ORIGINAL ARTICLE

A Rapid Review on the sensitivity of SARS-CoV-2 RT-PCR done on different clinical specimens

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The authors declare that the data presented are original material and has not been previously published, accepted or considered for publication elsewhere; that the manuscript has been approved by all authors, and all authors have met the requirements for authorship.

ABSTRACT

Background: RT-PCR using respiratory tract specimens, most commonly nasopharyngeal swab (NPS), has been used to confirm the diagnosis of COVID-19. NPS is a relatively invasive procedure that causes patient discomfort and risks viral transmission. Other specimens are therefore being investigated for the detection of SARS-CoV-2 RNA.

Objective: To determine the sensitivity of non-respiratory tract specimens in detecting SARS-CoV-2 RNA in patients with COVID-19.

Methodology: This review summarized the results of eight studies obtained from a literature search done in May 2020 in PubMed MEDLINE, Cochrane Library and MedRxiv. Two independent investigators reviewed and appraised the studies that were included, and pooled estimates of sensitivity for each specimen were determined using Stata's Metaprop function.

Results: The sensitivity in detecting SARS-CoV-2 RNA in non-respiratory tract specimens of diagnosed COVID-19 patients are as follows: Saliva 77% (95% CI 71-83%), stool/rectal swab/anal swab 22% (95% CI 22-37%), blood/serum/plasma 2% (95% CI 1-3%), and urine 22% (95% CI 18-25%).

Conclusion: SARS-CoV-2 RNA is detected in saliva, stool/rectal swab/anal swab, blood/serum/plasma and urine. Among these, saliva has the highest estimated sensitivity. However, more studies are needed to correct the heterogeneity brought about by factors such as timing of specimen collection, disease severity and treatment.

KEYWORDS: *COVID-19, nasopharyngeal, oropharyngeal, swabs and respiratory sample*

INTRODUCTION

SARS-CoV-2 was first identified in Wuhan, Hubei Province China in December 2019 and since then has spread throughout the world. It is a coronavirus (CoV) that has an enveloped positive-sense single-stranded RNA virus. Circulating coronaviruses in humans include two α-CoVs and two β-CoVs that cause the common cold. The SARS-CoV-2 is a human β-CoV. Other highly pathogenic human β-CoV that emerged in the past two decades include SARS-CoV-1 and MERS-CoV. Bats are considered the natural hosts for progenitors of highly pathogenic CoVs and transmission to humans involved intermediate animal hosts. Human-to-human transmission is via direct or indirect contact and primarily through inhalation of infectious respiratory droplets.

The viral particles enter the human body through the respiratory system. The glycoprotein spikes present on the outer surface of the virus are mostly responsible for attachment and entry to the host cell's respiratory epithelium to cause infection. Viral replication begins in the upper respiratory tract and peaks at day 5 of infection. This process is mediated by cleavage of the S1 and S2 regions of the viral protein and a myriad of symptoms such as high fever, sore throat, myalgia and fatigue may set in. In the lower respiratory tract, ACE II receptors bind to viral capsid antigens which facilitate viral entry into the epithelial cells lining the alveoli. Viral particles in lower respiratory secretions are expelled by coughing, sneezing or talking.[1] The presence of viral particles in respiratory secretions is the basis for using respiratory tract specimens for diagnosis through RT-PCR or viral load detection.

According to WHO guidelines published on March 19, 2020, in the laboratory testing for COVID-19 in suspected human cases, the decision to test an individual should be based on clinical and epidemiological factors and linked to an assessment of the likelihood of infection. Specimens should be collected from the upper respiratory tract: nasopharyngeal (NPS) and oropharyngeal swab (OPS) or wash in ambulatory patients and/or lower respiratory tract: sputum and/or endotracheal aspirate (ETA) or bronchoalveolar lavage (BAL) in patients with more severe respiratory disease, and sent for real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) to confirm the diagnosis of COVID-19.[2] A meta-analysis by Mohammadi et al. demonstrated the pooled sensitivity of OPS, NPS and sputum which are 43% (95% CI 34-52%), 54% (95% CI 14-67%), and 71% (95% CI 61-80%), respectively.[3] However, only 27% of patients diagnosed with COVID-19 have sputum production.[4] NPS and OPS swabs cause discomfort and may cause bleeding especially in patients with thrombocytopenia.[5]

This review summarizes the available evidence on the sensitivity of SARS-CoV-2 RT-PCR done on non-respiratory tract specimens of patients with COVID-19.

METHODOLOGY

Articles were selected based on the following inclusion criteria:

- **Population:** Suspect individuals based on history of exposure, and presence of signs and symptoms
- **Intervention:** Application of RT-PCR testing to detect SARS-CoV-2 nucleic acid in non-respiratory tract specimens
- **Outcomes:** Determination of diagnostic sensitivity of SARS-CoV-2 RT-PCR in non-respiratory tract specimen using a positive SARS-CoV-2 RT-PCR in any of the following respiratory specimens: NPS, OPS or wash, sputum, BALF or ETA, as reference standard.
- **Study designs:** observational (prospective and retrospective) cohort and case-control studies

Literature search was done in PubMed MEDLINE, Cochrane Library and MedRxiv. Study titles that did not satisfy the inclusion criteria were excluded. Review of abstracts was done on the remaining studies. Studies that were likely to be relevant based on review of the abstracts underwent full review and appraisal by two independent reviewers.

