High-Performance Swab Enhanced the qPCR Sensitivity in an *in vitro* COVID-19 N-Protein Gene Detection Test

Background

OPT Technologies has developed a new type of additive-manufactured swabs (InstaSwab™) with superior mechanical and technical properties. OPT's platform has the full freedom of defining swab microstructures to achieve desirable performances. The following report evaluates the elution properties of the OPT swabs as compared to leading flocked and polyester swabs for applications in molecular testing.

The properties were evaluated by comparing the qPCR Ct values of known initial quantities of DNA after absorption and elution by the swabs.

Materials

OPT Swabs: InstaSwab™ INS-T4BAA Standard anterior nasal swab
Polyester Swab: Omni Health Solution Specimen Polyester Swab (SKU#LAB-721)
Copan FLOQSwabs: Regular Flocked Swab with 80mm Breakpoint (FLOQSwabs® 502CS0)
Origene SARS-CoV-2 (COVID-19): N protein gene qPCR Template Standard (CAT#: HK212670)

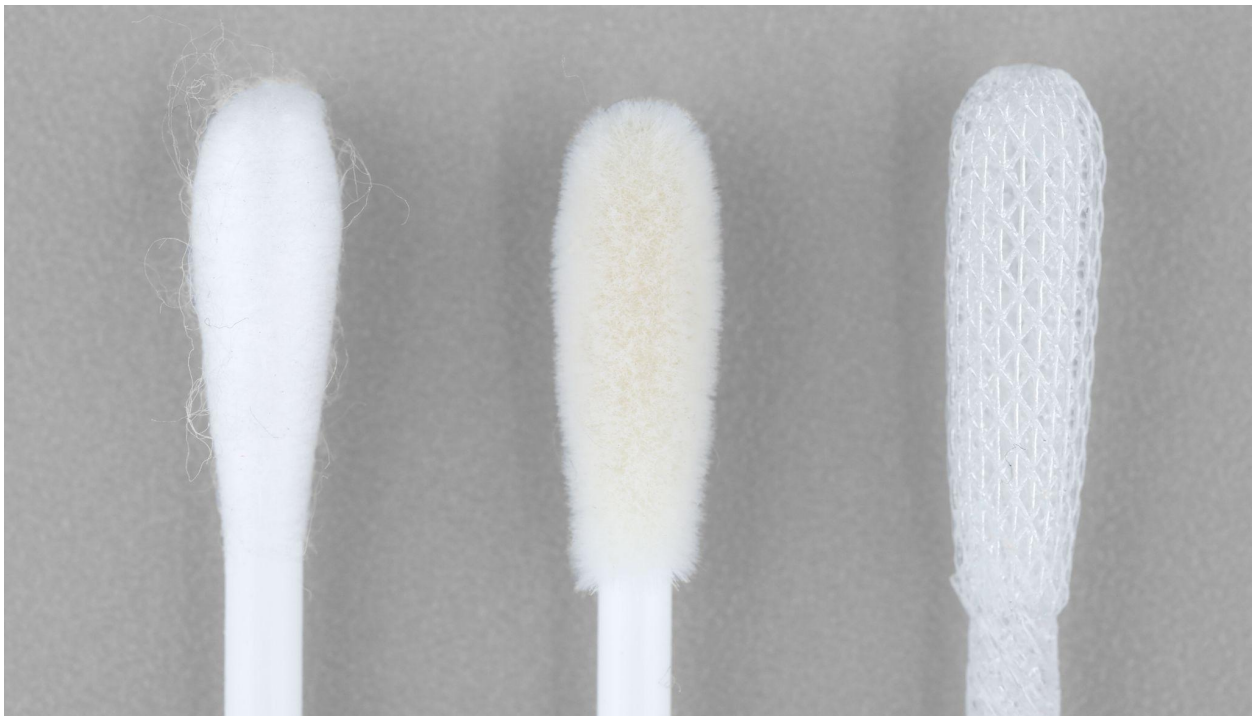

Figure 1. *Photographs of (from left to right) Polyester swab, Copan FLOQSwab, and OPT InstaSwab™.*

