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3D Printed Alternative to the Standard Synthetic Flocked Nasopharyngeal Swabs Used for COVID-19 testing.

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Summary: A 3D Printed swab was invented to address supply chain disruptions caused by COVID-19 resulting in shortages of the standard synthetic nasopharyngeal swab used for SARS-COV-2 testing. A clinical trial compared the novel 3D printed swab against the synthetic swab.

Abstract

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes COVID-19, can be detected in respiratory samples by Real-time Reverse Transcriptase (RT)-PCR or other molecular methods. Accessibility of diagnostic testing for COVID-19 has been limited by intermittent shortages of supplies required for testing, including flocked nasopharyngeal (FLNP) swabs.

Methods: We developed a 3D-printed nasopharyngeal (3DP) swab as a replacement of the FLNP swab. The performance of 3DP and FLNP swabs were compared in a clinical trial of symptomatic patients at three clinical sites (n=291) using three SARS-CoV-2 EUA tests: a modified version of the CDC Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel and two commercial automated formats, Roche Cobas and NeuMoDx.

Results: The cycle threshold (C(t)) values from the gene targets and the RNase P gene control in the CDC assay showed no significant differences between swabs for both gene targets ($p=0.152$ and $p=0.092$), with the RNase P target performing significantly better in the 3DP swabs ($p < 0.001$). The C(t) values showed no significant differences between swabs for both viral gene targets in the Roche cobas assay ($p=0.05$ and $p=0.05$) as well as the NeuMoDx assay ($p=0.401$ and $p=0.484$). The overall clinical correlation of COVID-19 diagnosis between all methods was 95.88% (Kappa 0.901).

Conclusions: 3DP swabs were equivalent to standard FLNP in three testing platforms for SARS-CoV-2. Given the need for widespread testing, 3DP swabs printed on-site are an alternate to FLNP that can rapidly scale in response to acute needs when supply chain disruptions affect availability of collection kits.

Keywords: SARS-CoV-2, COVID-19, 3D-printed swabs, molecular diagnostics, nasopharyngeal

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), that causes coronavirus disease 2019 (COVID-19), was first described in December 2019, in Wuhan, China and quickly spread globally. The World Health Organization (WHO) confirmed, 282 cases on January 20, 2020 and, as of June 30, 2020, confirmed over seven million cases[1], causing over ~500,000 deaths according to the database from the Center for System Science and Engineering (CSSE) at Johns Hopkins University.[2, 3]

SARS-CoV-2, the seventh coronavirus known to be transmitted between humans, has shown high transmission rates and is stable on surfaces and in aerosols.[4-6] Symptoms of COVID-19 range from mild to severe respiratory illness, with cough, fatigue, and fever being common. It is a progressive and severe disease in certain individuals. Those with underlying medical conditions and the elderly are most at risk of developing more severe disease.[4] In addition, high levels of SARS-CoV-2 shedding in the upper respiratory tract has been reported, even in asymptomatic patients,[7, 8] making individual testing to identify viral carriage even more critical for infection control. Initially, there were no available therapeutics approved for treatment or prevention of COVID-19 [9], however the investigational drug remdesivir received emergency use authorization on May 1, 2020 by the US Food and Drug Administration (FDA).[10] Containment depends on wide-spread testing, isolation of positive cases, and contact tracing.[11] The gold standard for testing respiratory viruses, including SARS-CoV-2, is collection from a patient's nasopharynx (NP) using a standard flocked NP swab (FLNP).[12]

COVID-19 diagnostic testing has expanded exponentially in an international effort fighting the pandemic. However, disruption in the medical supply chain[13] caused by this crisis resulted in test kit shortages, including FLNP swabs.[14] Furthermore, initial phases of the SARS-CoV-2 pandemic coincided with influenza season in many locations, compounding supply chain issues. NP swab global utilization relies on a limited number of manufacturers, who require significant lead time to ramp up production, and created major barriers to testing and containment of the disease. To address supply shortfalls, 3D printing is an effective stopgap technology for medical devices and supplies.[15] To combat critical NP swab shortages, we designed and clinically evaluated a 3D-printed swab (3DP) alternative to the FLNP swab used for nasopharyngeal sample collection.