We excluded studies that did not specify the following: what samples were taken, whether or not study participants were symptomatic, and those that did not clearly state the results.

Pooled estimates of sensitivity at 95% confidence interval (CI) for each specimen were obtained when possible using the Metaprop function of STATA®. In cases where there were no studies identified that included a prospective cohort of suspected patients with COVID-19, we just reviewed studies that included confirmed cases that reported the sensitivity of the different specimens.

RESULTS

Characteristics of Included Studies

The search keywords COVID-19, nasopharyngeal, oropharyngeal, swabs, and respiratory sample were used. A total of 130 search results were obtained from PubMed MEDLINE, Cochrane Library and MedRxiv last May 21, 2020. After title review, review of abstract was done on 35 articles. Subsequently, 20 articles remained for full paper review.

Majority of these studies investigated individuals with laboratory-confirmed COVID-19. With the presence of limited data, we pursued to analyze the diagnostic accuracy of SARS-CoV-2 RT-PCR on non-respiratory tract specimens in comparison to SARS-CoV-2 RT-PCR of a respiratory tract specimen in patients with laboratory-confirmed disease.

A total of 12 studies were excluded from the 20 studies reviewed. Three of the studies were excluded because they did not specify whether a respiratory tract specimen was used to confirm COVID-19 infection. Seven other studies were excluded because the presence or absence of symptoms were not clearly described. Two studies were excluded because the results were not clearly stated.

The studies included were five prospective and three retrospective observational studies. Five were done in China, and one study each in the United States, Japan, and Italy, between the months of January to March 2020.

The studies we found investigated individuals with laboratory-confirmed COVID-19 through a positive SARS-CoV-2 RT-PCR of a respiratory tract specimen (NPA, NPS, TS, and/or sputum). In these studies, the following specimen types were sent for SARS-CoV-2 RT-PCR: saliva, blood/serum/plasma, urine, and stool/rectal swab/anal swab. The diagnostic sensitivity for each specimen type was determined. Majority of these studies reported these sensitivities as positive rates.

The characteristics of the studies included is summarized in Appendix 1.

Outcomes

The pooled estimate of sensitivity of the different non-respiratory tract specimens are as follows:

1. saliva at 77% (95%CI 71-83%, n=4),
2. stool/rectal swab/anal swab at 22% (95%CI 22-37%, n=5),
3. blood/serum/plasma at 2% (95%CI 1-3%, n=4), and
4. urine at 22% (95%CI 18-25%, n=5).

There was significant heterogeneity in all the comparisons for the different specimen sites. See tables 1 and 2 in the Appendix for the summary of results.

