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Resin Straws: A System to Safely Store Nasopharyngeal Specimens Collected During the COVID-19 Pandemic in Ivory Coast

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Abstract **Original Research Article**

Background: Packaging in 0.5ml cryotubes and 0.3ml High Security straws was used to store more nasopharyngeal samples from Covid-19 pandemic surveillance in Ivory Coast. Previous studies have reported that there would not be, under experimental conditions, a significant difference in the survival rate values (per freeze-thaw cycle) of samples between cryotubes and High Security straws. However, storage in resin straws or High Security straws leads to a slight increase in the survival of microorganisms compared to cryotubes (Thammavongs *et al.*, 2004). ***Methods:*** Aliquoting is use like method to put nasopharyngeal specimens in cryotubes 0.5ml and in high security straws 0.3ml. ***Results:*** from April 2020 to December 2021, out of a total of 1,092,901 nasopharyngeal samples received at Pasteur Institute of Ivory Coast, 18.97 samples were placed in High Security Straws 0.3ml versus 9,441 in cryotubes 0.5ml. The results indicate that there are more nasopharyngeal samples put in the 0.3ml High Security Straws than in the 0.5ml cryotubes. ***Conclusion:*** Two of the conditions that could justify the safe cryopreservation of microorganisms contained in samples collected during a pandemic are the following: a secure closure by hermetic welding and a gain in terms of space for high-security straws compared to cryotubes.

Keywords: Cryopreservation, High Security Straws, COVID-19 pandemic, Cryotubes, Ivory Coast.

1. INTRODUCTION

Biobanks are physical spaces whose role is to guarantee the quality of the procedures for sampling, transport, processing, storage and provision of biological samples in compliance with the rules of good practice and regulations(Müller *et al.*, 2020). According to ISO 20387(*ISO - ISO 20387:2018*) relative to the general requirements for biobanks, processing refers to one of the activities important in preserving the integrity and value of the sample over time. The treatment or preparation includes all the manipulation operations of the biological material: extraction, multiplication, purification, aliquoting, allotment, packaging (*NFS 96-900 version 2011*). Aliquoting is a technique for dividing the initial volume of a sample into several series of tubes containing the same volume. This division of the sample into several secondary tubes increases the number of potential users and avoids contamination of the initial sample as well as repeated cycles of freezing and thawing.(Malm *et al.*, 2012)**.**This number would be higher when packaged in high security straws or resin straws than in cryotubes.

The high security straws and cryovials are the secondary tubes used as containers for stem samples intended for cryopreservation after their aliquoting, that is to say their division into small equal volumes.

Ionomer resin straws are currently used for the cryopreservation of biological materials such as bull semen(Thun *et al.*, 2002) and mammalian embryos (Chen *et al.*, 2013). Their long-term storage capacity of various types of eukaryotic cells has been widely explored and confirmed. Recently, a mouse blastocyst vitrification experiment using either conventional glass micropipettes or resin ionomer straws showed no significant difference in recovery rates after thawing(Rienzi *et al.*, 2016).

To our knowledge, very little is known about the cryopreservation of microorganisms see stem samples in ionomer resin straws (Guo *et al.*, 2020).

Since 2005, the Pasteur Institute of Ivory Coast has been committed to a standardized approach to the conservation of living organisms. This idea was born

following the express request of the WHO (World Health Organization) to confine the Polioviruses and numerous Biocollections: (Human Immunodeficiency Virus, Yellow Fever, Flu, etc.). Thus, Ivory Coast and the Pasteur Institute of Ivory Coast decided to create in 2009, a biobank to international standards. This biobank also called Center for Biological Resources has been hosting since April 15, 2018 the regional biobank of the member countries of ECOWAS (Economic Community of West African States). The collection, processing, storage and provision of biological samples are practices carried out in the broader context of organizations called biobanks (Vaught & Henderson, 2011). The Institute Pasteur of Ivory Coast in its mission of monitoring and supporting public health is participating in the response to the Covid-19 pandemic (coronavirus disease 2019) in Ivory Coast. It is involved in the diagnosis by RT-PCR (Reverse Transcriptase-polymerase chain reaction) of SARS COV2 (Coronavirus from severe acute respiratory syndrome 2). Through the Center for Biological Resources, it stores a priori the nasopharyngeal samples from the Covid-19 pandemic and the associated data.