Methods

3D-Printed Swab Design and collection kits

The 3D-printers selected for 3DP swab manufacturing were the Form2 and Form 3B (Formlabs, USA) due to pre-existing validation and FDA-cleared workflows using an autoclavable surgical grade resin (Surgical Guide, FormLabs) manufactured in an ISO 13485 certified facility. A detailed methodology used to develop the 3DP swab is available in an article in the journal, *3D Printing in Medicine* by Ford et al. [16] These printers are relatively affordable and ubiquitous in hospitals that maintain their own 3D printing labs. Also critical to the viability of this alternative swab, the surgical guide resin is manufactured in the US by FormLabs and the supply was deemed stable to ensure access to raw materials.

Because clinical sites had difficulties obtaining commercially supplied collection kits, 3DP swabs were usually provided with collection media in collection kits assembled in-house. Amongst the media used were WHO approved viral transport media,[17] in-hospital manufactured VTM following the Centers for Disease Control and Prevention (CDC)

recommended procedure for preparation of VTM (CDC SOP# DSR-052-03) [18] or, when available, either VTM or Universal Transport Media provided by commercial manufacturers (*BD*). In-hospital VTMs were manufactured and validated on-site and were accepted by our CLIA laboratories. Individual 3DP swabs were placed in peel-packs along with sterilization indicators and autoclaved. If needed, VTM collection media was packaged in sterile aliquots of 1.5 or 3 ml and provided as collection kits. Sites were also given detailed protocols so that they could produce collection kits on-site.[16]

Bench Lab Testing

To validate sample collection, the 3DP swab was first compared against the FLNP equivalent using respiratory syncytial virus (RSV) as a surrogate RNS virus. 1 ml of synthetic sputum[19] was spiked with 10^6 plaque-forming units (PFU) of RSV. FLNP and 3DPS were dipped in sputum and rotated for 5 seconds. Tips were cut off into 1 ml of *BD* universal transport media (UTM). Each tip was incubated in UTM for 15 minutes at 22° C. 140 µl UTM from each tube was subjected to RNA isolation with *Qiagen* viral RNA mini kit, elution in 50 µl. 10 µl of RNA was used to make cDNA (*iScript, BioRad*) in a 20 ml final volume. A qRT-PCR was performed with 2 sets of RSV primers for the N gene (RSV N Forward: AAGGGATTTTTGCAGGATTGTTT, RSV N Reverse: CTCCCCACCGTAGCATTACTTG) and the SH-G intergenic region (RSV SH-G Forward: TTAACATCCCACCATGCAAA, RSV SH-G Reverse: GCATTTGCCCCAATGTTATT). 2 µl of cDNA per well, in triplicate wells, was used in a 20 µl qRT-PCR reaction using SensiFAST SYBRgreen kit (*Bioline*). Cycle thresholds (C(t)) were recorded for RSV RNA detection.

Leeching tests were performed to ensure 3DPS stability after sterilization and to ensure swab materials did not interfere with downstream testing. *BD* UTM was spiked with

RSV (10^6 PFU/ml) and aliquoted (1 ml) into 1.8 ml cryotubes. Triplicate tubes either contained a sterile 3DPS tip or was left swab-less and incubated at 4° C. Samples (140 µl) harvested at 24, 48 and 72 hours and subjected to RNA isolation and qRT-PCR as above.

