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Design and Implementation of Nasopharyngeal Specimen Storage in Straws during the COVID-19 Pandemic in Côte d'Ivoire

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ABSTRACT

The Pasteur Institute of Côte d'Ivoire in its mission of surveillance and support to public health participates in the response to the COVID-19 pandemic in Côte d'Ivoire. It is involved in the diagnosis by RT-PCR of SARS-CoV-2. Through its biological resource center, which is the regional biobank of ECOWAS, the Pasteur Institute of Côte d'Ivoire keeps *a priori* or *a posteriori* nasopharyngeal samples of the COVID-19 pandemic and associated data. This study aimed to characterize the key issues driving the straw CBS™ storage of COVID-19 pandemic nasopharyngeal specimens in Côte d'Ivoire, the key determinants of the process and the final storage protocol. Planning was used to examine the technical, economic, logistical, and political aspects of the project. The study was designed to include 100,000 samples from Abidjan, the economic capital, and the interior of the country. In the interior of the country, the samples came from COVID-19 centers in the interior cities. The biobanking process of nasopharyngeal samples in straw CBS ™ or high security straws from a primary tube was carried out using two techniques: manual and semi-automatic. These straws are properly sealed with a guaranteed airtightness using the System of Manual Sealing (SYMS) and the semi-automated instrument (PACE) of the company CryoBioSystem (Saint Ouen sur Iton, France). The average monthly performance of the straws obtained is 1,685 for the manual technique versus 2,820 for the semi-automatic technique. This performance is in line with the objective set during the design of the study. The acquisition of the semi-automated technique (PACE) has allowed the biobank to reinforce biosafety measures concerning the handling of samples. In the event of a large-scale epidemic, the collection in a biobank requires a complete automated chain for stratification to facilitate the provision of samples to applicants and to strengthen the heritage collection.

Keywords: Biobanking, High Security Straws, System of Manual Sealing (SYMS), Semi-Automated Instrument (PACE), COVID-19 Pandemic.

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INTRODUCTION

Biobanks are research infrastructures for the collection, preparation, preservation and availability of different types of biological materials and associated data related to medical information or other characteristics [1]. These research structures are associated with specific studies and technological innovation centers, which are usually part of medical/academic complexes [2]. Biological samples are invaluable resources for understanding disease mechanisms and the relative contributions of genetic and environmental factors. These have been contributing to health care decision making since the introduction of evidence-based medicine [3]. In this sense, the establishment of a collection in a biobank is a key promoter of future research. Developed countries are ahead in this field of knowledge that biobanks represent.

Since the year 2000, Sub-Saharan Africa has been subject to an upsurge of epidemics of emerging or re-emerging pathologies such as Yellow Fever, Dengue, Rift Valley Fever, Chikungunya, Ebola, Zika, Lassa fever, COVID-19 etc. Insufficient local capacity to diagnose and preserve biological samples has led to the export of large quantities of these samples to developed countries. The latter have more technological tools to diagnose and preserve biological derivatives such as reference strains sometimes discovered in West Africa. The significance of collections is that they are useful in the design of future studies that will answer new scientific questions [2].

To address shortcoming related to sample storage, the Pasteur Institute of Côte d'Ivoire created in 2011 a Biological Resource Center or Biobank which hosts since April 15, 2018 the regional biobank of ECOWAS member countries. The collection, processing, conservation and availability of biological samples are practices carried out in the broader context of organizations called biobanks [4]. The Pasteur Institute of Côte d'Ivoire in its mission of surveillance and support to public health participates in the response against the pandemic in COVID-19 in Côte d'Ivoire. It is involved in the diagnosis by RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction) of SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). Through CeReB IPCI, it keeps *a priori* or *a posteriori* the nasopharyngeal samples of the COVID-19 pandemic and the associated data.

One of the primary concerns of the biological resources' conservation team during the pandemic at COVID-19 was how to build a collection of more samples that would occupy less space. The primary strategy governing the design and implementation of this collection was associated with two areas: developing local management expertise and having the necessary equipment to store the samples in straws [2].

Several authors from developed countries have reported the collection of samples from outbreaks in high-security straws or straws CBS^{TM} [5]. Unfortunately, these collection data are not collected in African countries, particularly in West African countries such as Côte d'Ivoire [6]. Few studies have described in detail the process of planning and implementing the collection of straws CBS^{TM} samples from epidemics. This study characterizes the main points that will drive the CBS^{TM} stratification of nasopharyngeal specimens from the COVID-19 pandemic in Côte d'Ivoire, the main determinants of the process (economic, political, and logistical), and the final storage protocol.

