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Thesis: Design of Saliva Collection Device

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<u>Υπεύθυνη δήλωση</u> για λογοκλοπή και για κλοπή πνευματικής ιδιοκτησίας:

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Abstract

Last years the need for saliva sampling has been radically increased. Especially due to the covid-19 pandemic, the need for saliva collection and examination was higher than ever. The existing methods for collecting saliva are limited and are characterized by a user-unfriendly way of usage. The aim of this project is to create a device that allows saliva collection at home, is user-friendly and helps the microbiologists have an easy sampling in the laboratory.

In this study, there was a conceptual design of the device based on the above specifications. The product was 3d printed and the final design of the product was based on many factors, but mainly on user experience. Then, the sealing of the product was tested, using different methods for it. Next, a biomarkers' detection test was made and the best solution of saliva: PBS was established, so that the microbiologists would be able to use it. Furthermore, the device was 3d printed with a medical resin type I and it got sterilized, in order to test its ability to collect saliva. Finally, an experiment designed to simulate the whole process, which starts from the user and ends to the microbiologist.

Περίληψη

Τα τελευταία χρόνια έχει αυξηθεί ραγδαία η ανάγκη για συλλογή σάλιου. Ειδικά με την πανδημία του κορονοϊού, η ανάγκη για συλλογή και εξέταση σάλιου είναι πιο επιτακτική από ποτέ. Οι υπάρχουσες μέθοδοι για τη συλλογή είναι περιορισμένες, ενώ ο χρήστης εισέρχεται σε μία αρνητική εμπειρία χρήσης. Στόχος της συγκεκριμένης εργασίας είναι η κατασκευή μιας συσκευής που επιτρέπει τη συλλογή σάλιου στο σπίτι, είναι φιλική προς τον χρήστη και βοηθάει ταυτόχρονα τους μικροβιολόγους να διεξάγουν με ευκολία τις αντίστοιχες εξετάσεις στο εργαστήριο.

Σε αυτή την εργασία, ο αρχικός σχεδιασμός της συσκευής βασίστηκε στις παραπάνω προδιαγραφές. Το προϊόν εκτυπώθηκε σε 3-Δ εκτυπωτή σε διαφορετικά στάδια και διαφορετικές μορφές και ο τελικός σχεδιασμός της συσκευής βασίστηκε σε πολλούς παράγοντες, αλλά κυρίως στην εμπειρία του χρήστη. Έπειτα, έγιναν τεστ για την στεγάνωση της συσκευής με χρήση διαφορετικών μεθόδων, ενώ ακολούθησε και μια διαδικασία εύρεσης βιοδεικτών, όπου διαπιστώθηκε και η καλύτερη δυνατή ανάμιξη σάλιου: διαλύτη, με στόχο να χρησιμοποιηθεί και από τους μικροβιολόγους. Επιπλέον, η συσκευή εκτυπώθηκε με μία βιοσυμβατή ρητίνη τύπου Ι και αποστειρώθηκε, για να διαπιστωθεί η ικανότητα της στη συλλογή σάλιου. Τέλος, σχεδιάστηκε ένα πείραμα που προσομοίωνε τη διαδικασία χρήσης της από τον χρήστη, ως τον μικροβιολόγο.

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1. Introduction

1.1 Reasons, why saliva collection is important

Saliva collection is a non-invasive procedure and safe source of information that could be a tool in the diagnosis of diseases and in the measurement of a variety of biomarkers. Especially now, with the covid-19 pandemic, the use of saliva samples to detect the disease is rapidly growing up. However, there are also other viral infections, that can be detected via diagnostic tests, which use salivary biomarkers, that are measured after following certain methods for saliva collection. Some of the most well-known diseases are hepatitis virus (A, B and C), measles, autoimmune diseases, diabetes, cardiovascular diseases, dental caries and HIV-1 [1]. The detection of these viruses is achieved through saliva-based antibody tests using the collected samples. Furthermore, saliva can not only be used for the diagnosis, but also for prognosing and monitoring of other diseases, such as type 2 diabetes and periodontitis. Obtaining saliva is rapid, simple, and painless, making this sample an uncomplicated tool for disease screening

1.1.1 Why is saliva collection better than blood collection?

Considering all the above facts, salivary tests can become the main tool for the measurement of specific biomarkers, that are mainly measured in blood nowadays. We should take into account the advantages that saliva collection carries compared to blood collection, mainly due to the collection procedure and the robustness of the sample. The need for highly trained personnel that is required in blood sampling, saliva can be self-collected via a painless process, reducing the discomfort most individuals endure from biopsies and repeated blood draws. In addition to these, saliva is easily collected, shipped, and stored, because saliva does not clot and requires less manipulation than blood, resulting in decreased overall costs for patients and health care providers. Considering all the above, saliva collection could be a way to revolutionize diagnostics at home with low cost and effective methods.

1.2.1 Why is saliva collection better than urine collection?

What is more, saliva collection can be also used as a tool for diagnostics instead of urine collection. Saliva testing is safe and quick, and there’s no need to plan for gender-specific observers, or dedicated rooms for collection since it can be performed anywhere. It’s also comfortable for both the donor and the observer or the person collecting the sample in a laboratory. The process is non-invasive, so there is no pain or even the slightest discomfort. For instance, the donor may experience a shy bladder during the urine collection, if the donor has difficulty urinating. Finally, the urine sample requires complicated logistics since it has to be preserved in defined conditions, while the saliva sample doesn’t have any need for special handling.

1.2 Existing methods

Taking into account all the above facts, it is important to list all the existing ways for saliva collection. It is important to mention than not every solution has yet been developed to a product, thus some of them are in an early stage and clinical experiments are conducted on them.

1.2.1 Method 1 – Passive drooling

First and foremost, the most usual way is the passive drooling method. The subject must let saliva come out of the mouth and flow into a tube. Passive drool, as shown in the figure 1.1, is considered by many researchers to be the gold standard when collecting saliva samples for biological testing, because it provides the purest sample possible and allows researchers to "biobank" samples for future testing. However, it is very user-unfriendly and that's why it is the less used method.

Figure 1.1: Passive drooling method using Salimetrics kit [2]

1.2.2 Method 2 – Spitting

Another acceptable method is spitting. In comparison to the previous method, it is user friendlier, however it is not acceptable by all biologists, since many of them believe that spitting destroys the biomarkers.

1.2.3 Method 3 – Swab

The other acceptable method by many laboratories, is the use of a special swab which is shown in figure 1.2. Its usage varies depended on the facilities of each company, but in the most cases, the donor must place the swab beneath the tongue, where the majority of saliva is produced, and collect passively saliva for 1-2 minutes. After that, the swab has to be placed inside a kit, which is sent to the laboratory. In the laboratory, the microbiologist has to centrifuge the swab to extract the collected saliva and then move into examining it. However, it should be mentioned, that the concentration of some biomarkers is altered during the centrifuge, since some proteins bind to the swab.

Figure 1.2: A kit for saliva collection via swab [3]

1.2.4 Method 4 – Suction

This method is used mainly by laboratories, which have the suitable equipment for this work. However, this method cannot be applied at home. More precisely, an aspirator with a pump is placed inside the donor's mouth and it collects the sample in a chamber as shown. An example of this device is shown in the figure 1.3.

Figure 1.3 Sunction machine sold by CA MI [4]

1.2.5 Method 5 – Foam

The company 'Salimetrics', which is a leader in saliva collection kits, uses a foam [5], mainly for children and animals. The donor places the foam, like the one shown in figure 1.4, inside the mouth and keeps it there for 2 minutes, until enough saliva has been absorbed. After the collection is finished, the foam is sent to the laboratory, where it gets centrifuged, so that the biologist can extract the sample.

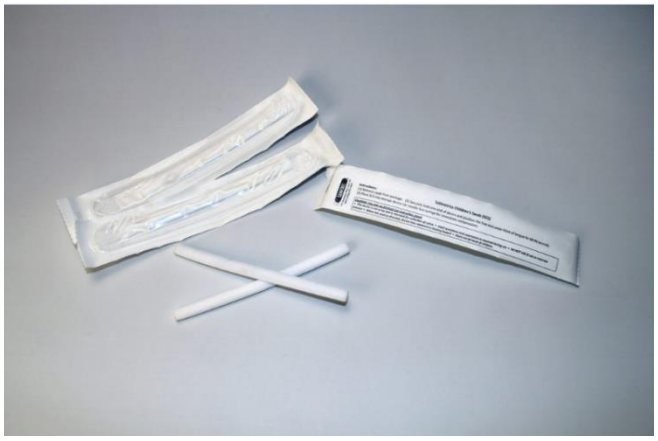

Figure 1.4 Foam-based saliva collection kit of Salimetrics

1.2.6 Combination of existing methods

Some companies also try to combine the existing methods to achieve better results. For example, the company 'neoteryx' has a kit with both a swab and a funnel [6]. The user collects initially saliva with the swab and then pushes the head of the swab to the funnel. By this way, the saliva is collected inside the chamber under the drooling. Another company uses the reverse procedure, where the user spits initially inside a chamber, and after that, he/she places swab inside the sample for 2 minutes. Although these solutions are sometimes user friendlier than the first ones, they still combine the disadvantages of the previous methods as far as biomarkers' detection is concerned.

1.2.7 Methods in process

Since all these methods have clearly some disadvantages, companies and institutions around the world try to develop new saliva collection devices. Many of the below listed devices are either in an initial stage of development, or they have been registered to the US patent office [7], but they haven't been yet developed into a product.

As a first example, we can name the patent No. US 6623298 B2, where the developer has created an active toothbrush, which collects saliva and has a biosensor system within a test channel for performing routine saliva tests. This device can be used for collection, aside from its testing aspect.

