Annals of Biomedical Engineering (© 2022)
https://doi.org/10.1007/s10439-022-03057-1

Original Article

Validation of 3D-Printed Swabs for Sampling in SARS-CoV-2 Detection: A Pilot Study

Verónica Bolaños-Suaréz,[1] Alma Villalobos-Osnaya,[1] José Antonio García-García,[2] Alma De León-Hernández,[3] Celia Sánchez-Pérez,[3] and Ana María Espinosa-García[1]

[1]Hospital General de México, "Dr. Eduardo Liceaga", Servicio de Farmacología Clínica, 06720 Ciudad de Mexico, Mexico; [2]Hospital General de México, "Dr. Eduardo Liceaga", Dirección de Educación y Capacitación en Salud, 06720 Ciudad de Mexico, Mexico; and [3]Instituto de Ciencias Aplicadas y Tecnología (ICAT), Universidad Nacional Autónoma de México (UNAM), AP 70-186, 04510 Ciudad de Mexico, Mexico

(Received 13 December 2021; accepted 15 August 2022)

Associate Editor Stefan M. Duma oversaw the review of this article.

Abstract—In this pilot study, we characterize and evaluate 3D-printed swabs for the collection of nasopharyngeal and oropharyngeal secretion samples for the SARS-CoV-2 detection. Swabs are made with the fused deposition modeling technique using the biopolymer polylactic acid (PLA) which is a medical-grade, biodegradable and low-cost material. We evaluated six swabs with mechanical tests in a laboratory and in an Adult Human Simulator performed by healthcare professionals. We proved the adequacy of the PLA swab to be used in the gold standard reverse transcriptase-polymerase chain reaction (qRT-PCR) for viral RNA detection. Then, we did *in vitro* validation for cell collection using the 3D-printed swabs and RNA extraction for samples from 10 healthy volunteers. The 3D-printed swabs showed good flexibility and maneuverability for sampling and at the same time robustness to pass into the posterior nasopharynx. The PLA did not interfere with the RNA extraction process and qRT-PCR test. When we evaluated the expression of the reference gene (RNase P) used in the SARS-CoV-2 detection, the 3D-printed swabs showed good reproducibility in the threshold cycle values (Ct = 23.5, range 19–26) that is comparable to control swabs (Ct = 24.7, range 20.8–32.6) with p value = 0.47. The 3D-printed swabs demonstrated to be a reliable, and an economical alternative for mass use in the detection of SARS-CoV-2.

Keywords—COVID-19, SARS-CoV-2, 3D-printed swabs, Polylactic acid (PLA).

Address correspondence to Ana María Espinosa-García, Hospital General de México, "Dr. Eduardo Liceaga", Servicio de Farmacología Clínica, 06720 Ciudad de Mexico, Mexico. Electronic mail: anaesga@hotmail.com

INTRODUCTION

The World Health Organization (WHO) declared COVID-19 a pandemic on March 11, 2020. In August 2021, there had been nearly 505 million confirmed cases worldwide and nearly 6.2 million deaths[13] related to this disease. Since then, the growing number of cases made necessary to increase the number of tests by swab technique, for diagnosis, follow-up, and control of cases. These triggered shortages of basic inputs to perform these tests, being the case of swabs. One of the most affected continents in the world was America, with the United States topping the list of cases and deaths, followed by Brazil and Mexico.[6] The greatest health and economic impact were in Latin America, due to the low development of health systems, the lack of materials to attend the pandemic, as well as comorbidities in the population. This had a greater impact in countries with commercial dependence or without the infrastructure installed to produce inputs for health care in order to supply their local markets.[10,24]

Researchers' efforts are focused on understanding the biology of the virus, as well as developing vaccines and drugs against COVID-19 that help to appease this pandemic,[18,26] but there is also a need for an adequate control, monitoring, and diagnosis of new cases.[21] Having nasopharyngeal (NP) and oropharyngeal (OP) swabs available for use in gold-standard qRT-PCR tests will help in the diagnosis of infected people, isolating them and minimizing person-to-person infection, thus mitigating the COVID-19 pandemic.[8,17]

Published online: 12 September 2022

Effective diagnostic tests are essential to control the SARS-CoV-2 coronavirus pandemic. The rapid spread of this coronavirus has led to a severe shortage of NP and OP swabs, due to a fast and sudden increase in detection tests that could not be fulfilled by the industry.[28] The shortage of swabs induced, in several health care centers, the use of other types of swabs for the collection of nasal epithelial cells; that in some cases were not suitable for rubbing the nasopharynx and could inhibit the qRT-PCR test.[9]

In response to this bottleneck, different groups worldwide proposed using 3D-printed swabs, representing a new way of production, at a low cost. In Mexico, the use of this 3D technology was already applied to the development of health care supplies with an expanding development for research and commercial purposes. The production of swabs using 3D-printing technology meets this basic need for the detection of SARS-CoV-2 due to its simplicity, capability for rapid prototyping and availability of several types of materials among which, there are biodegradable materials that meet clinical standards for the manufacturing of swabs. Another advantage is the feasibility of moving from small production to an industrial scale for large-scale volumes.[7,25] The fused deposition modeling (FMD) is the simplest and most popular technique in 3D-printing because it does not require postprocessing and has low-cost printers and materials. However, most of the 3D-printed swabs proposals have been done using stereolithography (SLA) which requires washing and curing cycles after printing, and special attention should be paid when the materials are exposed to direct light radiation.

The objective of this pilot study was the characterization and analysis of swabs manufactured by 3D-printing with the biopolymer polylactic acid (PLA), through functional and biological validation for the SARS-CoV-2 detection by the qRT-PCR test. The criteria for the selection considered flexibility, robustness, breaking point, comfort, the non-interference for isolation of the nucleic acid, the number of cells collected (comparing with swabs used in hospitals as a control), and the acceptance of volunteers and medical staff members.

MATERIALS AND METHODS

3D-Printed Swab Design

We designed in an iterative process, according to recommendations from health care staff, several prototypes of 3D-printed swabs using the software SolidWorks CAD 3D 2020 (Dassault Systèmes, France). We selected six prototypes for further testing in this pilot study, these were fabricated by a Flash Forge Creator Pro printer, using polylactic acid (PLA) filament of 1.5 mm.[20]

Sterilization

The 3D-printed swabs were then washed, sterilized with Ethylene Oxide (EO), and individually packaged in sealed bags before testing.[1]

Mechanical Testing

Mechanical testing for the 3D-printed and control swabs includes flexibility, breaking point, adaptability, and robustness for the sampling maneuver for three different batches. We tested the flexibility of the swabs' neck without breaking, first around a cylinder of 3.0 cm diameter, then with a 180° bend, and finally making a complete twist keeping this position for a minute, and then test the correct recovery. These tests were repeated for ten swabs of every prototype coming from each batch ($N = 30$).

