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Analysis of sputum/tracheal aspirate and nasopharyngeal samples for SARS-CoV-2 detection by laboratory-developed test and Panther Fusion system

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ABSTRACT

In this study, 127 sputum/tracheal aspirate specimens were evaluated by a laboratory-developed real-time RT-PCR method and Fusion SARS-CoV-2 assay. These specimens were collected from the patients who have nasopharyngeal swab (NPS) samples being used for SARS-CoV-2 detection previously or simultaneously. The overall agreement was 96% between the lower respiratory tract (LRT) and NPS samples, suggesting that LRT specimens could be an option for patients who develop a productive cough or those receiving invasive mechanical ventilation.

1. Introduction

Diagnostic testing of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from nasopharyngeal (NPS) and oropharyngeal (OP) swabs were the first FDA approved sample types to be used on laboratory-developed tests (LDT) or commercial platforms. The Centers for Disease Control and Prevention (CDC) stated that lower respiratory tract (LRT) samples, such as sputum, are permissible for patients with a productive cough or under special circumstances (e.g., invasive mechanical ventilation) (https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html). Testing sputum can also be helpful for patients under investigation (PUI), whose NPS swabs are negative for SARS-CoV-2. Previous studies have shown that SARS-CoV-2 RNA could be detected in non-NP sample types, such as sputum, bronchoalveolar (BAL) fluid, and saliva (Wang et al., 2020a; Mohammadi et al., 2020; Wang et al., 2020b).

Here, we examined the potential utilization of sputum/tracheal aspirate (collectively as sputum) samples for diagnosis of coronavirus disease 2019 (COVID-19) by real-time RT-PCR using LDT and Fusion SARS-CoV-2 assay (Hologic, San Diego, CA). The LDT had previously been established in our laboratory for NPS in March 2020. The Fusion assay was modified from the Emergency Use Authorization (EUA) approved protocol by using dithiothreitol treated sputum after 1:1 water dilution.

2. Material and methods

One hundred and twenty-seven retrospective sputum samples from 103 in-patients were collected. NPS samples from these 103 patients were tested for initial diagnosis of COVID-19, whereas sputum samples were collected at the time of NPS collection and/or during hospitalization. Out of 103 patients, 73 sputum samples were from 48 NPS-positive patients whereas 54 were from 54 NPS-negative patients (Table 1). Twenty-five out of 73 positive sputa were from the repeated collection. Sixteen of 48 NPS-positive patients had repeated sputa, collected at least twice during hospitalization. A majority of positive NPS (37) were tested by ID NOW COVID-19 (Abbott, Abbott Park, IL, USA), 5 by Panther Fusion® SARS-CoV-2 Assay (Hologic, San Diego, CA, USA), 2 by Abbott RealTi*m*e SARS-CoV-2 (Abbott Park, IL, USA), and 1 by Xpert® Xpress SARS-CoV-2 (Cepheid, Sunnyvale, CA, USA). All negative NPS were tested at least once by one of the RT-PCR platforms or tested multiple times by ID NOW COVID-19 assay.

All 127 sputum samples were evaluated by the LDT. Our LDT method utilized QIAamp Viral RNA Mini kit (Qiagen, Germantown, MD, USA) for

Abbreviations: NPS, nasopharyngeal swab; LDT, laboratory-developed test; LRT, lower respiratory tract; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; CDC, Centers for Disease Control and Prevention; PUI, patients under investigation; BAL, bronchoalveolar; COVID-19, coronavirus disease 2019; EUA, Emergency Use Authorization.

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Table 1
List of positive NPS and sputum samples with LDT and Fusion SARS-CoV-2 testing results.