Clinical testing

The clinical trial was approved by Western IRB for all sites under an umbrella protocol (No. 20200779). Participants (N=291) presenting for COVID-19 testing were verbally consented by health care providers. Three different medical centers participated in this initial study. Dual paired swabs (FLNP and 3DPS) were collected from each participant following CDC guidelines on COVID-19 NP collections. FLNP and 3DP swab order was not systematically tested. One swab, be it FLNP or 3DP, was used per nostril. The left or right nostril order was randomly selected. All sites printed and processed their own 3DP swabs. Site 1 (Northwell) samples were processed using a modified version of the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic EUA Panel with the N1, N2, and RNase P gene targets.[20] Site 2 (USF-Tampa General Hospital) samples were processed using the CDC assay and the automated Neumodx Sars-CoV-2 Assay (*NeuMoDx Molecular Systems*) which targets viral nonstructural protein 2 (NSP 2) and the N gene.[21] Samples were reported positive if either target was detected. Samples were reported negative if both targets were not detected. A sample was reported as indeterminate if repeated testing did not return as result. Site 3 (Thomas Jefferson University Hospital) samples were also processed using the CDC assay (subset samples for RNAseP only) and the automated Cobas 6800 SARS-CoV-2 assay, which targets the SARS-CoV-2 open reading frame 1 (ORF1) and the E gene (*Roche*).[22] A sample was reported positive if both targets (ORF1 and E) or only ORF1 was detected, and as presumptive positive if only E was detected. A sample was reported negative if both targets were not detected. Samples were recorded as positive, negative or inconclusive and used to calculate agreement between FLNP and 3DP swabs.

Statistical analysis

For bench lab testing, measures of central tendency were calculated for swab RSV detection as well as leeching. For clinical testing statistics, Wilcoxon Signed Rank Tests were conducted for C(t)s for both COVID-19 gene targets for positive cases for every testing platform. For CDC assays, RNase P (RP) were also compared via Wilcoxon Signed Rank Tests. A p-value ≤ 0.01 was considered statistically significant. Percent agreement and Kappa coefficient was also calculated to measure FLNP and 3DP agreement.

Results

3D swab design

Conventional FLNP are used for sampling the NP, requiring enough flexibility to access the NP while collecting sufficient material for diagnostic testing. Materials such as wood or cotton inhibit PCR reactions. The material used must be safe for humans, yet not inhibit down-stream diagnostic testing. The 3DP swab underwent several iterations before establishing a final design. Flocked swab geometry was referenced to influence ultimate 3DP design. Other influences included patient comfort, surface area maximization and the selected 3D-printer's capabilities.

Initial CAD 3DP swab drawings were created in *3-matic* (*Materialise*) with a "cattail" tip design, a rounded nose for patient comfort and lateral alternating nubs to maximize surface area. The total swab length is 150 mm, with a tip length of 15 mm and a 3.85 mm diameter. The neck is 20 mm in length with 1.5 mm in diameter and the shaft is 2.45 mm in diameter with a break-point at 70 mm, dimensions which meet most test tube requirements. The swab is printed without supports with 324-380 per batch (Figure 1). 3DP swab were tested for safety and comfort using volunteers. An independent study compared complications using the standard synthetic flocked swab and the 3DP swab and found they performed equally.[23]

Bench lab testing

To verify the swab material and collection kit VTM did not inhibit PCR reactions, initial lab testing was performed using a validated qRT-PCR assay RSV. It was not possible to directly test the stability of SARS CoV-2 due to lack of access to either a BSL3 facility or live virus. However, RSV is an enveloped RNA virus with a helical nucleocapsid and is biochemically very similar to SARS CoV-2. We used the standard WHO VTM recipe for human samples containing viruses, chlamydia and mycoplasma. Pilot studies examining the qRT-PCR assay sensitivity on RSV in VTM showed that 10^6 PFU/ml gave the best dynamic range to detect changes in viral RNA in the sample. Therefore, the 3D printed swab was tested with this concentration. For swab performance, C(t) values for RSV genome (SH-G) [7.23 ± 1.18 versus 8.04 ± 0.14 ($p = 0.3$, $N = 3$), 3DPS and FLNP, respectively] and N gene [5.89 ± 0.49 and 9.06 ± 0.70 ($p < 0.01$, $N = 3$), 3DPS and FLNP, respectively] were similar. In clinical labs, patient samples can be tested for up to 3 days after collection, so we determined the stability of the 3DPS over 3 days. 3DP leeching tests had C(t) values of RSV N gene for days 1, 2 and 3 were 25.00 ± 0.32, 25.42 ± 0.21, and 24.96 ± 0.58, respectively. Control (swabless vials) C(t) values for days 1, 2 and 3 were 24.79, 25.41 and 24.59 respectively.

