Prospective study comparing deep-throat saliva with other respiratory tract specimens in the diagnosis of novel coronavirus disease (COVID-19)

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Article Summary Line: Deep-throat saliva as a diagnostic specimen for COVID-19 has the advantage of being self-collected to minimize infectious exposure to healthcare workers. However, it is suboptimal in sensitivity. False-negative results may pose adverse impact on patient management and outbreak control.

Abstract--

Background

Self-collected specimens has been advocated to avoid infectious exposure to healthcare workers. Self-induced sputum in those with a productive cough, and saliva in those without a productive cough have been proposed, but sensitivity remains uncertain.

Methods

We performed a prospective study in two regional hospitals in Hong Kong

Results

We prospectively examined 563 serial samples collected during the virus shedding periods of 50 patients: 150 deep-throat saliva (DTS), 309 pooled-nasopharyngeal (NP) and throat swabs, and 104 sputum. DTS had the lowest overall RT-PCR positive rate (68.7% vs. 89.4% [sputum] and 80.9% [pooled NP and throat swabs]), and the lowest viral RNA concentration (mean log copy/mL 3.54 vs. 5.03 [sputum] and 4.63 [pooled NP and throat swabs]). Analyses with respect to time from symptom onset and severity also revealed similar results. Virus yield of DTS correlated with that of sputum (Pearson correlation index [95% CI]: 0.76 [0.62 – 0.86]). We estimated the overall false-negative rate of DTS could be 31.3%, and increased 2.7 times among patients without sputum.

Conclusion

DTS produced the lowest viral RNA concentration and RT-PCR positive rate compared to conventional respiratory specimens in all phases of illness. Self-collect sputum should be the choice for patients with sputum.

Keywords: COVID-19; SARS-CoV-2; coronavirus; novel infectious disease; saliva; diagnosis

Introduction

Since December 2019, the novel coronavirus disease (COVID-19) put healthcare systems all over the world under unprecedented stress. While an all-round control strategy consists of border control, quarantine measures, isolation, social distancing and contact tracing; an early case detection by sensitive laboratory diagnosis test is indispensable to achieve an effective control of pandemic [1].

International authorities recommend laboratory diagnosis of SARS-CoV-2 infection should base on real-time PCR (RT-PCR) detection of viral RNA in respiratory specimens [2, 3]. Conventional respiratory tract specimens consist of nasopharyngeal swab or aspirate, throat swab, sputum, and tracheal aspirate. However, collection of respiratory tract specimens carries a risk of inducing cough, and thus poses additional risk to healthcare personnel. Since COVID-19 patients do not always have productive cough [4], other types of self-collect specimens have been explored [5-8].

Saliva is a self-collect specimen. The collection process is non-invasive. It has long been explored for diagnosis of viral infections including HIV, hepatitis C, and dengue fever; but with variable degrees of success [9-12]. The diagnostic value of saliva for SARS-CoV-2 has recently been examined. Posterior oropharyngeal saliva was shown to be a good alternative to nasopharyngeal specimens for diagnosis [5-8], and for monitoring of viral load [13, 14].

While positive results aid prompt treatment and isolation, false-negative results carry a risk of inadvertent spread of infection in the community [15]. Here, we evaluated the diagnostic performance of deep-throat saliva (DTS) by comparing the positive rates and viral RNA concentrations with that detected with conventional respiratory specimens collected from confirmed cases of SARS-CoV-2 infection.

Materials and Methods

We performed a prospective study in two regional hospitals in Hong Kong. All recruited patients had SARS-CoV-2 infection confirmed by two RT-PCR targeting different regions of the RdRp gene performed respectively by the local hospital and Public Health Laboratory Service. Asymptomatic patients included in our study were admitted for isolation after testing positive for SARS-CoV-2 through active case finding as part of border control measures or contact investigations. All patients provided informed consent. The Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee approved the study. The study was registered with NCT (NCT04325919) database.