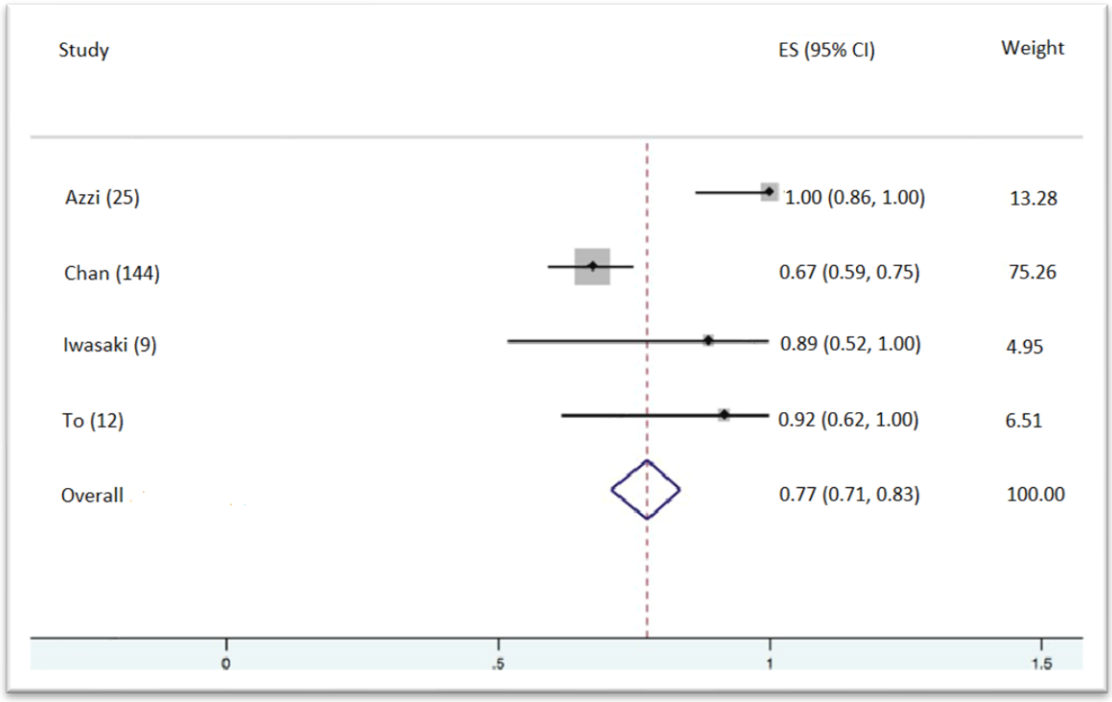

Figure 1: Pooled estimate of the sensitivity of saliva from four studies

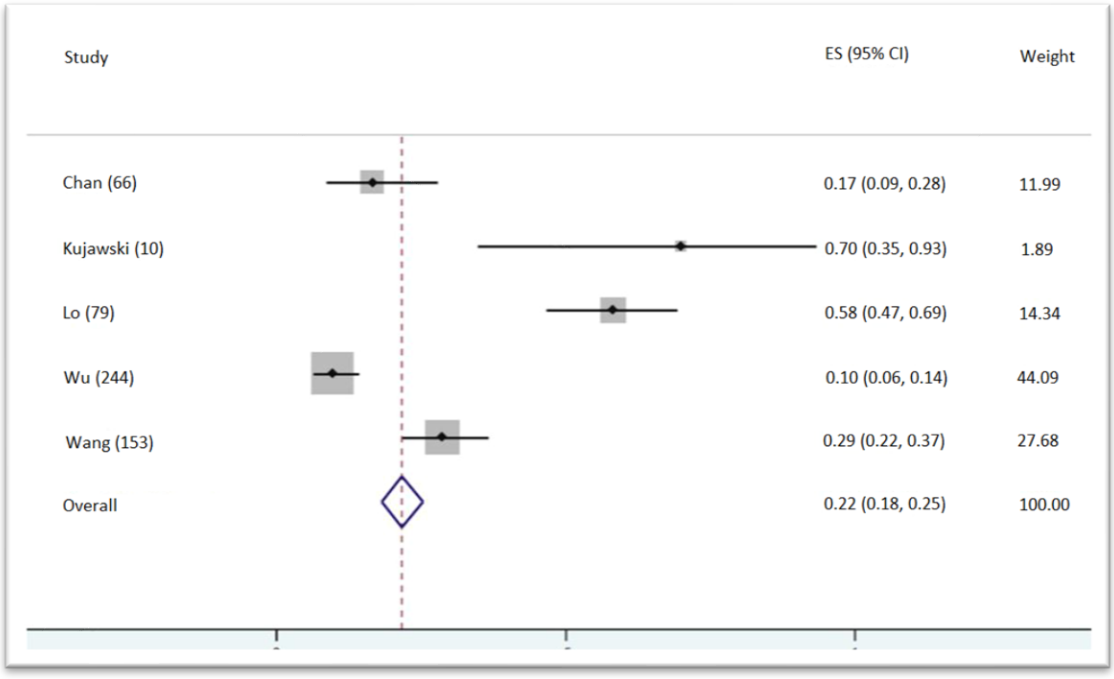

Figure 2: Pooled estimate of the sensitivity of stool/rectal swab/anal swab from five studies

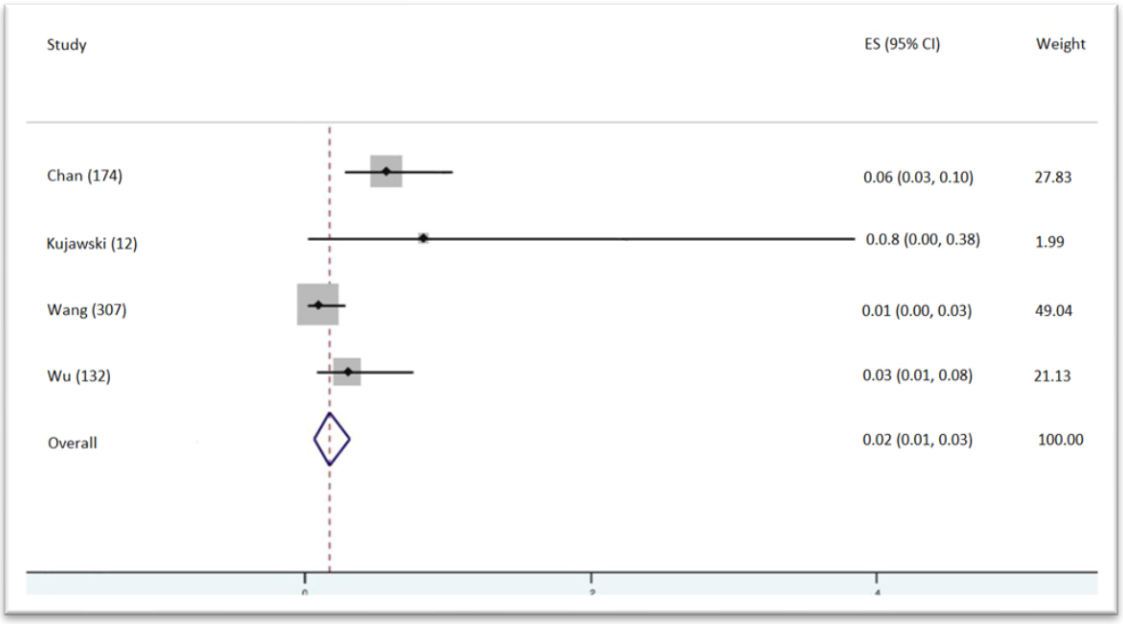

Figure 3: Pooled estimate of the sensitivity of blood/serum/plasma from four studies