Subject #	Specimen #	Assay used for initial NPS tested positive	Sputum collection days post NPS	Sputum results	LDT RT-PCR results (Ct value)				Fusion results (Ct value)
					E	N2	ORF8	RNase P (IC*)	ORF1ab
1	1	Abbott m2000	0	Pos[#]	32.07	34.29	34.13	24.61	NT[@]
2	2	ID NOW	0	Pos	16.19	17.02	17.07	36.34	14.3
3	3	ID NOW	0	Pos	17.53	18.04	17.93	24.86	NT
4	4	ID NOW	0	Pos	20.08	20.81	20.92	19.9	NT
5	5	ID NOW	0	Pos	23.38	24.83	25.13	19.61	NT
6	6	ID NOW	0	Pos	24.22	25.5	26.68	20.75	NT
7	7	ID NOW	0	Pos	25.13	26.05	26.6	20.3	NT
8	8	ID NOW	0	Pos	26.83	28.24	27.38	31.31	NT
9	9	ID NOW	0	Pos	29.27	31.24	31.16	24.04	NT
10	10	ID NOW	0	Pos	29.62	30.21	29.32	29.19	NT
11	11	ID NOW	0	Pos	30.22	31.39	31.5	27.23	NT
12	12	FUSION	0	Pos	24.15	25.32	24.81	32.4	NT
13	13	ID NOW	1	Pos	22.14	22.37	22.04	29.56	NT
14	14	ID NOW	1	Pos	22.34	24.33	22.86	25.6	21.2
15	15	ID NOW	1	Pos	25.9	27.23	26.66	29.33	NT
16	16	ID NOW	1	Pos	29.71	31.2	31.17	27.3	28.5
17	17	ID NOW	1	Pos		38.67	38.96	27.44	38.5
18	18	FUSION	1	Pos	33.18	34.8	34.49	19.35	34.1
19	19	NA^	3	Pos	27.46	29.09	28.89	22.16	NT
20	20	ID NOW	5	Pos	15.21	16.2	16.3	20.68	15
21	21	ID NOW	5	Pos	20.55	21.39	21.76	26.26	18.8
22	22	ID NOW	5	Pos	25.69	27.11	26.17	20.33	NT
23	23	ID NOW	5	Pos	27.5	29.1	28.93	23.4	26.8
24	24	FUSION	6	Pos	25.66	25.93	25.18	29.23	NT
25	25	ID NOW	7	Pos	27.72	28.05	28.5	24.11	NT
26	26	ID NOW	7	Pos	29.64	30.12	30.57	28.21	NT
27	27	ID NOW	7	Pos	32.65	33.91	34.39	22.95	NT
28	28	ID NOW	8	Pos	30.14	31.49	30.65	27.04	NT
29	29	ID NOW	8	Pos	33.47	33.92	34.16	24.51	NT
30	30	ID NOW	11	Pos	26.47	27.88	28.04	29.07	NT
31	31	ID NOW	11	Pos	29.35	30.33	30.1	20.76	NT
32	32	FUSION	15	Pos	36.72	37.25	36.44	26.28	NT
33	33	ID NOW	0	Pos	20.24	23.77	22.17	19.37	NT
	34		2	Pos	21.32	22.74	22.68	24.39	NT
34	35	ID NOW	0	Pos	23.56	25.96	25.02	28.12	NT
	36		6	Pos	36.72	35.37	35.47	24.05	NT
35	37	FUSION	4	Pos	27.36	28.6	28.16	25.3	27.4
	38		8	Pos	32.11	33.11	32.31	24.53	29.8
36	39	ID NOW	5	Pos	21.37	22.2	21.39	26.22	NT
	40		10	Pos	27.56	28.71	27.99	25.59	NT
37	41	Abbott m2000	9	Pos	29.93	31.25	31.29	24.03	NT
	42		13	Pos	36.74	38.67	37.39	21.16	NT
38	43	ID NOW	14	Pos	36.87	37.22	36.51	25.21	34.7
	44		17	Pos	39.02		40.15	22.01	NT
39	45	ID NOW	14	Pos	36.36	36.44	36.47	22.9	34.5
	46		20	Neg				25.25	Neg[&]
40	47	ID NOW	14	Pos	36.82	39.05	38.4	23.04	35.1
	48		21	Neg				23.57	Neg
41	49	ID NOW	20	Pos	32.92	33.5	32.43	22.56	NT
	50		23	Pos	36.04	37.77	37.07	25.9	NT
42	51	NA	30	Neg				24.31	Neg
	52		32	Pos	39.33		38.06	27.06	
43	53	ID NOW	0	Pos	35.45	36.77	36.75	28.15	38.1
	54		5	Neg				26.48	Neg
	55		8	Pos	39.76	41.66		25.71	NT
44	56	Xpert	0	Pos	24.95	26.16	25.62	28.42	23
	57		9	Pos	27.32	28.32	28.01	20.03	27.1
	58		13	Pos	32.4	34.25	33.39	19.79	32.4
45	59	ID NOW	12	Pos	36.22	38.42	39.01	20.41	38.1
	60		17	Pos		38.75	40.05	22.63	NT
	61		18	Pos		39.91		23.11	NT
46	62	ID NOW	3	Pos	23.92	24.61	25.12	20.45	23.6
	63		9	Pos	32.34	33.37	33.88	20.76	32
	64		19	Pos	39.34	42.04	39.53	24.54	38.5
	65		27	Neg				22.94	Neg
47	66	LDT	8	Pos	22.75	24.24	24.27	21.92	NT
	67		21	Pos	35.98	37.29	37.54	24.35	35.6
	68		26	Pos	36.46	37.29	37.5	23.31	NT
	69		32	Pos		41.48	40.89	23.15	NT