We collected serial conventional respiratory tract specimens including sputum and pooled nasopharyngeal (NP) and throat swabs. In addition, patients were asked to self-produce "saliva" according to a specific instruction to collect "deep-throat" secretions. Early morning samples before tooth brushing and breakfast were preferred. Patients were instructed to refer to a video tutorial produced by the Centre for Health Protection, HKSAR [16]. In brief, patients first cleared their throat by gargling with their own saliva, and then spitted out the DTS into a sterile bottle. Sputum samples were self-collected. Patients were asked to cough out sputum, and spitted into a sterile plastic bottle. Pooled NP and throat swabs were collected by healthcare workers under strict infection control precautions, using flocked swabs (FLOQSwabs, Copan, Italy) contained in a sterile bottle with viral transport medium.

RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). SARS-CoV-2 RNA was detected and quantified by real-time reverse-transcriptase polymerase chain reaction (RT-PCR), with primers and probes targeting the N gene of SARS-CoV-2 as described previously [17, 18].

The primer-probe set N1 (2019-nCoV_N1-F: 5'-GAC CCC AAA ATC AGC GAA AT-3', 2019-nCoV_N1-R: 5'-TCT GGT TAC TGC CAG TTG AAT CTG-3' and 2019-nCoV_N1-P: 5'-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3') were purchased from Integrated DNA Technologies, USA. The one-step real-time RT-PCR reaction contained 5 μL of the extracted preparation, 4 μL of T*aq*Man™ Fast Virus 1-Step Master Mix (Applied Biosystems, USA) in a final reaction volume of 20 μL. The primer and probe concentrations were 0.5 μM and 0.125 μM, respectively. The cycling conditions were set at 25°C for 2 min, 50°C for 15 min, and 95°C for 2 min; followed by 45 cycles of 95°C for 15 s, and 55°C for 30 s, and performed using the StepOnePlus Real-Time PCR System (Applied Biosystems, USA). The cycle threshold (Ct) values of real time RT-PCR were converted into viral RNA copies based on a standard curve prepared from 10-fold serial dilutions of know copies of plasmid containing the full N gene (2019-nCoV_N_Positive Control, Integrated DNA Technologies, USA). Samples were considered as negative if the Ct values exceeded 39.9 cycles. The detection limit of real-time RT PCR was 694 copies/mL.

According to the policy at that time, all patients were required to remain in isolation care until two consecutive RT-PCR-negative respiratory specimens were obtained at least 24 hours apart. We defined the respiratory viral shedding period as the period inclusive and between the first and last respiratory specimens tested positive by RT-PCR. Clinical severity was classified as previously described to mild, moderate, severe, and critically ill [4, 18]. SARS-CoV-2 viral RNA concentration data and positive rates were analyzed in relation to symptom onset date. For the purpose of analysis in this study, the day of diagnosis was taken as symptom onset for asymptomatic cases.

Data were presented as percentage, mean or median. Comparisons of positive rates and viral loads between groups were performed using Mann-Whitney U test and Wilcoxon signed-rank test as appropriate, with $p \leq 0.05$ considered as statistically significant. False-negative rate was calculated by

number of negative samples for each specimen type over the respiratory viral shedding period divided by the number of each type of specimen collected over the same period.

Results

Study patients

Between February 6 and April 10, 2020, a total of 50 patients, including 27 females and 23 males aged between 16 and 72 years old were recruited. One patient had critical illness, two had severe disease, 14 had moderate disease, 31 had mild disease, and two were asymptomatic.

Specimens examined

A total of 563 specimens collected within the respiratory shedding period of the corresponding patients were included for analysis. Specimens collected ≥ 23 days after symptom onset were excluded from this study, as those specimens were dominated by a few prolonged shedders. Each patient provided 2 to 32 specimens. All patients provided deep-throat saliva specimens (DTS) (range 1-10 per patient) and pooled NP and throat swabs (1-16 per patient). In addition, 26 patients provided sputum specimens (1-9 per patient). Samples were collected between one to 22 days (mean, standard deviation [SD]: 11.0 ± 5.3 days) after symptom onset. The mean number (standard deviation [SD]) of all types of samples provided by each patient was 11 ± 7.

SARS-CoV-2 RT-PCR positive rate

The overall RT-PCR positive rate for all specimen types combined was 79.2% (446/563). Sputum showed the highest positive rate of 89.4% (93/104), followed by pooled NP and throat swabs (80.9%, 250/309) and DTS (68.7%, 103/150).