Clinical testing

We tested both negative and positive samples at 3 different trial sites in 291 adults (age range 14-94) presenting to our emergency rooms or inpatients requiring SARS-CoV-2 testing (Table 1). CDC assay positive tests where PCR results for both gene targets matched ($N = 51$) showed no significant differences between FLNP and 3DP for either the N1 (FLNP C(t), 27.25 ± 6.02 3DP C(t), 27.85 ± 6.12) or N2 (FLNP C(t), 27.47 ± 5.91 C(t), 28.16 ± 6.18) viral gene targets ($p=0.18$, $p=0.103$ respectively). The 3DP performed significantly better for RNase P gene target detection with FLNP 26.03 ± 1.16, and 3DP 25.57 ± 1.12, (N

= 104, p <0.001). Results for the Roche Cobas assay where both gene targets matched (N = 11) showed no significant differences between FLNP and 3DP for either the ORF1 (FLNP C(t), 22.18 ± 6.02 3DP C(t), 23.26 ± 5.2) or E gene (FLNP C(t), 22.85 ± 5.55 C(t), 23.91 ± 5.63) viral gene targets (p=0.05, p=0.05 respectively). NeuMoDx assay positive tests where both gene targets matched (N = 8) showed no significant differences between FLNP and 3DP for either the NSP2 (FLNP C(t), 25.58 ± 6.36 3DP C(t), 26.64 ± 6.59) or N gene (FLNP C(t), 26.74 ± 6.41 C(t), 27.63 ± 6.29) viral gene targets (p=0.401, p=0.484 respectively). These results are graphically displayed in Figure 2A, B and C with numeric agreement in Tables 2, 3 and 4.

Overall clinical correlation of COVID-19 diagnosis between the two swabs was 95.88% (279/291) with Kappa = 0.901, a high level of agreement.[24] A detailed breakdown of the combined FLNP and 3DP swab agreement is in Table 5. Half of cases where swab results disagreed were inconclusive (i.e. a result could not be reported by the lab) and in a clinical setting would require patient resampling (6 times). There were two cases where the FLNP sample was positive but the 3D swab was negative. There were four cases where the FLNP sample was negative but the 3D swab was positive. In general, discordant cases were ones where C(t) values were high and less viral material was detectable. Throughout the trial's course, scant nasal bleeding after completion of both swabs was reported but no clinical intervention was needed in any of those cases and no other adverse reactions or breakage of either FLNP or 3DP swabs were reported.

Discussion

The SARS-CoV-2 pandemic created a burden on healthcare systems worldwide and interrupted supply chains used to fight against COVID-19. Diagnostic testing is critical to the response to the novel coronavirus. Current business models operate on "just-in-time" supply chain models, relying upon readily available supplies and delivery. Thus, stockpiling reagents

and supplies is atypical. Unfortunately, this efficient business model for production of FLNP swabs was disrupted by the SARS-CoV-2 health crisis, and manufacturers were unable to respond to the sudden world-wide surge in demand for FLNP.

3D-printed technology provides an alternate strategy for swab shortages by facilitating a local solution to FLNP shortages. The 3DPS displayed statistically identical results to standard FLNP in a head-to-head clinical trial, making it a viable option in testing for SARS-CoV-2 infections. The 3DP, which has a more rigid brush-like head compared to the standard FLNP, likely collected additional epithelial cells which may increase sensitivity in sample collections as seen by the 3DPS RPP gene C(t) values, which were significantly better than standard FLNP swabs. The fact that the FLNP and 3DPS comparison results were the same using three different assay methods (CDC assay, NeuMoDx and *Roche* Cobas) illustrates that the 3DP can be used reliably across platforms. At the time of this publication, Northwell Health moved to the Panther System (*Hologic*) for their clinical testing.

The 3DP swab was innovated to offset standard FLNP shortages not only at our local facilities but with the intention to share the design with any site throughout the world where 3D printer capabilities may be established. As 3D-printing allows for design alterations to be rapidly put into production, several iterations in swab design have been created and readily available by partner institutions. For example, the current swab being produced by Northwell contains a brush diameter that is 10% smaller compared to the USF swab. Furthermore, several other groups have utilized 3DP swabs in place of FLNP swabs. One group examined 160 designs from 48 different materials from 48 manufacturers, focusing on four designs in a clinical trial.[25] One of their main findings was to balance tip design to maximize surface are while balancing it for patient comfort. 3D printing technologies used in that clinical trial are not as readily available as the Form 2/Form 3B printer utilized in this study.