For the breaking point, we verified that the 3D-printed swabs easily snapped into a vial by placing them inside a vial and twisted the shaft with one hand against the edge proving that they break off correctly into two pieces. We performed ten attempts for every prototype from each batch ($N = 30$).

Finally, we validated the adaptability and stability of the swabs to easily pass into the posterior nasopharynx and oropharynx in a human adult simulator (Nasco Health Care Inc. Mod. 101-088FUALS. NY). Once in the interior of the OP and NP cavity, we also tested the sample collection maneuver by moving them in a circular motion in the interior for one minute verifying their integrity. This was consecutively repeated three times for each swab until completing ten tests for every prototype from each batch ($N = 30$). This test was done by the medical health care staff members trained to perform the specimen collection for the SARS-CoV-2 detection.

Collection for In Vitro Test

The 3D-printed and controls swabs were introduced for 30 s with continuous shaking in a suspension of lymphocytes isolated from peripheral blood using the ACK lysing buffer (NH_4Cl 150 mM, $KHCO_3$ 10 mM, Na_2EDTA 0.1 mM) and subsequently placed in a viral transport medium. The number of cells collected by each swab was determined using a hemocytometer and an automated cell counter instrument (Countess™ II Automated Cell Counter, Thermo Fisher Scientific, Waltham, MA) to validate the quantification. This was done repetitively until completing ten tries for each prototype. We repeated this for the three batches ($N = 30$).

Collection Test in Volunteers

A total of 10 presumptive COVID-negative volunteers were chosen for testing based on the Clinical Evaluation of New Diagnostics recommendations, emitted from the Instituto Nacional de Diagnóstico y Referencia Epidemiológica (INDRE) from the Mexican Health Ministry under a protocol approved by the committee for ethical, biosecurity, and research of the Hospital General de Mexico (HGM) (DI/20/501/04/52). The inclusion criteria were for adults over 18 years old which underwent a verbal consent process and were questioned about technical aspects of the swabs. All samples were processed in a biosafety class II laboratory under Good Manufacturing Practices (GMP) standards.

Volunteers Sample Collection

Sample collection in volunteers was performed by trained physicians to ensure a high-quality sample. The control swab was first inserted into either the right or left nostril (based on medical and volunteers' preference) until the posterior nasopharynx wall was reached. Then, the swab was gently rotated for some seconds and withdrawn. This process was used for both 3D-printed swabs and control swabs. In a subsequent test, the choice of the nostril was random. Control samples were collected with the nylon swabs type Copan®. The swabs with the sample were inserted into a universal viral transport medium (Virseen, VCS-1031). After collection, the samples were promptly delivered to the laboratory and if not immediately processed *via* RT-PCR, they were stored for less than 48 h at 4 °C until processing.

Adverse Event Reporting and Acceptability Questionnaire

Immediately after the swabs were used, each participant reported the pain score, as well as any adverse events. An acceptability questionnaire was then delivered recording the participant's perception of the comfort of the 3D-printed and control swabs, as well as acceptability for future testing.

RNA Isolation and Quantification

RNA was extracted from each sample using the Quick-RNA™ Viral Kit (Zymo Research) according to the manufacturer's protocol. RNA obtained was quantified in the DS-11 FX + spectrophotometer (Denovix) three times. RNA was stored at − 80 °C until use. A method of isolation of nucleic acids by TRIzol™ reagent (Invitrogen, Carlsbad CA) was also used following to manufacturer's instructions. The integrity of the RNA extracted by TRIzol™ was evaluated by agarose gel electrophoresis.

Real-Time Quantitative Retrotranscription PCR (qRT-PCR)

To remove possible genomic DNA residues, RNA samples were treated with QuantiNova gDNA Removal Mix (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. After the removal of genomic DNA, 20 ng of RNA were used for cDNA synthesis using the QuantiNova™ Reverse Transcription Kit (Qiagen GmbH) according to the manufacturer's protocol. Samples were stored at − 20 °C until analysis.

The amount of mRNA of RNAse P gen was measured by qRT-PCR using the Syber green method (iQ™ SYBR Green Supermix, BIO-RAD). The following pairs of forward and reverse primers were used for RNAse P: *5′AGATTTGGACCTGCGAGCG-3′* and *5′-GAGCGGCTGTCTCCACAAGT-3′*. The primers were synthesized in the Institute Biotechnology (IBT), UNAM. qRT-PCR analyses were performed using a CFX96 Touch Deep Well (BIO-RAD) and the reactions were subjected to the following protocol: 95 °C for 3 min; 40 cycles at 95 °C for 15 s, annealing at 58 °C for 40 s, and elongation at 72 °C for 40 s. The melting curve was obtained to determine the specificity of the reactions. In each run, we included reactions without a template as a control. Transcript relative values (ng/μL) were obtained by interpolating fluorescent values with standard calibration curves constructed with tenfold serial dilutions (500–0.05 ng/μL) from a single pool of lymphocytes cDNAs obtained from volunteers. Any sample with an exponential fluorescent curve and threshold cycle (Ct) value less than 40 were considered. The experiments were run three times.[16]

Data Analysis

All statistical analyses were performed using GraphPad Prism (GraphPad Software, La Jolla, California). Cell counting, RNA quantification, and data qRT-PCR were compared using *Student's t test* or *one-way analysis of variance (ANOVA)* as required. χ^2 test 2 × 2 was used to compare frequencies. p value significance was set at ≤ 0.05.

RESULTS

3D-Printing

We tested 6 prototypes of the 3D-printed swabs with a cylinder-shaped collection head (CH), generated by a

hollow revolutionized profile. They all are 170 mm long with the breakpoint placed at 97 mm from the swab tip for the collection head. Swab printing consumes 0.41 m of PLA (filament of 1.5 mm) using a layer height of 0.12 mm giving a printing time of 14 min. The CH surface of the prototypes had a smooth finish, by controlling the printing parameters. The sixth 3D-printed swabs had its CH in piriform shape, which was like that of a classical swab. The CHs of the 6 prototypes had lateral and upper holes to facilitate the storage of the sample inside it and were manufactured with different dimensions in length and width, to evaluate their performance and their suitability for sample collection in the oropharynx and nasopharynx (Fig. 1).

The shaft of the 3D-printed swab was the same in the 6 prototypes with a triangular shape to facilitate maneuverability and a narrowed area at 97 mm from the CH for the breaking point, to facilitate the cut without requiring any instrument once it is inserted into the corresponding vial (Figs. 1a and 1b).