Another device, which actively collects saliva, is the patent No. 6022326, which is an aspiration device, and it has a mouthpiece on a wand. The wand is connected to an interface section via a flexible conduit. Saliva is transported by aspiration into the device. Bulk air is removed, and saliva is collected in a collection chamber. For the collection of volatile components, air flow, vacuum, conduit diameter and length, and collection times are controlled and limited, to reduce loss of volatile components.

Moreover, there is a device, which demands spitting from the end user, but succeeds in a better storage of the sample and it has a patent No. 9113850. More specifically, it is a mouthpiece with a fluid inlet connected to a collection chamber. The collection chamber includes a collecting vessel, a venting outlet, and an access port. The venting outlet may be covered by a liquid-impervious or resistant membrane, such as a hydrophobic membrane, and the access port is suitable for removing some or all ~~of~~ the collected fluid. This arrangement allows a saliva donor to continuously spit saliva and blow air into the closed collection chamber, without pressure build-up in the collection chamber, and without the need for the donor to release the device until the desired oral fluid volume is collected. A valve, including a check valve may be in the saliva flow stream and baffles and structure creating a tortuous path may be utilized to keep saliva away from the membrane.

Instead of active solutions, other developers use passive methods for saliva collection. Particularly the patent No. 6440087 B1, uses an apparatus, which has a fluid resistant shield adjacent one portion or side of the absorbent to support the collection of oral fluid, while excluding the collection of mucosal transudates.

Last but not least, there is a device which uses a swab like many others, but the developer tries to keep the sample in a better condition, since storing and transporting wet saliva can be expensive and problematic. Additionally, the biomarkers of disease are subject to degradation during storage and delivery. For this reason, the patent No. 8998824 B2 achieves to collect and transfer saliva in a better way, in comparison to the existing methods that use swabs.

1.3 What do these solutions lack

However, these methods have some disadvantages that cannot be left without a comment. First of all, the passive drooling method is considered to be one of the most user-unfriendly methods, since the subject has to let saliva flow outside the mouth using the gravity force and aim correctly inside the funnel, which leads many times to the salivation of the donor. The spitting method is considered to be an untrustworthy method, because the spitting destroys many biomarkers. A far as the swab is concerned, it takes a lot of work from the biologists to extract the saliva from the swab and the lab should be equipped with the necessary devices that will enable the biologists to do this extraction, and in the majority of the cases many proteins are held by the swab. The devices that are currently under development, and especially the active toothbrush, can measure only a couple of biomarkers that in certain cases are not suitable for the subject's case, while other methods still require complex handling from an average user.

1.4 The proposal

Taking all the above facts into account, it is clear that there is a need in the market for a user friendlier collection method, which doesn't destroy any biomarkers of the donor. For this reason, we tried to create a device that will provoke positive feelings to the subject and will be non-invasive in any case. What is more, it should not demand a complex handling by the user, unlike the aspiration device and it should be eligible for use at home. There is also a need for low manufacturing costs, and it should be easily transported by transportation companies without destroying the biomarkers. That's the main reason, why the collection is based mainly on whole saliva.

Hence, we decided that this device should have the shape of a lollipop, which is a shape that most donors are familiarized with during their childhood and is shown in figure 1.5

Figure 1.5 The device

The sealing will be achieved by a lid, which is visible in figure 1.6, with an O-ring and they create and assembly that is in figure 1.7.

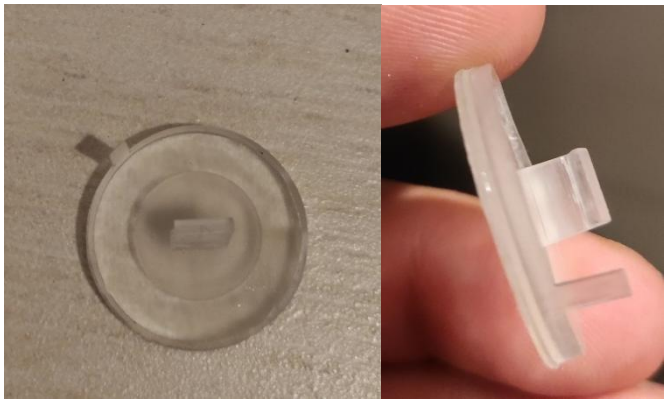

Figure 1.6 The lid without the O-ring

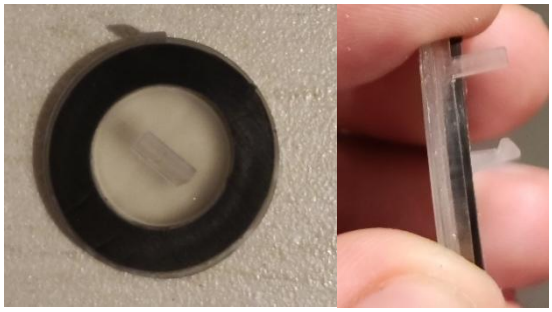

Figure 1.7 The lid with the O-ring

After the transportation of the whole assembly, as shown in figure 1.8, to the laboratory, the microbiologist will have to place it to the centrifuge, in order to get the sample into the third and last part of the device, which is the stick of the lollipop. The stick has on top a threading, so that it can be connected to the main part of the device, the head of the lollipop, where saliva is collected.

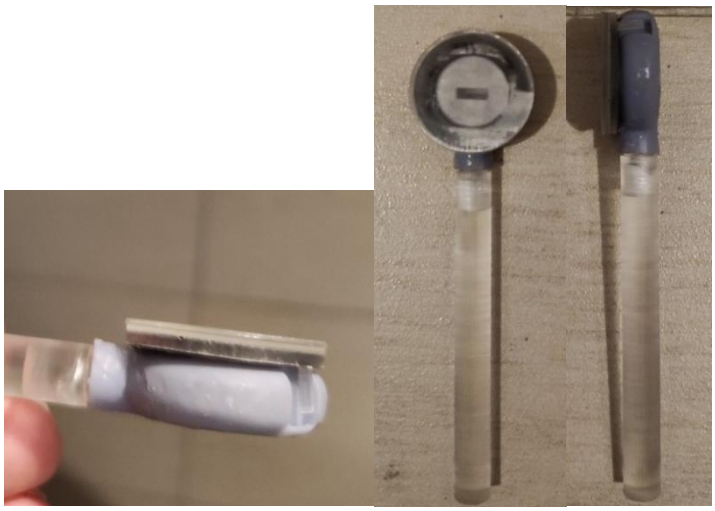

Figure 1.8 The device sealed

After the centrifugation, the saliva will be placed on the chamber of the stick shown in figure 1.9, which can be removed just by unthreading the tube from the main container of saliva.

Figure 1.9 The stick and the chamber of the stick

2. Design of the device

2.1 Specifications of the device

In the first place it is necessary to state the specifications of the device. For this reason, before starting with the design, we set the specifications, as shown below.

Main design parameter	The device should be lollipop alike as familiar to the donor as possible, so that he/she can feel comfortable when using it. Thus, the dimensions should be as close as possible to a typical lollipop, presented in figure 2.1.
Collection	At least 200 uL of saliva should be collected inside the chamber of the tube.
Sealing	The device should seal tightly to ensure that no sample will be lost during transportation. It is important that the donor closes the lid without any complex handling and without any danger of being wounded
Processing from the microbiologist	The microbiologist should perform as few operations as possible to process the sample. Furthermore, the device should be able to fit in a SPL tube, which is designed to be placed inside this centrifuge. However, it should not have a big radial clearance, so that the oscillation during the centrifugation is reduced. The dimensions of the SPL clinical tube are to be found in the Annex.
Collection from the microbiologist	The chamber on the top of the tube, where the sample is led after its centrifugation, should have a diameter big enough to allow the tip of the 200 [µL] pipette to fit in.
Transportation	The device should be transportable in a small packing, to reduce the transportation costs.
Manufacturing	The manufacturing costs should be as low as possible. Considering that there will be demand for the product, we consider injection molding as the most effective solution for our case.

Figure 2.1 Dimensions of classic lollipop

2.2 Stages of the Design

It's obvious that it was not easy to end up to the final design of the device since the main parameter of it was the user-friendliness and the donor's safety. That's why the design demanded a lot of time, since the brainstorming and the prototyping of all these ideas was time consuming.

2.2.1 Conceptual Design-Early Stages-Brainstorming

In the early stages, there were many alternatives on how to design the device.

The first idea consisted of a 2-part lollipop and its assembly is presented in the picture below (figure 2.2), while we can also see the 2 separates parts (figure 2.3) of this assembly.