When the whole viral shedding period was considered, DTS showed the lowest RT-PCR positive rate per individual patient compared to those of sputum and pooled NP and throat swabs (mean positive rate, 95% confidence interval [CI] of DTS: 72.3% [62.6% - 81.8%]; sputum: 91.7% [83.8%- 99.6%]; pooled NP and throat swabs: 82.6% [76.7% - 88.6%] (Figure 1A). Further subgroup analyses according to specimen collection periods of early (1-5 days), mid (6-10 days) and late (11-15 days) phases after symptom onset also revealed that DTS provided the lowest positive rate compared to sputum and pooled NP and throat swabs throughout specimen collection periods (Figure 1B). The positive rate of DTS relative to sputum and pooled NP and throat swabs decreased progressively with time, and the differences reached statistical significance in mid (6-10 days) and late (11-15 days) phases after symptom onset (Figure 1B). We did not include samples collected beyond Day 15 from illness onset for this time interval analysis due to the small sample size.

DTS viral RNA concentration and disease severity

The viral RNA concentration detected from DTS of all patients combined (mean [95% CI]: 3.54 [3.09 – 3.99] log copy/mL) was significantly lower than those of sputum (5.03 [4.55 – 5.52], $p < 0.001$) and pooled NP and throat swabs (4.63 [4.30 – 4.97], $p < 0.001$) (Figure 2A). Although the difference between sputum and pooled NP and throat swabs did not reach statistical significance ($p = 0.24$). Further subgroup analyses according to clinical outcome revealed a similar pattern among patients with mild and moderate diseases, for whom the positive rates of DTS remained the lowest compared to sputum and pooled NP and throat swabs. Of note, among patients with severe disease, no

significant differences between DTS and other specimens were observed. Although the positive rate of DTS was still lower than that of sputum; whereas patients with severe disease had significantly higher viral load in sputum when compared with pooled NP and throat swabs (6.83 [5.41 – 8.23)] vs 4.45 [3.39 – 5.52], $p = 0.039$) (Figure 2B).

Head-to-head viral RNA concentration comparison

In order to provide a head-to-head comparison on viral RNA concentrations among specimen types, we focused at specimen sets with all three types of specimen collected on the same day from the same patient, hence referred as "synchronized" specimens. A total of 52 synchronized specimen sets were collected from 21 patients whom provided 52 sputum, 65 pooled NP and throat swabs, and 53 DTS samples. Among these 21 patients, six provided more than one specimen of the same type on the same day. These specimens were collected between 4 and 22 (mean ± SD: 11.7 ± 5.4) days after symptom onset, and were grouped into four time frames (Days 1-5, 6-10, 11-15, and 16-22) for the purpose of analysis. The peak of viral RNA concentration was observed on day 6-10 for all specimen types, and the differences with other time intervals were statistically significant (Figure 3). These synchronized specimen sets reproduced the previous observation that DTS had the lowest RT-PCR positive rate compared to sputum and pooled NP and throat swabs in all four time-intervals examined.

Pearson correlation index showed DTS had higher correlation to sputum (0.76: 95% CI 0.62 – 0.86) than to pooled NP and throat swabs (0.67; 95% CI 0.48 – 0.79) (Figure 4A). The correlation between DTS and sputum dropped over time from day 1-5 (0.83: 95% CI 0.22-0.97) to day 16-22 (0.42: 95% CI 0.14 – 0.79) (Figure 4B). Of note, sputum also exhibited a good correlation with pooled NP and throat swabs.

Given the observed correlation between sputum and DTS in viral RNA concentration, we further examined the synchronized specimen sets by grouping them according to the viral RNA concentrations of sputum (Figure 5). For the 17 sets of synchronized specimens collected from 13 patients that had a medium level of viral RNA concentration in sputum (4.13-6.91 log viral copy/mL), 3 (17.6%) had their DTS showing false-negative RT-PCR results. Whereas the false-negative rate for the 18 sets collected from 10 patients that had a low level of viral RNA concentration in sputum (0-3.95 log viral copy/mL) were even higher (44.4%, 8/18). Of note, similar false-negative rates were observed for pooled NP and throat swabs (23.5%, 4/17 and 44.4%, 8/18) for patients with medium and low level of viral RNA in sputum, respectively.

