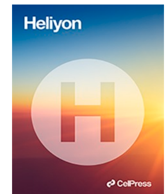
Heliyon 9 (2023) e20965

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Comparison of "hock-a-loogie" saliva versus nasopharyngeal and oropharyngeal swabs for detecting common respiratory pathogens

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ARTICLE INFO

Keywords:
Nasopharyngeal swab
Oropharyngeal swab
Hock-a-loogie saliva
Respiratory pathogen

ABSTRACT

Self-collection of saliva samples has attracted considerable attention in recent years, particularly during the coronavirus disease 2019 pandemic. However, studies investigating the detection of other common respiratory pathogens in saliva samples are limited. In this study, nasopharyngeal swabs (NPS), oropharyngeal swabs (OPS), and "hock-a-loogie" saliva (HLS) were collected from 469 patients to detect 13 common respiratory pathogens. Overall positivity rates for NPS (66.1 %), HLS (63.5 %), and OPS (57.8 %) were statistically different ($P = 0.028$), with an overall concordance of 72.7 %. Additionally, detection rates for NPS (85.9 %) and HLS (83.2 %) for all pathogens were much higher than for OPS (73.3 %). Coronavirus and human rhinovirus were most frequently detected pathogens in NPS ($P < 0.001$). *Mycoplasma pneumoniae* was significantly more prevalent in the HLS group ($P = 0.008$). In conclusion, NPS was a reliable sample type for detecting common respiratory pathogens. HLS was more easily collected and can be used in emergencies or specific conditions. Mixed NPS/OPS and NPS/HLS specimens have the potential to improve detection rates, although OPS testing alone has a relatively high risk for missed detection.

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https://doi.org/10.1016/j.heliyon.2023.e20965
Received 18 May 2023; Received in revised form 10 October 2023; Accepted 12 October 2023
Available online 12 October 2023
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1. Introduction

Acute respiratory tract infections are commonly caused by various pathogens and may lead to severe morbidity and hospitalization in adults and children, posing a serious threat and great burden to human health [1]. The detection of respiratory pathogens provides valuable information for the clinical diagnosis of infection, helping to direct the proper management of patients and prevent nosocomial transmission [2]. Identifying respiratory pathogens is highly dependent on the sample type [3,4]. Nasopharyngeal swabs (NPS) are considered to be the most practical sample type for detecting respiratory pathogens due to the high concentration of infected cells, viral load, and detection rate [5–7]. In addition, oropharyngeal swabs (OPS), which are convenient to collect, are usually collected for clinical detection. However, collecting NPS and OPS is an uncomfortable and invasive procedure that requires trained healthcare professionals to obtain high-quality samples [6]. In addition, the airborne particles released by infected individuals during cough, sneezes, respiration, speaking, which contains infectious viruses and aerosols, lead to increased contamination and infection risks in frontline healthcare workers [6,8,9].

In recent years, the use of self-collected saliva in pathogen infection diagnosis has attracted interest because of the ease of sample collection [10–12]. In Hong Kong, saliva has been used for pathogens in accident and emergency conditions and for screening of travelers at airports [12]. Recently, enhanced saliva samples, such as deep-throat saliva and saliva from sputum after stimulation, have been used for clinical detection. Patients are instructed to sniff strongly to elicit a cough, and saliva samples are collected [13–15]. Many studies have highlighted the importance and advantages of saliva for the clinical detection of respiratory pathogens, particularly during the coronavirus disease 2019 (COVID-19) pandemic [14,16–18]. Carrouel et al. detected and analyzed coupled NPS and self-collected saliva from outpatients with confirmed COVID-19 and asymptomatic to mild symptoms and indicated that results of saliva and NPS were comparable, with an agreement of 74.1 % [14]. In addition, other studies have reported saliva to be a reliable sample type for detecting adenoviruses [2] and influenza viruses [6]. However, whether saliva is reliable for identifying other common respiratory pathogens remains unclear.