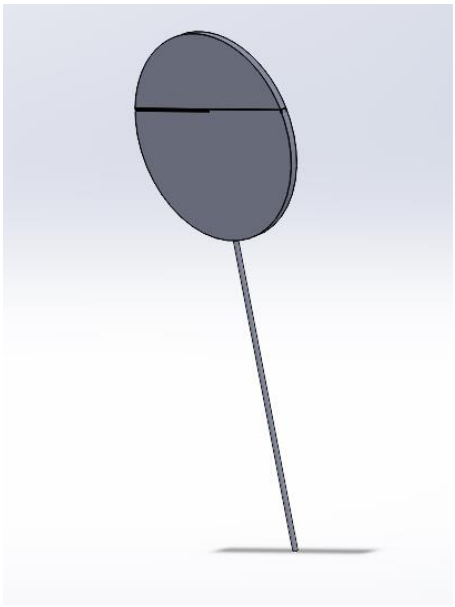

Figure 2.2 The assembly of the device

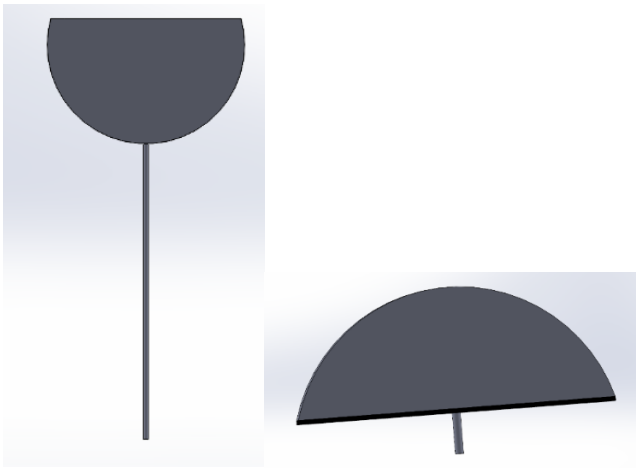

Figure 2.3 The 2 separate parts of the device

The donor would use the second part as a lollipop, and then he/she would cover the sample with the first part, which had a squared section seal on the bottom, to ensure that no collected saliva would be lost. The saliva would be stored inside the inlet channels of the second part device, which is shown below in figure 2.4.

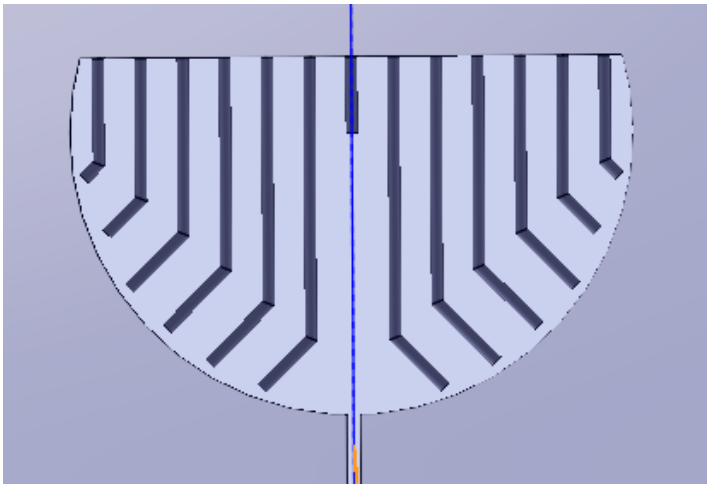

Figure 2.4 Cross section of the first option

However, this solution, despite being user- friendly, it would require a lot of work from the microbiologist, because it's difficult to extract the sample. What is more, we are not sure that the collected sample would be enough, because the viscosity of saliva is very high to allow the flow in these small diameter inlet channels. Finally, it should also be mentioned that there is a little danger for the user to get wounded in case he/she used in a totally wrong manner the second part and its slightly cutting edge. For all the above reasons, this solution was rejected.

The second option was a solution that was consisted of 6 parts, and the shape of the main container was pizza-alike, as you can see in the figure 2.5 below.

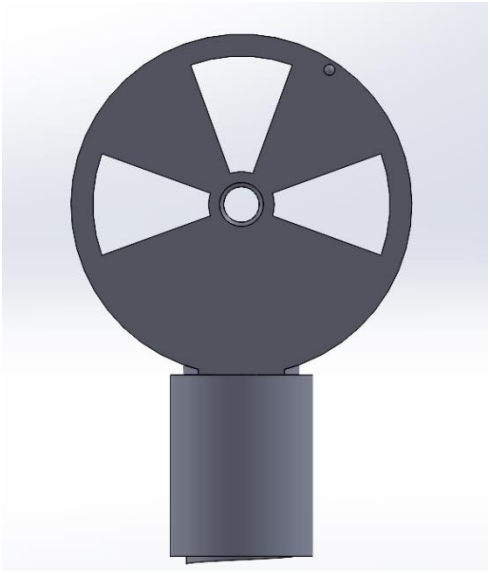

Figure 2.5 Container of the second option

The 6 parts would be used as followed. The stick (figure 2.6) connects to a tube (figure 2.7), which is connected to the main container, where the sampling takes place. These two parts were used so that the geometry would smoothly change.

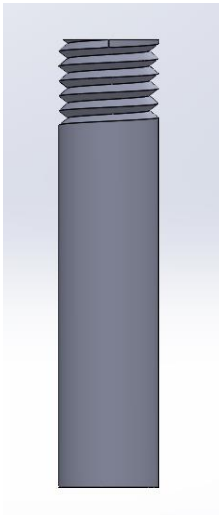

Figure 2.6 Stick of this device

Figure 2.7 Tube of this device

After the collection of the saliva has been completed, the donor would have to screw only for 45 degrees both lids (figure 2.8) of the container, to ensure that no sample would be lost. The thread and the lid were designed in this way, so that the open sections of the container would align with the closed sections of the lid, as shown in the 2 figures below, in figure 2.9. The dimple that exists there would ensure that the user wouldn't bolt the lid furthermore.

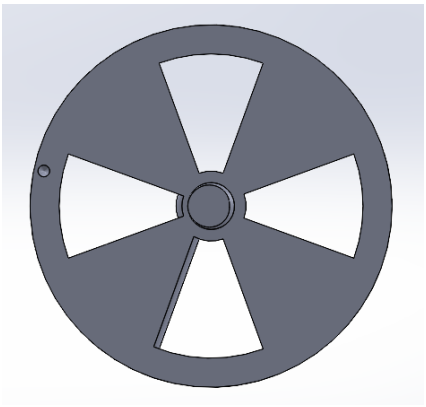

Figure 2.8 Lid of the device

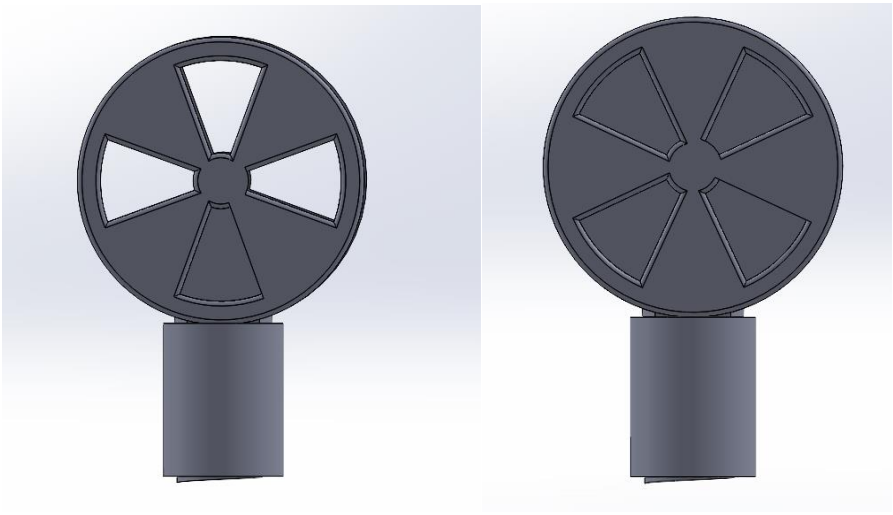

Figure 2.9 The device before (on the left) and after (on the right) the collection of saliva

After the sealing of the device, the saliva would flow to the lower tube. In the laboratory, the microbiologist would collect the sample from the stick, after centrifuging it, using the designed lid to seal the tube, as shown in the figure 2.10 below.

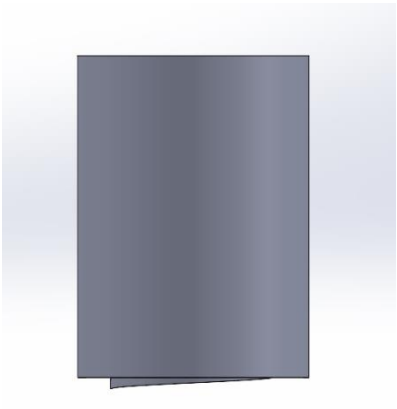

Figure 2.10 The lid for the tube

However, this solution wasn't feasible either, since there were some problems. First and foremost, due to the high viscosity of saliva, we are not sure if the sample would fall into the tube. What is more, the end user could rotate the lid more than 45 degrees, which means that the sample wouldn't be sealed. Finally, the device was user- unfriendly due to its geometry (both at the bottom of the container and at the collection process).

The third and last option, which led us to the final design, was a device similar to the previous one, with the main difference on the container of the device. As you can see in the pic below, the container in figure 2.11 has some holes on its surface, which enable the saliva to get into the chamber, using the capillary force.

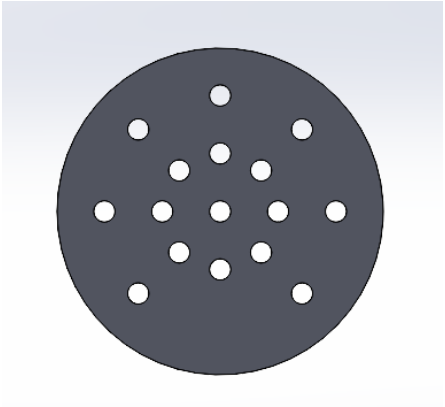

Figure 2.10 Container of third option

The hole on the center of the container is used for threading the lid to the device. The lid (figure 2.12) is designed in a way, that the user rotates it using the small bulge so that the holes of the lid and the holes of the container are totally misaligned. In this way we achieve the sealing of the device.