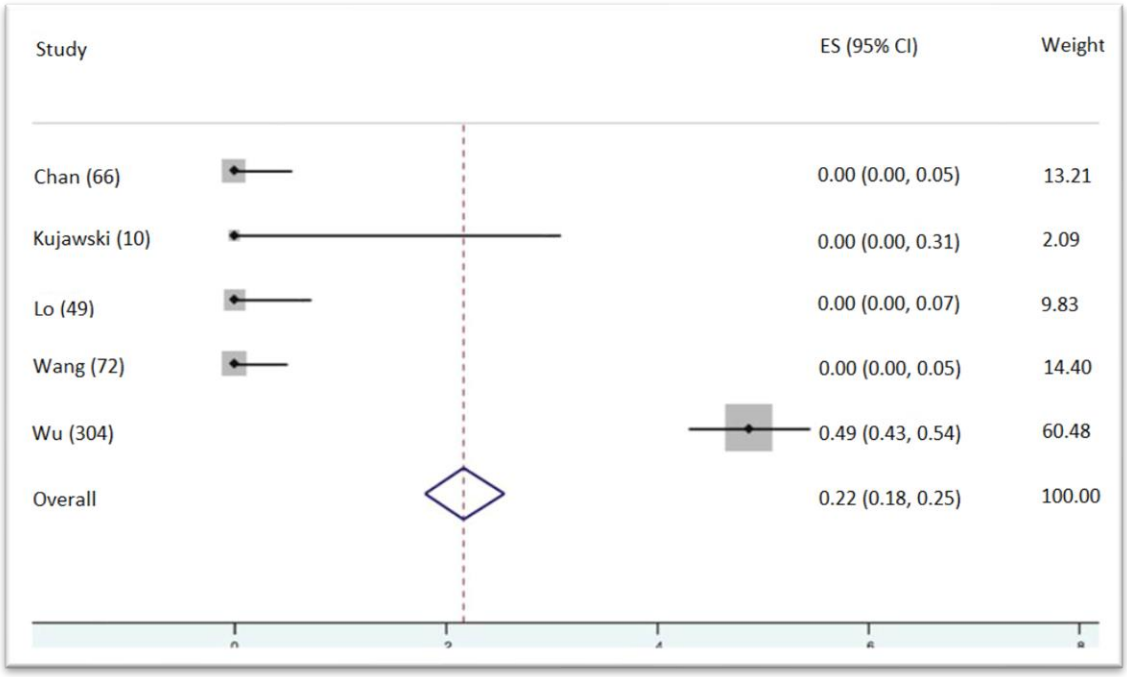

Figure 4: Pooled estimate of the sensitivity of urine specimen from five studies

Table 1: Summary of results of individual studies

Author	Saliva	Stool/Rectal swab/Anal swab	Blood/Serum/Plasma	Urine
Azzi	25/25			
Chan	97/144	11/66	10/174	0/66
Iwasaki	8/9			
Kujawski		7/10	1/12	0/10
Lo		46/79		0/49
To	11/12			
Wang		44/153	3/307	0/72
Wu		24/244	4/132	148/304

Critical Appraisal

Ideally, to examine the predictive value of RT-PCR on non-respiratory tract specimen to detect SARS-CoV-2 nucleic acid, the test should be performed on suspected cases. However, due to the urgency with which this test would need to be validated, majority of the studies used confirmed cases with a positive SARS-CoV-2 RT-PCR of a respiratory tract specimen – which at the onset of the pandemic, was considered to have the highest diagnostic yield, and pre-COVID samples which are the kind of samples utilized in phase IIC validation studies of diagnostic tests..

Although there was independent definition of the index and reference tests, blinding of independent comparison with the reference standard for study participants was not feasible since they were already patients with laboratory-confirmed disease. Specificity and likelihood ratios, therefore, could not be calculated. Some studies reported on the correlation of disease severity and timing of specimen collection with the diagnostic yield. However, this was not done with majority of the studies. Over-all, these studies are deemed to have a high risk for bias.

The methodologies of these studies are easily reproducible. All studies employed standardized methods of specimen storage, nucleic acid extraction and RT-PCR assays.

DISCUSSION

Pooled Sensitivity

Testing for COVID-19 currently makes use of NPS swab to detect for the presence of SARS-CoV-2 nucleic acid. This is based on previous experience with the MERS-CoV and SARS-CoV epidemics. However, this method of specimen collection has been a subject of discussion since it is uncomfortable to the patient and puts the health care worker collecting the specimen at an increased risk of exposure.

The pooled sensitivity of non-respiratory tract specimens of confirmed COVID-19 patients were as follows: Saliva 77%, stool/rectal swab/anal swab 22%, blood/serum/plasma 2% and urine 22%. However, there is significant heterogeneity in between studies which are expected in studies on diagnostic accuracy.

Saliva specimen

One of the most investigated specimens is saliva since patients can easily collect samples by themselves. Studies have shown that SARS-CoV-2 RT-PCR of saliva has been evaluated to have sensitivity comparable or higher than NPS.[5,6] Based on our review, we were able to get a pooled sensitivity of saliva at 77% (95% CI 71-83%) from four studies. The timing of specimen collection and severity of disease were heterogenous in these studies. Azzi et al. studied patients with severe disease. Majority of patients in the study of Chan et al. had stable medical condition. Iwasaki et al. included patients with mild to moderate disease treated with favipiravir, and while the disease status of the population studied by To et al. was not described, all patients were hospitalized.

Three of the four studies reported that saliva has a sensitivity of more than 90%, while only Chan showed a lower sensitivity of 67%. This can be attributed to Chan's methodology where they used two different RT-PCR assays – COVID-19-RdRp/Hel and RdRp-P2 probes – and where the study's results showed that there is significant difference between the two assays favoring COVID-19-RdRp/Hel assay ($p<0.001$). If Chan's study was excluded in the pooled estimation of sensitivity, we would yield an estimated sensitivity of 98% (95% CI 90-100%). More studies would be needed in order to correct

the heterogeneity brought about by this difference in methodology.