The present investigation enrolled 469 patients and collected OPS, NPS and a type of "enhanced" saliva—the so-called "hock-a-loogie" saliva (HLS)—from each patient. This study evaluated these three sample types to detect 13 common viral and bacterial respiratory pathogens using a capillary electrophoresis-based multiplex PCR (CEMP) assay. This assay is based on multiplex PCR amplification and the capillary electrophoresis to separate specific PCR products based on length and is able to analyze several sequences simultaneously in a single tube within 4 h [19,20]. It would be of great benefit to have a rapid and precise diagnosis and to guide clinical treatment decisions for patients with respiratory infections and would also have the potential to both simplify and increase public health surveillance capacity in the future.

2. Materials and methods

2.1. Study design and participates

This study was conducted from November 2019 to January 2020. A total of 469 outpatients were enrolled from the following centers in China: First Affiliated Hospital, Zhejiang University School of Medicine (Hangzhou, Zhejiang); Ningbo Beilun People's Hospital (Ningbo, Zhejiang); Huzhou Central Hospital (Huzhou, Zhejiang); Yiwu Central Hospital (Yiwu, Zhejiang); and Zhoushan Hospital (Zhoushan, Zhejiang). The study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and approved by the Research Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University (reference number, 2019-1346). The inclusion criteria were at least one of the following: focal or systemic symptoms, cough, rhinorrhea, nasal congestion, sore throat, chest pain, fever, headache, muscle soreness, and feeling unwell; screening for respiratory pathogens; illness for no more than seven days; and willingness to participate in the study procedures [21]. Individuals not able to provide qualified specimens, those who refused to provide samples, and those unable to provide written informed consent were excluded.

2.2. Specimen collection

OPS, NPS, and HLS of each patient were collected. Briefly, OPS were collected first, followed by NPS and HLS, at an interval of 5 min for each sampling method. The OPS and NPS were collected by experienced professional workers using flocked oropharyngeal swabs (502CS01; FLOQSwabs, Copan, Italy) and nasopharyngeal swabs (503CS01; FLOQSwabs, Copan, Italy). The NPS was inserted into one nostril until it reached the back of the nasopharyngeal cavity, rotated, and maintained for approximately 10 s to collect fluid and epithelial cells. The OPS was directed toward and wiped at the posterior pharyngeal wall and left and right tonsillar arches 3 times separately before removal. The swabs were dipped in 3 mL of universal transport medium (330C, Copan, Italy) until analysis [1]. For HLS collection, the patient was asked to wear a mask, cough 3–5 times, and spit saliva into a disposable sterile container behind the mask, with a sample volume of 0.5–1.0 mL. The specimens were stored at 4 °C and delivered within 1 h.

2.3. Extraction of nucleic acid

Total nucleic acids were extracted in the confines of a biosafety cabinet. Swab specimens were vortexed for 1 min. HLS samples were mixed with 0.4 mg/mL Protease K buffer and vortexed for 20 s. Then, a 200 μL sample, a negative control, or positive control were taken. Nucleic acid extraction was performed on a commercially available Smart LabAssist-32 system (ANBead, Taiwan, China) using Nucleic Acid Extraction or Purification kit (Ningbo HEALTH Gene Technologies Ltd., Ningbo, China). Ultimately, 80 μL of eluted

nucleic acid was collected.

