al., 2019; Christoffels and Abayomi, 2020; Adepoju, 2019). Several studies in developed countries have shown that the quality of samples should be assessed as part of a routine procedure (Zhang et al., 2019).

The increasing number of biospecimens collected for multicentre and international

clinical studies and diagnostics points to the need to optimise the management of these biospecimens so that research biobanks can ensure that samples made available to academic or industrial researchers are of good quality and without intrinsic institute-dependent bias (Betsou, 2017).

In Africa, and particularly in Côte d'Ivoire, very little work has been done on the evaluation of the conservation quality of biological samples. The aim of this work was to evaluate the quality conservation of biological samples at the biobank of Pasteur Institute of Côte d'Ivoire (PICI) by taking as examples the samples of nasopharyngeal for Influenza and Cerebrospinal fluid for *Streptococcus pneumoniae* meningitis.

MATERIALS AND METHODS

Material

Study framework

Since 2010, the IPCI has created a biobank to improve the conservation of surveillance and diagnostic samples for research. The main mission of biobank of PICI is to provide assistance to researchers and other practitioners wishing to build up and/or use collections of human biological samples for biomedical, basic or biotechnological research projects. Biobank of PICI has several conservation methods: cold storage with +4°C, -20°C, -80°C and nitrogen liquid storage (-196°C); and conservation at room temperature by freeze-drying and encapsulation of nucleic acids.

Equipment for RNA extraction from nasopharyngeal samples

RNA extraction was performed using the Qiagen viral RNA mini kit (Qiagen, # 52904). In addition to the kit, other equipment used included a Type II Microbiological Safety Station (MSS II), a refrigerated centrifuge, sterile 2 ml Eppendorf tubes, a vortex mixer, micropipettes (P1000, P200, P100, P20, P10, P5), sterile cones (P1000, P200, P100, P20,

P10, P5), sterile gloves, waste bags, absorbent paper and 95-100% ethanol and personal protective equipment.

Equipment for DNA extraction from CSF samples

The equipment used for the extraction of DNA from the samples is : the -20°C freezer, a heated shaker, micropipettes (P1000, P200, P100, P20, P10, P5), sterile cones (P1000, P200, P100, P20, P10, P5), sterile gloves, waste bags, absorbent paper and 95-100% ethanol, a Type II Microbiological Safety Station (MSSP II), a centrifuge, sterile 2 ml Eppendorf tubes and a vortex shaker, personal protective equipment.

Equipment used for RT-PCR of nasopharyngeal influenza samples

RT-PCR of nasopharyngeal samples was performed using a Microbiological Safety Station Type II (MSS II), a 7500 Software Version 2.0 Thermocycler. 6 (Fast Real Time PCR Systems), a centrifuge with a rotor for 96-well microtitre plates, a vortex mixer, micropipettes (P1000, P200, P100, P20, P10, P5), sterile cones (P1000, P200, P100, P20, P10, P5), sterile gloves, waste bags, absorbent paper, 96-well plate, N95 mask and influenza A amplification and Qiagen kit, primers (InfA-F, InfA-P), InfA-R probe, 25X RT-PCR Enzyme, Detection Enhancer, Nuclease-free Water, 2X RT-PCR Buffer, personal protective equipment.

Equipment used for conventional PCR of DNA from CSF samples

The equipment used consisted of PSM II (Sterilgard, Class II typeA/B3, the Baker Company), GenAmp PCR System 9700 thermocycler (Applied Biosystems), refrigerated centrifuge, vortex mixer, (P1000, P200, P100, P20, P10, P5), sterile cones (P1000, P200, P100, P20, P10, P5), sterile gloves, waste bags, absorbent paper, 96 microtube racks, PCR buffer (pH 8. 8) 5X (supplied with Promega Taq polymerase), MgCl2 50 mM (supplied with Promega Taq polymerase), dNTP (dATP, dCTP, dGTP, dTTP), DMSO (Dimethyl Sulfoxide), PROLIGO primer, Promega Taq polymerase, Personal Protective Equipment (PPE).