Physical Tests

To evaluate the mechanical performance of the 3D swabs, an analysis was performed using adult human simulators, thus emulating the sampling through the nasopharyngeal and oropharyngeal routes. This consisted of introducing and turning the swab, and evaluating the ease of use. Tests were performed for each prototype, in both the OP and NP cavity consecutively (Figs. 1c and 1d).

In sampling, it was important that the 3D-printed swabs bend to get through the NP and OP cavity without breaking, thus flexibility and breaking point tests were performed (Figs. 1e–1g). We labeled the 3D-printed swabs from 1 to 6 and the control swabs for the OP and NP cavities, as 7 and 8 respectively (Fig. 1b). The six prototypes of the 3D-printed swabs resisted bending and broke adequately at the breaking point therefore they were stable for use. The tests for the adult simulators were performed in 10 swabs for each prototype three times for different batches. The best maneuverability for the NP cavity was obtained by the swab 3 and for the OP cavity was by the swab 4 (Table 1).

Functional Tests

To evaluate the cell collection for the six 3D-printed swabs, a cell suspension of lymphocytes was used. Results plotted in Fig. 2a show that there were no significant differences in the cell collection among the prototypes. However, we found that, *in vitro*, control swabs collected more cells compared with 3D-printed swabs. The highest cell collection was done by the swab 7 that doubled that of the NP control swab 8.

The extraction of RNA from lymphocytes was done using a commercial kit (see "Materials and Methods") with a volume of 100 L for the initial extraction. The amount of RNA extracted with the prototypes did not show a significant difference between the 6 models, being almost the same as for the control NP swab (Fig. 2b). Another way for the extraction that is easier for reproduction in any laboratory due to its simplicity is the extraction with TRIzol™. In this case, the results showed differences in the concentrations, proportional to the dimensions of the head, resulting in a greater amount of RNA extracted with the control OP swab (data are not shown). An interesting way to evaluate the integrity of the biological material obtained (RNA) with the 3D-printed swabs was through TRIzol™. When we analyzed the RNA isolated from lymphocytes, we observed that the quality of the RNA extracted from the cells taken with our swabs was like that obtained with the control swabs (Fig. 2c). This indicates that 3D-printed swabs do not degrade and are compatible to obtain RNA.

With these results, we determined that the 6 prototypes had no significant differences in obtaining RNA, so the selection of the swabs was mainly based on the mechanical tests, selecting prototypes 3 and 4, as candidates to be tested in the next step with the volunteers.

We implemented in a group of healthy volunteers the sampling of epithelial cells in the OP and NP cavity with prototypes and control swabs. Before their use, the 3D-printed swabs in OP and NP models were certified by the Research and Technical Assistance Laboratory of the National Polytechnic Institute (Mexico City), to ensure their sterility and the absence of skin reactivity. The sampling was carried out by trained physicians from the HGM, and in addition, to know the perception of the participants about the use of 3D-printed swabs, at the end of the procedure an evaluation questionnaire was done (Table 2). The results indicate that swab 3 was more comfortable, and they were easily introduced with minimum or no pain. However, 2 volunteers referenced a burning sensation with 3D-printed swabs. The perception of the participants concerning for to the OP prototype was that it was similar to the control. In addition, a questionnaire was also carried out for the physicians, who performed the sampling (Table 2), in which packaging, design, and use were evaluated. They mentioned that the identification was better on the 3D-printed swabs as well as maneuverability, and the facility of the breaking point which was absent in control swabs. In general, the opinion of the experts in the sampling was for the use of 3D-printed swabs and that the processing of samples in the laboratory was conducted with less runoff, and better aliquot intake to perform the RNA extraction and the qRT-PCR test.