2.4. Multiple PCR amplification and electrophoresis

A commercially available CEMP-compatible Respiratory Pathogen Multiplex Detection Kit (Ningbo HEALTH Gene Technologies, Ltd., Ningbo, China) was used for pathogen identification. Detected pathogens included influenza A virus (Flu A), Flu A/H3N2, Flu A/pdm 09 (H1N1), influenza B virus (Flu B), human rhinovirus (HRV), adenovirus (ADV), human parainfluenza virus (HPIV, including HPIV1, HPIV2, HPIV3, HPIV4), Boca virus (BOCA), *Mycoplasma pneumoniae* (Mp) and chlamydia (CH, including *Chlamydia pneumoniae* [Cp] and *Chlamydia trachomatis*), coronavirus (CoV, including HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1), human metapneumovirus (HMPV) and human respiratory syncytial virus (HRSV) [19]. Multiple PCR were performed using a C1000 thermocycler (Bio-Rad, Hercules, CA, USA) in accordance with manufacturer's instructions. The total volume of the PCR reaction was 20 μL, including 1 μL RT-PCR enzyme (reverse transcriptase, UDG enzyme, thermo-activated DNA polymerase), 14 μL ResP premix (dNTPs, primers and buffer) and 5 μL nucleic acid sample. The RT-PCR program was set as follows: 25 °C for 5 min, 50 °C for 15 min, 95 °C for 2 min; 94 °C for 30 s, 60 °C for 30 s, 72 °C for 60 s (6 cycles [annealing from 65 °C to 60 °C]; annealing interval, 1 °C); followed by 94 °C for 30 s, 60 °C for 30 s, 72 °C for 60 s (29 cycles); 72 °C for 10 min, then hold at 4 °C.

After amplification, PCR products were separated by capillary electrophoresis using an ABI 3500Dx gene sequencer (Thermo Fisher Scientific, Waltham, MA, USA). The electrophoretic sample system contained AB Master Mix (Hi-Di Formamide including 0.25 % SIZE-500Plus) and 1 μL PCR product. After capillary electrophoresis, the different PCR products were separated according to migration rate. The fluorescence intensities of the labelled PCR products were measured to obtain detection data for further analysis.

2.5. Statistical analysis

Patients were considered to be positive if the results were positive for any sample type. Statistical analysis was performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Graphs were drawn using GraphPad Prism version 9.0.0 (GraphPad Inc, San Diego, CA, USA) and spreadsheet software (Excel, Microsoft Corp., Redmond, WA, USA). The positivity and detection rates for the different sampling methods were compared using Pearson's chi-squared test if no expected counts were <5; otherwise, the results were analyzed using Fisher's exact test. Differences with $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. Patient characteristics

In total, 469 patients (age range, 2–86 years; median age, 28 years; 39.2 % male) were included in this study (Table 1). All patients were outpatients with respiratory symptoms who required screening for respiratory pathogens. The most common symptoms included

Table 1
Demographic features and clinical presentations of the participants (n = 469).

Characteristic	Finding
Age (yr)	
Range	2–86
Median (IQR)	28 (22–37)
Gender [no. (%)]	
Male	184 (39.2)
Female	285 (60.8)
Symptoms [no. (%)]	
Fever	451 (96.2)
Cough	288 (61.4)
Muscle soreness	260 (55.4)
Headache	246 (52.5)
Weakness	239 (51.0)
Sore throat	226 (48.2)
Xerostomia	178 (38.0)
Rhinorrhea	175 (37.3)
Nasal congestion	116 (24.7)
Dizziness	106 (22.6)
Nausea	79 (16.8)
Sputum	68 (14.5)
Vomiting	35 (7.5)
Chest pain	30 (6.4)
Sample collection date (days after onset)	
Range	0–7
Median (IQR)	1 (1–3)

Abbreviations: IQR, interquartile range.

fever (96.2 %), cough (61.4 %), muscle soreness (55.4 %), headache (52.5 %), and weakness (51.0 %). OPS, NPS, and HLS samples were collected within 7 days of disease onset, with a median of 1 day (interquartile range [IQR] 1–3 days).

3.2. Comparison of positivity rates among the different sample types

In total, 1407 OPS, NPS, and HLS samples were collected for pathogen detection. Patient distribution according to the number of pathogens detected using the CEMP assay is summarized in Table 2. The overall positivity rates for OPS, NPS, and HLS samples were 57.8 % (271 of 469), 66.1 % (310 of 469), and 63.5 % (298 of 469), respectively; the difference was statistically significant (P = 0.028). The positivity rates for OPS, NPS, and HLS for detecting 2 pathogens were 0.9 % (4 of 469), 4.7 % (22 of 469) and 2.6 % (12 of 469), respectively (P = 0.001). The positivity rates for one or three types of pathogens in the samples were not significantly different.