One of the main concerns of the team in charge of the conservation of biological resources during the Covid-19 pandemic was how to build a collection of a larger number of samples that would occupy less space.

Several authors from developed countries have reported the collection of samples from epidemics in high security straws(Bingham & Riboli, 2004). Unfortunately, these collection data are not collected in African countries, particularly West African countries including Ivory Coast.(Andreasson *et al.*, 2013)

This study report describes the packaging in straws and cryotubes of nasopharyngeal samples collected during Covid-19 pandemic surveillance in the Republic of Ivory Coast. The main objective is to put in the long-term cryobanking of the maximum aliquots obtained while saving available storage capacity.

2. MATERIAL AND METHOD

2.1. Setting and Type of Study

This is a descriptive study carried out at the Biological Resource Center or Biobank of the Pasteur Institute of Ivory Coast located on the Adiopodoumé site during the Covid-19 pandemic. The Biological Resource Center has a cryobiology room with an eventual capacity of 44 cryoconservatives with cryotubes and straws (Table I). The cryotube cryoconservatives (RCB 660 L and RCB 1000L) contain 44 racks and 40 racks respectively. This is equivalent to a storage capacity of 52800 cryotubes (44 racks of 12 cryoboxes of 100 cryotubes each) and 48000 cryotubes (40 racks of 12 cryoboxes of 100 cryotubes each). As for the straw cryoconservatives (RCB 660 L and RCB 1000L), they 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 216 straws each). These cryoconservatives with cryotubes or straws are particularly suitable for storing large quantities of biological samples for extended periods of time. Thus, these large reservoirs offer enough space for a large number of samples while the narrow opening reduces nitrogen consumption.

Table I: Advantage of storage in straws compared to storage in cryotubes

Type of Cryoconserver and Capacity	Number of Rack/Canister	Number of Cryobox/Cryogenic Cup Per Rack/Canister	Number of Cryotube/Straw Per Cryobox100/Cryogenic Cup	Total
With Cryotubes (660l)	40	12	100	48,000
With Cryotubes (1000l)	44	12	100	52,800
With Straws (660l)	165	4	165	108,900
With Straws (1000l)	216	4	165	142,560

2.2. Material

The 0.5ml cryotubes and the 0.3ml high security straws (Figure 1) were supplied respectively by Thermo Fisher Scientific (MA, USA) and Cryo Bio System (ZI n°1 Est, 61300 L'Aigle, France). Sterile filter tips 200µl, 1000µl and micropipette 200µl-1000µl were supplied by EppendorfT^M (Germany). The SYMS (system of manual sealing) and the PACE were purchased from Cryo Bio System (ZI n°1 Est, 61300 L'Aigle, France). For the labeling and recording of the aliquots, a Brady device (ZI n°1 Est, 61300 L'Aigle, France) and an Excel file were used throughout the study. The cryoconservatives with cryotubes or straws (RCB 660 L and RCB 1000L) were supplied by Air Liquide Ivory Coast (3 Boulevard de Marseille, Abidjan).

Figure 1: High security straws (0.3ml capacity with weighted ID rod 30mm)

2.3. Methods

Aliquoting in Cryotubes

This method makes it possible to start again in cryotubes containing 0.5ml each of the raw sample or strain. The latter is a nasopharyngeal sample contained in a preservation solution (Viral Transport Medium). Using a micropipette connected to a 1ml cone, 0.5ml of each raw sample is withdrawn from the primary tube and transferred into 0.5ml cryovials identified with a Brady label. Cryotubes are used as much as necessary so as not to throw away the core sample. The aliquots obtained are stored in cryoboxes of 100 wells because of 100 cryotubes per cryobox. The cryoboxes are stored in racks with 12 cryoboxes per rack and cryopreserved at -196°C.