Materials and reagents for RNA agarose gel electrophoresis

Preparation of the 2% migration gel was carried out using agarose, a precision balance, sterile distilled water, measuring cylinder, combs, Tris Acetate EDTA (TAE) buffer, Syber Safe, PPE and a microwave. Subsequently, deposition of samples into wells and agarose gel electrophoresis of DNA extracts was performed using a migration vessel (Cleaver scientific Ltd, England), aluminium foil, Tris Acetate EDTA (TAE) buffer, Syber Green, sterile gloves (Delta Plus Group, France), PPE, micropipettes (P20, P10), sterile filter cones (P20, P10). Visualisation of the DNA bands was obtained using the band visualisation device (Gel DocTM EZ Imager, USA).

Methods

Type of study

Our quality control study was a paired series study (or pairwise method) which took place from August to October 2017 i.e. over 2 months. This study used samples that had been analysed and stored. The pairwise method was used for each element of the sample considered, we have two values observed at different periods (before and after conservation).

Sample collection and processing

This study used samples that had been analysed and preserved. We performed random sampling using the "random()" function in Microsoft Excel 2003, which handles random numbers between 0 and 1. We drew 25 positive (only serotype A) and negative influenza virus samples (Table 1) and 25 positive and negative CSF samples (Table 3) stored at -80°C and -196°C from the data extracted from the sentinel influenza and meningitis surveillance specimen storage databases collected from 2009 to May 2014, with PCR results of influenza A virus and *Streptococcus pneumoniae* from the CSF. The data for this

study were entered and analysed using Microsoft Excel.

Biological tests for preservation quality assessment

RNA extraction from nasopharyngeal influenza samples

RNA extraction was performed using the Qiagen viral RNA mini kit (Qiagen, # 52904). Lysis, washing and purification of RNA extracts were obtained according to the manufacturer's instructions. The resulting RNA extracts were used for RT-PCR.

Preparation of the RT-PCR mix and reaction

In the Mix room, 20 µl of each mix is distributed to the corresponding wells and 5 µl of water is added to the well corresponding to the negative control (NC). In the RNA extract addition room, 5 µl of RNA extracts are added to the corresponding wells. Similarly, 5 µl of RNA from the positive control is added. The 96-well reaction tray is then sealed with a suitable optical adhesive film and the reaction tubes are sealed with suitable lids. Then the 96-well reaction plates are centrifuged in a rotor centrifuge in the form of a microtitre plate for 30 seconds at approximately 3000 rpm. Finally, the 96-well plate is placed in the thermal cycler for amplification.

Extraction of DNA from CSF samples

Pre-analytical phase: Under the hood (PSM Type II), 500 µl of the identified CSF sample is taken and transferred to a sterile 2 ml Eppendorf tube. The heated shaker is switched on (programmed to 95°C).

Analytical phase (heat shock): CSF is frozen at -20°C for 30 minutes, then the microtubes are transferred to the heated shaker at 95°C for 10 minutes. Centrifuge at 12,000 rpm for 10 minutes to remove cellular debris.

The supernatant is collected and transferred to a new microtube and the deposit is removed.

Post-analytical phase: The supernatant is stored in the refrigerator at +4°C or at -20°C in a freezer in the storage room if the amplification reaction is delayed.

Conventional PCR of DNA from CSF samples

The test mix is prepared from the stock mix by adding Taq polymerase and the rack is transferred to the DNA extract addition room. Then the mixture is homogenised by vortexing, centrifuged using the mini centrifuge and transferred to the thermal cycler room. The PCR tubes are placed in the thermal cycler and the amplification program corresponding to the genes to be amplified is started.

2% agarose gel electrophoresis

The preparation of the 2% agarose gel can be done while the gene amplification is running. 2 g of agarose is weighed and 100 ml of TAE buffer is added to a 500 ml Erlenmeyer flask, and the mixture is homogenised by hand shaking. The agarose is dissolved by heating in a microwave oven. Then cool to 65°C and add 10 µl of 10% syber safe. The gel is poured into the gel holder where the combs are aligned. The gel is allowed to harden and the combs are carefully removed. The gel is then placed in an electrophoresis tank containing 1X TAE and 10 µl of each sample is placed in each well of the gel.

Statistical analysis

The descriptive and comparative analyses used were performed in Microsoft Excel. The main comparison of interest was between the parameters (positive and negative) before storage and after storage. To describe the comparison between these 2 periods, we performed Chi-2 paired series tests for these categorical variables. Our Null Hypothesis was that the proportions of positive or negative are the same between two populations or 2 periods, the observed difference comes only from sampling fluctuations. The results were considered significant when $p \leq 0.05$.