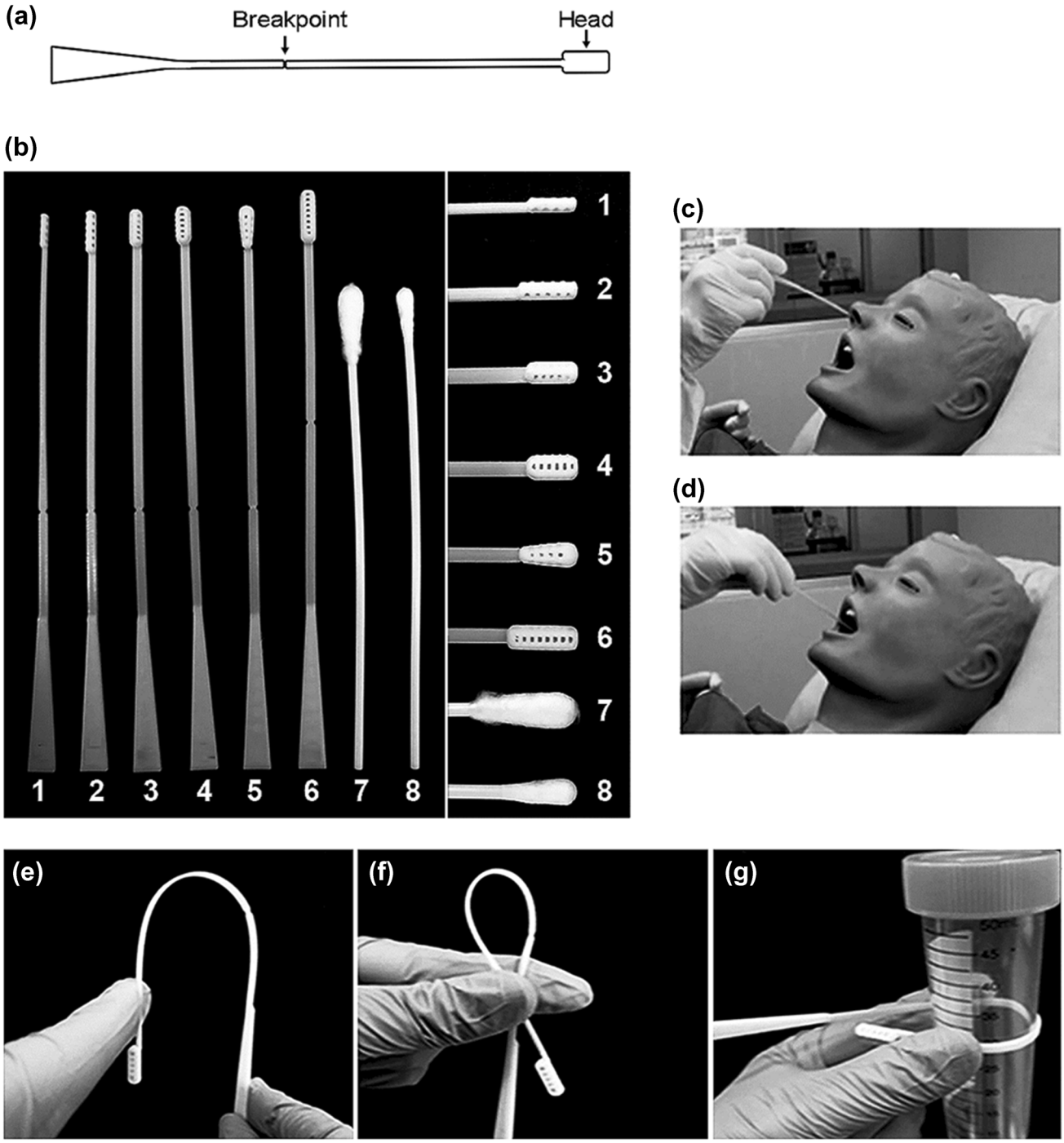

FIGURE 1. Design of 3D-printed swabs and physical tests. (a) Schematic of the 3D-printed swabs. (b) Six prototypes of 3D-printed (1–6) and control (7–8) swabs including a zoom for the head of the swab. (c and d) Repeatedly sampling maneuver for the insertion and extraction of the 3D-printed and control swabs, from top to bottom, in the nasopharynx and the oropharynx for the Adult Human Simulator. (e–g) Flexibility test for the 3D-printed swabs, from left to the right, for the head, the neck, and the breaking point.

The RNA collected from the volunteers was extracted with the commercial kit (see Fig. 2d). We must point out that the concentrations of RNA obtained with the 3D-printed swabs were higher than the control swabs except for volunteers 2 and 10. The purification with the TRIzol™ method presented the same behavior (data are not shown).

We performed qRT-PCR testing to obtain the expression profiles of the RNAse P gene for each isolated sample (Fig. 3). For this purpose, a standard

TABLE 1. Mechanical testing for 3D-printed prototypes[a].

Swabs		Flexibility[b]	Breaking point[c]	Adaptability NPcavity[d]	Adaptability OPcavity[e]	Stability NPcavity[f]	Stability OPcavity[g]
3D-printed swabs	1	Yes	Approved	Inadequate	Inadequate	Unstable	Unstable
	2	Yes	Approved	Inadequate	Inadequate	Unstable	Unstable
	3	Yes	Approved	Adequate	Inadequate	Stable	Unstable
	4	Yes	Approved	Adequate	Adequate	Stable	Stable
	5	Yes	Approved	Adequate	Adequate	Stable	Unstable
	6	Yes	Approved	Inadequate	Adequate	Stable	Stable
Control swabs	7	Yes	Not approved	Inadequate	Adequate	Stable	Stable
	8	Yes	Not approved	Adequate	Adequate	Stable	Stable

NP nasopharynx, *OP* oropharynx.
[a]Swabs were tested by triplicate. This test was repeatedly done for ten swabs of each prototype coming from each batch (N=30).
[b]Flexion of the swabs around a cylinder of 3.0 cm diameter for 1 min without breaking.
[c]Swabs were tested to snap at the breakpoint when placed into a vial.
[d&e]Adaptability to pass into the posterior nasopharynx and oropharynx respectively without deviation to another anatomical part in the adult human simulator.
[f&g]Stability to replicate insertion and retraction into the nasopharynx and oropharynx respectively in the adult human simulator.

curve of 5 orders of magnitude (known concentrations of RNA) was constructed. The 3D-printed swabs had a mean Ct = 23.5 (range of values 19.6–26) (Figs. 3a and 3c) and the mean of transcript relative values was 57.3 ng/μL (Table 3); the mean Ct values obtained for control swabs were Ct = 24.7(range of values: 20.8–32.6) (Figs. 3b and 3c) and the mean transcript relative values was also 57.3 ng/μL (Table 3); both measurements indicate acceptable values for the detection of the reference gene that are values comparable with those commonly obtained in the SARS-CoV-2 detection. We point out that in some of the samples taken with control swabs it was not possible to obtain the amplification of the gene (Table 3). The amplification graphs of RNAse P are sigmoidal for all swabs and, it is relevant to mention that the dissociation curves show the presence of a single amplicon, indicating that the PLA material does not inhibit nor interfere with the qRT-PCR test (Figs. 3a and 3b).

To rule out the possibility that the RNA could be degraded in contact with the PLA when the samples were not processed immediately. We did the extraction after 1, 24, and 48 h after collection,[23] according to the guidelines from WHO. The amplification of the gene RNAse P was carried out by the qRT-PCR and the results (Fig. 3d) indicated that there was no significant difference in the Ct values for 1 h (Ct = 24.3±1.8), 24 h (Ct = 24.2±0.4) and 48 h (Ct = 23.5±0.9); $p > 0.5$.

DISCUSSION

In this pilot study, we proposed a methodology for the validation and use of 3D-printed swabs for NP and OP sampling, The use of this technology allowed to generate, comfortable, and effective designs for sampling with an adequate collection for the diagnosis of COVID-19. The possibility of implementing swabs using 3D technology in Mexico arose from the need for local production and rapid distribution of this supply. The current industrial capacity is insufficient to cover the needs in most places where swabs are required as well as other inputs to combat the COVID-19 pandemic.[14,30]

This pandemic created a burden on health systems around the world and disrupted supply chains needed to fight COVID-19. 3D-printing has an increasingly important role in the biomedical area, enabling interdisciplinary work to achieve a positive impact on patient care with an emphasis on the real needs during and after the pandemic.[4] The production of 3D-printed swabs with PLA takes advantage of the use of printers and materials that can be implemented by any country, at a low cost.[19]

Diagnosis and monitoring of recovery of patients from the virus requires continuous testing that include adequate sampling with swabs being an indispensable input for this arduous task. The sampling of patients with suspected COVID-19 is usually performed by NP and OP route.[27] All outcomes are important positive, or negative, for relevance and clinical involvement. A false-negative result could result in contagion, so accuracy in diagnosis is imperative and the number of false-positive results must be minimized.[12]

This article proposes a methodology to determine the functionality of the manufactured swabs, easy-to-implement in the laboratory making 3D-printed swabs an alternative that has the potential to generate a simple, and efficient manufacturing process to have potential lower production costs (< 0.07 USD in our study). 3D-printing technology involves different materials, which allows for different applications,