Additionally, material costs of an individual 3DP swab ranges from $0.25-$0.46 depending on whether an institution has an in-house printing team and sterile processing unit with the necessary equipment and materials. Comparatively, commercial FLNP swabs cost approximately $1.00 per unit. Regarding the effort required to print and process 3DP swabs, sites were able to shift staff with available extra capacity due to decreased workloads caused by a decrease in surgical caseloads. This further illustrates the role the 3DP swab can play in filling the supply gap that is cost efficient and rapidly deployable. The initial cost of a Formlabs system is approximately $3,600 (Form 2) to $6,000 (Form 3B). This includes washing and curing stations for post processing. Each Surgical Guide resin cartridge is $249. Print times range from 11-15 hours per batch.

Our clinical trial showed that 3DP swabs were an efficient and reliable alternative to standard FLNP swabs. The data supports the feasibility of medical teams across the US to deploy 3DP swabs at local sites allowing for rapid local production and distribution that can utilize the same production designs and protocols, facilitating supply maintenance and data comparison. FLNP swabs are also routinely used to collect nasopharyngeal samples testing for other respiratory viruses including influenza and RSV. Our preliminary results suggest that 3DP may be used for diagnosis of other respiratory viruses, but our clinical study was conducted when the incidence of these infections was low in our respective geographic regions, so future studies will need to be conducted to evaluate whether 3DP swabs can be used for all indications that FLNP swabs are used. If so, it is possible that 3DP swabs may be deployed in under resourced or remote settings to enable local manufacturing of swabs.

Some study limitations include sample size and lack of pediatric testing. As this study was developed in response to a critical shortage of testing swabs, our hospitals were unable to provide large FLNP swab samples to be used for clinical trial. Once we were able to

demonstrate the validity of the clinical results, the data was presented to the hospitals' advisory committees and were accepted as standard of care swabs due to the crisis. Additionally, this trial focused on adults. While a pediatric version of the swab was created, the current clinical trial only included one subadult.

As a result of this study, all three sites and others participating in our clinical trial have moved forward using 3DP swabs as the alternate to the standard of care swab in patient testing when commercial swabs and kits are not available. As demand for swabs increases, validated designs, such as the one described here, can be designed on multiple printing platforms, assisting healthcare facilities worldwide with diagnosis testing for SARS-COV-2. In addition, combining in-house UTM production[17, 18] and 3DP swabs may provide an effective, cost-efficient, and fast alternative to standard FLNP and viral transport media kits used for COVID-19 testing and with the potential to alleviate a major supply chain hurdles, while increasing testing capabilities.

NOTES

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Role of funding source

No funding was received to perform this study.

Conflict of Interest

Drs**.** Decker, Ford, Goldstein, Hazelton, Kim, Sinnott, and Teng hold the provisional patent for 3D-Printed Swab for Diagnostic Testing.

Dr. Kim serves on the Editorial Board of the Sanford Guide to Antimicrobial Therapy and is site director for COVID-19 clinical trials funded by Regeneron, Romark and Abbott.

Dr. Wilson reports grants and personal fees from Gilead Pharmaceuticals, Pfizer, Janssen, and Portola outside of the submitted work.

All other authors have no potential conflicts to disclose.