Table 1: Results of the random draw of influenza nasopharyngeal samples.

Years	Total number of samples	Number of samples selected
2009	1486	4
2010	1682	4
011	1106	4
2012	1509	5
2013	2076	4
2014	2417	4

Table 2: PCR results before and after storage of randomly selected Influenza A nasopharyngeal samples.

Years	Number of samples tested	PCR before conservation		PCR after conservation	
		Positive	Negative	Positive	Negative
2009	4	2	2	2	2
2010	4	2	2	1	3
2011	4	2	2	2	2
2012	5	3	2	3	2
2013	4	2	2	1	3
2014	4	2	2	2	2

Table 3: Concordance of PCR results before and after storage of randomly selected Influenza A nasopharyngeal samples.

	Before conservation	After conservation	Concordance (%)
Results	13 positives	11 positives	84.6%
		2 negatives	15.4%
	12 negatives	0 positive	0%
		12 negatives	100%

RESULTS

Nasopharyngeal influenza samples

Table 2 shows that of the 25 influenza samples, 13 samples were positive and 12 samples negative before storage. Of the 13 positive samples, 11 (84.6%) remained positive and 2 (15.4%) became negative after some years of storage at biobank of PICI. All the negative samples at the beginning (before conservation) remained negative after conservation, i.e. a percentage of 100%.

The results obtained in this study showed a discrepancy of 15.4% in the Influenza A nasopharyngeal samples. Figure 1 shows the fluorescent signals showing sigmoid curves representing the positive control (red) and positive influenza nasopharyngeal samples and those showing straight lines representing the negative controls and negative influenza nasopharyngeal samples.

CSF samples for *Streptococcus pneumoniae* meningitis

In Table 4, for the 18 *Streptococcus pneumoniae* CSF samples, after PCR there were 11 positive samples and 7 negative samples before storage at biobank of PICI. Of the 11 positive samples, 7 (63.6%) remained positive and 4 (36.4%) became negative after a few years of storage at biobank of PICI. The 7 samples that were negative before storage remained negative after storage, i.e. a percentage of 100%.

Regarding agarose gel electrophoresis after conventional PCR of *Streptococcus pneumoniae* DNA, Figure 2a shows that lane M is the molecular weight marker, lane 1 is the positive control, lane 3 is the negative control, lanes 4, 8, and 11 are the positive samples and lanes 2, 5, 6, 7, 9 and 10 are the negative samples. And Figure 2b shows that the M tracks on the right and left are the molecular weight markers, track 1 is the positive control, track 3 is the negative control, tracks 4, 6, 11 and 12 are the positive samples and tracks 2, 5, 7, 8, 9, 10, 12, 13 and 14 are the negative samples. For *Streptococcus pneumoniae* CSF samples, the discordance was 36.4%.