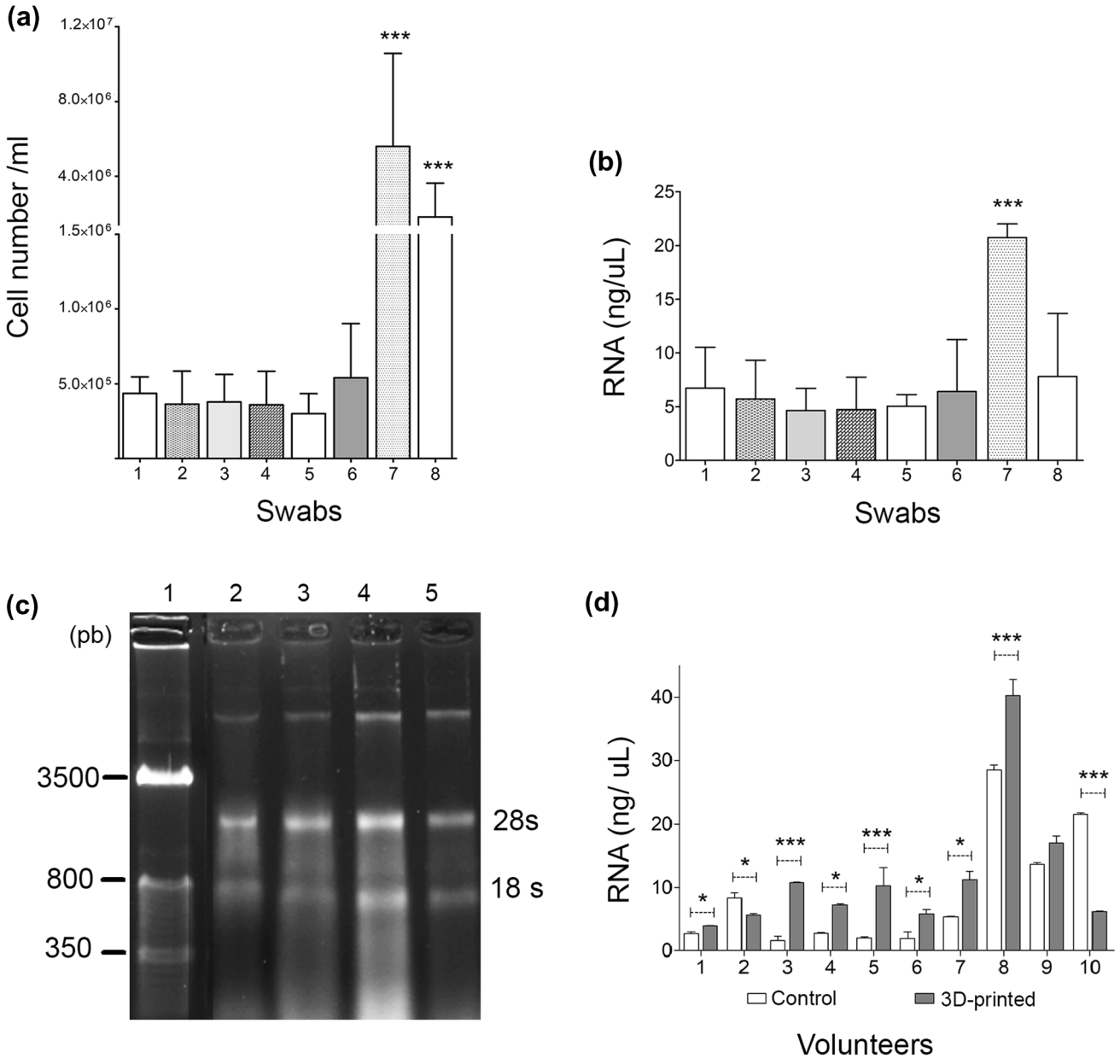

FIGURE 2. Cell collection tests and RNA quantification. (a) Graph of cell count. The bars represent the total number of cells collected for each prototype of 3D-printed (1–6) and control (7–8) swabs. (b) The RNA obtained from lymphocytes taken with 3D-printed swabs and control, expressed in ng/μL. (c) Gel electrophoresis of total RNA samples. Visualization of total RNA isolated of lymphocytes of the six 3D-printed swabs prototypes and control swabs run on 1% agarose gel electrophoresis of total RNA stained with ethidium bromide. Line (1) marker molecular reference; lines 2 and 3, RNA obtained with 3D-printed; lines 4 and 5, control swabs. The bands are demonstrated by the presence of intact ribosomal RNA (rRNA), with the 28 s band twice as intense as the 18 s band.28 and 18 s ribosomal, is indicated, referring to the quality of the RNA. D) RNA recovered from nasopharynx (NP), and oropharynx (OP) samples from volunteers taken with the 3D and control swabs. A one-way ANOVA test was performed with the Graph Pad Prism 5 program, statistical differences are indicated by asterisks: *$p<0.05$, **$p<0.005$, ***$p<0.0005$.

including in medicine.[3,15] We proposed the use of a biocompatible, biodegradable, non-toxic material called PLA to produce swabs for biological sampling for the detection of coronavirus.[20,22] Before the clinical use of the 3D swab prototypes, medical requirements were met, such as a sterilization process with ethylene oxide and tests for the absence of skin reactivity.[2,23]

It is important to mention that in this study we considered as controls, the swabs used in the HGM for sampling patients with suspected COVID-19, these swabs are manufactured with rayon on the collecting head and are commonly used in public hospitals in the country. 3D-printed swabs are products that can be positioned in the market because they are easy to

TABLE 2. Acceptability and opinion of the use of 3D and commercial swabs reported by participants and healthcare workers.

	Nasopharyngeal swabs(*n*%)			Oropharyngeal swabs(*n*%)		
Variable	3D-printed	Commercial	*p* value[a]	3D-printed	Commercial	*p* value[a]
Participants (*n*=10)						
The swab was introduced easily						
Yes	9 (90)	4 (40)	**0.019**	10 (100)	10 (100)	0.990
No	1 (10)	6 (60)		0	0	
The participant felt comfortable with the swab						
Yes	9 (90)	4 50)	**0.019**	10 (100)	10 (100)	0.990
No	1 (10)	6 (50)		0	0	
Procedure caused pain						
Yes	1 (10)	3 (30)	0.260	0	1 (10)	0.300
No	9 (90)	7 (70)		10 (100)	9 (90)	
Participants felt a burning sensation						
Yes	6 (60)	5 (50)	0.650	3 (30)	0	0.060
No	4 (40)	5 (50)		7 (70)	10 (100)	
Participant felt pressure						
Yes	0	3 (30)	0.060	0	0	0.990
No	10 (100)	7 (70)		10 (100)	10 (100)	
Epistaxis						
Yes	0	0	0.990	0	0	0.990
No	10 (100)	10 (100)		10 (100)	10 (100)	
Healthcare workers (*n*=2)						
Swab was flexible						
Yes	2 (100)	2 (100)	0.990	2 (100)	2 (100)	0.990
No	0	0		0	0	
The swab was broken at the breakpoint when placed into the vial						
Yes	2 (100)	0	**0.045**	2 (100)	0	**0.045**
No	0	2 (100)		0	2 (100)	
The swab resisted bending						
Yes	2 (100)	2(100)	0.990	2 (100)	2 (100)	0.990
No	0	0		0	0	
Collection was adequate						
Yes	2 (100)	0	**0.045**	2 (100)	2 100)	0.990
No	0	2 100)		0	0	
Participants felt comfortable with the swab						
Yes	2 (100)	0	**0.045**	2 (100)	2 (100)	0.990
No	0	2 (100)		0	0	