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Tables

Table 1: Site Demographics

Site	Male	Female	Age Range
Northwell Health	47	57	14-86
Jefferson Health	45	56	20-86
Tampa General Hospital	43	43	19-94

Table 2: Table of Agreement for CDC Assay

		3DP +	3DP -	3DP inconclusive	Total
FLNP	+	**54**	0	2	56
FLNP	-	2	**50**	0	52
FLNP	inconclusive	0	2	**0**	2
Total		56	52	2	110

*** 94.55% Agreement, Kappa 0.895**

Table 3: Table of Agreement for Roche Cobas

		3DP +	3DP -	3DP inconclusive	Total
FLNP	+	**11**	2	0	13
FLNP	-	1	**87**	0	88
FLNP	inconclusive	0	0	**0**	0
Total		12	89	0	101

*** 97.03% Agreement, Kappa 0.863**

Table 4: Table of Agreement for NeuMoDx

		3DP	3DP	3DP	Total
		+	-	inconclusive	
FLNP	+	**9**	0	1	10
FLNP	-	1	**66**	1	68
FLNP	inconclusive	0	0	**2**	2
Total		10	66	4	80

*** 96.25% Agreement, Kappa 0.867**

Table 5: Table of Agreement for All Methods

		3DP	3DP	3DP	Total
		+	-	inconclusive	
FLNP	+	**74**	2	3	79
FLNP	-	4	**203**	1	208
FLNP	inconclusive	0	2	**2**	4
Total		78	207	6	291

*** 95.88% Agreement, Kappa 0.901**

Figures and Figure Legends

Figure 1. 3D Model of 3DP swabs and two batches of 324 swabs

Figure 2. Graphical comparisons of the FLNP and 3DP C(t) targets for A) CDC Assay, B) Roche Cobas and C) NeuMoDx.