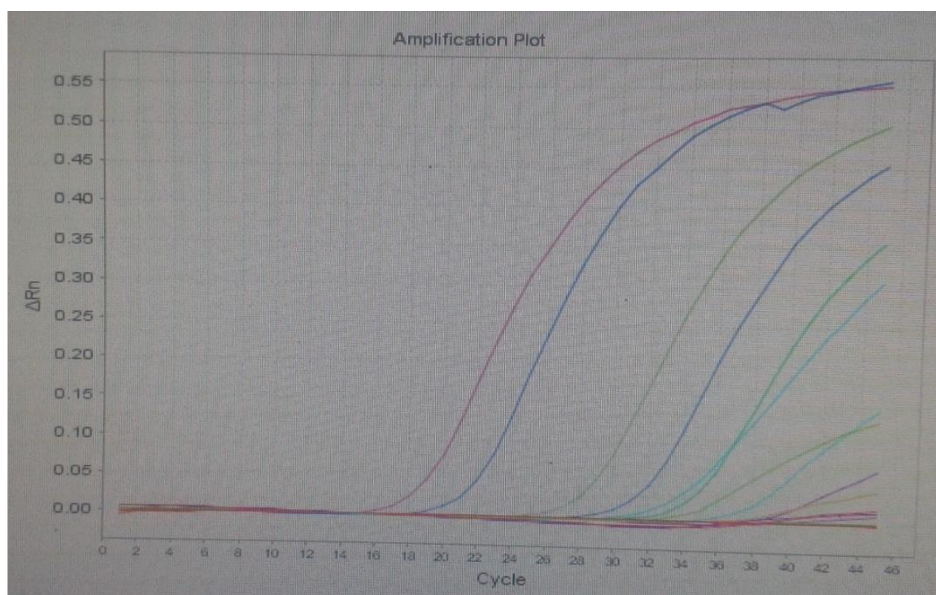

Figure 1: Real-time PCR amplification plot of randomly selected influenza nasopharyngeal swab samples.

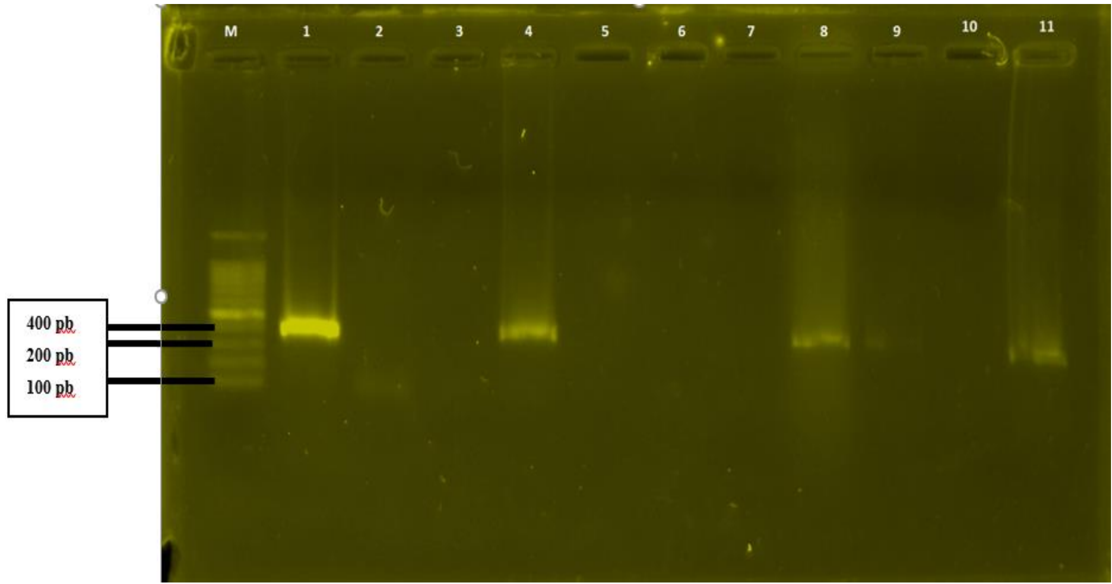

Figure 2a: 2% agarose gel electrophoresis containing Syber Safe at 10 µl of single PCR amplification products of *Streptococcus pneumoniae* DNA in CSF samples.