Sputum producers and non-sputum producers

Given the observation that DTS RT-PCR positive rate correlated with viral RNA concentration in sputum, we further analyzed DTS RT-PCR false-negative rates among sputum producers and non-sputum producers. In this analysis, we focused on events occurred during the first week from illness onset, as this is the most critical period for diagnosis. We divided patients into sputum producers (N = 15) and non-producers (N = 35) according to their symptoms reported during the first week of illness. Of note, there were an additional 11 patients who developed productive cough after the first week, making a total of 26 patients had experienced productive cough when the whole study period was considered. Among the RT-PCR results of 48 DTS collected during day 1-7 after symptom onset, we found that the DTS RT-PCR false-negative rate of non-sputum producers were 2.6 times higher than that of sputum producers (22.2%, 8/36 vs. 8.3%, 1/12).

Discussion

Countries have strived to improve both accessibility and capacity of laboratory testing for SARS-CoV-2. Drive-thru testing is implemented in the US and South Korea [19, 20], where individuals drive their vehicle to the testing sites, which are usually open, naturally ventilated areas for healthcare workers to collect nasopharynx plus throat swabs in full gear personal protective equipment (PPE) of surgical mask, face shield, gloves, and gown. Hong Kong has adopted an alternate approach of home-based screening utilizing self-collect DTS specimens.

There are several advantages of self-collected DTS specimens. It alleviates the demand of PPE during specimen collection and negative pressured rooms or open space required during nasopharyngeal specimen collection by healthcare workers. This is especially valid during the hike of pandemic period where supply of PPE is scarce in many parts of the world. In Hong Kong, the Centre for Health Protection implemented a policy using early morning DTS (also referred to as posterior oropharyngeal saliva) collected at home to test for SARS-CoV-2 among persons who do not require hospitalization, and inbound travelers at the Hong Kong International Airport [16, 21].

The appropriateness of the type of samples to be collected for diagnosis of COVID-19 should be further investigated. In two previous studies [13, 22], the authors analyzed 173 respiratory specimens collected from 23 patients throughout the disease course. Ten patients had severe symptoms and 13 had mild symptoms. They found that RT-PCR was negative in the saliva specimens of 3/23 (13%) patients. In another study by the same research group [23], they tested archived nasopharyngeal swabs and posterior oropharyngeal saliva specimens from 58 confirmed COVID-19 patients using Xpert® Xpress SARS-CoV-2 assay. They found that 6/58 (10.3%) of saliva specimens were falsely negative. However, in these two studies, the authors did not scrutinize clinical severity and time of specimen collection. Another group studied two hundred sample pairs of nasopharyngeal and throat swabs and saliva samples from 200 individuals attended an acute respiratory infection clinic. Nineteen patients

were diagnosed with COVID-19. The sensitivity and specificity of RT-PCR using saliva specimen were 84.2% and 98.9% respectively, when using nasopharyngeal and throat swabs as reference standard [24]. In another retrospective analysis, 95 patient-matched DTS and nasopharyngeal swab specimens from 62 patients tested for SARS-CoV-2 by RT-PCR were analyzed. The authors concluded that the performance of DTS was equivalent to nasopharyngeal swab specimens [25].

To date, our current study provides the largest number of patients with prospectively collected saliva specimens throughout the clinical course and with head-to-head comparison of DTS to both upper and lower tract respiratory samples. We involved two major acute hospitals and included patients from a wide spectrum of illness ranging from asymptomatic, mild, moderate, to severe and critical; and specimens were collected at different time from symptom onset..

Our findings are in concordance with others, where we observed that viral RNA concentration was highest during early phase of illness, and gradually decreases over time. This observation is also consistent with our previous findings [18]. More importantly, DTS produced the lowest viral RNA concentration and lowest RT-PCR positive rate compared to conventional respiratory specimens throughout the early, mid and late phases following symptom onset. We therefore do not recommend the use of DTS when other specimen types are feasible, namely hospitalized patients and designated screening centers where PPE can be provided and infection risk can be controlled.

We found that sputum, an option of self-collect specimen, provided the highest diagnostic yield. However, not all SARS-CoV-2-infected patients are sputum producers. As in our study cohort, there were patients with very mild symptoms without cough at all; or those with cough but could not produce sputum. Under these circumstances, other choice of specimen is needed.