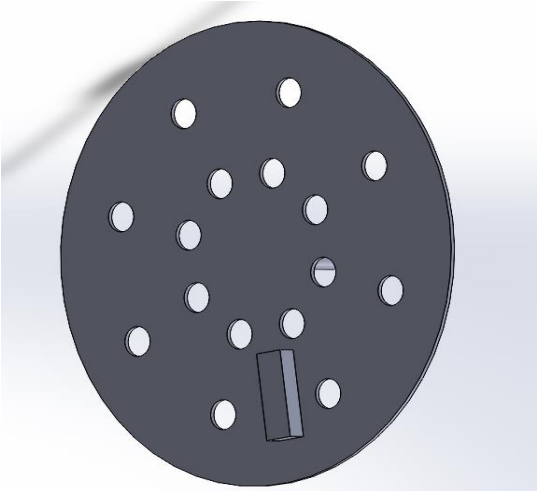

Figure 2.11 Lid of the third option

The tube that is shown in figure 2.13 on the lower end of the container would be used as in the previous option, but this device has got the same disadvantages with the second one due to the handling of the end-user and the viscosity of saliva.

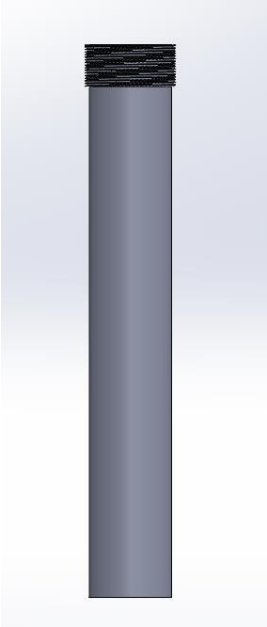

Figure 2.12 Tube of third option

2.2.2 Final Solution – how the user uses the device

After all the above solutions were examined, we managed to design the final solution for the end-user.

As you can see in the figure 2.14 below, the container is very similar to the one used in the last option, since it has the same operating principle for the collection. However, the container collects saliva only from one side, hence we need to seal it only from this side.

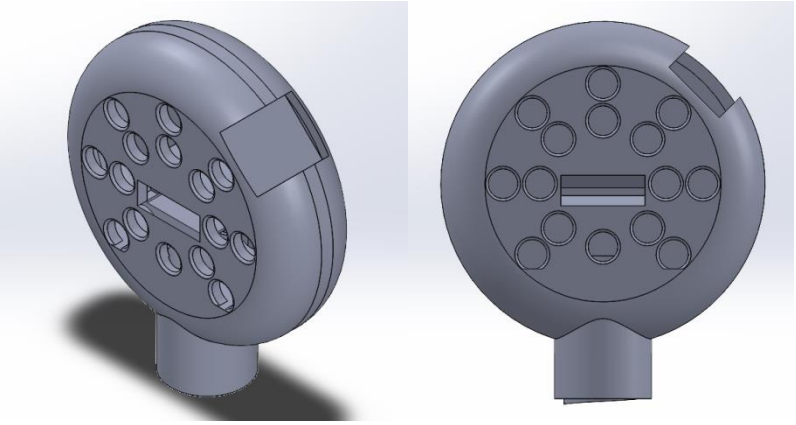

Figure 2.13 The container of final solution

However, there are also some significant changes for the sealing.

At first, the sealing is achieved via a snap-fit mechanism, which is very functional in our case. More specifically, the snap-fit mechanism is used to ensure that the lid and the container will remain tightly sealed. The dimensions of the snap-fit and the experimental procedure that was followed, in order to get these dimensions, will be analyzed in chapter 2.3. As you can see in the figure 2.15 below, the lid has a male geometry, connected to its corresponding female geometry, which is designed inside the container.

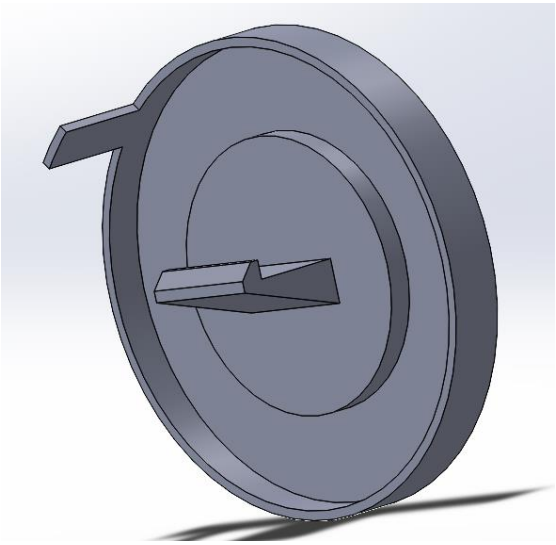

Figure 2.14 Design of the lid of the device

This design has also some other significant advantages from the aspect of the end user in comparison with the solutions that were analyzed on chapter 2.2.1.

First and foremost, the end user doesn't have to make a circular movement of the sealing mechanism, but a linear one, which is more ergonomic. Furthermore, the existence of a small extrusion, as shown in the figure 2.15, helps the end user determine the orientation of the snap-fit mechanism, so that he/she doesn't place it in the opposite way. The corresponding female recess exists on the upper part of the container.

What is more, the circular geometry that exists on the inner part of the lid ensures that there is enough room for the O-ring to be placed correctly and to be squeezed, in order to work properly as a seal. The reason why an O-ring sealing was chosen instead of a PDMS will be analyzed in chapter 2.4

Finally, the design of the tube shown in figure 2.16, where the sample will be gathered is also user- friendlier, since it has a fixed and constant diameter across the tube, in order to ensure that the donor will be more familiarized with it.

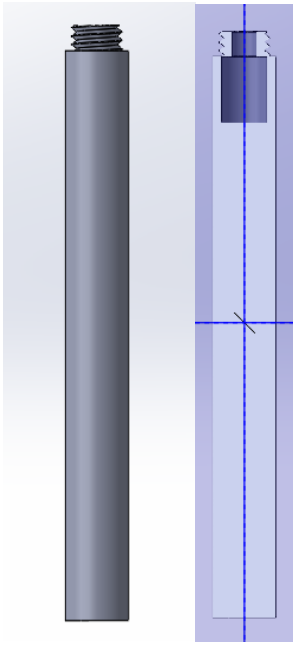

Figure 2.15 Tube of the device

The microbiologist could also be helped a lot by this design, since after the centrifugation of the sample, he/she would be able to collect the sample with a 200 [µL] pipette.

2.2.3 Prototyping.

In order to get to the most viable solution and be sure that the donor will have a good experience, we prototyped the majority of the solutions, or -to be more accurate- the solutions that were more feasible.

At the figure 2.17 below, we can see the solution number 2 that got proposed. The prototyping of this solution helped us understand how user - unfriendly it was, due to the variable diameter. What is more, the thread was not working on this solution for the sealing, hence it was rejected.

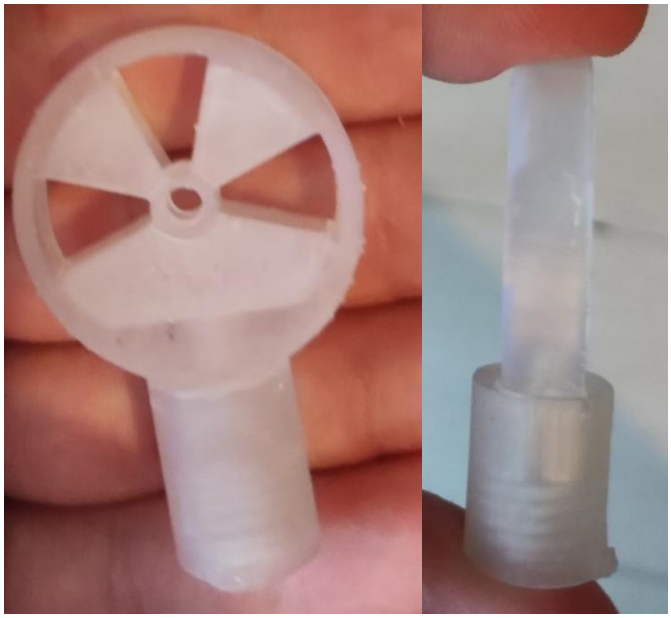

Figure 2.16 Prototype of the second option

Another solution which is shown in figure 2.18, which was assessed while the design of the final solution took place, was one, which resembles the upper picture, but uses a snap-fit mechanism as a locking mechanism. However, the result of this was not aesthetic at all and the collection of saliva was in doubt, hence this solution was rejected as well.