Table 1 *(continued)*

Subject #	Specimen #	Assay used for initial NPS tested positive	Sputum collection days post NPS	Sputum results	LDT RT-PCR results (Ct value)				Fusion results (Ct value)
					E	N2	ORF8	RNase P (IC*)	ORF1ab
48	70	ID NOW	9	Pos	28.99	29.05	28.67	24.86	NT
	71		10	Pos	29.39	30.32	30.14	20.39	NT
	72		10	Pos	32.35	33.07	32.18	25.62	NT
	73		14	Pos	34.32	34.63	33.72	25.42	NT

^NA: Not available.
* IC: internal control.
# Pos: Positive.
& Neg: Negative.
@ NT: Not tested.

nucleic acid extraction and Reliance One-Step Multiplex Supermix (Bio-Rad, Hercules, CA, USA) for RT-PCR reaction. The assay targeted SARS-CoV-2 E, N2, and ORF8 genes with the Ct cutoff at 42. The human housekeeping gene RNase P was used as an internal control. RT-PCR was performed on the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). Briefly, sputum was diluted with molecular grade water in a 1:1 ratio as mentioned in Branche et al. (Branche et al., 2014), and the mixture was vortexed and settled for 15–30 min; 140 μL of diluted samples were processed for the downstream RNA extraction and RT-PCR reactions.

Due to the limitation in Fusion reagents, only 50 specimens (27 positives and 23 negatives) out of 127 were tested by Fusion. The diluted (1:1) sputum samples from above were mixed with an equal volume of freshly prepared 10 mM dithiothreitol (DTT) dissolved in sterile PBS (pH = 7.2), and the mixture was incubated at room temperature with intermittent mixing until mucus was liquefied (up to 30 min), according to the CDC's sputum processing protocol (https://www.cdc.gov/coronavirus/2019-ncov/downloads/processing-sputum-specimens.pdf). The liquified sample mix was then transferred to the Fusion lysis tube per manufacturer's instruction (Hologic Panther Fusion® SARS-CoV-2, 2020). The limit-of-detection (LoD) analyses for both methods were performed by spiking the whole genome of SARS-CoV-2 viral RNA, provided by The World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) at the University of Texas Medical Branch (UTMB). Twenty replicates were tested at the estimated lowest detection levels to achieve minimal 19 positive results, based on the FDA guidelines.

Statistical analysis was performed using Medcalc stats (https://www.medcalc.org/calc/diagnostic_test.php). Cohen's kappa coefficient (κ) was also calculated using the Graphpad Prism QuickCalcs website (https://www.graphpad.com/quickcalcs/) as a measure of the overall agreement, with values representing levels of agreement that are categorized as almost perfect (0.81–1.00), substantial agreement (0.61–0.80), moderate agreement (0.41–0.60), fair agreement (0.21–0.40), and slight agreement (0.00–0.20) (Landis and Koch, 1977).