Stool/rectal swab/anal swab specimen

The pooled sensitivity of detecting SARS-CoV-2 from stool/rectal swab/anal swab specimens from five studies was determined to be 22% (95% CI 22-37%). In the study by Kujawski et al., only three out of 12 patients reported to have diarrhea, and two patients with vomiting. Lo et al. reported that 80% of patients had diarrhea and 50% with nausea. Like saliva, the timing of specimen collection, severity of illness and treatment given were factors that may be causes of heterogeneity. In the study by Wang et al., live SARS-CoV-2 virus was observed in the stool sample of two patients who did not have diarrhea. Transmission via exposure to fecal material is yet to be established. The utilization of stool SARS-CoV-2 RT-PCR as aide in the decision for hospital discharge or discontinuation of self-quarantine has not yet been evaluated.

Blood/serum/plasma specimen

SARS-CoV-2 nucleic acid had also been detected in blood/serum/plasma specimen. However, this has not been correlated with viremia, severity of illness, and treatment given. From four studies, the pooled estimate of sensitivity is 2% (95% CI 1-3%) which was the lowest among the non-respiratory specimens. The samples taken were from stored blood of patients which may have affected the yield of the tests.

Urine specimen

Urine specimen had a pooled sensitivity of 22% (95% CI 18-25%) from five studies. However, it is only in the study by Wu et al. that SARS-CoV-2 nucleic acid was actually detected in urine specimens. In this study, 61% of the patients had non-severe (common type) disease, 33% had severe disease and 6% had critical illness. The detection of viral nucleic acid in urine was not correlated with the timing of specimen collection or the patients' disease course. There was also no explanation provided for the paradoxical results.

Correlation of timing of specimen collection and diagnostic yield

Azzi et al. reported that there was no significant difference of the Ct values of the initial saliva specimens sent for SARS-CoV-2 RT-PCR with regards to the period elapsed after the onset of symptoms. Iwasaki et al. reported a median day of sampling of 10 days (range 7-19 days) after onset of symptoms. In this study, when the viral load was correlated with the duration from onset of symptoms to timing of sampling, the viral load was seen to be equivalent between the NPS and saliva samples at earlier time points but declined in saliva at later time points. To et. al. reported that saliva specimens were collected at a median of two days after hospitalization (range 0-7 days). In their cohort of patients, six had viral load analysis of serial saliva specimens. It was seen that the viral load was highest in the earliest available schedule for five patients, and for one patient, viral load was slightly higher on day one after hospitalization than on the specimen taken on the day of admission.

In the study by Kujawski et al., serial testing to determine the duration of viral shedding was done and showed that SARS-CoV-2 nucleic acid was detected at a maximum of 26 days for NPS and OPS, 29 days in sputum, and 25 days in stool. It was reported that the duration of nucleic acid detection from the onset of symptoms did not differ by hospitalization status or supplemental oxygen requirement. All 12 patients in this study reported symptom resolution. The median duration of symptoms was 14 days (range 6-20 days). SARS-CoV-2 RNA was detected after reported symptom resolution in 11 patients who had cough as the last symptom, including six from NPS, two from OPS, one from sputum, and three from stool specimens. Lo et al. also did serial specimen collection in 10 patients to determine duration of viral shedding. Viral RNA was detected in the NPS and stool samples of these patients and the viral RNA conversion time in both NPS and stool were 18.2 days (SD 4.6) and 19.3 days (SD 3.4), respectively. No viral RNA was detected in the serial urine specimens of these patients. In the study by Wu et al, stool and anal swab were analyzed separately. Anal swab revealed a sensitivity of 10%.

Limitations of the study

The timing of specimen collection, severity of disease and treatment given to the study population were vastly heterogenous in these studies and would certainly affect the estimated sensitivity results. Some studies have reported the sensitivity of samples taken from a single person at different points in time from the onset of symptoms to monitor viral shedding in these sites, contributing also to the heterogeneity of the results.

Recommendation from Other Guidelines

The recommendation from the Centers for Disease Control and Prevention as of July 8, 2020 for collection and testing of specimens for SARS-CoV-2 include the following: (1) Nasopharyngeal specimen collected by a health care provider, (2) Oropharyngeal specimen collected by a health care provider, (3) Nasal mid-turbinate swab collected by a healthcare provider or a supervised onsite self-collection (using a flocked tapered swab) (4) Anterior nares (nasal swab) specimen collected by a healthcare provider or by onsite or home self-collection (using a flocked or spun polyester swab) (5) Nasopharyngeal wash/aspirate or nasal wash/ aspirate specimen collected by a health care provider.

CONCLUSION

The pooled sensitivity of detecting SARS-CoV-2 nucleic acid in non-respiratory tract specimens of patients was highest for saliva at 77% (95%CI 71-83%). However, the pooled sensitivity was unacceptably low for stool/rectal swab/anal swab 22% (95% CI 22-37%), blood/serum/plasma 2% (95% CI 1-3%), and urine 22% (95% CI 18-25%).

DECLARATION OF CONFLICT OF INTEREST

No conflict of interest.