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85 Hicks Ave, Medford, MA 02155
info@optindustries.com
www.optindustries.com

Sample Preparation

A vial of lyophilized SARS-CoV-2 (COVID-19) N protein gene qPCR Template Standard containing $50x10^7$ copies of double stranded DNA was received from Origene. The template was reconstituted in 0.5 mL dilution buffer (Origene) to create a stock solution containing $1x10^9$ copies/mL. The stock solution was further diluted using Origene's dilution buffer into 2 mL Eppendorf tubes containing 150 µL aliquots with concentrations ranging from $1x10^8$ to $1x10^2$ copies/mL. Each swab was placed into a tube and gently rotated 10 times. After that, each swab was transferred in a 2mL Eppendorf tube containing 1 mL PBS and placed on a shaker plate for 5 min to perform the elution. The swabs are then removed and the samples are capped. 5µl of each sample was then transferred to a 96-well plate for the assay. Figure 1 shows the entire workflow for the sample preparation.

Assay

The amplification of eluted samples was done with the combination of Origene SybGREEN qPCR primer (CAT#: HP234775) and SensiMix SYBR master mix with Hi-ROX (CAT#: QP100001), following the instructions provided by the manufacturer. DI water that was used for the reagent reconstitution was used as the negative control and the dilution series of the standard template positive control. A Life Technologies 7900 HT Fast Real-Time PCR was used to perform the qPCR with the following conditions 5µl of each of the eluted samples, 1µl qPCR primer pair mixture (10µM), 6.5µL of DI water, and 12.5µL of SYBR master mix] in a96-well plate. The qPCR cycling conditions were as follows: 2 minutes at 50°C, 10 minutes at 95°C, followed by 40 cycles of denaturing at 95°C for 15 seconds, and annealing and extension at 60°C for 1 minute. Data collection was enabled at the annealing and extension step. The melt curve protocol followed with 15 seconds at 95°C and then 15 seconds each at 0.2°C increments between 60°C and 95°C. Data collection was enabled at each increment ofthe melt curve.

PCR Amplification and data analysis

For Ct value over 35, the data was discarded as unreliable.

Results and Discussion

We test the swabs by sampling with each of them from a fixed volume of solutions (150 uL) containing known concentrations of COVID-19 N protein gene. We prepared a series of seven dilutions, ranging from $1x10^8$ to $1x10^2$ copies/mL. Three types of swabs were included in this study. We selected them based on the materials and microstructures they used: polyester tipped swabs, flocked fiber tipped swabs, and OPT InstaSwab™ (Fig. 2).

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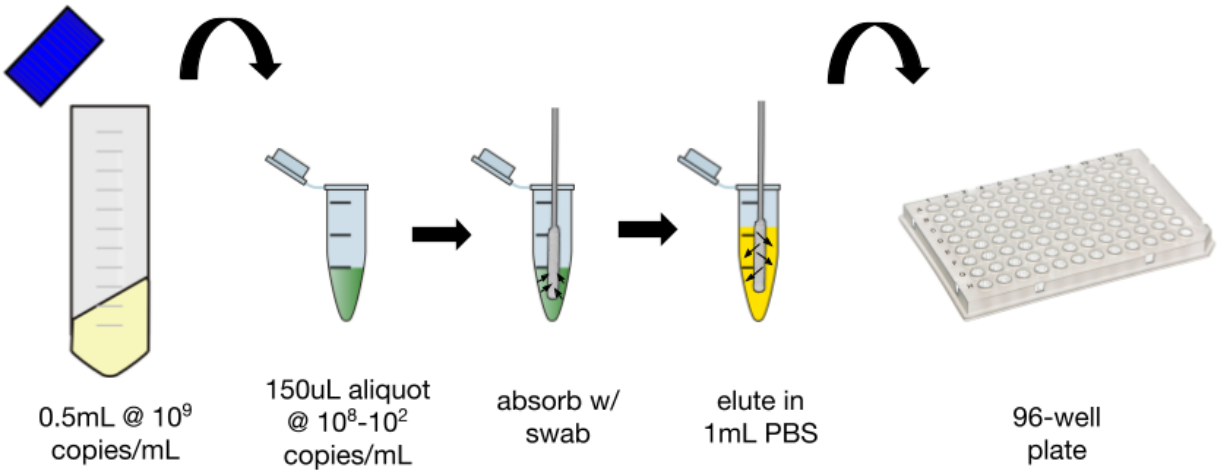