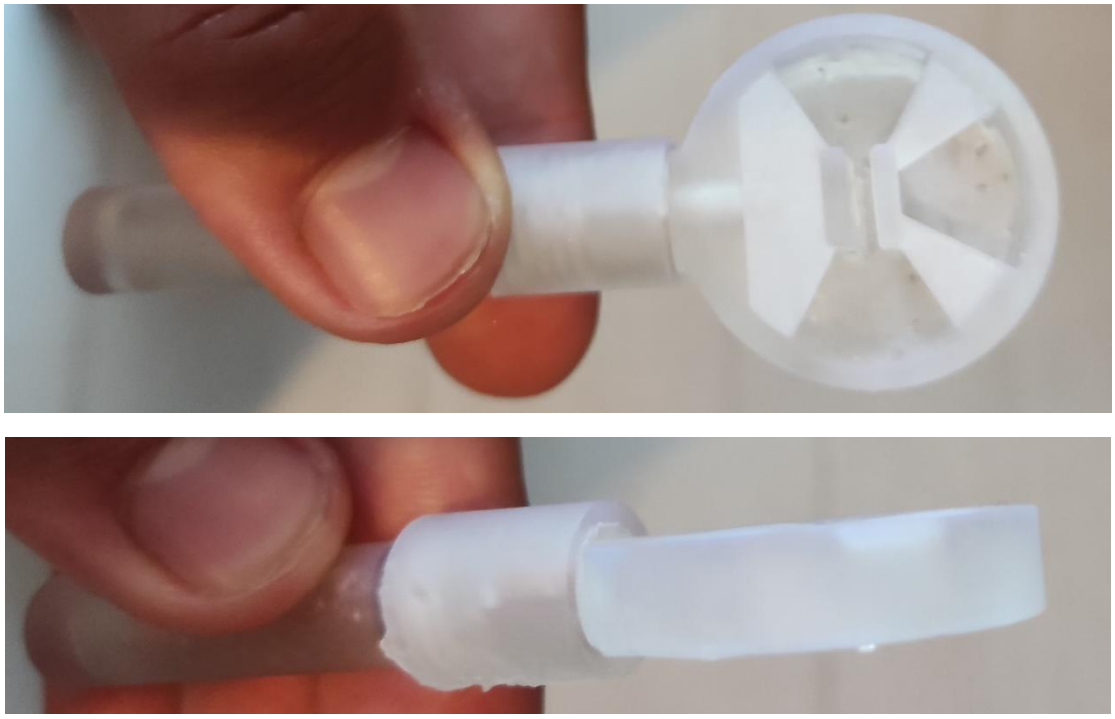

Figure 2.17 Prototype of solution described above

The final design is in the figure 1.5.

This design was based very much on prototyping because it helped us understand the needs of the user and the difficulties that may have been occurred. For example, the protrusion was initially on the surface of the container, but this ended up creating discomfort to the user, when the sealing of the device was about to happen. In the figure 2.19, you can see this solution, where the protrusion is marked with a red circle and the defect that was caused on the surface of the container due to the difficulty in placing the lid.

Figure 2.18 Prototype of the solution with the protrusion on the surface of the device

All in all, the prototyping helped us define and assess the solutions. The final design was initially 3d printed with a normal grey resin (figure 1.5) and then was 3d printed with the use of biomedical resin type 1. This resin was then sterilized so that the experiments could be conducted.

2.3 Locking mechanism of the device

It's obvious that there was a need of a locking mechanism for the device, in order to ensure that the lid would lock with the container, so that the device would be able to get transported to the laboratory. As it was stated in the previous paragraphs, the main parameter was the ease of use from the donor.

2.3.1 Possible Solutions

The first solution that was examined, as stated in the paragraph 2.2.1 was a lid that was using a thread, to ensure that the lid would close. However, this solution was rejected for 2 reasons.

First and foremost, the rotary movement demanded is not easy for the donor. In many cases, he/she would not be able to lock the lid correctly, because the donor wouldn't be able to find the stop, or it would result in a feeling of discomfort.

Furthermore, the resin prototypes couldn't work appropriately, especially due to the small diameter of the thread. Thus, this solution was rejected on its early stages after the prototyping.

2.3.2 Snap-fit mechanism

However, it occurred that a snap-fit solution could be a much easier and user-friendlier solution. The difficulty in this solution was to prototype it with a resin 3d printer. All the snap-fit mechanisms that exist in the market are made by plastics (e.g., ABS) and there was no such a paper, in which the author has made research on this kind of mechanisms produced with resin. This happens because resin is relatively brittle, thus it's not recommended for 3d printed snap-fit mechanisms [8]. But these problems do not apply in our project because the locking mechanism will be used only once by the user.

2.3.2.1 Early stages of the design

At the first stages of the design, there was an effort to find an existing snap-fit mechanism, in order to see the dimensions and the ratios between dimensions. Despite the fact that, the bibliography on already made snap-fit mechanism was not extended, we found a paper [9], in which the authors wanted to alter the dimensions, in order to change the behavior of the mechanism in mass production scale. In the figure 2.20 we can see a typical cantilever snap-fit and on table 2.1 we can see its nominal dimensions.

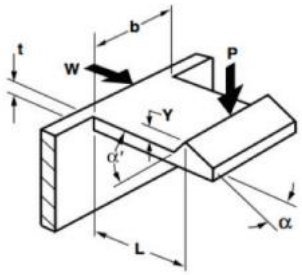

Figure 2.20 Parameters of typical cantilever snap-fit

Parameter	**Nominal Working Dimension**
Lead angle α, [degree]	45.04
Return angle α', [degree]	61.41
Beam thickness t, [mm]	2.00
Beam length L, [mm]	16.73
Deflection Y, [mm]	2.73
Beam width b, [mm]	6.00

Table 2.1 Dimensions of a typical working cantilever snap-fit

Because the geometry of the container was not subjected to change, except of course the middle of it, which was designed for the locking mechanism, it was obvious that we had to scale down these dimensions.

The 3d modelling took place in SolidWorks, and the upper dimensions were scaled down with a factor of 3.

2.3.2.2 From plastic to resin 3d print process

However, these dimensions were designed for plastics (e.g ABS) and not for parts that were aimed to be 3d printed. Nevertheless, we had to prototype the solutions to see if they are feasible and if the donor has a good user experience.

For all the above reasons, it was necessary to convert the properties of the classic plastic snap-fit mechanism into a resin-based structure. For our application, durability was not an important factor, because the user locks the device only once. Furthermore, we used ABS-like resin for the locking mechanism, in order to be as close as possible to the material properties that a normal snap-fit mechanism has. The ABS-like resin has high stiffness and can withstand greater stress and strain than normal resins [10]. What is important, however, is the young modulus of the resin, so that we can find the deflection and if it is inside the permissible limits. The young modulus for the resin we used is 1882 [MPa] according to the official site of Elegoo [11]. We cho~~o~~se to select the lower boundary for the mathematical operations, because the snap-fit was printed vertically, in order to have a better surface without support.

As a next step we already have an initialization from the scaling of the existing geometry and we have the properties of the resin we used, we can set as parameter the beam thickness t, in order to be sure that our resin snap-fit won't break at the bending moment.

For our resin, we have as data:

Permissible strain: ε = 2% [12]

Friction coefficient resin μ = 0.6 [13]

We set a safety factor of 2, hence we want to reach maximum a 1% of strain.

$$t = \frac{1.09 * \varepsilon * L^2}{Y}$$

This equation derives from the applied Castigliano theorem. The result is that the minimum thickness is above 0.35 mm for our scaled device, which complies with the initialization of dimensions that we have, where our beam is 0.67 mm.

After ensuring that our device won't break at elongation due to excessive stress, we continued with the prototyping, in order to find the best solution

2.3.2.3 Steps of the design, Prototyping

Getting to the right geometry was not an easy task. For this reason, we worked using an iterative algorithm for the design process, which is shown on the flowchart below.

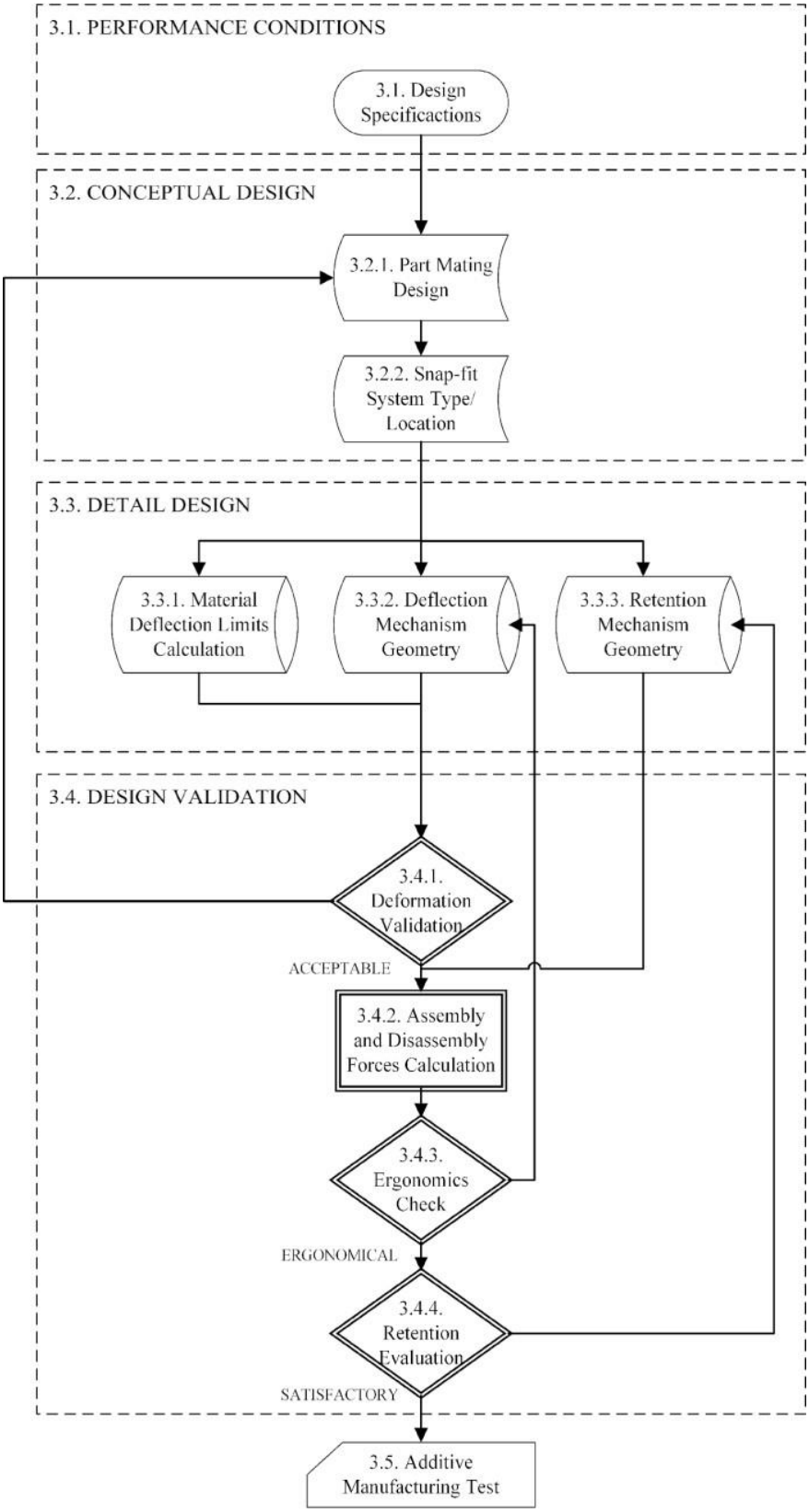

Figure 2.21 Flowchart with the Design process

The prototypes consisted of simple geometries, in order to reduce the printing time and test faster the mechanism. An example of the first prototypes is shown in figures 2.22 and 2.23 below.