3.3. Comparison of concordance among OPS, NPS, and HLS

A total of 341 patients had concordant results in OPS, NPS and HLS, including 218 (63.9 %) concordant positive results and 123 (36.1 %) concordant negative results (Fig. 1). However, 128 patients exhibited discordant results for NPS, OPS, and HLS. Among those with discordant results, 33 (25.8 %) had only concordant NPS and OPS, 32 (25.0 %) had only concordant NPS and HLS, 55 (43.0 %) had only concordant OPS and HLS, and 8 (6.3 %) had discordant results for either of the two types of samples. The detailed discordant results are summarized in Table S1. The most common discordant results were negative OPS and HLS and positive NPS (29 of 469 [6.2 %]), particularly with only positive NPS for HRV (8 of 29 [27.6 %]), followed by positive NPS and HLS for the same pathogen and negative OPS (23 of 469 [4.9 %]), especially positive Flu B in NPS and HLS (9 of 23 [39.1 %]).

3.4. Comparison of performance of the different sampling methods based on pathogen type

A comparison of detection rates using different sample types according to pathogen type is presented in Table 3. A total of 375 pathogens were identified in 469 patients using any of the sampling methods: 275, 322, and 312 pathogens were identified in OPS, NPS, and HLS, with overall detection rates of 73.3 % (275 of 375), 85.9 % (322 of 375) and 83.2 % (312 of 375), respectively (P < 0.001). Influenza virus (265 of 375) was the most common pathogen detected, of which type A (H1N1pdm09 and H3N2) was dominant (143 of 256). The overall detection rate improved to 93.9 % (352 of 375) when combining NPS and OPS. The overall detection rate improved to 99.2 % (372 of 375) when combining NPS and HLS. The detection rate of each pathogen using different sampling methods was also analyzed. For most pathogens, the detection rate for NPS was equal to or greater than those for OPS and HLS, with significant differences in CoV and HRV (P < 0.001). Flu A/pdm 09 (H1N1), Flu B, and Mp were detected more frequently in patients with HLS, and the difference in the prevalence of Mp was significant (P = 0.008). The detection rate for OPS for all pathogens was lower than that for NPS and HLS. In addition, the detection rate for most pathogens was 100 % with combined NPS and HLS, except for influenza B and HMPV (98.4 % and 90.9 %, respectively).

4. Discussion

A total of 469 outpatients with respiratory symptoms (fever and cough) were enrolled in the present study. A significantly higher overall positivity rate for NPS (66.1 %) was found, as well as the positivity rate in detecting two different pathogens (4.7 %). These results indicate that NPS demonstrated the best efficiency in detecting respiratory pathogens among the three sample types. In fact, NPS is usually considered to have the highest detection rate for respiratory viruses [22] and is the reference sampling method for clinical detection [23,24]. Other studies have also demonstrated that the detection rate for NPS is higher than that for OPS [25,26] and saliva [22] in identifying common respiratory viruses, which is consistent with our findings.

The present study demonstrated high overall concordance (72.7 %) among OPS, NPS, and HLS, and the concordance between OPS/NPS, OPS/HLS, and NPS/HLS was 79.7 % (374 of 469), 84.4 % (396 of 469), and 79.5 % (373 of 469), respectively, which was similar to some previous studies [12,21,22]. However, another study found that the concordance between NPS and saliva was 95.5 %, which is higher than in our study [2]. One study published by Wyllie et al. suggested that NPS and saliva have similar sensitivities for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) during the course of hospitalization [27]. These differences may be due to differences in the spectra of detected pathogens, patient enrolment, and detection systems. The relatively lower overall detection rate for OPS and saliva, compared with NPS, is probably due to lower pathogen loads, according to previous studies

Table 2
Distribution of patients according to the number of respiratory pathogens detected from different sample types (n = 469).