METHODOLOGY

Setting and Type of Study

The study is a prospective study focused on the establishment of a straw biocollection of nasopharyngeal samples from the COVID-19 pandemic. It was conducted at the Sample Management Unit (SMU) of the Biological Resource Center (or Biobank of the Pasteur Institute of Côte d'Ivoire (Adiopodoumé site) from April 2020 to December 2021. The Sample Management Unit (SMU) is managed by two biologists and one technician, and trainee staff. Each staff member has received detailed information on the sample collection and storage process. The unit has consumables, equipment and materials for biosafety, sample processing and preparation [microbiological safety level II (MSL II), personal protective equipment (PPE), pipette, welding machine, etc.]. A room is dedicated to sample processing.

Study Planning

The study was designed to include 100,000 samples from the Infectious and Tropical Diseases Department (SMIT) of the Treichville University Hospital in Abidjan, the economic capital, and from the 10 COVID-19 screening centers in each commune of the Abidjan district. For the interior of the country, the samples came from COVID-19 centers in the interior cities (Figure 1).

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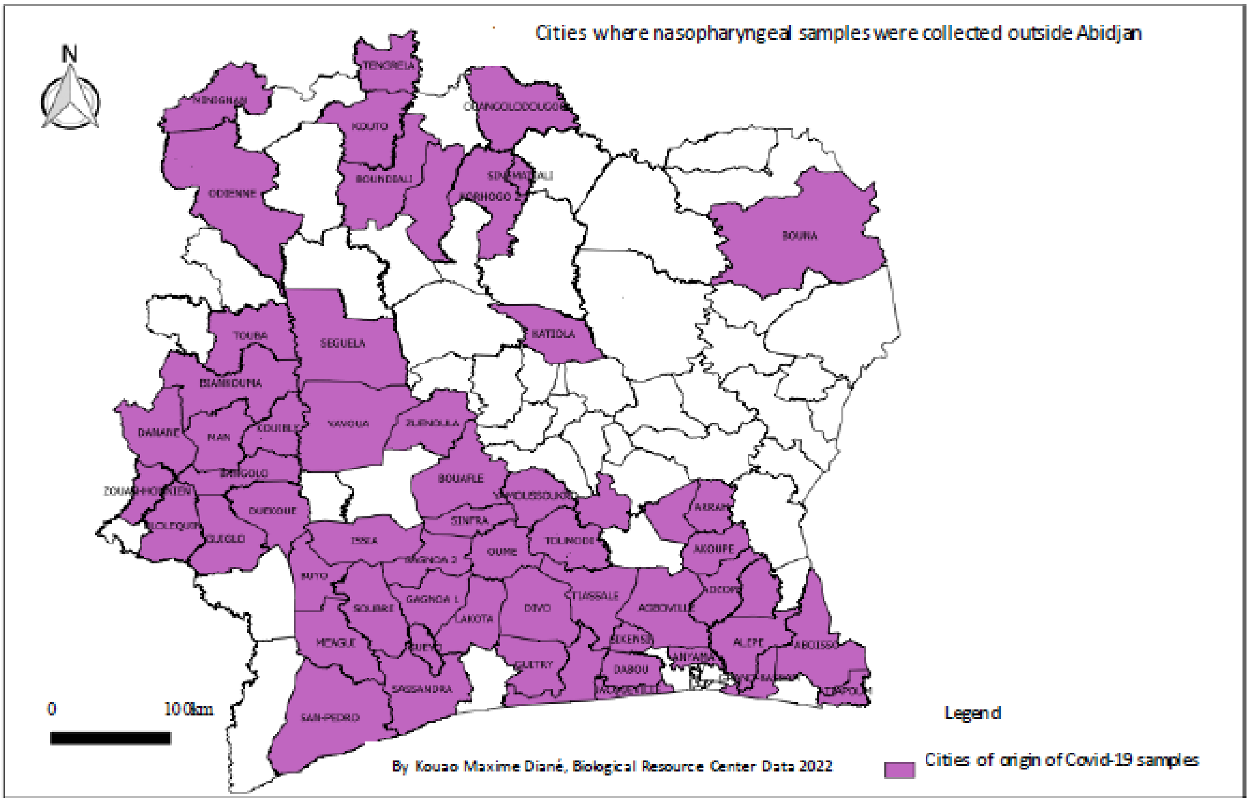