It would be advisable to be specific on the terminology used for "saliva" specimens. We suggest DTS to be differentiated from saliva specimens collected from anterior buccal cavity, or those collected by the "drooling technique" where saliva was collected intraorally with the use of a pipette [26]. We found that saliva specimens were more closely correlated to sputum than to pooled NP and throat swabs. In addition, the consistency of DTS was often observed to be thick and mucoid and sputum-like. These findings suggest DTS represents lower respiratory tract secretions that are propelled up from lower airway by the ciliated cells of the respiratory epithelium. Although we found that the diagnostic performance of DTS was improved in sputum producers, it is not clinically relevant as for these patients a self-collect sputum would be a better choice.

Our findings caution the use of DTS. One must be fully aware of its limitations before advocating its widespread use. DTS contains lower viral RNA concentration and is less sensitive in detecting SARS-CoV-2 infection than sputum and pooled NP and throat swabs. Collection of good DTS specimens demands certain technique which could vary between individuals, especially for home-collected specimens. Sensitivity of DTS is likely to be influenced by factors including the volume of saliva collected, the time of day of specimen collection, collection technique, and being a sputum producer or not. Clear instructions must be given to ensure acceptable specimen quality.

Our study had several limitations. Firstly, out of the 563 samples analyzed, only 170 were from synchronized samples. The comparison could be more meaningful if more synchronized samples were available. Secondly, we did not have data on the time of collection for the DTS samples. We postulate the timing could affect the sensitivity of DTS, and with a higher viral RNA concentration from early morning specimens than at later times of the day. Also, we did not directly supervise the collection of DTS to ensure adherence to instructions, and this might influence the diagnostic accuracy of DTS. Nevertheless, this means our results reflect the expected performance of self-collect DTS. Lastly, only

two patients in our study were asymptomatic. Asymptomatic persons are more likely to be the target of screening, and their DTS specimens could give an even lower sensitivity.

In conclusion, with the highest overall detection rate and viral RNA concentration, we believe self-collected sputum is the specimen of choice for sputum-producing patients. DTS has an obvious advantage of being self-collected and with a low infectious risk during specimen collection. However, since DTS has both the lowest viral RNA concentration and detection rate amongst the three specimen types analyzed in the current study, one must bear in mind its limitations before advocating its widespread use.

Footnote

The content of this manuscript has not been previous presented in meeting or conferences.

Conflict of interest statement

All authors declared no conflict of interests

Funding

The study was supported by the Health and Medical Research Fund - Commissioned Research on the Novel Coronavirus Disease (COVID-19) (reference no. COVID190107) from the Food and Health Bureau, Hong Kong SAR Government.

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Figure Legends

Figure 1. SARS-CoV-2 RNA RT-PCR positive rate among specimen types collected from 50 COVID-19 patients. (A) Violin plot showing positive rates of individual patients within their viral shedding periods according to specimen types. The box widths indicate the kernel densities of number of patients. The dots indicate mean positive rates, with error bars showing 95% confidence intervals. **(B)** Positive rates over three collection periods from illness onset (day 1-5, 6-10, and 11-15). The dots indicate mean positive rates, with error bars showing 95% confidence intervals. NPSTS: pooled nasopharyngeal and throat swabs; DTS: deep-throat saliva.

Figure 2. SARS-CoV-2 viral RNA concentrations among specimen types collected from 50 COVID-19 patients. (A) Violin plot showing viral RNA concentrations according to specimen types of all patients. **(B)** Viral RNA concentrations of different specimen types according to clinical severity. The violin plot box widths indicate the kernel densities of number of samples. The dots indicate mean viral concentrations, with error bars showing the 95% confidence intervals. Negative samples with Ct value >39.9 were set as 0 for calculation. NPSTS: pooled nasopharyngeal and throat swabs; DTS: deep-throat saliva.

Figure 3. SARS-CoV-2 viral RNA concentrations among specimen types according to time of collection. 52 sets of "synchronized" specimens from 21 patients were included. The violin plot box widths indicate kernel densities of number of specimens. The dots indicate mean viral concentrations, with error bars showing 95% confidence intervals. Negative samples with Ct value >39.9 were set as 0 for calculation. NPSTS: pooled nasopharyngeal and throat swabs; DTS: deep-throat saliva.