No. of pathogens detected	No. (%) of positive samples			*P*
	OPS (n = 469)	NPS (n = 469)	HLS (n = 469)	
Positive for at least one pathogen	271 (57.8)	310 (66.1)	298 (63.5)	**0.028**
1	267 (56.4)	288 (61.4)	285 (60.8)	0.323
2	4 (0.9)	22 (4.7)	12 (2.6)	**0.001**
3	0 (0.0)	0 (0.0)	1 (0.2)	1.000

Abbreviations: OPS, oropharyngeal swab; NPS, nasopharyngeal swab; HLS, hock-a-loogie saliva.

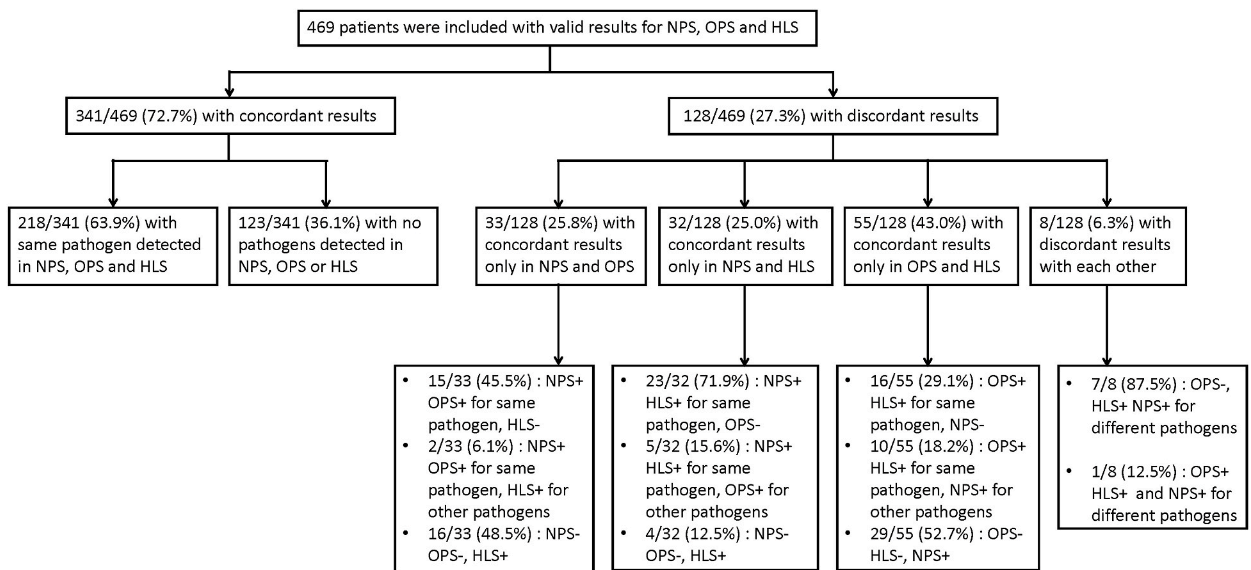

Fig. 1. Summary of nasopharyngeal swab (NPS), oropharyngeal swab (OPS), and "hock-a-loogie" saliva (HLS) results in 469 patients.

Table 3
Comparison of detection rates for oropharyngeal swab (OPS), nasopharyngeal swab (NPS), and "hock-a-loogie" saliva (HLS) samples according to pathogen type.