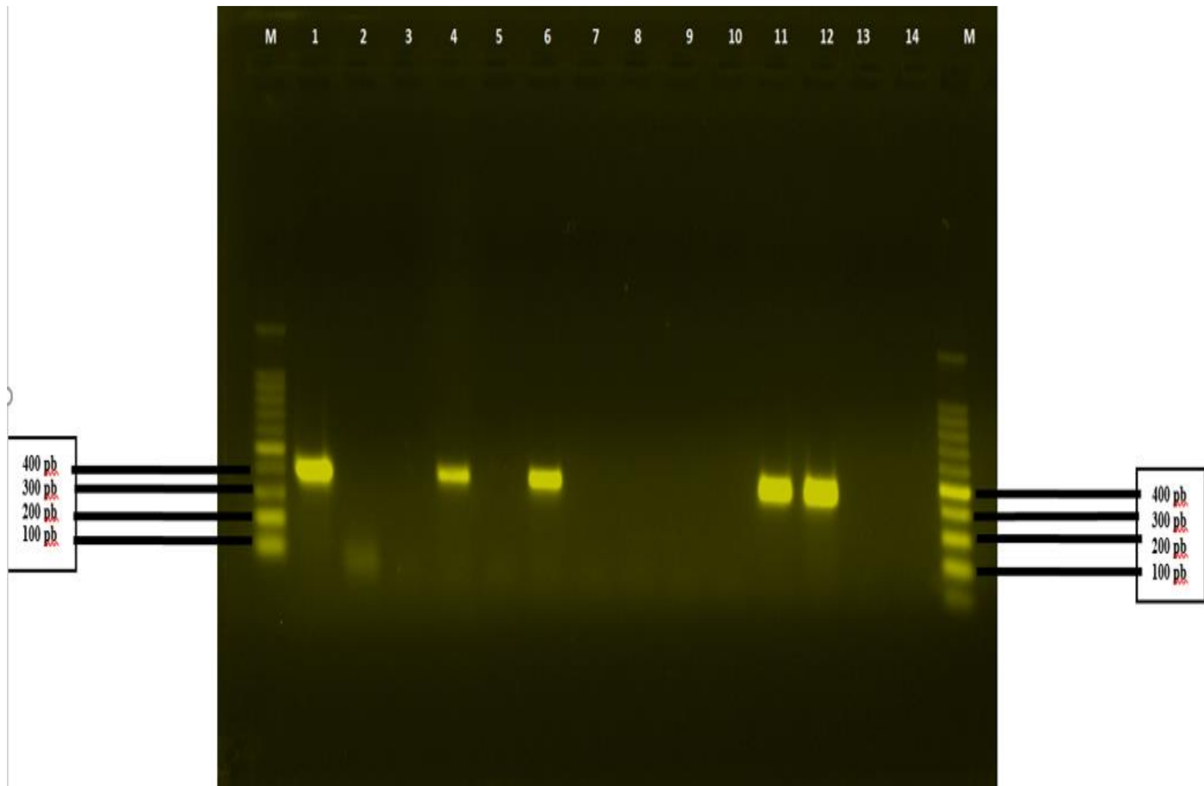

Figure 2b: Electrophoresis of 2% agarose gel containing Syber Safe at 10 µl of single PCR amplification products of *Streptococcus pneumoniae* DNA in CSF samples.

Table 4: Results of the random draw of CSF samples for *Streptococcus pneumoniae* meningitis.

Years	Total number of samples	Number of samples selected
2012	404	1
2013	59	8
2014	33	9

Table 5: Pre- and post-storage PCR results of randomly selected *Streptococcus pneumoniae* CSF samples.

Years	Number of samples tested	PCR before conservation		PCR after conservation	
		Positive	Negative	Positive	Negative
2012	1	1	0	1	0
2013	8	6	2	3	5
2014	9	4	5	3	6

Table 6: Concordance of PCR results before and after storage of randomly selected *Streptococcus pneumoniae* CSF samples.

	Before conservation	After conservation	Concordance (%)
Results	11 positives	7 positives	63.6%
		4 negatives	36.4%
	7 negatives	0 positive	0%
		7 negatives	100%

DISCUSSION

The availability of high-quality biological specimens for research purposes requires the development of standardised methods for collection, long-term storage, retrieval and distribution of specimens that will enable their future use (Shabihkhani et al., 2014). However, given the practical difficulties in the clinical setting, placing the specimen on ice immediately and then freezing the specimen within 20 minutes will mitigate against unnatural gene expression and phosphorylation aberrations. If biological specimens are to be used primarily for DNA, current data suggest that long-term storage of -70°C or -80°C is satisfactory but that RNA may degrade by 5 years (Shabihkhani et al., 2014).

The high discordance of 15.4% obtained in the Influenza A nasopharyngeal samples could be explained by the fact that his samples were weakly positive (lower RNA copy number) after analysis in the respiratory virus laboratory. His samples were then stored at -20°C for 2 years at the Department of Epidemic Viruses before being admitted to the Institut Pasteur biobank in Côte d'Ivoire where they were stored at -80°C. However, the Institut Pasteur de Côte d'Ivoire at the

Adiopodoumé site is confronted with a frequency of electricity cuts of 3 times a week and an average duration of cut of around 2 hours. This would explain why its samples have undergone numerous repeated freeze-thaw cycles, which could have an impact on the integrity of the RNA given their low yield of RNA before conservation. Several studies have found that numerous repeated freeze-thaw cycles result in significant decreases in RNA integrity (Ma et al., 2012; Sherwood et al., 2011; Atz et al., 2007). In some cases, up to 2 thawing events were sufficient to reduce RNA quality. However, other studies have shown a reduction in RNA integrity in samples stored at -70°C or -80°C for 5 years or more (Imrali et al., 2020; Chu et al., 2002).