Figure 1: Geographical map showing cities (sites) of origin of nasopharyngeal samples outside Abidjan collected during the COVID-19 pandemic

A project team made up of the biobank's SMU team was set up by the administration with the mandate to design and implement the storage of samples from the collection sites in straws. At the first meetings of the team, the main points of the biobanking activity were discussed. The basic biobanking protocols were drafted by the SMU members. The meetings started two months after the collection of samples and data. The main determinants for the implementation of the biobanking were:

1. Store all or part of samples (RNA extract, source samples), taking into account the quantity of samples to be collected and the minimum requirements for monitoring and surveillance.
2. Ensure the best possible storage conditions for all samples collected.
3. Ensure that quality control, aliquoting, sample tracking and long-term storage protocols are in place.

Economic aspects have always regulated the study protocol in the design and implementation of the biocollection. In particular, decisions based on the number of samples, storage conditions and the development of an information system to monitor collection, transport and quality control have always been driven by economic aspects [2].
The initial decision to include 100,000 specimens had to be abandoned due to lack of knowledge of the magnitude of the pandemic at COVID-19 and insufficient personnel requisitioned for preservation.

Samples were transferred in triple UN 2814 (human infectious material) secure packaging to dedicated vehicles at the Pasteur Institute of Côte d'Ivoire (Adiopodoumé site) 24 hours a day, 7 days a week by the national epidemic disease surveillance and response network. Figure 1 is showed the map of sample collection sites outside of Abidjan.

After consensus was reached on the key points of the biocollection set-up, the main issue to be decided by the curation team was the evaluation of the need to store the stem cell

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samples and the nucleic acid extracts (RNA). Arguments for and against were considered, and in the end a consensus was reached to store only the stem cell samples. The reasons for this decision were related to the fact that splitting the stem cell samples would increase the biological resources available for assays and future research [2]. The storage protocol should have been simple enough to ensure the reproducibility of the study. This was not only about developing expertise in aliquoting and storage, but also about the needs of the Sample Management Unit (SMU) not exceeded its capacity to maintain a steady flow of samples.

Assets of Biological Resource Center of the Pasteur Institute of Côte d'Ivoire

Biological resource center of the Pasteur Institute of Côte d'Ivoire has a large cryobiology room (480 m^2 with a capacity of 44 cryoconservers) with a series of advanced technological installations necessary for conservation at -196°C: external reservoir (capacity of 21,000 liters of nitrogen), super insulated vacuum lines, large capacity cryoconservers to store samples for long periods of time in vapour or liquid phase of nitrogen (Espace 661 or RCB 10001) [. The staff of the biological resource center is dedicated and permanent. Nitrogen supply is provided by two private companies based in Côte d'Ivoire. Maintenance and supply companies for cryopreservation equipment and consumables are locally represented. Several contracts for the maintenance of the installations and equipment are signed with the private companies coupled with the supply of cryopreservation consumables.

The select straw cryoconservatives (RCB 660 L and RCB 1000L) for this study contain 165 canisters and 216 canisters respectively. This corresponds to a storage capacity of 108900 straws (165 canisters of 4 cryogenic cups of 165 straws each) and 143560 straws (216 canisters of 4 cryogenic cups of 165 straws each).

Biobanking Process for Nasopharyngeal Samples

Figure 2 shows the biobanking process for nasopharyngeal samples in straws. Upon arrival of the samples at the Pasteur Institute of Côte d'Ivoire, reception and processing was performed by the Nasopharyngeal Specimen Reception, Selection and Decontamination Unit. This unit then forwarded the samples to the Epidemic Virus Department (EVD) and the Sample Management Unit (SMU) for diagnosis and biobanking respectively, according to predefined criteria for accepting a sample [8].