Figure 4. Correlation analysis of viral RNA concentrations among specimen types of "synchronized" samples. (A) Pearson correlation test of viral RNA concentration between paired specimen types. The dot colors indicate different collection periods from illness onset (day 1-5, 6-10, 11-15, and 16-22). Viral RNA concentration shown is in log copy/mL. Negative samples with Ct value >39.9 were set as 0 for calculation. NPSTS: pooled nasopharyngeal and throat swabs; DTS: deep-throat saliva. **A.** Overall. **B.** According to different time frames.

Figure 5. Pairwise correlation of SARS-CoV-2 viral RNA concentration between specimen types of "synchronized" samples with different viral load. Samples were divided into three equally spaced quantile groups based on the viral RNA concentration values (log copy/mL) of sputum specimens, and classified as high (7.01-9.71), medium (4.13-6.91) and low (0-3.95). Negative samples with Ct value >39.9 were set as 0 for calculation. NPSTS: pooled nasopharyngeal and throat swabs; DTS: deep-throat saliva.

Figure 1

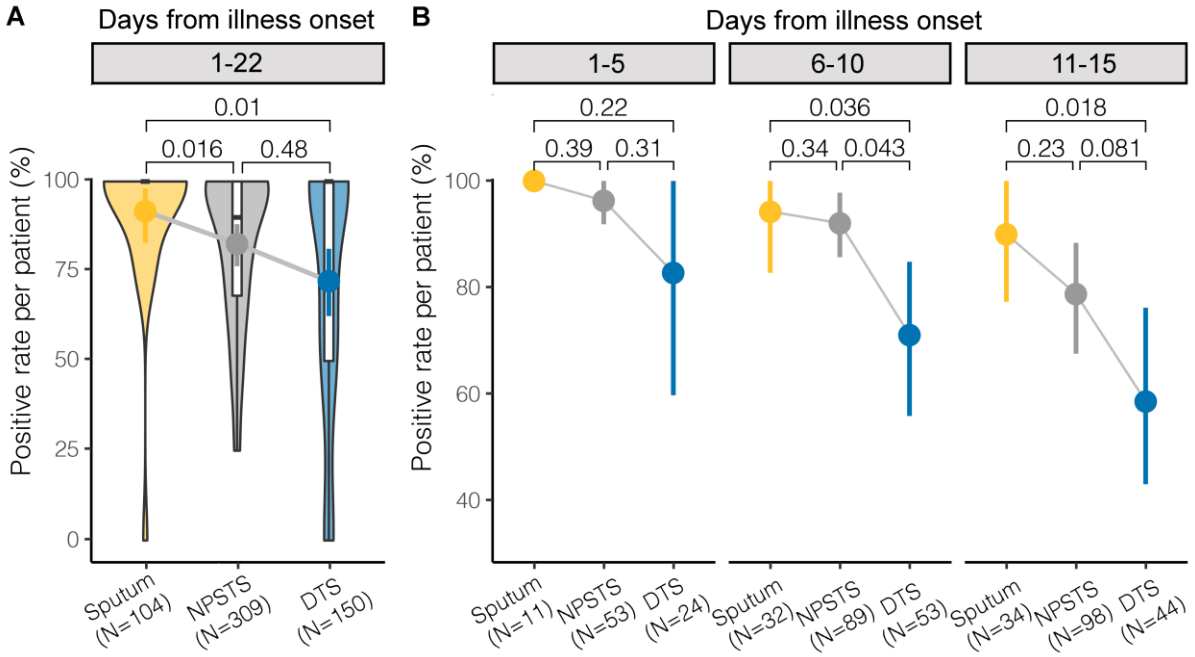

Figure 2

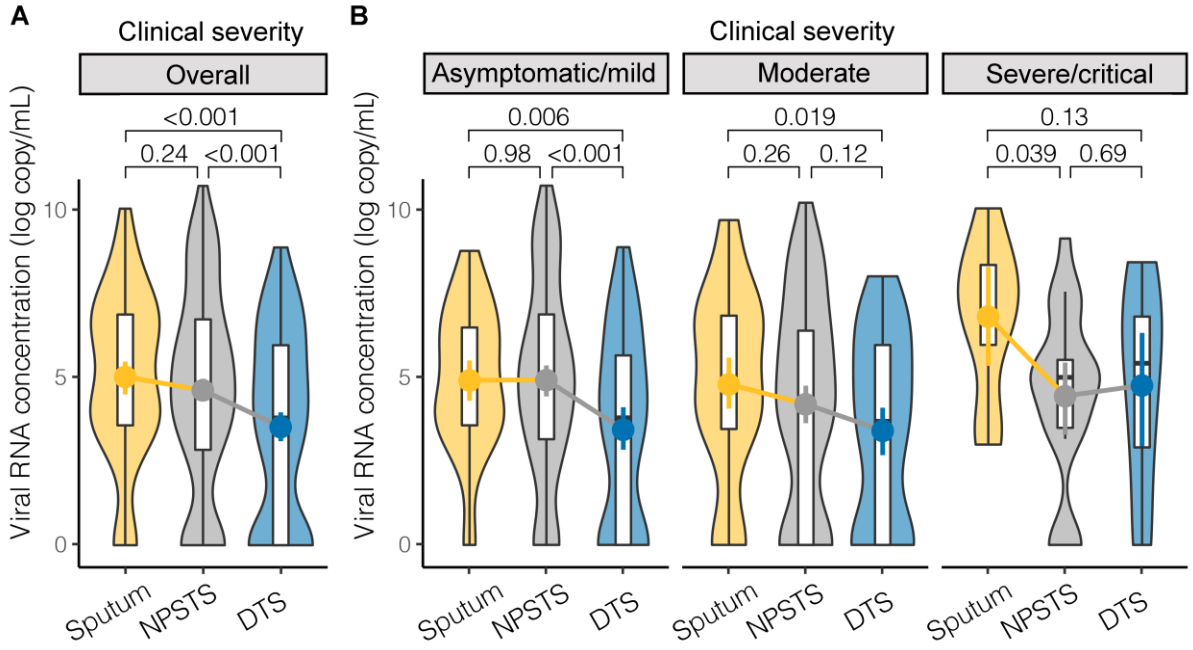

Figure 3

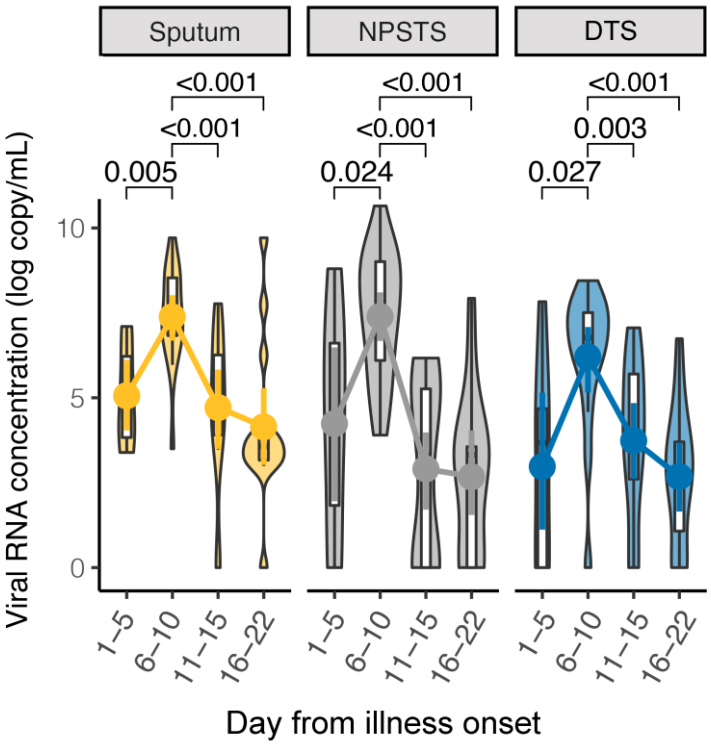