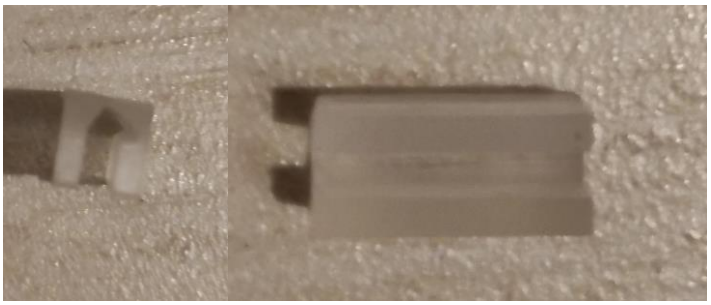

Figure 2.22 Female geometry of snap-fit mechanism produced during prototyping

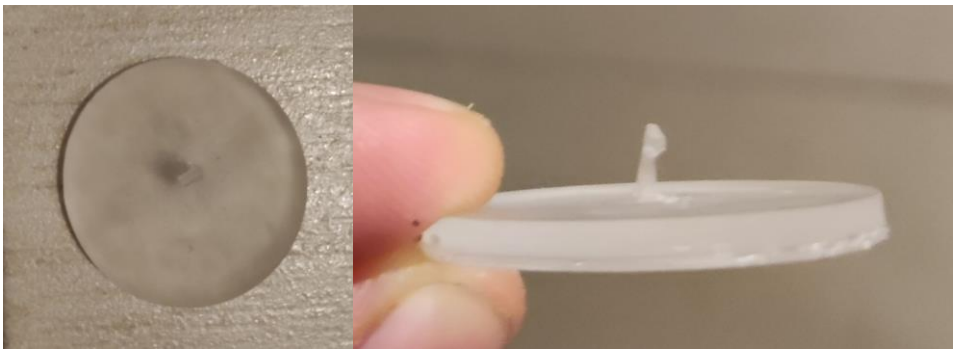

Figure 2.23 Male geometry of snap-fit mechanism produced during prototyping

It was observed that the locking was not that much efficient, as we wished it was, because we wanted that the user would have difficulties in unlocking the mechanism. For this reason, the return angle was set to 90 degrees [14].

Furthermore, as it was previously mentioned, there was a need for a good surface roughness. Thus, the male geometry was vertically placed on the build platform of the resin 3d printer. However, it was observed that the nominal dimensions were different in comparison to the dimensions of the prototype. More specifically, the length (L) of the prototype was supposed to be equal with 5 mm, but it was measured to be equal with 4 mm. After conducting some experiments with 20 samples, and measurements of each sample, it was derived that using this orientation on the 3d printing platform, compressed the male geometry of the snap-fit by 20%. Thus, all the parameters that will be on the chapter 2.3.2.4 of the device have a deviation of 20% from the measured ones.

2.3.2.4 Final design

After following all the steps of the flowchart that were presented in the previous chapter, the final design was the one that is shown in figure 2.24 for the female geometry and on figure 2.25.

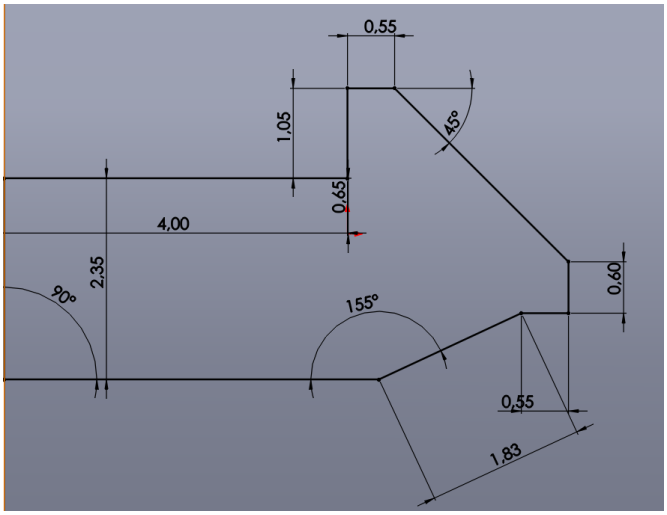

Figure 2.24 Female Snap-fit geometry

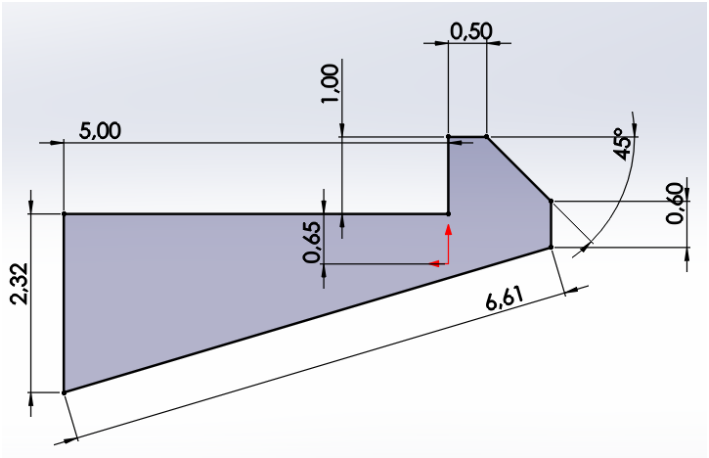

Figure 2.25 Male Snap-fit geometry

Finally, we wanted to calculate the force that the end user must apply, in order to lock the device with the snap-fit mechanism. Hence, we have that the deflection force P is, for b = 6.5 [mm]:

$$P = \frac{b * t^2}{6} * \frac{E * \varepsilon}{L} = 19.95\ [N]$$

Therefore, the mating force W equals with:

$$W = P * \frac{\mu + tan(a)}{1 - \mu * \tan(a)} = 29.92\ [N]$$

It is obvious that this force can be applied by any user, since it's about 3 kgs, hence we understand that even children can use the device.

2.4 Sealing

The sealing of the device was also an issue that concerned us because its transportability was very crucial. To achieve this, we examined 2 solutions. The first one consisted of a classic O-ring solution, while the second one was with a PDMS coating on the top of the lid. Both solutions were feasible, however the O-ring solution was preferred for its better sealing capacity, as the experiments showed. These experiments will be demonstrated on part 3.1.

2.4.1 O-ring sealing

In the first stage, we calculated the deformation that the ring will have due to the applied force by the user, using the Poisson's ratio of the material (rubber). After that, we created a space inside the lid, so that the O-ring could be placed there and attached to it with the help of a glue.

In this point it should be mentioned, that the design of the container changed a little due to the difficulty on sealing the device. Specifically, the existing holes that were in the perimeter of the container on figure 2.26 were moved inside as you can see in figure 2.27, so that they could be sealed. We chose a common 3/4 rubber O-ring for the device, which matches the dimensions of our device.

Figure 2.26 First design of the container with bigger holes

Figure 2.27 Final design of the container

2.4.2 PDMS sealing

The second option was consisted of a PDMS coating. The coating was applied with the spinner WS-650 SERIES SPIN PROCESSOR of Laurel Technologies.

Before preparing the PDMS, we needed to find the most appropriate settings for the spinner. At first, based on the lower diagram [15] of figure 2.28, we chose the time of spinning to be equal with 30 seconds.

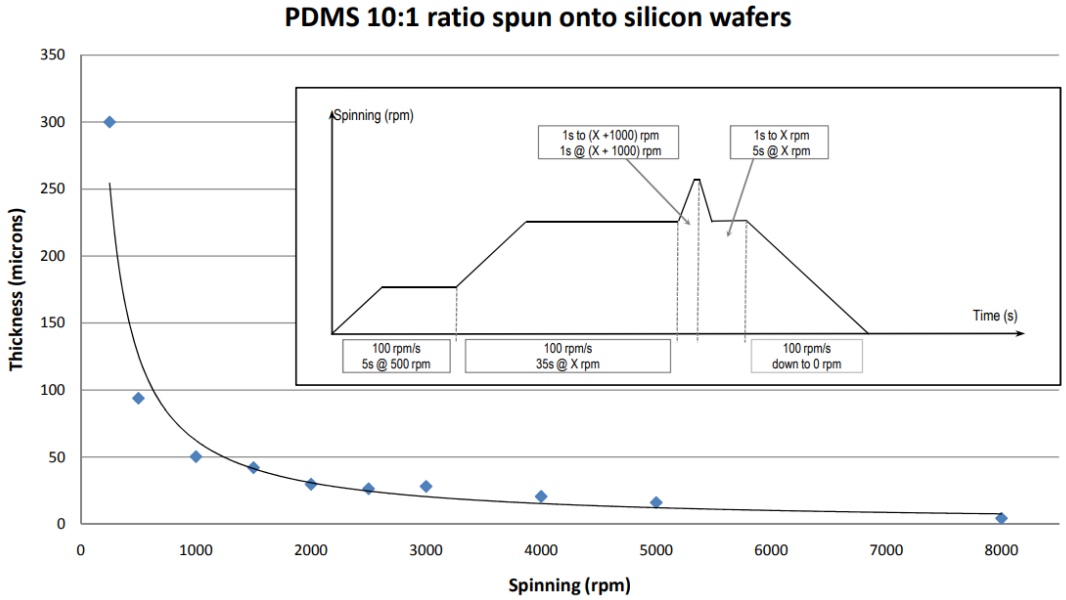

Figure 2.28 Spinning based on time diagram

After setting the time, we had to set the rpm. Based on the rpm, we could define the thickness of the coating in each case. For this reason, we used the diagram [16] below on figure 2.29 to choose the desired thickness.