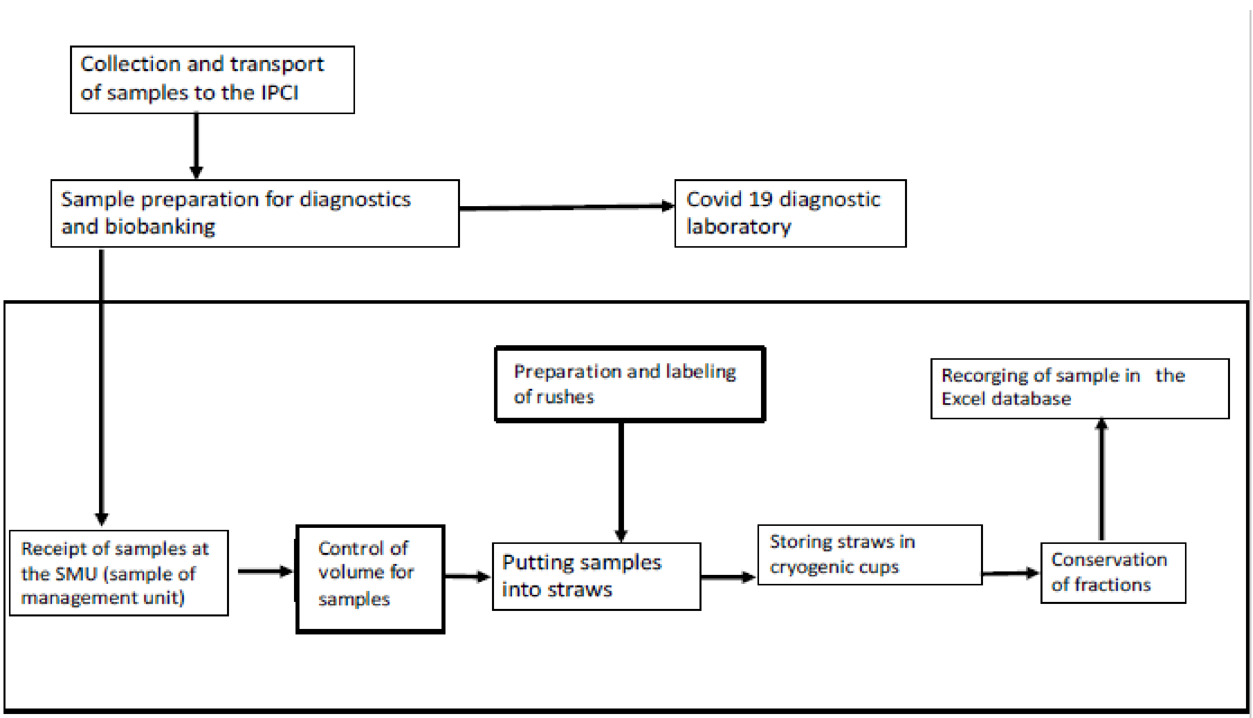

Figure 2: Flow diagram of the circuit of strawed samples from collection to storage during the COVID-19 pandemic

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At the SMU level, the volume of each sample was recorded to know the exact number of 0.3ml aliquots to be performed. Limited access spreadsheets were also created, linking the BRADY BMP 53 printer labels (Fisher Scientific SAS, Strasbourg, France) to a certain participant, preserving the anonymity of the sample. These labels were wrapped on rushes and inserted into the short end of the straws for identification.

Sample pelleting from a primary tube was performed using two techniques: manual and semi-automatic. In the manual technique, a volume of 0.3 ml of sample is aspirated by a 1 ml insulin syringe and injected into the free end of the High Security straw. The two ends of the straw were successively introduced into the reserved area of the SYMS (SS1: http://getjournal.org/wp-content/uploads/2022/12/Supplementary-GJOBOH-2022-030.pdf) (CryoBioSystem, Saint Ouen sur Iton, France) for thermal welding using a foot pedal and removed as soon as the green light appears. The operation is repeated until the last sample fraction is pelleted (i.e. until the primary tube is empty).

As for the semi-automatic technique, the PACE machine (CryoBioSystem, Saint Ouen sur Iton, France) (SS2: http://getjournal.org/wp-content/uploads/2022/12/Supplementary-GJOBOH-2022-030.pdf) allowed the biological samples to be put into straws CBS™ or High Security straws. In the PACE hopper, the required number of 0.3 ml High Security straws was introduced by placing the end of the straws containing the rushes on the side corresponding to the aspiration nozzle. The sample contained in a primary tube was conveyed into the free end of the straws through a sterile CBS™ blue long injection nozzle. Then, both ends of the straws were heat-sealed simultaneously [9].

Each straw (Plate 1) were physically arranged inside visiotubes (at a rate of 15 straws per visiotube) contained in counterclockwise cups. The cryogenic beakers (Plate 2) were subdivided with visiotubes that represent a variety of colors to facilitate rapid localization of the specimens (CryoBioSystem, 2006). The specimens were then gently placed from the bottom to the top in the canisters of the RCB1001 and Espace 661 liquid nitrogen cryoconservaters (SS3: http://getjournal.org/wp-content/uploads/2022/12/Supplementary-GJOBOH-2022-030.pdf).