REFERENCES

1. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19): A Review. JAMA. 2020 Aug 25;324(8):782-793.
2. World Health Organization. (2020). Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases: interim guidance, 2 March 2020. World Health Organization. https://apps.who.int/iris/handle/10665/331329
3. Mohammadi A, Esmaeilzadeh E, Li Y, Bosch RJ, Li J. SARS-CoV-2 Detection in Different Respiratory Sites: A Systematic Review and Meta-Analysis. medRxiv [Preprint]. 2020 May 19:2020.05.14.20102038. doi: 10.1101/2020.05.14.20102038. Update in: EBioMedicine. 2020 Jul 18;102903.
4. Zheng S, Fan J, Yu F, Feng B, Lou B, Zou Q, et. al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. BMJ. 2020 Apr 21;369:m1443.
5. To KK, Tsang OT, Yip CC, Chan KH, Wu TC, Chan JM, et. al. Consistent Detection of 2019 Novel Coronavirus in Saliva. Clin Infect Dis. 2020 Jul 28;71(15):841-843.
6. Azzi L, Carcano G, Gianfagna F, Grossi P, Gasperina DD, Genoni A, et. al. Saliva is a reliable tool to detect SARS-CoV-2. J Infect. 2020 Jul;81(1):e45-e50.
7. Iwasaki S, Fujisawa S, Nakakubo S, Kamada K, Yamashita Y, Fukumoto T, et. al. Comparison of SARS-CoV-2 detection in nasopharyngeal swab and saliva. J Infect. 2020 Aug;81(2):e145-e147.
8. Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19). March 2020. Center for Disease Control and Prevention. Interim Guidelines for Clinical Specimens for COVID-19 | CDC
9. Chan JF, Yip CC, To KK, Tang TH, Wong SC, Leung KH, et. al. Improved Molecular Diagnosis of COVID-19 by the Novel, Highly Sensitive and Specific COVID-19-RdRp/Hel Real-Time Reverse Transcription-PCR Assay Validated In Vitro and with Clinical Specimens. J Clin Microbiol. 2020 Apr 23;58(5):e00310-20.
10. COVID-19 Investigation Team. Clinical and virologic characteristics of the first 12 patients with coronavirus disease 2019 (COVID-19) in the United States. Nat Med. 2020 Jun;26(6):861-868.
11. Lo IL, Lio CF, Cheong HH, Lei CI, Cheong TH, Zhong X, et. al. Evaluation of SARS-CoV-2 RNA shedding in clinical specimens and clinical characteristics of 10 patients with COVID-19 in Macau. Int J Biol Sci. 2020 Mar 15;16(10):1698-1707.
12. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, et. al. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. JAMA. 2020 May 12;323(18):1843-1844.
13. Wu J, Liu J, Li S, Peng Z, Xiao Z, Wang X, et. al. Detection and analysis of nucleic acid in various biological samples of COVID-19 patients. Travel Med Infect Dis. 2020 Sep-Oct;37:101673.

Appendix 1: Characteristics of included studies

First Author Article Title Month-Year Country	Study Design	Sample Population	Intervention	Outcome Measured	Population Characteristics	Study Results
Azzi, Lorenzo Saliva is a reliable tool to detect SARS-CoV-2 Apr-20 Italy	Prospective observational study	25 SARS-CoV-2 infected patients who underwent hospital admission after the diagnosis of COVID-19 provided by rRT-PCR on NPS	Saliva collected through the drooling or pipetting technique, analyzed by rRT-PCR.	Prevalence of positivity in saliva and association between clinical data and the cycle threshold as a semiquantitative indicator of viral load were considered	Male: female ratio 2.1:1; age range of 39-85 years (mean 61.5 years +/- 11.2 years); all were admitted in the ICU; included severe and very severe disease	Positive rate for saliva 25/25 (100%), Ct values (range 18.12–32.23, mean value 27.16 + / − 3.07); no differences in the Ct values with regards to the period elapsed after the onset of symptoms; inverse correlation between the LDH values recorded and the Ct values (p=0.04); no significant correlation between usRCT and the Ct values (p=0.07); Ct values were not influenced by the patient's age (p=0.34), sex (p=0.31) or comorbidities; Eight patients underwent a second salivary swab after 4 days and results were consistent with the initial analysis.
Chan, Jasper Fuk-Woo Improved Molecular Diagnosis of COVID-19 by the Novel, Highly Sensitive and Specific COVID-19-RdRp/Hel Real-Time Reverse Transcription-PCR Assay Validated In Vitro and with Clinical Specimens May-20 China	Prospective observational cohort study	15 patients with laboratory-confirmed COVID-19 in Hong Kong whose NPA/NPS/TS, and/or sputum specimens tested positive for SARS-CoV-2 RNA by the RdRp2 assay	120 respiratory tract (NPA/NPS, TS, saliva, and sputum) and 153 non-respiratory tract specimens (plasma, urine, feces/rectal swabs) were collected and sent for COVID-19-RdRp/Hel and RdRp-P2 assays	Comparison between the COVID-19-RdRp/Hel and RdRp-P2 real-time RT-PCR assays for the detection of SARS-CoV-2 RNA in different types of clinical specimens	Male:female ratio of 1:1.4; age range of 37-75 years (median 63 years); all had clinical features of acute community-acquired atypical pneumonia and radiological evidence of ground-glass lung opacities; 11 were in stable condition, 3 in critical condition, 1 expired	Among 273 specimens collected from these 15 patients, 77 (77/273, 28.2%) were positive by the RdRp-P2 assay; COVID-19-RdRp/Hel assay were positive for all these 77 patients, in addition to 42 other specimens including 29/120 (24.2%) respiratory tract specimens, and 13/153 (8.5%) non-respiratory tract specimens that were negative in the RdRp-P2 assay (119/273, 43.6%) (p < 0.001)
Iwasaki, Sumio Comparison of SARS-CoV-2 detection in nasopharyngeal swab and saliva	Prospective observational cohort study	9 COVID-19 patients diagnosed by a positive NPS SARS-CoV-2 RT-PCR	Paired nasopharyngeal swab and saliva samples were taken and sent for RT-qPCR	Comparison of the efficacy of PCR detection of SARS-CoV-2 between paired NPS and saliva samples	Median age 70.5years (range 30-97 years); most had mild to moderate disease; all patients received favipiravir	Specimens were sampled within 10 days (range, 7-19 days) after symptom onset. SARS-CoV-2 was detected in all 9 patients in nasopharyngeal samples and in 8/9 (89%) patients in

