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Evaluation of the quality of SARS COV 2 positive nasopharyngeal samples stored in liquid nitrogen at the biobank (Pasteur institute of Côte d'Ivoire)

Julie José Rita Bouagnon[*1], Marcelle Money[1], Kouao Maxime Diané[1], Bahou Roger Dehé[1], Souleymane Cissé[1], Kouadio Ayébé Edwige Aka[1], Kouamé Ambroise Kintossou[1], Litio Sinali Coulibaly[1], Hervé Alberic Kadjo[1], Jean David N'Guessan[2], Alico Joseph Djaman[1,2], Mireille Dosso[1]

[1]*Pasteur Institute of Côte d'Ivoire, Abidjan, Côte d'Ivoire*

[2]*Félix Houphouët Boigny University, Abidjan, Côte d'Ivoire*

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Abstract

The SARS Cov2 positive samples from the COVID 19 pandemic surveillance were subjected to quality control after storage at the biobank of the Pasteur Institute of Côte d'Ivoire. They were evaluated one year after their cryopreservation by a PCR test (polymerase chain reaction) and the threshold values of the cycle were recorded. PCR test results indicated 100% concordance of all nasopharyngeal specimens that tested positive for SARS CoV2. It appears that nasopharyngeal samples packaged in high security straws and stored at -196°C are still viable for detecting SARS-CoV-2 after one year of storage. A reproducibility of the preservation protocol can be carried out to standardize the preservation technique. An annual program for evaluating the conservation of samples can be instituted at the biobank as a form of quality control.

* **Corresponding Author:** Julie José Rita Bouagnon ✉ bouagnonrita@yahoo.fr

Introduction

According to the ISO 20387 standard, the biobank or the legal entity of which it is part must determine, control and maintain the dedicated facilities/areas in order to provide the conditions required for compliance with the defined quality control (QC) criteria. During the COVID-19 pandemic, biobanks provided clinical samples (nasopharyngeal swabs from the anterior nares and middle turbinate and saliva) taken from patients with clinical signs in order to carry out scientific studies (Frediani *et al.*, 2022). Several factors, including the longitudinal stability of the samples provided, are required to guarantee their quality. These samples are collected and transported according to Biosafety rules and stored in liquid nitrogen.

Authors such as (Perera *et al.*, 2020) reported that blood plasma samples from COVID-19 patients largely tested positive for SARS-CoV-2 antibodies in serological testing. Furthermore, archived blood samples, some of which were taken years before the epidemic, have long been used by researchers to trace early human exposure to new pathogens (Anderson *et al.*, 2012) (Bone *et al.*, 2012). As for the (nasopharyngeal) samples collected since the appearance of the first SARS-CoV-2 positive case in West Africa, they are cryopreserved in the biobank of ECOWAS member countries (housed at the Pasteur Institute in Côte d'Ivoire) for further research (Bouagnon, 2022).

Early work examining the validity of saliva samples after long-term storage for detection of SARS-CoV-2 found that positivity remained stable for up to 12 months at -80 ℃ (Frediani *et al.*, 2022). To our knowledge, there are no data on the work carried out in sub-Saharan Africa concerning the validity of nasopharyngeal samples after a storage time for the detection of SARS-CoV-2. The objective of this study is therefore to assess the stability of nasopharyngeal samples from patients with SARS-CoV-2 after one year of cryopreservation (-196°C in liquid nitrogen) at the biobank of the Pasteur Institute of Côte d'Ivoire.

Materials and methods

Setting and population of the study

Quality control focused on SARS COV2 positive samples stored in April 2020 cryopreserved in high security straws in the cryobiology room of the IPCI biobank (Adiopodoumé site). This cryobiology room with a long-term capacity of 44 cryopreservatives is dedicated to the long-term safeguarding of biological resources by cold. It contains personal protective equipment and is equipped with a digicode system to ensure the biosafety and biological safety of the samples.

Sampling

64 aliquots (one aliquot per sample) were selected in April 2020, i.e. at the start of the COVID 19 pandemic in Côte d'Ivoire. These were found on the basis of a record linked to the data associated with them in Excel software.

Equipment used for extraction of RNA from nasopharyngeal samples

RNA extraction was performed using the Qiagen Viral RNA Mini Kit (Qiagen, #52904). Equipment such as a type II microbiological safety cabinet (PSM II), a refrigerated centrifuge, 2 ml sterile 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 were used for the extraction of the RNA.

Equipment used for RT-PCR of nasopharyngeal samples

RT-PCR testing of nasopharyngeal samples was performed using a type II microbiological safety cabinet (PSM II), a LightCycler® 480 real-time thermal cycler (Roche), a centrifuge with a rotor for 96-well microtiter plates and a vortex mixer. Small equipment such as micropipettes (P1000, P200, P100, P20, P10, P5), sterile tips (P1000, P200, P100, P20, P10, P5), sterile gloves, waste bags, absorbent paper, a 96-well plate, N95 mask were essential. Qiagen SARS COV 2 amplification kit, primers, enzymes, detection enhancer, nuclease-free water and buffer were used. Personal protective equipment served as a biosecurity measure during handling.

Extraction from nasopharyngeal 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 room under type II microbiological safety cabinet (PSM II), 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 reaction plate (96-well) is centrifuged in a rotor centrifuge in the form of a microtitre plate for 30 seconds at approximately 3000 rpm. Finally, the 96-well's plate is placed in the thermal cycler "LightCycler® 480 (Roche)" for amplification.