Figure 4

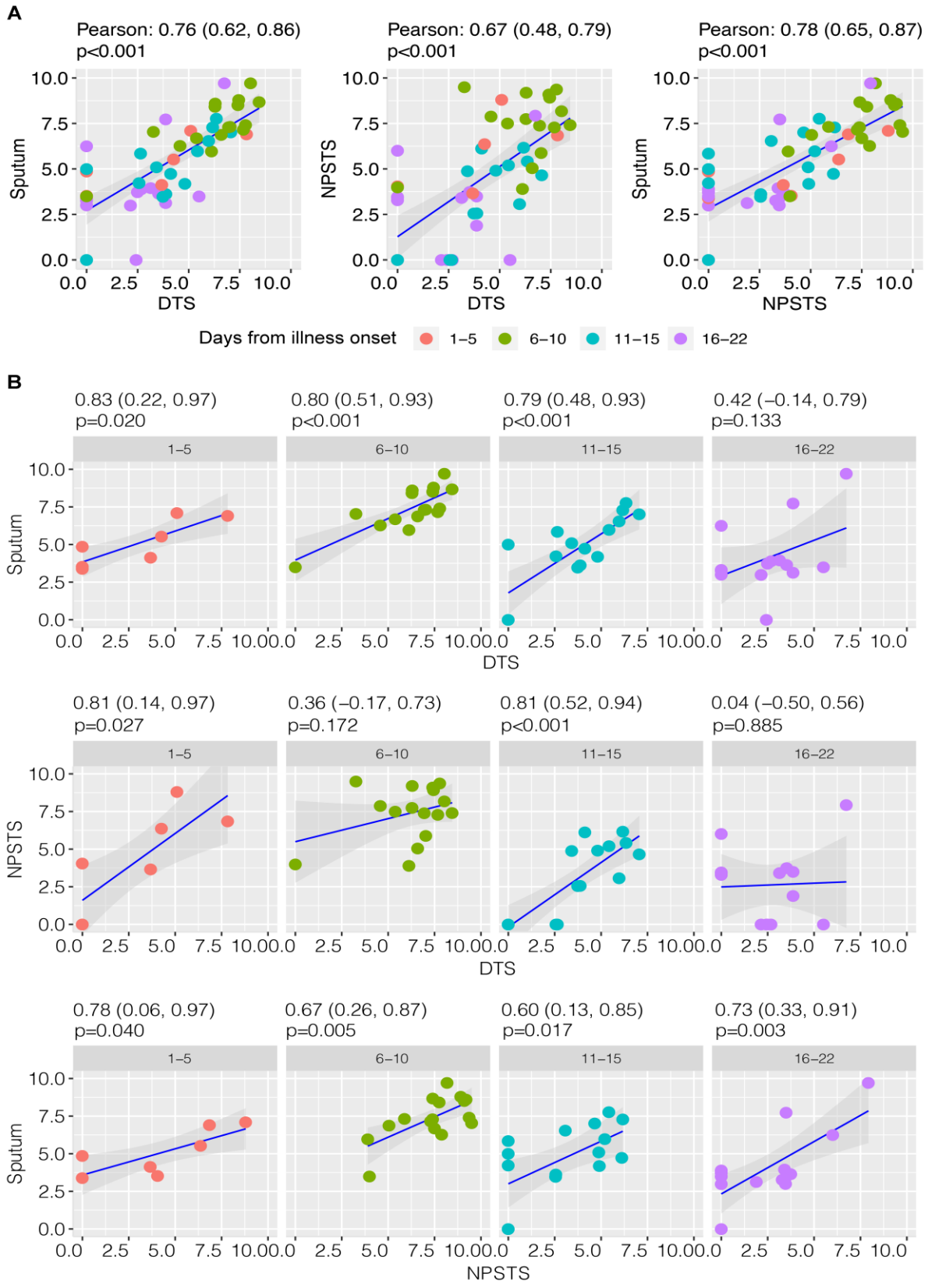
A
Pearson: 0.76 (0.62, 0.86)
p<0.001
Pearson: 0.67 (0.48, 0.79)
p<0.001
Pearson: 0.78 (0.65, 0.87)
p<0.001
Sputum
NPSTS
Sputum
DTS
DTS
NPSTS
Days from illness onset
1–5
6–10
11–15
16–22
B
0.83 (0.22, 0.97)
p=0.020
0.80 (0.51, 0.93)
p<0.001
0.79 (0.48, 0.93)
p<0.001
0.42 (–0.14, 0.79)
p=0.133
0.81 (0.14, 0.97)
p=0.027
0.36 (–0.17, 0.73)
p=0.172
0.81 (0.52, 0.94)
p<0.001
0.04 (–0.50, 0.56)
p=0.885
0.78 (0.06, 0.97)
p=0.040
0.67 (0.26, 0.87)
p=0.005
0.60 (0.13, 0.85)
p=0.017
0.73 (0.33, 0.91)
p=0.003
Sputum
NPSTS
DTS
NPSTS

Figure 5