May-20 Japan			when symptoms were relieved to determine the timing of discharge			saliva samples. The mean ± SD of the CT values were 24.2 ± 4.4 and 30.4 ± 4.9 in nasopharyngeal and saliva samples, respectively, and significantly higher in saliva samples (P=0.018). The CT values were equivalent between the two samples at earlier time points but higher in saliva at later time points; All 11 samples taken within 2 weeks from the onset of symptoms were positive in both NPS and saliva. After 2 weeks, some samples tested negative.
Kujawski, Stephanie A First 12 patients with coronavirus disease 2019 (COVID-19) in the United States Mar-20 USA	Prospective observational study	12 patients diagnosed with COVID-19 who were confirmed by CDC during Jan 20- Feb 5,2020 by a positive SARS-CoV-2 rRT-PCR in >/= 1 respiratory tract specimen (NP, OP or sputum)	Respiratory, stool, serum, and urine specimens were submitted for SARS-CoV-2 rRT-PCR testing every 2-3 days for the first 17 days of illness for SARS-CoV-2 virologic testing	Report the epidemiology, clinical course, clinical management and virologic characteristics of the first 12 patients with COVID-19 diagnosed in the US	5 patients received only out-patient care and were isolated at home, 7 were hospitalized; male: female ratio of 1.5:1; median age 53 years (range 21-68 years); 4/5 patients with >/= 1 underlying medical conditions were hospitalized; 10 patients travelled to mainland China 2 weeks before onset of illness, 2 other patients reported exposure with a previously infected patient with COVID-19; Over the course of illness, patients reported cough (n=12), subjective or measured fever (n=9), diarrhea (n=3), and vomiting (n=2). Three patients who did not report fever were never hospitalized and remained on home isolation.	398 specimens were collected and tested from the 12 patients throughout the course of illness. All 12 patients had SARS-CoV-2 RNA detected in at least one NP swab, 11/12 in an OP swab, 6/6 in sputum, 1/12 in serum, 7/10 in stool, and 0/10 in urine (Figure 3). Among 98 pairs of simultaneous NP and OP specimens, 58 (59%) had concordant results. Among 27 discordant pairs with one positive specimen, the NP specimen was positive in 70%; the remaining 13 discordant pairs had one negative and one inconclusive specimen. Two patients provided sputum specimens when NP and/or OP specimens tested negative, and sputum continued to be positive in both patients. In Patient 7, viral RNA was detected in sputum 17 days after the last positive OP specimen and ≥2 weeks after reported symptom resolution. In seven patients who had SARS-CoV-2 RNA detected in stool, most detections occurred when viral RNA was still detectable in the respiratory tract. Among three patients who reported diarrhea, all had viral RNA detected in stool. Mean Ct

						values in positive specimens were 17.0–39.0 for NP, 22.1–39.7 for OP, and 24.1–39.4 for stool. Ct values were lower in the first week of illness than the second in most patients; in some patients, low Ct values continued into the 2nd and 3rd week of illness. There was no apparent relationship between Ct values in the upper respiratory tract and disease progression. SARS-CoV-2 rRT-PCR results turned positive in serum of Patient 9 in the second week of illness at the time of rapid clinical deterioration; Serial testing to determine duration of RNA detection and viral shedding. SARS-CoV-2 RNA has been detected at a maximum of day 26 in NP specimens, day 26 in OP, day 29 in sputum, and day 25 in stool. The duration of viral RNA detection did not differ by hospitalization status or supplemental oxygen requirement.
Lo, Iek Long Evaluation of SARS-CoV-2 RNA shedding in clinical specimens and clinical characteristics of 10 patients with COVID-19 in Macau Feb-20 China	Retrospective observational study	Ten COVID-19 patients enrolled in the Centro Hospitalar Conde de São Januário (CHCSJ) between Jan 21-Feb 16, 20, who were diagnosed through detected RNA signals in NPS and sputum specimen	Serial qRT-PCR for SARS-CoV-2 were performed for different specimens, including NPS, urine, and stool	Evaluation of SARS-CoV-2 RNA shedding in clinical specimens and clinical characteristics	Male: female ratio 1: 2.3; median age 54 years (range 27-64 years); 5 patients had comorbid medical conditions; 2 had mild disease, 4 had moderate and another 4 had severe disease; all patients received treatment with lopinavir and ritonavir	There were positive SARS-CoV-2 RNA signals in all patients' NPS (100%) and stool specimens (100%) but negative in all urine specimens (0%). The average viral RNA conversion time in both NPS and feces were 18.2 days (SD:4.6) and 19.3 days (SD:3.4), respectively.
To, Kelvin Kai-Wang Consistent Detection of 2019 Novel Coronavirus in Saliva	Prospective observational study	12 patients with laboratory-confirmed 2019-nCoV infection by a positive NPS or sputum SARS-	Saliva were collected for SARS-CoV-2 RT-PCR	Detection of SARS-CoV-2 nucleic acid in saliva	Male: female ratio of 1.4:1 ; median age of 62.5 years (range 37-75 years); all were hospitalized	Saliva specimens were collected at a median of 2 days after hospitalization (range 0-7 days); SARS-CoV-2 nucleic acid was detected in the initial saliva specimens of 11 patients (91.7%)

