Self-collected saliva for SARS-CoV-2 detection: evaluation of Copan Lollisponge™

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INTRODUCTION

Starting from February 2020, the SARS-CoV-2 outbreak has caused an unprecedented burden on healthcare system worldwide and testing, tracking and tracing operations were of crucial importance in fighting the pandemic.

The gold standard SARS-CoV-2 detection is based on nasopharyngeal swab (NPS), performed by a healthcare worker, subsequently tested with quantitative reverse transcription polymerase chain reaction (RT-qPCR). Molecular tests on NPS are usually performed within 24 – 36 hours. However, NPS are associated with patient's discomfort or complications as well as an increasing HCW's exposure to SARS-CoV-2[1,2].

Saliva is a non-invasive and attractive solution for SARS-CoV-2 diagnosis and sampling enables self-collection without pain and discomfort. Saliva has inserted in the list of clinical samples to which apply the laboratory tests since recent studies have shown that molecular tests performed on saliva had sensitivity and specificity comparable to those observed with NPS[3,4,5]. Moreover, the self-collection sampling significantly reduces the exposure to HCW by cough or aerosolization during sampling. The value of using saliva for viral RNA detection has been variable between different studies, especially how to concern the type of collection and the samples processing[3].

Copan Italia has developed, in collaboration with the University of Milan, a new medical device that enables saliva collection: Copan Lollisponge™. It consists of sponges able to absorb saliva. Sponges are inserted on a plastic shaft, fixed on the cap, that allow to collect saliva samples without the sample coming into contact with the hands or the tube.

HOW TO USE COPAN LOLLISPONGE

Copan Lollisponge is safe and easy to be used for saliva collection, following sample rules before to start:

- To wait for 30 minutes after eating, drinking smoking or brushing your teeth and
- To wash or sanitize your hands before using the device.

To use the device for saliva collection:

- Open the tube paying attention that sponges do not touch surfaces or parts of the body other than the mouth.
- Kept the device for one minute in the mouth allowing for the sponge to soak pure saliva. It is not needed to bite the sponge or to insert saliva spitting into the test tube.
- After one minute, return sponges into the tube and close the device.

Samples collected in Copan Lollisponge can be stored before testing up to 3 days at room temperature or refrigerated.

HOW TO PROCESS IN THE LAB COPAN LOLLISPONGE™

In the laboratory, the device must be centrifuged for 60 seconds at 450g in order to extract saliva from sponges. After centrifugation, holding the device in a vertical position, take the device and unscrew the cap with the attached stick and sponges. Using a micropipette, mix the specimen at the bottom of the test tube to homogenize and to dissolve pellets eventually formed as effect to the centrifugation step and use it following instruction provided with the assay.

Copan Lollisponge was tested for SARS-CoV-2 detection with the PCR method described by Borghi *et al*[6], the Seegene Allplex SARS-Cov-2 assay and the Cepheid Xpert® Xpress SARS-CoV-2/Flu/RSV[7].

USABILITY AND PERFORMANCE EVALUATION

Volunteers were enrolled in Copan during a weekly, voluntary-based, screening for SARS-Cov-2 detection by rapid antigenic assay. For the usability study, Copan Lollisponge™ was provided to volunteers together with instructions on how to collect saliva samples, asking to fill a questionnaire at the end of the collection procedure. Samples collected during the test were used to investigate average volume of saliva released from sponges.

A total of 100 saliva samples were collected by Lollisponge™, with an average volume of saliva released from sponges equal to 0.524 ml (95% CI 0.452 - 0.596), with a 6% of invalid samples (i.e. dry sponges). Data from the usability questionnaire showed a high acceptability, safety and ease of the self-collection procedure:

- 92% declared no discomfort during the collection procedure (no answer: 7%)
- 97% found the How to Use provided with the device clear
- 96% declared that it was not necessary to ask for additional explanations during the self-collection procedure
- 98% found no difficulties to perform the self-collection procedure described in the How to Use
- 96% declared they preference for the self-collection procedure, instead of HCW
- 100% of people that have children declared the procedure as easy to be performed by a child in a possible national screening program.

During the study, 3 people were found positive for SARS-CoV-2 when tested with the rapid antigen (Biosensor) in use for the company-based screening. Positive results were confirmed when saliva samples collected with Lollisponge were tested using the Cepheid Xpert® Xpress SARS-CoV-2/Flu/RSV.

PERFORMANCE COMPARISON BETWEEN SALIVA AND NPS

In a study performed by the University of Milan[8], authorized by the Ethical Committee, volunteer adult subjects were enrolled at an authorized facility between people were under screening with NPS for SARS-CoV-2 detection. In addition, children and young people were enrolled at V. Buzzi children's hospital in Milan, and with parent agreement, to be tested for SARS-CoV-2. Saliva samples were sent to the central laboratory and processed within 72 hours. Saliva was recovered by centrifugation to release saliva from sponges. For the test, 50 µl of saliva were transferred in a sterile tube and treated with proteinase K, followed by a heat inactivation step at 95°C for 5 minutes. Five microliters were tested by direct amplification as per protocol described by Borghi *et al*[6].

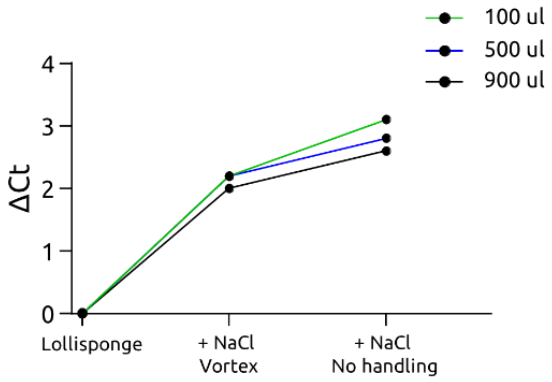
More than 50% of people enrolled were female and an age range of 4-73 years (more the 25% lower than 18 years old). Data obtained from the study showed a 90% of agreement between results from both samples collected, with substantial concordance between data. Discordant results, less than 10% of samples tested, were observed for both sample types (NPS and saliva) and mainly related to people tested at a very early (saliva positive, NPS negative) or late stage (NPS positive, saliva negative). This agrees with articles showing that self-collected saliva testing is effective for COVID-19 detection, especially in early stages of disease progression[9].

PERFORMANCE OF A DILUTION STEP USING SALINE SOLUTION INTO THE COLLECTION TUBE

Test were internally performed to evaluate the impact of saline solution added to the Lollisponge tube to skip the centrifugation step. A negative saliva matrix was spiked with Influenza A virus (ATCC® VR-1679™) to achieve a Ct value of around 20 when tested with Cepheid Xpert FLU/RSV test. Different volume from the spiked saliva matrix were spotted onto the Lollisponge: 100 µl, 500 µl and 900 µl. Each sample volume was tested in triplicate as follows:

- No saline solution into the tube, sample extracted from sponge by a centrifugation step
- 2.5 ml of saline solution added into the tube, sample extracted from sponges by a vortexing step
- 2.5 ml of saline solution added into the tube, sample extracted from sponges by an upside-down inversion step

The average difference between Ct values was 2 – 2.2 and 2.6 – 3.1 respectively for centrifugation vs vortex and centrifugation vs inversion.



As conclusion, the addition of saline solution, as expected, impacts the Ct due to the dilution effect on the sample. In the most conservative scenario, the Ct is delayed by a max of 3 cycles. The Lollisponge is thus confirmed as an extremely robust collection device.

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