In the *Streptococcus pneumoniae* CSF samples, the discordance of 36.4% was very high. This discrepancy could be explained by the fact that there could be an error in reading DNA bands on agarose gel in the testing laboratory before storage. Indeed, the identification size of *Streptococcus pneumoniae* is slightly closer to that of *Haemophilus influenzae* type b by multiplex PCR on agarose. The size of *Streptococcus pneumoniae* is 390 bp and *Haemophilus influenzae* type b is 343 bp. So there could be confusion about the size of the DNA when identifying *Streptococcus pneumoniae*. This is one of the drawbacks of conventional PCR. Apart from the misreading for the identification of *Streptococcus pneumoniae* and *Haemophilus influenzae* type b, the discrepancy in CSF samples could also be explained by the fact that *Streptococcus pneumoniae* does not possess either catalase or peroxidase, which induces the accumulation of hydrogen peroxide that is partly responsible for its autolysis.

The results obtained show that freeze-thawing should be avoided as much as possible by appropriate aliquoting. Where possible, placement of samples on dry ice or wet ice during sampling should be considered. Sherwood et al found that aliquots stored in RNAlater (Qiagen, Valencia, CA) produced better quality RNA than snapshot samples and were more resistant to freeze-thawing (Sherwood et al., 2011).

It should be noted that the size of our study samples was small and the number of mismatches less than 10 (<10), so our results have a lot of caveats and cannot be generalized. However, our results show that there are significant discordances between the results of samples before and after preservation for *Streptococcus pneumoniae* CSF samples.

Conclusion

Quality control testing of influenza nasopharyngeal and Spneumococcal CSF specimens was performed by RT-PCR and conventional PCR respectively. The results showed significant discrepancies between the results of the samples before and after preservation for the *Streptococcus pneumoniae* CSF samples. This study validates the need to write procedures for sample conservation at the level of the analysis laboratories and the Biobank. Therefore, a reflection must be carried out to standardise the conservation techniques and a programme of evaluation of conservation of samples.

COMPETING INTERESTS

The authors declare that they have no competing interests related to this manuscript.

AUTHORS' CONTRIBUTIONS

AKK performed the biological analyses and wrote the article; MKD performed the sample collection and data analysis; HAK participated in writing the biological analysis protocol; SC, RJB, EAA, LSC and SG provided corrections and suggestions to the manuscript; MM and MD designed the study.

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REFERENCES

Adepoju, Paul. 2019. Africa's First Biobank Start-up Receives Seed Funding. *Lancet (London, England),* **394** (10193): 108.

Atz M, Walsh D, Cartagena P, Li J, Evans S, Choudary P, Overman K, Stein R, Tomita H, Potkin S, Myers R, Watson SJ, Jones EG, Akil H, Bunney WE. 2007. Methodological considerations for gene expression profiling of human brain. *Journal of Neuroscience Methods*, **163**: 295–309.

Betsou F. 2017. Quality Assurance and Quality Control in Biobanking. *Biobanking of Human Biospecimens*, 23‑49.

Christoffels A, Abayomi A. 2020. Careful Governance of African Biobanks. *Lancet (London, England)* **395** (10217): 29-30.

Chu TY, Hwang KS, Yu MH, Lee HS, Lai HC, Liu JY. 2002. Une banque de tissus tumoraux basée sur la recherche d'oncologie gynécologique: caractéristiques des acides nucléiques extraits de tissus normaux et tumorales de différents sites. *International Journal of Gynecological Cancer*, **12** : 171-176.

Clément B, Chêne G, Degos F. 2009. A national collection of liver tumours: lessons learnt from 6 years of biobanking in France. *Cancer Lett*., **286**: 140–144.

de Menil V, Hoogenhout M, Kipkemoi P, Kamuya D, Eastman E, Galvin A, Mwangasha K, de Vries J, Kariuki SM, Murugasen S, Mwangi P, Singh I, Stein DJ, Abubakar A, Newton CR, Kirsten AD, Robinson E. 2019. The NeuroDev Study: Phenotypic and Genetic Characterization of Neurodevelopmental Disorders in Kenya and South Africa. *Neuron,* **101** (1): 15‑19.