Feb-20 China		CoV-2 RT-PCR, in Hong Kong				
Wang, Wenling Detection of SARS-CoV-2 in Different Types of Clinical Specimens Mar-20 China	Retrospective observational study	205 patients with COVID-19 diagnosed based on symptoms and radiology and confirmed by SARS-CoV-2 detection in NPS	Pharyngeal swabs were collected from most patients 1 -3 days after hospital admission. Blood, sputum, feces, urine, and nasal samples were collected throughout the illness. Bronchoalveolar lavage fluid and fibrobronchoscope brush biopsy were sampled from patients with severe illness or undergoing mechanical ventilation. Specimens were sent for SARS-CoV-2 RT-PCR	Detection of SARS-CoV-2 in different types of clinical specimens	68% were male; mean age 44 years (range 5-67 years); 19% had severe illness	Bronchoalveolar lavage fluid specimens showed the highest positive rates (14 of 15;93%), followed by sputum (72 of 104; 72%), nasal swabs (5 of 8; 63%), fibrobronchoscope brush biopsy (6 of 13; 46%), pharyngeal swabs (126 of 398; 32%), feces (44 of 153; 29%), and blood (3 of 307; 1%). None of the 72 urine specimens tested positive
Wu, Jianguo Detection and analysis of nucleic acid in various biological samples of COVID-19 patients Apr-20 China	Retrospective observational cohort study	132 patients diagnosed with COVID-19 in East Section of Renmin Hospital of Wuhan University from Jan 31-Feb 29, 20, in accordance with relevant epidemiological and clinical manifestations and a positive SARS-CoV-2 RT-PCR	Nasopharyngeal swabs, sputum, blood, feces and anal swabs were sent for 2019-nCoV nucleic acid detection	Detection and analysis of nucleic acid in various biological samples of COVID-19 patients	Male: female ratio of 1.2:1; mean age of 66.7 years +/- 9.1 years; 33% had severe disease, 6% had were critical cases	Positive rate of 2019-nCoV nucleic acid test of oropharyngeal swab is 38.13% (180/472 times), the positive rate of 2019-nCoV nucleic acid test of sputum is 48.68% (148/304 times), the positive rate of blood 2019-nCoV nucleic acid test is 3.03% (4/132 times), and the positive rate of 2019-nCoV nucleic acid test of feces is 0.83% (24/244 times) The positive rate of 2019-nCoV nucleic acid detection in anal swabs is 10.00%（12/120 times) Positive rates of 2019-nCoV nucleic acid test were determined from all specimen types

Appendix 2:

Table 1: Summary of results of individual studies

Author	Saliva	Stool/Rectal swab/Anal swab	Blood/Serum/Plasma	Urine
Azzi	25/25			
Chan	97/144	11/66	10/174	0/66
Iwasaki	8/9			
Kujawski		7/10	1/12	0/10
Lo		46/79		0/49
To	11/12			
Wang		44/153	3/307	0/72
Wu		24/244	4/132	148/304

Table 2: Summary of pooled sensitivity

Specimen	Sensitivity	95% CI	Number of studies	Number of participants
Saliva	77%	71-83%	4	190
Stool/Rectal swab/Anal swab	22%	22-37%	5	552
Blood/Serum/Plasma	2%	1-3%	4	625
Urine	22%	18-25%	5	501

Appendix 3:

Literature search

DATABASE	SEARCH STRATEGY / SEARCH TERMS	DATE AND TIME OF SEARCH	RESULTS	
			Yield	Eligible
Medline	Search (("Coronavirus Infections"[Mesh] OR "Coronavirus"[Mesh] OR coronavirus OR novel coronavirus OR NCOV OR "COVID-19" [Supplementary Concept] OR covid19 OR covid 19 OR covid-19 OR "severe acute respiratory syndrome coronavirus 2" [Supplementary Concept] OR severe acute respiratory syndrome coronavirus 2 OR SARS2 OR SARS 2 OR SARS COV2 OR SARS COV 2 OR SARS-COV-2)) AND nasopharyngeal AND oropharyngeal AND swabs AND respiratory sample	May 21,2020 21:00:00	24 (23 observational studies, 1 meta-analysis)	6
Cochrane Library	COVID-19 AND nasopharyngeal AND oropharyngeal AND swabs AND respiratory sample	May 22, 2020 14:00:00	1	0
MedRixv	COVID-19 AND nasopharyngeal AND oropharyngeal AND swabs AND respiratory sample	May 21, 2020 22:00:00	105 including 2 meta-analysis	2