Plate 1: Straws already labeled with rods and sealed ready to be stored in the visiotubes

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Plate 2: Arrangement of visiotubes containing straws inside a cryogenic beaker at the Pasteur Institute in Côte d'Ivoire

A storage sheet is used to record the information related to the traceability of the straws, i.e. their position in the canisters and the position of the canisters in the cryoconservers. This same information was recorded in limited access spreadsheets, saved on an external hard drive, and secured by the biological resources data management unit.

RESULTS

Concerning implementation, the training and accreditation of the team dedicated to the biobanking of nasopharyngeal samples was carried out at the sample management unit by the two biologists and the technician. When PACE (semi-automated system) was acquired, two representatives from CryoBioSystem installed and trained the semi-automated system. The initial decision to create aliquots until the available samples were exhausted was made during the manual technique. However, it had to be rejected after switching to the semi-automate technique due to the re-start of aliquoting cycles caused by vacuum suction in the tube bottoms.

From April to November 2020, the hand-stacking technique was used and out of a total of 178,405 specimens received at the Pasteur Institute of Côte d'Ivoire, 3,496 nasopharyngeal specimens were placed in 13,475 straws. This represents 1.9% of the samples retained compared to the samples received at Pasteur Institute of Côte d'Ivoire. The number of aliquots obtained by the manual technique ranged from one (1) to nine (9) depending on the volume available (Table 1).

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Table 1: Assessment of samples put in straws by manual technique

Number of aliquots	Number of samples	Number of straws
1	58	58
2	265	530
3	1 159	3477
4	1 626	6504
5	33	165
6	67	402
7	80	560
8	93	744
9	115	1035
Total	3 496	13475

One sample equals one or several straws (aliquots); one aliquot equals one straw

The period of January through December 2021 was marked by semi-automated pandemic specimen stratification at COVID-19. The total number of specimens received at Pasteur Institute of Côte d'Ivoire from January to December 2021 was 914,496 nasopharyngeal specimens. 15,474 of the 914,496 specimens received were stratified into 33,840 straws. This represents a rate of 1.69% of samples retained compared to samples received at Pasteur Institute of Côte d'Ivoire. The number of aliquots obtained by the semi-automated technique ranged from one (1) to five (5) due to sample loss caused by vacuum suction upon reaching the bottoms of tubes (Table 2).

Table 2: Assessment of samples put in straws by semi-automatic technique

Number of aliquots	Number of samples	Number of straws
1	5 088	5 088
2	2 715	5430
3	7 437	22 311
4	159	636
5	75	375
Total	15 474	33840

One sample equals one or several straws (aliquots); one aliquot equals one straw

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The number of straws made is obtained by the following calculation:

Number of straws made = number of samples put in straws x number of aliquots.

The average monthly straws performance obtained was 1,685 for the manual technique versus 2,820 for the semi-automatic technique. This performance is in line with the objective set during the design of the present study. Indeed, the rates of 1.9 and 1.69 obtained by the manual and semi-automatic technique correspond well to the objective of preserving one to two samples out of 100 nasopharyngeal samples collected (Table 3).

Table 3: Summary of samples put in straws by the manual and semi-automatic technique during the COVID-19 pandemic (2020/2021)

Period	Number of collected samples	Number of samples put in straws	Number of straws made	(Number of samples put in straws / Number of collected samples)*100	Monthly average performance (number of de straws / number of months)
Avril à November 2020 (manual technic)	178405	3 496	13475	1,9	1685
Janvier à December 2021 (semi-automatic technic)	914 496	15 474	33840	1,69	2820

DISCUSSION

Samples stored in straws in the specific context of biobanks have several advantages. Straws are smaller and save more space. They have a sealing system that prevents the infiltration of nitrogen into the samples (liquid nitrogen does not penetrate the sealed straws). The System of Manual Sealing (SYMS) and the semi-automated instrument (PACE) have a special impulse heat sealing device that has allowed the straws to be sealed correctly with a guaranteed seal according to the specification [10]. In addition, a wrapping of the labels onto identification rushes inserted into the short end of the straws is required regardless of the technique used. Also, these straws typically contain 0.3 ml each representing the volume of material needed for an assay as this has decreased in recent years [6].