Bold values indicate statistically significant ($p<0.05$).
n=Total volunteers or healthcare workers.
[a]χ^2 test 2×2.

produce and can improve costs by being competitive and functional for OP and NP sampling, considering they are a good option to meet this need worldwide.[29]

We designed swabs that fit the anatomical dimensions of the nose and throat. The mechanical analysis performed on the 3D-printed swabs in the laboratory and in adult simulators showed that they have characteristics of hardness and flexibility that make them viable and safe for sampling.

The 3D-printed swabs showed statistically similar results in cell collection and RNA concentration in lymphocyte samples for the six prototypes tested. The control swabs collected a greater number of cells *in vitro*, probably because they are more absorbent and for this test, they were completely immersed in the solution. The 3D-printed swabs labeled 3 and 4 were proved as the best candidates for the test in volunteers. Interestingly, the 3D-printed swabs presented a better performance when sampling in volunteers compared to the control swabs, this can be attributed to their design and dimensions, which allowed them to be introduced into the nasal cavity to obtain a sufficient amount of sample to perform the RNA extraction and to obtain the amplification of the RNAse P gene by qRT-PCR.[5] This can be attributed to the design of the 3D swabs allowing the collection head to have a larger contact area with the NP cavity than the control swabs in which only the widest area of the collection head (piriform shape) makes contact for the collection of

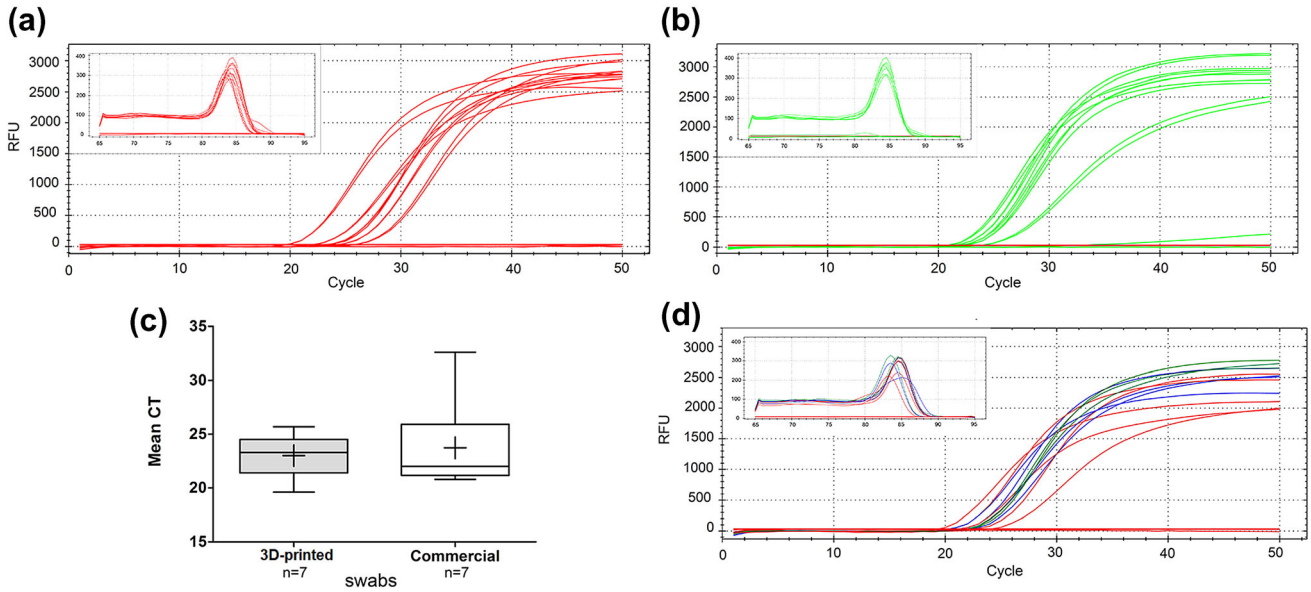

FIGURE 3. qRT-PCR tests. Shows the amplification curves and high-resolution melting curves (box up) of the RNase P gene from OP and NP samples of volunteers taken with: (a) 3D-printed swabs (red) and (b) control swabs (green). (c) The Box plot shows the mean Ct of RNAse P gene obtained from 3D-printed vs control swabs. The upper and lower boundaries of the boxes represent the 75th and 25th percentiles, respectively. The black line within the box represents the median value, the cross the mean and the whiskers represent the minimum and maximum values that lie within 1.5 × the interquartile range from the end of the box. (d) Stability test of 3D-printed swabs. Amplification curves of the RNAse P gene at 1 h, Ct 24.3 ± 1.8, (red line); 24 h, Ct 24.2 ± 0.4 (green line), and 48 h, Ct 23.5 ± 0.9 (blue line), $p > 0.5$ (ANOVA test).

TABLE 3. Quantitation of gene expression RNase P by qRT-PCR with 3D-printed swabs and control swabs.