Figure1

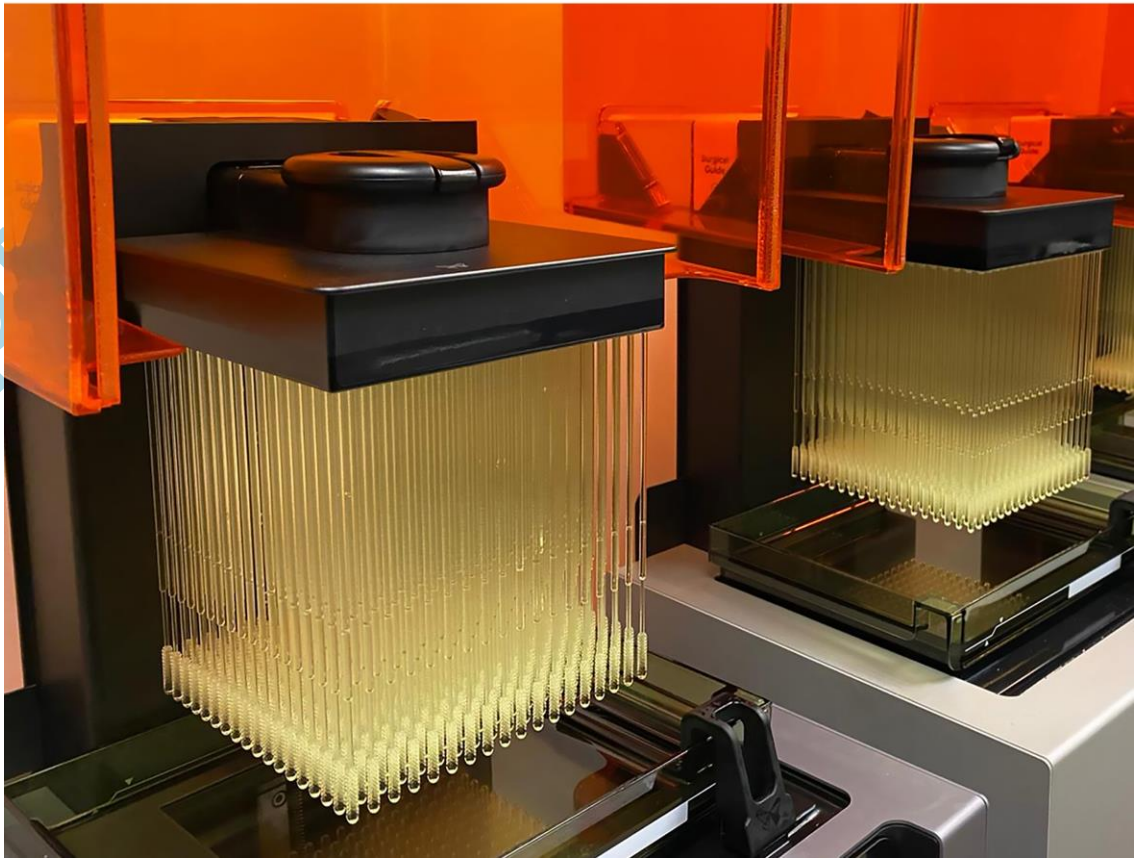
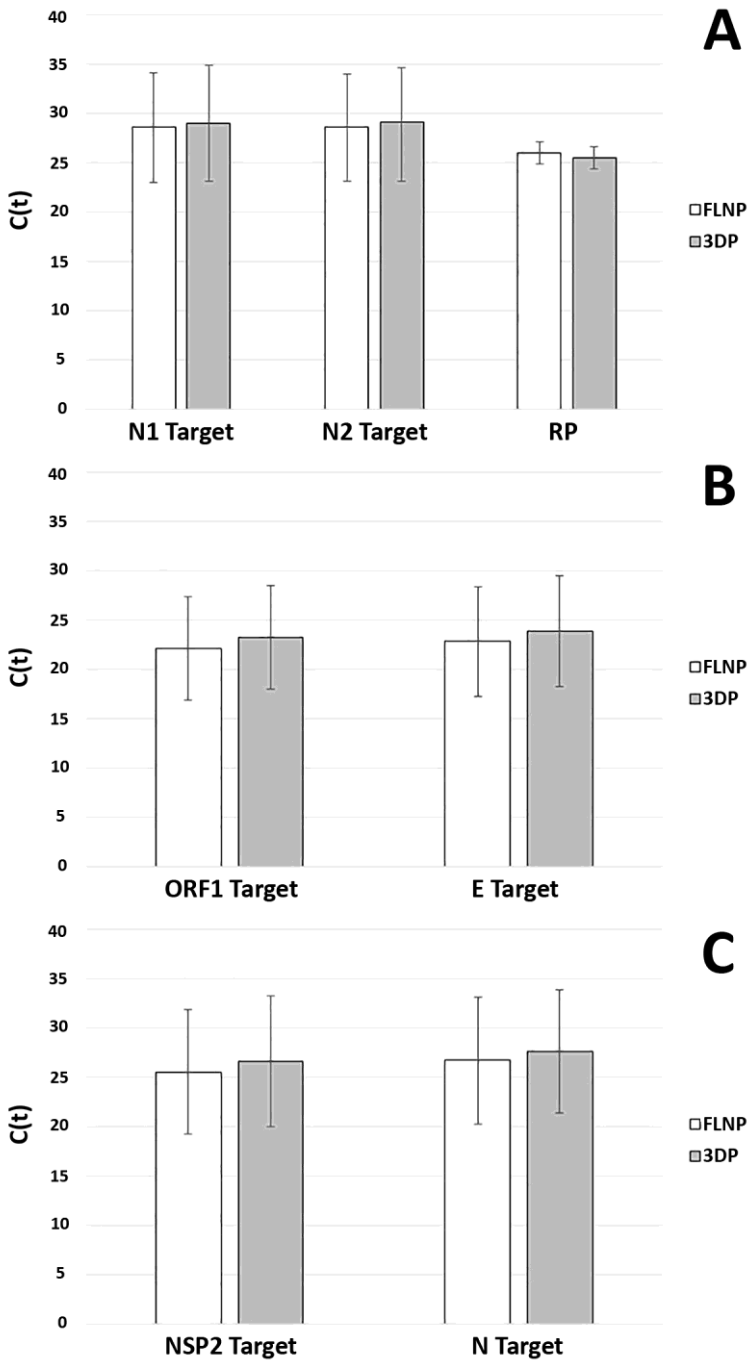


Figure2


A
40
35
30
25
20
15
10
5
0
C(t)
N1 Target
N2 Target
RP
FLNP
3DP
B
40
35
30
25
20
15
10
5
0
C(t)
ORF1 Target
E Target
FLNP
3DP
C
40
35
30
25
20
15
10
5
0
C(t)
NSP2 Target
N Target
FLNP
3DP