Aliquoting in Straws

This method is carried out using two techniques: manual and semi-automatic. Aliquoting in straws by manual technique begins with the insertion of a filling tip with aseptic precautions at the free end of a straw. The capped end is then connected to the suction system using a 1ml insulin syringe, and the liquid sample is pumped upwards. As soon as the sample reaches the cotton plug, the straw is disconnected from the suction system. The filling tip is removed from the straw and the free end is sealed with the SYMS (System Manual Sealing) machine. A colored identification rod (or identification ring) with a label is inserted at the inside the straw in the compartment located behind the cap before sealing. The operation is repeated until the last fraction of the sample is put in straw (that is to say until the primary tube is empty).

As for using the semi-automatic technique to put in straw, this is done using the "PACE" (Cryo Bio System, ZI n°1 Est, 61300 L'Aigle). It is a semi-automatic system for packaging biological samples in High Security straws. From a raw sample primary tube, PACE fills the free end of the High Security straws previously identified by a colored identification rod (or identification ring) with a label inserted inside the compartment located behind cap. It follows an automatic welding of the two ends. The PACE includes a filling and sealing unit as well as a control box. Unlike the manual technique, PACE requires the use of additional consumables including the use of a sterile CBS^{TM} long blue injection nozzle for the aspiration of each sample and a sterile CBS^{TM} aspiration nozzle for each use of the device.

The high security straws obtained are placed in colored compartments (called visiotubes) which are available in several colors within the cryogenic cups. A cup contains 11 visiotubes and 15 straws per visiotube (Figure 2). This makes a total of 165 straws per cup.

The aliquots contained in the visiotubes of the cups are stored in canisters due to 4 cryogenic cups per canister and cryopreserved at -196°C.

Figure 2: Arrangement of the straws in the visiotubes within a cryotube beaker

Statistical Analysis

Data entry and analysis were performed using Excel software.

3. RESULTS

From April to November 2020, the number of samples received at the Pasteur Institute of Ivory Coast was 178,405. During this period, a total of 3,496 nasopharyngeal samples were placed in 13,476 straws by manual technique and 2,668 were divided into 26,680 cryotubes manually (Table III).

The period January to December 2021, the number of samples received at the Pasteur Institute of Ivory Coast was 914, 496. During this period, 15,474 nasopharyngeal samples were placed in 33,840 straws by semi-automatic technique and 6,773 were divided into 67,730 cryotubes manually (Table II).

The monthly performance of the number of cryotubes produced compared to that of straws produced by manual technique is estimated at 334 against 1685 from April to November 2020. As for the monthly performance of the number of cryotubes produced compared to that of the number of straws carried out by semi-automatic technique, it is estimated at 565 against 2820 from January to December 2021(Table III).

Table II: results of samples placed in cryotubes and straws during the Covid 19 pandemic (period April 2020 to December 2021)

Period	Number of collected samples	Number of samples put in straws	Number of straws made	Number of samples put in cryotubes	Number of cryotubes made
April to November 2020	178,405	3,496	13,476	2,668	26,680
January to December 2021	914,496	15,474	33,840	6,773	67,730

Table III: Performance of the number of cryotubes produced per month VS that of straws during the Covid-19 pandemic (period April 2020 to December 2021)

Period	Number of collected samples	Number of samples put in straws	(Number of samples put in straws / Number of collected samples)*100	Monthly average performance (number of straws / number of months)	Number of samples put in cryotubes	(Number of samples put in cryotubes / Number of collected samples)*100	Monthly average performance (number of cryotubes / number of months)
April to November 2020	178,405	3,496	1.9	1,685	2,668	1.49	334
January to December 2021	914,496	15,474	1.69	2,820	6,773	0.74	565

4. DISCUSSION

This study experimented with the benefits of long-term banking of nasopharyngeal specimens collected during Covid-19 pandemic surveillance in the Republic of Ivory Coast while saving available storage capacity. The samples thus stored in straws in the specific context of biobanks have several advantages over those stored in cryovials. Straws are smaller and save more space than cryovials. They have a sealing system which prevents the infiltration of nitrogen into the samples (the liquid nitrogen penetrates through the screws of the cryotube, but not through the sealed straws).