Figure 2. *Schematic of the elution experiment.*

As shown in Fig. 3, when collecting samples from simulants with high viral gene loads ($1x10^8$ to $1x10^4$ copies/mL), all the swabs were capable of eluding enough samples for PCR amplifications. The Ct values for polyester swabs across concentrations were consistently lower than that for the Copan FLOQSwabs and InstaSwabs. Among the three, InstaSwabs showed lowest Ct values, signifying a higher concentration of DNA recovered. InstaSwab™ was the only swab type to produce results at a lower concentration of $1x10^4$ copies/mL.

In order to quantify the elution, samples were run by directly diluting stock solution in the same quantity of elution buffer to represent the ideal case of 100% elution. By comparing against the 100% eluted concentration, we established an average conversion efficiency of 63% for the OPT InstaSwab™, 36% for the Copan FLOQSwabs, and 14% for the Polyester swab. The average elution efficiencies were calculated only for those concentrations within the detectable limit of each particular swab. Notably, at $1x10^4$ copies/mL, when other types of swabs failed to elute enough samples for qPCR amplification, InstaSwab™ had demonstrated an elution efficiency of over 50% (Fig. 4)

These results signify the importance of collection and elution efficiency of swabs for diagnostics. Although PCR is a sensitive method, the limits of detection (LODs) of a specific assay are influenced by multiple factors. Our results show that even within relatively medium concentration of viral load ($1x10^4$ copies/mL), low elution efficiency may result in inadequate concentration of the target in the final analyte, therefore leading to false negative results.

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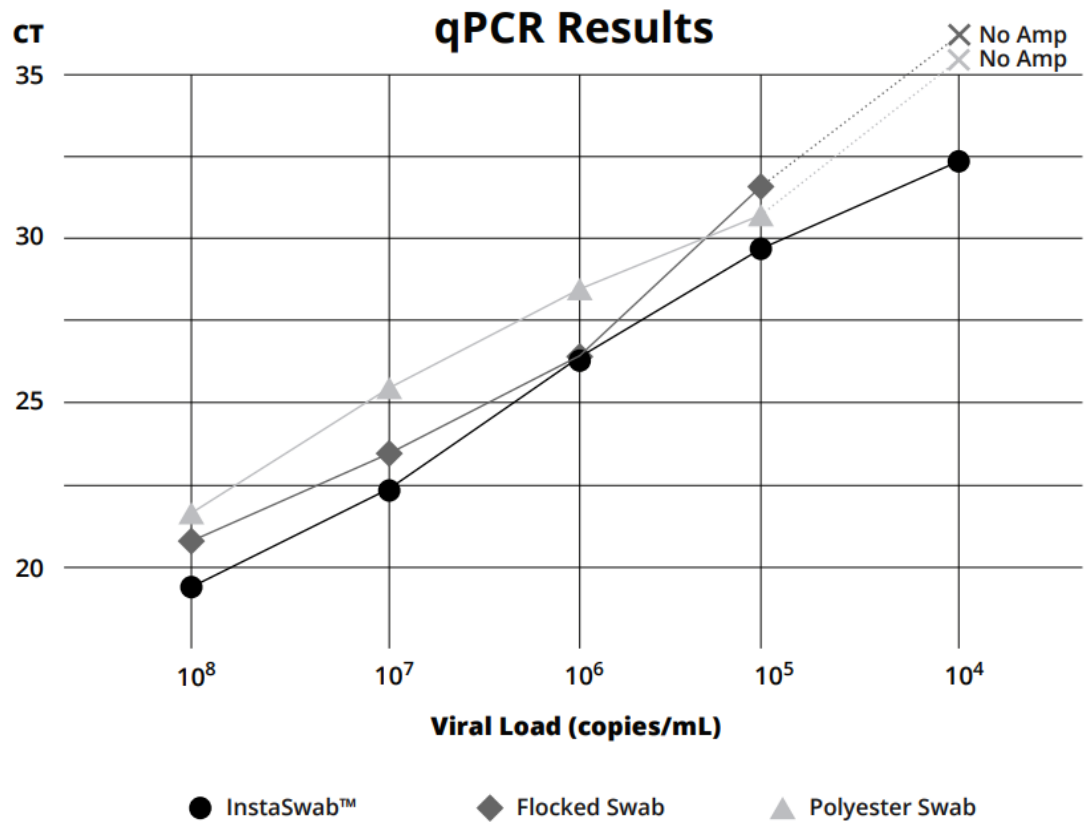