De Paoli P. 2005. Biobanking in microbiology: From sample collection to epidemiology, diagnosis and research. *FEMS Microbiology Reviews,* **29**: 897–910.

Imrali A, Hughes CS, Coetzee AS, Delvecchio FR, Saad A, Roberts R, Chelala C, ChinAleong JA, Kocher HM. 2020. Validation of a Novel, Flash-Freezing Method: Aluminum Platform. *Curr Protoc Essent Lab Tech,* **21**(1): e46.

Julio CM, Ignacio B, Alejandra H, Alberto G, 2012. Biobanques : Une stratégie exigeante et essentielle pour la conservation des échantillons biologiques. *Acta Médica Colombiana*, **37**(3) : 158-162.

Ma Y, Dai H, Kong X. 2012. Impact of warm ischemia on gene expression analysis in surgically removed biosamples. *Analytical Biochemistry*, **423**: 229–235.

Massett HA, Atkinson NL, Weber D, Myles R, Ryan C, Grady M, Compton C. 2011. Assessing the need for a standardized cancer Human Biobank (caHUB): findings from a national survey with cancer researchers. *J Natl Cancer Inst Monogr*, **2011**: 8–15.

Mwaka, Erisa, et Lyn Horn. 2019. Researchers' Perspectives on Informed Consent and Ethical Review of Biobank Research in South Africa: A Cross-Sectional Study. *Journal of Empirical Research on Human Research Ethics: JERHRE,* **14** (4): 307‑17.

Nnamuchi, Obiajulu. 2016. Biobank and Genomic Research in Uganda: Are Extant Privacy and Confidentiality Regimes Adequate? *The Journal of Law, Medicine & Ethics: A Journal of the American Society of Law, Medicine & Ethics,* **44** (1): 85‑95.

Shabihkhani Ma, Lucey GM, Wei B, Mareninov S, Lou JJ, Vinters HV, Singer E, Cloughesy TF, Yong WH. 2014. The procurement, storage, and quality assurance of frozen blood and tissue biospecimens in pathology,

biorepository, and biobank settings. *Clinical Biochemistry*, **47**(4-5).

Sherwood KR, Head MW, Walker R, Smith C, Ironside JW, Fazakerley JK. 2011. RNA integrity in post mortem human variant Creutzfeldt-Jakob disease (vCJD) and control brain tissue. *Neuropathology and Applied Neurobiology*, **37**: 633–642.

Soo, Cassandra Claire, Freedom Mukomana, Scott Hazelhurst, et Michele Ramsay. 2017. « Establishing an Academic Biobank in a Resource-Challenged Environment ». *South African Medical Journal = Suid-Afrikaanse Tydskrif Vir Geneeskunde,* **107** (6): 486‑92.

Vaught J, Rogers J, Myers K, et al. 2011. An NCI perspective on creating sustainable biospecimen resources. *J Natl Cancer Inst Monogr*, **2011**:1–7.

Whyte B. 2003. National tumor bank set up in United kingdom. *J Natl Cancer Inst*, **95**: 706.

Yakubu, Aminu, Nchangwi Syntia Munung, et Jantina De Vries. 2020. How Should Biobanking Be Governed in Low-Resource Settings? *AMA Journal of Ethics,* **22** (2): E156-163.

Zawati, Ma'n H. 2013. Biobanques Destinées à La Recherche: Introduction et Défis Majeurs (Research Biobanks: Introduction and Major Challenges). SSRN Scholarly Paper ID 2634644. Rochester, NY: Social Science Research Network. https://papers.ssrn.com/abstract=2634644.

Zhang, Xia, HanQiu-Yue, ZhaoZhang-Sheng, ZhangJian-Gang, ZhouWen-Jun, et LinAifen. 2019. Biobanking of Fresh-Frozen Gastric Cancer Tissues: Impact of Long-Term Storage and Clinicopathological Variables on RNA Quality. *Biopreservation and Biobanking*, **17**(1): 58-63.