	RNAse P (CT) [Mean ± SD][a]		RNAse P (ng /L) [Mean ± SD][b]	
Volunteers	3D-printed[c]	Control[d]	3D-printed[c]	Control[d]
1	23.4 ± 0.1	21.9 ± 0.3	22.5 ± 1.8	68.7 ± 12.7
2	19.6 ± 0.2	32.6	365.2 ± 1.1	0.0
3	24.1 ± 0.2	22.1	13.3 ± 2.3	56.3 ± 1.8
4	23.2 ± 0.2	20.8 ± 0.2	25.3 ± 2.4	153.8 ± 25.8
5	26.0	21.3 ± 0.1	3.2	105.1 ± 5.7
6	25.7 ± 0.1	23.7 ± 0.2	3.9 ± 0.3	17.4 ± 2.3
7	22.0	30.5	63.3 ± 0.3	0.1
8	25.1 ± 0.1	ND	6.4 ± 1.5	ND
9	23.4 ± 0.1	ND	22.2 ± 1.3	ND
10	22.4	ND	47.2 ± 0.2	ND
Mean (range)	23.5 (19.6–26)	24.7 (20.8–32.6)	57.3 (3.2- 365.2)	57.3 (0–153.8)
p value[e]	0.47		0.98	

Ct threshold cycle, *SD* standard deviation, *ND* undetermined.
[a]Gene expression of RNAse P, the values of the mean are shown ± SD of three independent experiments.
[b]Absolute quantification with a standard curve, the results are shown as the mean+SD of three independent experiments.
[c]3D-printed swabs.
[d]Control swabs.
[e]Student t-test.

cells. Around 500,000 was considered a minimum cell number sufficient to perform the qRT-PCR.

PLA material also proved to be a good candidate for manufacturing because there was no evidence that it could degrade RNA and therefore the qRT-PCR assays were satisfactory, demonstrating that it does not inhibit the reaction in a two-step system for qRT-PCR. The results with volunteers' samples showed the same behavior observed in the *in vitro* assays for the qRT-PCR test, obtaining a Ct = 23.5 (range 19.6–26), which agrees with values reported in the literature.[11]

The 3D-printed swabs showed statistically similar results in cell collection and extraction of RNA in lymphocyte samples. We statistically expected that

there should not be significant differences ($p \geq 0.05$), but if there are, these should not compromise the performance of the swabs for the detection by qRT-PCR. That is, the 3D-printed swabs did not perform significantly less than the control swabs, in terms of the evaluation of RNA extraction.

The 3D-printed swabs had better performance *in vivo* than control swabs. We demonstrated that the proposed prototypes are more efficient and could be used in countries with low resources (technology dependency) with which reliable results could be obtained in the diagnosis of SARS-CoV-2 infection by qRT-PCR. Therefore, could be a viable option for the diagnosis of COVID-19, which also allows proposing the use of swabs for the collection and analysis of other medical and biological applications. The use of bioactive material for 3D printing is expected to open enormous possibilities in handling future pandemics as well.

This pilot study was carried out at a time of acute crisis in the Mexican health system in which the capacities for patient care collapsed causing that the tests for COVID-19 detection were done with the limitation in shortage supplies due to the impossibility of purchases abroad. Under these conditions, this pilot study was conducted with a limited sample giving a statistical power (calculated using G*Power) of 73.7% just below of the conventional value of 80%, thus the statistical inference is valid. The performance we have obtained is a promising result for a pilot study so we can move forward to the clinical validation with patients affected by COVID-19 including inter and intra-operator variability as a parameter in the tests. The swab design and validation methodology could be used by other countries to reduce the negative impact of the shortage in supplies for the SARS-CoV-2 detection.

CONCLUSION

We designed and validated 3D-printed swabs for sample collection in the detection of SARS-CoV-2 in a pilot study. The 3D printing technique used is easy to be implemented and the material used is low cost. In mechanical tests, they showed good flexibility and recovery to bending. Also, they had robustness in the emulation of the nasopharyngeal and oropharyngeal sampling with good acceptance by healthcare personnel in terms of ease of maneuverability and stability in adult human simulators. The functionality was molecularly demonstrated by taking samples from a small group of volunteers, obtaining good extraction of RNA comparable to that of the commercial control swabs. The non-inhibition of the 3D-printed swabs in the qRT-PCR test was demonstrated, in which the expression of the reference gene (RNase P) was equivalent to that obtained by the control swabs. We believe that this innovative design proposal and the methodology used for its validation can be easily implemented in low-resource settings countries to solve in a short time the shortage of swabs in the pandemic.

ACKNOWLEDGMENTS

This research was conducted with support from Universidad Nacional Autónoma de México under Grant UNAM-PAPIIT IV100320 and the Secretaría de Educación, Ciencia, Tecnología e Innovación de la CDMX under grant SECTEI/090/2020, UNAM. Resources of Hospital General de México "Dr. Eduardo Liceaga": Authors thanks for their valuable contribution to: Guadalupe Mercedes Lucía Guerrero Avendaño, MD (General director); Ricardo Juan García Cavazos, MD. Mariano A. Capurso García, MD. Fabiola Serratos Canales. M. Sc. (Directorate of Health Education and Training); Jesús Carlos Briones Garduño, MD, and Olga Martha Rodríguez Peñeyro, MD. (Directorate of Support for Diagnosis and Treatment), Ana Isabel García Jerónimo, MD. Anayeli Rodríguez Castillo, MD, and Daniel Amaral Villaseñor, MD. (Epidemiological Surveillance Unit).

CONFLICT OF INTEREST

The authors declare no conflicts of interest regarding this study.

REFERENCES

[1]Archer, E., H. Allen, A. Hopwood, and D. Rowlands. Validation of a dual cycle ethylene oxide treatment technique to remove DNA from consumables used in forensic laboratories. *Forensic Sci. Int. Genet.* 4:239–243, 2010.

[2]Arjunan, A., S. Zahid, A. Baroutaji, and J. Robinson. 3D printed auxetic nasopharyngeal swabs for COVID-19 sample collection. *J. Mech. Behav. Biomed. Mater.* 114:104175, 2021.

[3]Armijo, P. R., N. W. Markin, S. Nguyen, D. H. Ho, T. S. Horseman, S. J. Lisco, and A. M. Schiller. 3D printing of face shields to meet the immediate need for PPE in an anesthesiology department during the COVID-19 pandemic. *Am. J. Infect. Control*. 49:302–308, 2021.

[4]Benmassaoud, M. M., C. Kohama, T. W. B. Kim, J. A. Kadlowec, B. Foltiny, T. Mercurio, and S. I. Ranganathan. Efficacy of eluted antibiotics through 3D printed femoral implants. *Biomed. Microdevices*. 21:1–10, 2019.

[5]Bwire, G. M., M. V. Majigo, B. J. Njiro, and A. Mawazo. Detection profile of SARS-CoV-2 using RT-PCR in different types of clinical specimens: a systematic review and meta-analysis. *J. Med. Virol.* 93:719–725, 2021.