Pathogen	OPS	No. (%) of pathogens detected					*P*
		NPS	HLS	OPS or NPS	NPS or HLS	OPS or NPS or HLS	
Flu A	125 (87.4)	129 (90.2)	129 (90.2)	136 (95.1)	143 (100)	143	0.677
pdm09 H1N1	33 (89.2)	33 (89.2)	34 (91.9)	36 (97.3)	37 (100)	37	1.000
H3N2	92 (86.8)	96 (90.6)	95 (89.6)	100 (94.3)	106 (100)	106	0.659
Flu B	101 (82.8)	109 (89.3)	111 (91.0)	118 (96.7)	120 (98.4)	122	0.119
ADV	6 (60.0)	8 (80.0)	8 (80.0)	8 (80.0)	10 (100)	10	0.668
CoV	2 (15.4)	13 (100)	5 (38.5)	13 (100)	13 (100)	13	< **0.001**
HRSV	8 (57.1)	13 (92.9)	8 (57.1)	14 (100)	14 (100)	14	0.080
HRV	15 (37.5)	38 (95.0)	24 (60.0)	40 (100)	40 (100)	40	< **0.001**
Mp	7 (63.6)	4 (36.4)	11 (100)	5 (45.5)	11 (100)	11	**0.008**
HMPV	5 (45.5)	8 (72.7)	8 (72.7)	9 (81.8)	10 (90.9)	11	0.473
HPIV	5 (83.3)	6 (100)	5 (83.3)	6 (100)	6 (100)	6	1.000
CH	1 (33.3)	2 (66.7)	2 (66.7)	2 (66.7)	3 (100)	3	1.000
BOCA	0 (0.0)	1 (50.0)	1 (50.0)	1 (50.0)	2 (100)	2	1.000
Total	275 (73.3)	322 (85.9)	312 (83.2)	352 (93.9)	372 (99.2)	375	< **0.001**

Abbreviations: OPS, oropharyngeal swab; NPS, nasopharyngeal swab; HLS, "hock-a-loogie" saliva"; Flu A, influenza A virus; Flu B, influenza B virus; ADV, adenovirus; CoV, coronavirus; HRSV, human respiratory syncytial virus; HRV, human rhinovirus; Mp, mycoplasma pneumoniae; HMPV, human metapneumovirus; HPIV, human parainfluenza virus; CH, chlamydia.

[21,28,29]. Therefore, a patient with high clinical suspicion of respiratory infection but negative saliva or OPS results should be tested using NPS [21]. Furthermore, some studies have recommended a combination of ≥2 specimen types as the most effective approach for pathogen detection [4]. A study by Lieberman et al. reported that the combination of NPS and OPS improved the overall detection rate for respiratory viruses to 84.5 %, which is consistent with our results (93.9 %) [26]. In practice, OPS samples are usually collected together with NPS for the clinical detection of respiratory viruses, effectively reducing missed diagnosis in clinics [30]. In the current study, markedly improved overall detection rate of combined NPS and HLS (99.2 %, 372 of 375) was found. Similar results were found in our previous study, which revealed that the detection rate of combined NPS/HLS for detecting SARS-CoV-2 was increased to 98.1%–100 % [28]. Another study, using multiplex RT-PCR to detect common respiratory viruses, also demonstrated that detection rate of combined NPS/saliva samples was 86.4 %, which was markedly higher than that by detecting NPS (77.5 %) and saliva samples (76.3 %) alone [2]. These findings highlighted the great importance of detecting HLS as a complement when highly suspected patients have negative NPS results, which would effectively reduce missed diagnosis in clinics.

In addition, the detection rates for each pathogen using NPS, OPS, and HLS were analyzed. The influenza virus (265 of 375) was the most common pathogen detected. This may be explained by the sample collection season, which was influenza season between November 2019 and January 2020 [4]. We found that CoV and HRV were detected much more frequently in NPS than in OPS and HLS ($P < 0.001$, respectively). These findings are consistent with a Korean study, which also found more frequently detected HRV in NPS

(detection rate, 34.4 %) [2]. According to the current literature, HRV has a relatively low optimal temperature for growth (33 °C), which may reflect its adaptation to the human nasopharynx [31], supporting NPS as the best sampling method for HRV detection. In this study, Mp was more frequently detected in the HLS group (P = 0.008). Mp can effectively replicate in the lung and cause lower respiratory tract symptoms even lung injury [32,33], and is considered to be the most common and important pathogen causing community-acquired pneumonia (CAP) in children 5–15 years of age [34,35]. Komatsu et al. revealed that saliva was useful and promising for detecting Mp by PCR as well as sputum, which supported our results [36]. Some studies have reported that OPS affords a higher sensitivity and specificity in detecting Mp compared with NPS [37,38], which is consistent with our findings (63.6 % in OPS versus 36.4 % in NPS). An obviously higher detection rate for Mp in HLS samples (100 %) was found in this study, which may be of great benefit for collecting samples from children. However, the higher detection rate of CoV differed from our previous study on SARS-CoV-2, in which the detection rate using HLS (80.4%–86.5 %) was the highest [28]. This may be due to the relatively low pathogenicity of human CoVs detected in this study, which were mainly found in the upper respiratory tract and had higher viral loads in the NPS [39,40].