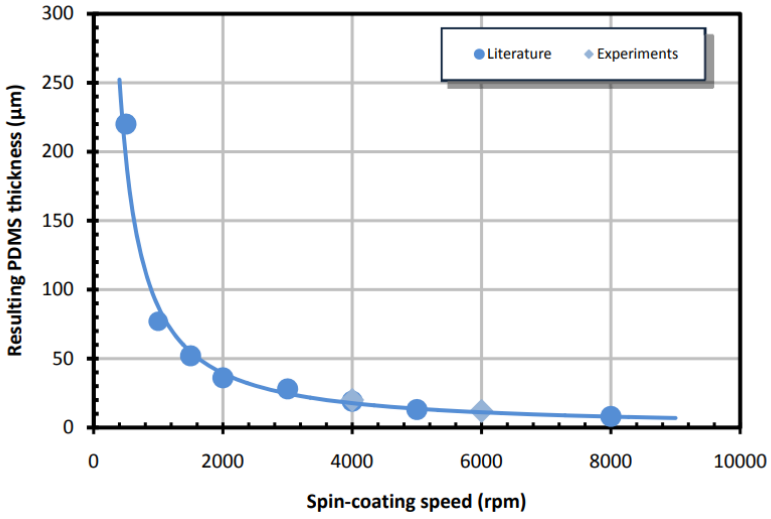

Figure 2.29 Diagram of thickness of PDMS- spin coating speed

Finally, we measured the assembly's height of container and lid and the height of each part separately, to find the dimension of the existing gap.
The results of these measurements are shown on the table 2.2.

Assembly	10,41	mm
Container	8,07	mm
Lid	2,21	mm
Gap	0,13	mm

Table 2.2 Measurements of the assembly

The measured gap was not taking into account the compression of the PDMS layers. For this reason, we decided to create 4 different lids, each of one had a different layer height, in order to find the best solution. The speed of the rpm was set between 200 and 500 rpm. All the alternatives were assessed with the experiments that are shown on chapter 3.1.

3. Experiments and configuration of the device

After finishing with the design parameters and having a prototype, we moved on to the configuration of the device, in order to find if it is in accordance with the specifications. It was necessary to prototype the container of the device with a biomedical resin type 1. Since the resin is certified for it, the donor is allowed to keep the device inside his/her mouth for 30 minutes.

3.1 Sealing experiments

The first experiments had to do with the sealing of the device. They were very significant because their result would determine the type of sealing that we would use. The aim was to find the flow rate that each type of sealing had in different conditions of pressure (measured in [cm] of water) and the equilibrium level for each type, which is the water level, where the flow rate is zero.

3.1.1 Experimental procedures

Before starting the experiment, a pipeline was fabricated from translucent material, so that we can see and measure the water level each time. A volumetric tube of 1 [L] was used as a tank. We connected the one side of the pipeline with the container of the device and its other side was placed inside the tank, as you can see below in figures 3.1 and 3.2.

Figure 3.1 Experimental setup for the sealing. In the middle of the figure, we can see the pipeline

Figure 3.2 Connection of the pipeline with the container

The container of the device was placed inside a small volumetric tube Corning, which you can see in figure 3.3, so that we can measure the flow rate. As it is obvious, every time we wanted to test each sealing, we changed the type of the lid (PDMS layers or O-ring), without changing anything else on the experimental setup.

Figure 3.3 Container inside a Corning tube

After these steps had been completed, we could proceed with the experiment. The water column was set on 46, 77 and 100 [cm] respectively, considering the position of the container as the zero-pressure level. The flow rate was measured in each case in [mL/min]. After finding the flow rate, the first end of the pipeline, which was inside the big volumetric tube, was taken out and was placed in a vertical position as shown in figure 4.4, so that we could find the equilibrium level in each case. All the measurements took place with the help of a timer and a meter.

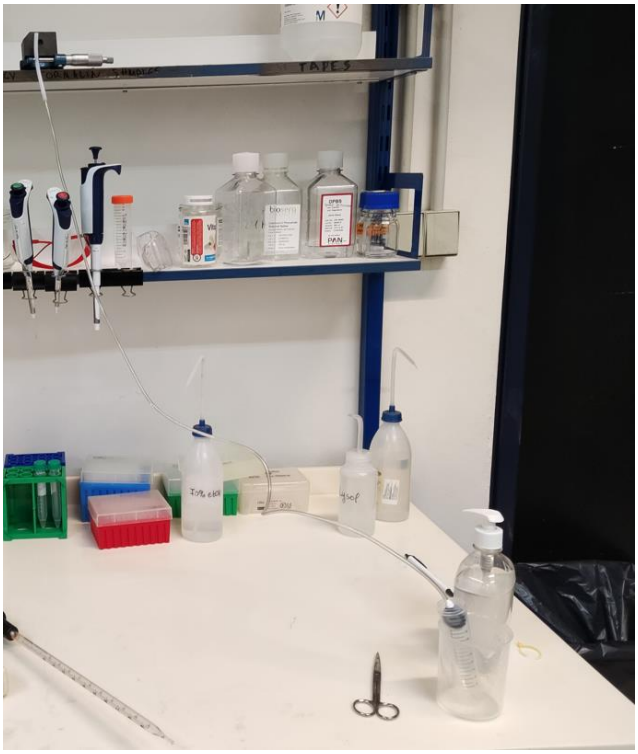

Figure 3.4 Procedure for measuring the equilibrium level

3.1.2 Results

The results of the experiments are shown on the table 3.1 below. It is obvious, that the best sealing is accomplished with the use of the O-ring, and that's the main reason why we chose this as a sealing solution.

Type of Sealing	Equilibrium level [cm]	Flow rate [mL/min]	Pressure [cm of water]
O-Ring	14	20	46
		37	77
		58	100
350 µm PDMS (200 RPM on the spinner)	3	60	46
		85	77
		135	100
200 µm PDMS (300 RPM on the spinner)	5	45	46
		65	77
		110	100
135 µm PDMS (400 RPM on the spinner)	2	68	46
		96	77
		150	100
100 µm PDMS (500 RPM on the spinner)	NONE	87	46
		112	77
		200	100

Table 3.1 Results of sealing experiments

3.2 Establishment of the best solution

The main objective of this experiment was to find the solution between saliva and solvent that should be used to dissolve saliva, so that the measured protein can always be inside the curve. We have done this experiment in order to give guidance to the microbiologist, what the ratio between saliva and solvent should be.

3.2.1 Experimental procedure

The whole procedure is described on the SOP protocol that is on the Annex of this thesis.

What is more, the work order that was followed is also given on the Annex. The work order is the report of the steps made before analyzing the results

3.2.2 Results

To deepen into the results, we need to present the plate used, which is the one presented on figure 3.5.

	1	2	3	4	5	6
A	2000	2000	2000	Sample 1 1:1	Sample 1 1:1	Sample 1 1:1
B	1500	1500	1500	Sample 1 1:2	Sample 1 1:2	Sample 1 1:2
C	1000	1000	1000	Sample 1 1:5	Sample 1 1:5	Sample 1 1:5
D	750	750	750	Sample 1 1:10	Sample 1 1:10	Sample 1 1:10
E	500	500	500	Sample 1 1:20	Sample 1 1:20	Sample 1 1:20
F	250	250	250	Sample 1 1:50	Sample 1 1:50	Sample 1 1:50
G	125	125	125	BLANK	BLANK	BLANK
H	0	0	0			

Figure 3.5 Design Template of our experiment

The 3 first columns are made for the creation of the control line, which shows the boundaries of the concentration of saliva. The columns 4:6 are used for the determination of the best solution of our saliva sample. The sample of saliva is the same in each well, but the solution is different. For example, the 1:50 ratio means 1 mol of saliva to 50 moles of solvent. The block BLANK contains only solvent, which is used for denoising of our result.

The result is shown in the figure 3.6. As we can see in figure 3.7, there is a trendline, which has an $R^2 = 0.99$, which means that the control line is very well calculated.