[6]Callejas, D., J. M. Echevarría, Y. Carrero, A. J. Rodríguez-Morales, and R. Moreira. The SARS-CoV-2 pan-

demic in Latin America: the need for multidisciplinary approaches. *Curr. Trop. Med. Rep.* 7:120–125, 2020.
[7]Decker, S. J., T. A. Goldstein, J. M. Ford, M. N. Teng, R. S. Pugliese, G. J. Berry, M. Pettengill, S. Silbert, T. R. Hazelton, and J. W. Wilson. 3D printed alternative to the standard synthetic flocked nasopharyngeal swabs used for COVID-19 testing. *Clin. Infect. Dis.*, 2020.
[8]Dramé, M., M. Tabue Teguo, E. Proye, F. Hequet, M. Hentzien, L. Kanagaratnam, and L. Godaert. Should RT-PCR be considered a gold standard in the diagnosis of Covid-19? *J. Med. Virol.* 92:2312–2313, 2020.
[9]Freire-Paspuel, B., P. Vega-Mariño, A. Velez, P. Castillo, E. E. Gomez-Santos, M. Cruz, and M. A. Garcia-Bereguiain. Cotton-tipped plastic swabs for SARS-CoV-2 RT-qPCR diagnosis to prevent supply shortages. *Front. Cell. Infect. Microbiol.* 10:356, 2020.
[10]Halpern, B., M. L. C. da Louzada, P. Aschner, F. Gerchman, I. Brajkovich, J. R. Faria-Neto, F. E. Polanco, J. Montero, S. M. M. Juliá, and P. A. Lotufo. Obesity and COVID-19 in Latin America: a tragedy of two pandemics—Official document of the Latin American Federation of Obesity Societies. *Obes. Rev.* 22:e13165, 2021.
[11]Ishige, T., S. Murata, T. Taniguchi, A. Miyabe, K. Kitamura, K. Kawasaki, M. Nishimura, H. Igari, and K. Matsushita. Highly sensitive detection of SARS-CoV-2 RNA by multiplex rRT-PCR for molecular diagnosis of COVID-19 by clinical laboratories. *Clin. Chim. Acta*. 507:139–142, 2020.
[12]Ji, T., Z. Liu, G. Wang, X. Guo, C. Lai, H. Chen, S. Huang, S. Xia, B. Chen, and H. Jia. Detection of COVID-19: a review of the current literature and future perspectives. *Biosens. Bioelectron.* 166:112455, 2020.
[13]Johns Hopkins University & Medicine. No Title. , 2021. https://coronavirus.jhu.edu/map.
[14]Karthik, K., R. P. A. Babu, K. Dhama, M. A. Chitra, G. Kalaiselvi, T. M. A. Senthilkumar, and G. D. Raj. Biosafety concerns during the collection, transportation, and processing of COVID-19 samples for diagnosis. *Arch. Med. Res.* 51(7):623–630, 2020.
[15]Kumar, K. P. A., and M. Pumera. 3D-printing to mitigate COVID-19 pandemic. *Adv. Funct. Mater.* 31:2100450, 2021.
[16]Li, D., J. Zhang, and J. Li. Primer design for quantitative real-time PCR for the emerging Coronavirus SARS-CoV-2. *Theranostics*. 10:7150, 2020.
[17]Li, H., Z. Liu, Y. He, Y. Qi, J. Chen, Y. Ma, F. Liu, K. Lai, Y. Zhang, and L. Jiang. A new and rapid approach for detecting COVID-19 based on S1 protein fragments. *Clin. Transl. Med.* 10:e90, 2020.
[18]Lurie, N., M. Saville, R. Hatchett, and J. Halton. Developing Covid-19 vaccines at pandemic speed. *N. Engl. J. Med.* 382:1969–1973, 2020.
[19]Manoj, A., M. Bhuyan, S. R. Banik, and M. R. Sankar. 3D printing of nasopharyngeal swabs for COVID-19 diagnose: past and current trends. *Mater. Today Proc.* 44:1361–1368, 2021.
[20]Murariu, M., and P. Dubois. PLA composites: from production to properties. *Adv. Drug Deliv. Rev.* 107:17–46, 2016.
[21]Nguyen, V. T., P. Rivière, P. Ripoll, J. Barnier, R. Vuillemot, G. Ferrand, S. Cohen-Boulakia, P. Ravaud, and I. Boutron. Research response to coronavirus disease 2019 needed better coordination and collaboration: a living mapping of registered trials. *J. Clin. Epidemiol.* 130:107–116, 2021.
[22]Oland, G., O. Garner, and A. de StMaurice. Prospective clinical validation of 3D printed nasopharyngeal swabs for diagnosis of COVID-19. *Diagn. Microbiol. Infect. Dis.* 99:115257, 2021.
[23]Pajot, T., L. Benichou, E. Moreau, V. Tallon, J.-P. Meningaud, R. H. Khonsari, and S. Ketoff. Implementation of a digital chain for the design and manufacture of implant-based surgical guides in a hospital setting. *J. Stomatol. Oral Maxillofac. Surg.* 121:347–351, 2020.
[24]Pollard, A. J., R. D. Reeves, and A. J. M. Baker. Facultative hyperaccumulation of heavy metals and metalloids. *Plant Sci.* 217:8–17, 2014.
[25]Rybicki, F. J. 3D printing in medicine: COVID-19 testing with 3D printed nasopharyngeal swabs. *Clin. Infect. Dis.*, 2020.
[26]Siemieniuk, R. A., J. J. Bartoszko, L. Ge, D. Zeraatkar, A. Izcovich, E. Kum, H. Pardo-Hernandez, B. Rochwerg, F. Lamontagne, and M. A. Han. Drug treatments for covid-19: living systematic review and network meta-analysis. *Bmj*. 370:m2980, 2020.
[27]Tahamtan, A., and A. Ardebili. Real-time RT-PCR in COVID-19 detection: issues affecting the results. *Expert Rev. Mol. Diagn.* 20:453–454, 2020.
[28]Thwe, P. M., and P. Ren. How many are we missing with ID NOW COVID-19 assay using direct nasopharyngeal swabs? Findings from a mid-sized academic hospital clinical microbiology laboratory. *Diagn. Microbiol. Infect. Dis.* 98(2):115123, 2020.
[29]Wang, X., L. Tan, X. Wang, W. Liu, Y. Lu, L. Cheng, and Z. Sun. Comparison of nasopharyngeal and oropharyngeal swabs for SARS-CoV-2 detection in 353 patients received tests with both specimens simultaneously. *Int. J. Infect. Dis.* 94:107–109, 2020.
[30]Yang, Y., L. Zhang, X. Huang, Y. Zhou, Q. Quan, Y. Li, and X. Zhu. Response of photosynthesis to different concentrations of heavy metals in Davidia involucrata. *PLoS ONE*. 15:e0228563, 2020