Although high positivity rate and detection rates for NPS were found, most patients were uncomfortable with the NPS process. By contrast, collecting HLS data can avoid patient discomfort [16]. During the COVID-19 pandemic, To KK et al. reported that saliva is a promising noninvasive for diagnosis and monitoring in patients with SARS-CoV-2 infection [41]. It is particularly suitable for patients with severe bleeding tendencies, in whom collection of NPS samples is not recommended [22]. Other advantages of saliva have been described in some studies, including reduced risk for infection of professional healthcare staff, finishing sample collection with minimal equipment, and the convenience of POCT [7,12,21,28]. Emerging platforms will increasingly empower individuals to obtain samples (such as saliva and urine) and monitor their health status at home [7,42]. However, one of the technical challenges of HLS is the presence of mucus with sometimes high viscosity, which makes detection difficult. Therefore, the non-viscous part of HLS should be used for detection and correct sample preprocessing methods, such as homogenization, require further development, which will also be of benefit for at-home testing [16,43].

The present study had some limitations. First, the enrolled cohort comprised outpatients without severe disease(s). Given that viral load may be lower in patients with mild symptoms, and patients with more severe symptoms would be more likely to benefit from laboratory confirmation, further studies should include inpatients with more severe disease. Second, the samples were collected during the influenza season, which may have biased the results regarding the proportion of pathogens detected in this study. Thus, future studies should consider more patients throughout the year, comprising different seasons. Third, considering the small number of BOCA-, HPIV-, HMPV-, and CH-positive samples in this study, a larger sample size may be needed in future studies to determine significant differences in these pathogens.

In conclusion, although saliva sampling has been widely recommended during the COVID-19 pandemic, NPS remains a reliable option for the detection of common respiratory pathogens. Given the convenience of collection, HLS samples can be used during emergencies or under specific conditions. Furthermore, the combination of samples can greatly improve the detection rate for diagnosis, and mixed NPS/OPS and NPS/HLS specimens may be valuable for future clinical detection of common respiratory pathogens. Testing OPS samples alone, however, is not recommended.

Ethical approval

The study was reviewed and approved by the Research Ethics Committee of the First Affiliated Hospital College of Medicine, Zhejiang University, with the approval number: 2019-1346.

Research funding

The National Natural Science Foundation of China (grant numbers 81803290, 81971919 and 82072377); the China National Mega-Projects for Infectious Diseases (grant number 2017ZX10103008-001, 2017ZX10103008-005, 2017ZX10103008-006, 2017ZX10103008-007, 2017ZX10103008-008).

Data availability statement

Data included in article/supp. material/referenced in article.

Informed consent

Informed consent was obtained from all individuals included in this study.

CRediT authorship contribution statement

Renke Chen: Data curation, Methodology, Writing – original draft. **Jiaqi Bao:** Data curation, Writing – original draft, Writing – review & editing. **Xiaojuan Huang:** Methodology. **Qianna Chen:** Methodology. **Maowen Huang:** Methodology. **Min Gao:** Methodology. **Fanghao Yu:** Methodology. **Jiayao Chen:** Methodology. **Weihua Zou:** Methodology. **Lumei Shi:** Methodology. **Xiao Chen:** Conceptualization. **Bo Feng:** Writing – review & editing. **Ruonan Wang:** Methodology. **Baihuan Feng:** Software. **Shufa Zheng:** Conceptualization, Funding acquisition, Software. **Fei Yu:** Conceptualization, Data curation, Funding acquisition, Methodology,

Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We acknowledge the contributions of other clinical and laboratory staff in the First Affiliated Hospital, Zhejiang University School of Medicine.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20965.

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