Blank subtracted	1	2	3	4	5	6
A	1,295	1,243	1,234	0,3453	0,3424	0,3748
B	1,102	1,084	1,010	0,1846	0,1532	0,1974
C	0,7636	0,8016	0,6444	0,1533	0,1088	0,1307
D	0,6616	0,5422	0,6540	0,09279	0,1022	0,1152
E	0,3666	0,3885	0,3812	0,07813	0,06091	0,07349
F	0,2184	0,2155	0,1982	0,04025	0,02359	0,03059
G	0,1281	0,1437	0,1420	-0,001005	-0,0002867	0,001292
H	0,001021	-0,001755	0,003884			

Figure 3.6 Results of the experiment where protein is measured in [μg/mL]

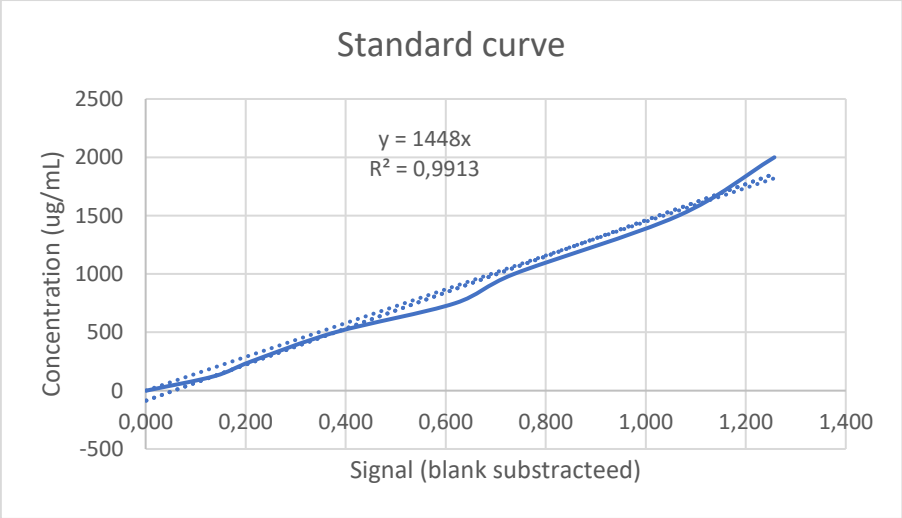

Figure 3.7 Graph of standard curve and the function of concentration-signal

As it is obvious, the best solution is the 1:2. We chose this solution as the best option, because the donor of this saliva has not eaten for more than 12 hours, hence his protein was really low and in previous experiments has been observed that other donors could have a much bigger protein concentration (which was overflowing the curve) if they have eaten one hour prior to the examination. Taking all the above facts into account, we decided that the saliva should be in a ratio of 1 saliva mol: 2 solvent mols. As solvent we used DPBS, as it's obvious on the report in the Annex.

3.3 Collection detection

The main objective of this experiment was to find whether our device collects sample and if so, how much time the subject must place it inside his/her mouth before collection. What is more, we found the settings of the centrifugation of the sample, so that we could ensure that the microbiologist could always collect the sample.

3.3.1 Experimental procedure

The experimental procedure was based on two parts. During the first part, the donor spitted inside the container, in order to find the settings of the centrifuge that would allow us to collect the sample on the chamber of the tube. We started doing this with an initialization at 10000G for 30 minutes, which, according to an article [17], would help us also remove the cellular debris. However, we saw that it was too much for our application and that's why we used an iterative algorithm to find the most appropriate settings.

During the second part of the experiment, we used the device for collection of saliva. The donor would have to put the device into his mouth and use it as the end-user would, so that we can see if and how much saliva we can collect.

3.3.2 Results

As far as the first part of the experiment is concerned, we extracted through many iterations that the best conditions of the centrifuge is to set the time at 2 minutes and the force at 1000G.

For the second part, we managed to find out that the collection is indeed taking place. The results are shown on table 3.2 below. We should however mention that it is necessary for our device to be kept in an horizontal position (figure 3.8) for 45 minutes after the collection, so that the saliva can flow inside with the use of capillary force. After that, our sample is ready for centrifugation.

Sampling time [minutes]	Sample collected [μL]
1	20
2.5	100
3.5	200
5	300

Table 3.2 Sample collected in a specific time

Figure 3.8 Position that the device should be placed

3.4 Biomarkers' detection

In this experiment we tried to find the difference of the concentration of protein between a sample that comes from passive drooling and a sample, where the collection is made with our device. The collection of both samples was made at home, and they were transported the next day to the laboratory, after keeping both samples inside the refrigerator for the rest of the day, before making the transportation. It is also important to mention, that the samples were not placed in a vertical position inside the bag, so that we could simulate the transportation process.

3.4.1 Experimental procedure

The procedure that was followed was based on the same SOP and the same work order that we followed in the chapter 3.2. However, we changed the design of the plate. More concisely, the plate layout was the one in figure 3.9. The sample 1 derives from our device, while the sample 2 from the passive drooling. As it's obvious, we used the solution that we found when we conducted the experiment on chapter 3.2.

	1	2	3	4	5	6
A	2000	2000	2000	Sample 1 1:2	Sample 1 1:2	Sample 1 1:2
B	1500	1500	1500	Sample 2 1:2	Sample 2 1:2	Sample 2 1:2
C	1000	1000	1000	BLANK	BLANK	BLANK
D	750	750	750			
E	500	500	500			
F	250	250	250			
G	125	125	125			
H	0	0	0			

Figure 3.9 Plate layout of this experiment

3.4.2 Results

The results of this experiment are in the figure 3.10. We can see that our samples have a concentration of total protein with an average value of 1.488 [μg/mL] and standard deviation equal with 0.028, which is very small. It is impressive then, that our method shows the same effectiveness in saliva collection as the most established method (passive drooling) and the protein concentration of saliva is almost the same, while we achieve this in a much friendlier way.

Blank subtracted	1	2	3	4	5	6
A	2,116	2,139	2,091	1,485	1,528	1,452
B	1,636	1,685	1,569	1,507	1,492	1,461
C	1,287	1,256	1,244	-0,0008416	0,0006253	0,0002163
D	0,8800	0,9687	0,8065			
E	0,6672	0,6215	0,6536			
F	0,3665	0,3557	0,3380			
G	0,2306	0,2300	0,2352			
H	0,007993	0,005214	0,01135			

Figure 19 Results of the concentration of this experiment

4. Discussion and conclusions

4.1 Current limitations

Although our device has some remarkable attributes, which were analyzed on the previous chapters, there are some limitations, which should be mentioned. These limitations must be overcome in case we want this device to be used in further applications.

The first and bigger problem is the taste of the device. After the 3d printing with the biomedical resin type 1, the device has an odor of plastic, which is repulsive for the end user and deteriorates a lot the user experience. We should mention here that after the sterilization of the device with the use of autoclavable machine (figure 4.1) the odor of resin has been almost disappeared. Nonetheless, if we do want a better user experience, it would be better to apply a taste on our container, which provokes positive feelings, when the donor uses it.

Figure 4.1 Autoclavable machine

Furthermore, it would be recommended that the product should be tested for its toxicity before starting the clinical trials on a large scale. Of course, the resin that was used for the experiments is biomedical resin type 1 that is certified for the ISO 10993-5:2009, which describes test methods to assess the in vitro cytotoxicity of medical devices and for the ISO 10993-10:2021, which specifies the procedure for the assessment of medical devices and their constituent materials with regard to their potential to induce skin sensitization.

Last but not least, the roughness of the surface which is caused from the supports applied on 3d printer, should be improved. One solution would be to change the orientation of the 3d printed device. However, we tested many alternatives about the orientation, none of which worked. The best solution in terms of roughness is the one applied on the final prototype. The surface could be improved with the use of a sandpaper as it was proven through some trials.

4.2 Future Development

4.2.1 Technical

In case we want to develop this product for the market, we should take into consideration some of the following points. As a first step, we should create a cast for injection molding. Injection molding is the recommended solution for a mass production of this device; hence we will have to create the prototype with a better surface roughness, in order to have the better result possible.

What is more, there should be found a flavor enhancer, which should be combined with the injection molding. The solution of a coating is not recommended since the coating might alter the concentration of biomarkers. A suggested solution would be the mold of a material, which is biocompatible and has a good taste, however we are not sure if such a material exists and how much it costs.

4.2.2 From prototype to product

Going from prototype to product demands many aspects, which are not taken into account by many engineers. First and foremost, a fully functional business plan should be demonstrated and ~~be~~ implemented. This demands highly trained personnel, which will take account of all the perspectives of the device.

The first aspect is the biocompatibility of the device. It is very significant that our product is complied with FDA and MDR regulations for type I medical devices. An engineer should be responsible for the validation and the verification of the device. The plastic that is used for injection molding should be carefully selected as mentioned in chapter 4.1 and the taste of the container should be taken into consideration in order to maximize the user experience.

Furthermore, the logistics are an important part. After the collection of saliva, the sample should be stored inside a refrigerator to ensure minimal destruction of biomarkers, in case the sampling doesn't take place in a laboratory. The transport company must collect and deliver the device to the laboratory as soon as possible and of course in a relatively low cost, so as to make the product marketable.

Last but not least, it would be an advantage for our product to measure a certain biomarker and not just the concentration of protein, as we have done in our experiments. This could help to the marketability of the device, and revolutionize at-home-diagnostics, in case the biomarker could be measured with a lateral flow test from the collected sample. The device could also be used for biomarkers' detection, which are not altered through the time and can keep their concentration steady on the sample for a long time regardless from outdoor conditions.

Finally, a patent attorney should be hired to make the research for the Intellectual Property and write a proposal for our product to the European Patent Office.

4.3 Conclusions

As a conclusion for our device, we could mention that it could revolutionize saliva collection and diagnostics. The user-friendly experience that our device can offer, in combination to the existing solutions in the market, can make our product attractive. Especially now, after the covid-19 pandemic, where the need for at home diagnostics is rising, this device could be a useful tool.

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6. ANNEX

1. [TDS] SPL Conical Tube
2. SOP000_BCA_ASSAY
3. Jacob-BCA_Protein_Assay_Biosys_Labs
4. Mechanical Drawings of the device