3. Results

We demonstrated a 100% negative agreement for NPS-sputum negative pairs (54/54) and a 93% (95%CI: 85–98%) positive agreement for NPS-sputum positive pairs (68/73) by LDT (Table 2). The overall agreements between NPS and sputum pairs were 96% (95% CI: 91–99%) and 90% (95%CI: 78–96%) for LDT and Fusion, respectively. Due to a limited supply of Fusion reagents, the sample size for Fusion was smaller than LDT, which likely resulted in a slightly lower agreement than LDT. The κ values were 0.92 (95% CI: 85.2–98.9%) and 0.80 (95% CI: 64–96%) for LDT and Fusion, respectively, indicating that the agreements were almost perfect to substantial. The results from all samples tested on Fusion correlated with LDT. Five discrepant samples (i.e., NP positive but sputum negative) (Samples# 46, 48, 51, 52, and 65) tested by LDT were repeated on Fusion and resulted as negative, which was consistent with the LDT results. Among those discrepant sample sets, 2 were from 2 different patients (#51 and 54) and were most likely poor quality since the repeat testing of recollected sputum within 2 days (#52 and 55) resulted in positive. The remaining discrepant samples (#46, 48, and 65) were from 3 different patients (Subject #39, 40, and 46), who had at least two sputum specimens collected throughout their hospitalization.

The LoD were determined to be 10,000 and 3,000 copies/mL for LDT and Fusion method on sputum samples, respectively.

Table 2
Sputum vs. NPS samples results by LDT and Fusion.

Sputum analyses by		Nasopharyngeal swab samples	
		Positive	Negative
LDT	Positive	68	0
	Negative	5	54
Fusion	Positive	22	0
	Negative	5	23

4. Discussion

We demonstrated that sputum is one of the alternative specimen types for SARS-CoV-2 diagnosis in the hospitalized patient population. The discrepancy (i.e., positive NPS and negative sputum) could be attributed to i) poor quality of sputum and ii) sample collection at the disease recovery stage.

One of the potential issues with sputum is the presence of mucus which poses difficulty during sample preparation, particularly on automated platforms. For Fusion SARS-CoV-2 assay, the manufacturer discourages the mucus part of LRT samples being transferred into Fusion lysis tubes. Despite the 1:1 dilution of sputum in sterile water (leftover samples from LDT performance), the persistent viscosity deterred successful handling by the Panther Fusion instrument. We have overcome these issues, by applying the CDC guidelines for sputum preparation and successfully demonstrated that sputum can be validated on any platform, whether it is a manual or an automated instrumentation system.

Most clinical sites utilize two consecutive negative NPS as an indication of infection clearance. However, some patients who develop a productive cough at the later course of the disease may still be positive with SARS-CoV-2 RNA in the sputum despite the NPS became negative, a finding described in Chen et al. (Chen et al., 2020). Wolfel et al. (Wölfel et al., n.d.) have demonstrated the presence of infectious viral particles in LRT, the detection of SARS-CoV-2 RNA in sputum, particularly those with low to moderate Ct values, likely indicate the possibility of persistent infection. Therefore, testing of sputum may be necessary for a certain patient population indicated below. A testing algorithm for sputum may need to be developed with the support of infectious disease clinicians. We believe that our findings are beneficial in cases of i) hospitalized individuals with endotracheal intubation before the NPS samples could be taken for COVID-19 evaluation (endotracheal aspiration may be indicated in this situation); ii) patients with a traumatic fracture to the facial/nasal area or anatomic anomaly; iii) symptomatic patients who have a productive cough with negative NPS results. One pitfall of sputum collection is the generation of aerosols; therefore, induced sputum is not recommended by the CDC. However, endotracheal sputum aspirates or self-collected sputum (with proper instructions) in

a defined patient population would provide several advantages over NPS, such as the discomfort associated with NPS sampling.

5. Conclusions

To our knowledge, this is the first study analyzing the use of sputum on LDT and Fusion platforms. Overall, we have demonstrated that both platforms are validated and can be utilized for the diagnosis of COVID-19 from sputum samples in symptomatic patients.

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Conflict of interest

All authors have no conflict of interest.

Ethical approval

The project is approved by the UTMB Institutional Review Board (IRB) under an expedited review process with IRB # 20–0143.

Informed consent

Not applicable.

CRediT author statement.

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Ping Ren: Conceptualization, Resources, Formal analysis, Project administration, Writing – Reviewing and Editing.

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