Likewise, the hand-stacking method requires the handler, external disinfection of each straw and sterilization of the heat sealer between samples. CryoBioSystem recommends disinfection with a less volatile disinfecting agent such as hypochlorite, followed by a sterile water rinse. This made the manual work tedious. These disinfection actions reduce the risk of contamination by skin commensals or other microorganisms that could contaminate the cryopreservation tank [11].

As for the semi-automatic technique, it frees the user from the constraints related to the disinfection of the straws and instruments used. The main limitations to the use of PACE (the semi-automatic technique) are the cost and the need to purchase additional consumables including sterile CBS™ long blue injection nozzles for the aspiration of each sample and sterile CBS™ aspiration nozzles for each use of the device [9].

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The eleven color visiotubes (yellow, orange, pink, gray, red, black, fluorine green, brown, blue, light green, purple) per cup were used to arrange counterclockwise 15 straws per visiotube starting with yellow (Figure 6) at a rate of 165 straws per cup. These beakers were then arranged in fours in a bottom box inside the cryoconservaters.

The acquisition of the semi-automatic (PACE) has allowed the biobank to strengthen biosafety measures regarding sample handling. PACE was obtained thanks to funding from a partner in the COVID-19 pandemic response plan, thus reinforcing the biobank's equipment. The SYMS has become a back-up of the latter (semi-automated) for the optimization of put in straws biological samples.

Two pre-storage facilities at -80^{o}C were provided at the SMU and designed to safely store selected biological samples temporarily, ensuring that their integrity was maintained pending aliquoting followed by long-term storage in liquid nitrogen. Pre-stored samples were maintained in their original tubes and were accurately identified in a simple manner. Complete cryogenic preservation is able to store in straws biological samples because the liquid nitrogen storage system has many advantages. The liquid nitrogen system has a significantly lower maintenance cost and includes fewer mechanical and electrical components compared to freezers. This makes it less vulnerable to mechanical or electrical failure, generally reduces the need for regular upgrades, and simplifies long-term system maintenance [12]. Despite this, large banks using liquid nitrogen must be carefully planned to ensure that samples are kept in an adequate long-term storage environment and that teams work under safe conditions in the facilities [2].

Problems and Challenges to Implementation

During the implementation phase, there were some technological and logistical problems.

Manual pelletizing during the outbreak was tedious, slow, and caused some errors in volumes. Problems caused by temperature increase and noise level could also be reported. With respect to logistical problems, procurement barriers and the need for budgetary changes, among others, that could be caused by the procurement system for consumables and durable goods for cryopreservation, required additional planning. The shortage of personnel during this major epidemic was noted in the stratification team, requiring additional personnel planning.

Lessons Learned

Creating a high security cryogenic straws biocollection in a biobank from pandemic samples is not a simple task. The creation of the biocollection must be planned and approved, not only taking into account the demands of fieldwork, but also anticipating the known and unknown challenges ahead. The acquisition of a semi-automatic (PACE) of put in straws biological samples has improved the performance of the biocollection. Moreover, every small choice made at the planning stage can have serious scientific, economic, logistical and even political consequences.

In the event of a large-scale epidemic (such as COVID-19), collection in a biobank requires a full automated sequencing line to facilitate the provision of samples to requestors and to strengthen the legacy collection.

Planners must consider the costs of setting up, implementing and maintaining the facilities.

Planning for a cryogenic straws biocollection usually competes with other key study issues in the implementation phase of the project, such as the number of cases to be recruited.

Maintenance costs are often difficult to predict and have been minimized in the planning stage of the study. The choice of cryogenic technology type has a significant impact on future costs.

CONCLUSION

This study allowed the design and implementation of the conservation of nasopharyngeal samples in CBS™ High Security straws from the COVID-19 pandemic (2020-2021) in Côte d'Ivoire. It was carried out at the biological resource center (or Biobank) of the Pasteur Institute of Côte d'Ivoire. It allowed the biobanking of 47,315 straws from 18,970 samples. In addition to the technical aspects, budgetary, human resources and bureaucratic issues were taken into account to determine the success or failure of the implementation of biobanking of samples.

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AUTHORS' CONTRIBUTIONS

BJJR designed, performed the methodology and data analysis. The manuscript was written and edited by BJJR and DKM. CL, NF and KKA collected the data. DKM made the map of the sampling sites outside Abidjan. All authors contributed to the article and approved the submitted version.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal

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relationships that might appear to influence the work reported in this article.

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