Figure 3. Ct values of qPCR amplification results for different swabs. Note that the viral load refers to the DNA concentrations of the aliquot solutions, from which the swab collected samples. Each data point is the average of 3 independent replicates.

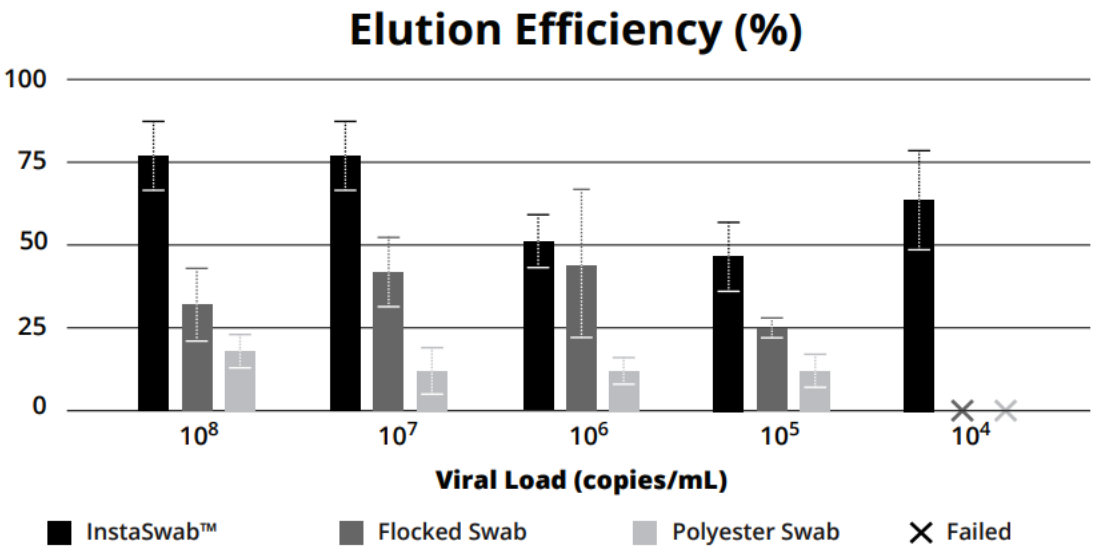

Figure 4. Elution efficiency of the swab.

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Conclusions

Under the experimental conditions in this study, OPT InstaSwabs™ show significantly improved collection and elution efficiency as compared to both Copan FLOQSwabs and more traditional polyester swabs. The InstaSwabs™ enhanced efficiency has the potential to increase the limit of detection and sensitivity of molecular assays by up to an order of magnitude thereby improving assay performance and reducing the likelihood of false negatives.

This report was written by Liangqi Ouyang (OPT Industries, Inc.), Kaveh Milaninia (Planobo), and Matthew Au (Boston University). OPT and Planobo designed the experiments. OPT and BU performed the experiments. All three participated in the data analysis.

Liangqi Ouyang

Senior Materials Developer
OPT Industries, Inc.

Kaveh Milaninia

Founder
Planobo

Matthew Au

Associate Director of the AIC
AIC, Boston University