Cryotubes are bottles made of polypropylene with polypropylene or polyethylene screw caps. Many questions arise regarding their integrity during cryonics(Adiga *et al.*, 2020),(Mortimer, 2004), even for cryovials with internal thread which, in conjunction with the silicone gasket, provides the best possible seal. It has been reported that despite strict laboratory technique when filling and closing cryotubes, 45% of Nunc cryotubes without an O-ring (Nalge Nunc product no.340711) and 85% of Iwaki cryotubes with an O-ring (Asahi Techno Glass Corporation Scitech Division, Tokyo, Japan) leaked up to 1 ml of liquid nitrogen(Mortimer, 2004).

Mortimer(Mortimer, 2004) summarized the arguments for and against secondary containment as follows. When cryotubes are immersed in liquid nitrogen, the air inside contracts as it cools, causing a reduction in pressure that will draw liquid nitrogen into the air space if the seal is faulty. During thawing, liquid nitrogen quickly turns into gaseous nitrogen whose volume is multiplied by 700, which can cause the explosion of the cryotube. A leaking seal is a breach of biological containment between the specimen and the liquid nitrogen inside the cryopreserver.

All straws are made from a chemically inert, biocompatible resin ionomer and have a number of important advantages. They have physical characteristics of resistance to ultra-low temperatures and pressures created by expanding liquids and liquid nitrogen. The straws are heat sealable using a special heat-sealing device (the SYMS sealer). Properly sealed CBS^{TM} straws are guaranteed absolutely leak-proof at pressures up to 150 kg/cm-2. They are mechanically strong and unbreakable even at -196°C and can be subjected to significant bending even when frozen. They are also resistant to bacteria, viruses and have special filling nozzles so that none of the materials loaded into the straw come into contact with the outside of the straw (Santiago-Moreno *et al.*, 2011). They are then placed in a secondary packaging called a visiotube, the color coding of which facilitates the identification of the straws when they are recovered from the cryogenic cups.

Before proceeding with the cryopreservation of straws or cryotubes in their secondary packaging, disinfection actions make it possible to reduce the risk of contamination by skin commensals or other microorganisms likely to contaminate the cryopreservation tank. During thawing, the exterior of all containers may be contaminated with organisms present in liquid nitrogen, even if vapor storage has been used(Bajerski *et al.*, 2021). Disinfection of specimen exteriors after thawing should be common to all cryobank standard operating procedures (“SOPs”). Straws typically hold 0.3ml, compared to cryovials which typically hold 2.0ml or even 0.5ml. As the volume of material needed for an assay has decreased in recent years, it is better to store six straws of 0.3 ml each than one cryovial of 2.0 ml(Pereira *et al.*, 2013)or 4 cryotubes of 0.5ml.It should be specified that the0.5 ml capacity cryotubes occupy the same space as those of 2ml in a cryobox.

5. CONCLUSION

This study was carried out in order to keep as many nasopharyngeal samples as possible from the Covid-19 pandemic (2020 -2021) in Ivory Coast, precisely the biological resource center (Biobank) of the Institute Pasteur of Ivory Coast. The results indicated the advantages of aliquoting samples in 0.3ml straws over aliquoting samples in 0.5ml cryotubes. The collection of biological resources from the pandemic to Covid-19 placed in cryotubes or straws is subdivided into a heritage collection and an open collection. The open collection will be evaluated in the long term to verify the impact of cryopreservation in accordance with the requirements of ISO 20387. It will also be made

available to interested parties for research, training, technical validation and other purposes.

DECLARATION OF COMPETING INTERESTS

The authors declare that they have no known competing financial interests or personal relationships which might appear to influence the work reported in this article.

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