Statistical analysis

The comparative analysis used was carried out in Microsoft Excel thanks to the value of the cycle threshold (Ct) of the samples positive for SARS COV2.

Results

All positive nasopharyngeal samples (64 in number) before storage remained positive one year after storage in liquid nitrogen. This represents a percentage of 100%. Fig. 1 through a graph shows the Ct values obtained by samples All samples evaluated have Ct values below 33 (Table 1).

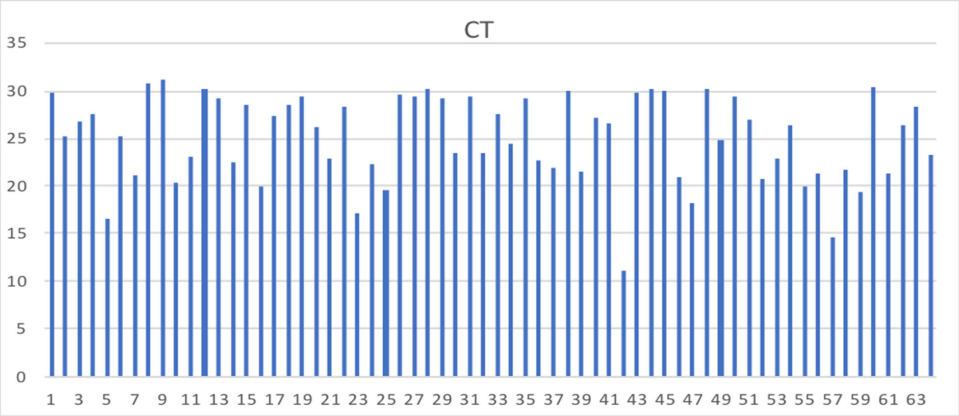

Fig. 1. Graph representing the Ct values of the positive samples

Table 1. Interpretation of Ct values

Real-time thermal cycler	Ct value	On 64 samples evaluated by RT-PCR	Interpretation
LightCycler 480 Roche Diagnostics	Ct ≤ 33	64	Significant viral shedding
	Ct > 33	0	Moderate to very low viral shedding
	Ct ≥ 37	0	

Source: Evaluation reports of RT-PCR kits- CNR Respiratory Viruses Institut Pasteur, Paris / CNR Respiratory Viruses Lyon on the website of the French Society of Microbiology (SFM); https://www.sfm-microbiologie.org/covid-19-reactifs-evaluations.

Discussion

The present study demonstrated the availability of high-quality nasopharyngeal specimens one year after cryopreservation. All nasopharyngeal specimens contained in the high security straws and tested after thawing had their SARS-CoV-2 detectable.

Indeed, SARS-CoV-2 reverse transcription polymerase chain reaction (RT-PCR) cycle threshold (Ct) values have been used as a means to quantify viral load during the Covid 19 pandemic (He *et al.*, 2020).

Although difficult to implement, investment efforts have been made by the state of Côte d'Ivoire with the aim of constituting a systematic collection of samples from the Covid 19 pandemic. These efforts supported by donors funds have enabled the cryopreservation of said samples in order to accelerate research and deepen knowledge on Covid 19 (Vaught, 2020).

The failure to save the threshold values (Ct) of the positive samples at the start of the pandemic (April 2020) constitutes a limitation of this study which should be mentioned. The result returned for this technique was purely qualitative, namely negative or positive depending on the absence or presence of viral RNA in the sample analyzed. All Ct values below 33 indicate a large quantity of viral particles and by extrapolation suggest high contagiousness of patients carrying the virus. This confirms the quality of the nasopharyngeal samples analyzed which originally depended on the authorization and experience of the sampler (Bullard *et al.*, 2020).

This could be explained by the strong pressure to report diagnostic results on time and by apprehensions about the outcome of the pandemic. This gap did not make it possible to compare the Ct of the positive samples before and one year after their storage in liquid nitrogen. However, these stored samples are needed to improve diagnostic designs, advance therapeutic research and vaccine development against SARS-CoV-2 (LaVergne *et al.*, 2021).

Faced with the growing demand for high-quality biological samples, it would be important to reduce the volume of biological resources made available due to research activities in genomics, post-genomics and personalized medicine (Coppola *et al.*, 2019). Packaging in 0.3ml high security flakes would be appropriate for the samples used for the research mentioned above. The fundamental reason which would explain this option is the reduction in recent years of the volume of material necessary for an assay (Rita *et al.*, 2023).

Conclusion

This study performed a quality control test of nasopharyngeal specimens on SARS COV2 by RT-PCR. The results indicated concordance between the results of the samples before and after storage. This study thus validates the procedure for maintaining the integrity of nasopharyngeal samples in high security straws from the Covid 19 pandemic after one year of cryopreservation. The same exercise will be carried out after two, three, four and five years of storage of the samples in order to set up a five-year standardized method of quality control at the Biobank of the Pasteur Institute of Côte d'Ivoire.

Competing interests

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

Ethics approval statement

Regarding the 'Statement of Ethics Approval', we the author means that the article submitted to your journal does not require approval from the ethics committee because no personal data has been processed.

Authors contributions

JJRB supervised the biological analyzes and wrote the article; MM contributed to the storage and traceability of the samples; MKD performed the data analysis; BRD carried out the biological analyses; LSC and FN made the samples available; SC, EAAK, AKK and HAK made corrections and suggestions to the manuscript; JDN, JAD and MD designed the study.

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